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Foreword

THE FOOD AND NUTRITION BOARD, while primarily concerned over the years with nutritional problems in the United States, has manifested its increasing interest in such problems throughout the world by the organization of the Committee on Protein Malnutrition and, more recently, the Committee on International Nutrition Programs.

THE COMMITTEE ON PROTEIN MALNUTRITION was created to organize and supervise a world-wide research program on high-protein foods for the improvement of the nutrition of populations, particularly of growing children, in food-deficient countries. The work of the Committee has been supported by a grant of $550,000 from the Rockefeller Foundation. An important factor in the progress of the Committee's program has been an allocation of $300,000 by the Executive Board of UNICEF for laboratory tests, process development, and support of acceptability trials of protein-rich foods.

The great progress made in research on protein foods as a result of the work supported by the Committee in many different parts of the world made it desirable to convene this conference for the purpose of reviewing the present state of our knowledge, exchanging information and ideas between research workers, surveying the areas of greatest promise for future work, and planning both new research activities and the practical utilization of present knowledge.

Paralleling in the United States the world-wide concern of the Committee on Protein Malnutrition is the work of the Board's Committees on Infant Nutrition and on Amino Acids. Both of these committees participated in and profited greatly from this conference.

The Food and Nutrition Board is most grateful to the National Institutes of Health for the support (Grant A-3844) and participation of the Nutrition Study Section which made possible this important conference and the opportunity it provides for evaluating the progress made through the cooperative efforts of a notable group of workers and organizations, and for glimpsing the areas where further work is needed.

The responsibility for editing of the Proceedings was assigned to LeRoy Voris, Executive Secretary of the Food and Nutrition Board, who has had the able assistance of Mrs. Rose Hardy, Mrs. Jeanette Pelcovits, and Mrs. Marianna Nelson.

Glossary of Abbreviations

BV	**Biological value**
CD	**Coefficient of digestibility**
CS	**Chemical score**
Hb	**Hemoglobin**
INCAP	**Institute of Nutrition for Central America and Panama**
IWL	**Insensible weight loss**
LPC	**Leaf protein concentrate**
MCH	**Mean corpuscular hemoglobin**
NAS-NRC	**National Academy of Sciences—National Research Council**
NBI	**Nitrogen balance index**
NPU	**Net protein utilization**
PBI	**Protein-bound iodine**
PER	**Protein efficiency ratio**
PM	**Protein malnutrition**
PRP	**Provisional reference protein**
PS	**Protein score**
SD	**Standard deviation**
SPA	**Safe practical allowance**

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The Use of Soy Products in the Treatment of Protein Malnutrition

J. E. Dutra de Oliveira, Norberto de Oliveira Netto, Luiz Scatena, Geraldo G. Duarte and Renato J. Woiski

THE PRESENT REPORT deals with the results of treatment of children with protein malnutrition, receiving initially a diet having soy milk as its only protein supplement and later changed to one having 75% of its protein content derived from soy milk, soy flour and ground common beans, and the remaining 25% from the usual food of the children's home diet.

The results of this treatment were to be compared with those from a similar group of children treated initially with skim milk and afterwards receiving 75% of the diet protein content from skim milk, meat and eggs and the 25% from the same food as in the preceding group.

A third group of children was to start the treatment with skim milk and, in a second period of treatment, to be changed to a vegetable protein diet as in the first group (soy milk, soy flour, ground beans and home diet). It was thought to be of interest to see if the children who would not respond to the initial treatment with vegetable milk could be treated initially with animal milk and then changed to a mixed vegetable diet to consolidate the cure.

We consider our project as starting in November 1959, although some preliminary studies have been done before.

As we have studied only 14 children with protein malnutrition under this project, we do not believe that we could draw general conclusions from our results. We intend to explain our plan of study and show the results and we hope to have them fully discussed. The general plan of study is shown in table 1, with the balance periods indicated by black blocks.

PLAN OF STUDY

The children selected for our studies were from 12 to 48 months of age, all suffering from protein malnutrition.

We consider our children as belonging to 2 major groups: 1) severe malnutrition, mainly with protein deficiency, with a fair caloric intake (kwashiorkor or

TABLE 1
PLAN OF STUDY

GROUPS	MILK PERIOD	MIXED DIET PERIOD
GROUP I	SOYMILK	BASIC DIET + Soyflour, Ground Beans, Soymilk
GROUP II	COW'S MILK	BASIC DIET + Cow's Milk, Meat, Egg
GROUP III	COW'S MILK	BASIC DIET + Soyflour, Ground Beans, Soymilk

21 days 21 days

pluricarencial hydropigenic dystrophy); 2) global malnutrition, with low caloric and protein intake (marasmus).

In this report both groups will be presented together. Their diagnosis is shown in table 2.

TABLE 2
SUMMARY OF SEX, AGE, DIAGNOSIS AND TREATMENT OF CHILDREN

Subject	Case	Sex	Age (months)	Diagnosis	Observations
			GROUP I. VEGETABLE PROTEIN		
JD	1	M	19	Kwashiorkor	
RF	2	F	21	Marasmus	"Recuperation syndrome" on 23rd day
MAC	3	F	26	Kwashiorkor	
MLD	4	F	15	Kwashiorkor	
			GROUP II. ANIMAL PROTEIN		
JM	5	M	14	Marasmus	
JCV	6	M	19	Kwashiorkor	
RB	7	M	13	Kwashiorkor	
SAA	8	F	21	Kwashiorkor	Blood transfusion on 14th day
			GROUP III. ANIMAL PROTEIN-VEGETABLE PROTEIN		
AL	9	M	32	Kwashiorkor	
CAA	10	M	15	Marasmus	
HA	11	M	17	Marasmus	
AF	12	F	21	Kwashiorkor	
VF	13	F	28	Kwashiorkor	Blood transfusion on 12th, 13th and 14th days
WTB	14	F	48	Kwashiorkor	

To standardize the diagnosis, we classified the clinical and laboratory data according to their importance as major and minor signs of malnutrition, as follows:

Major: dietary history showing protein deficiency; skin lesions; reduction of subcutaneous tissue; muscular atrophy; low serum albumin (below 2.5 gm %).

Minor: low weight (below 30% of a Brazilian table of Emma de Azevedo;) low height (6 months less than actual age, according to the same table); underdevelopment of the neuro-motor system; hair changes; diarrhea; loss of appetite.

A child to be admitted for study must show at least two major and three minor signs.

When admitted, the child entered a metabolic unit, air conditioned, under strict supervision. A special kitchen was established to prepare food for the children in the metabolic unit only.

During the first 24 hours a thorough physical examination was performed; the collection of material for laboratory tests was started. The children received an electrolyte solution on the basis of 100 ml/kg/day and a caloric intake of 100 cal/kg/day, from a calculated amount of carbohydrates (dextrimaltose). As a prophylactic measure, during the first 10 days all of them received penicillin and sulfaguanidine. A daily supplement of vitamins (4,800 IU vitamin A, 960 IU vitamin D_2, 4.8 mg vitamin B_1, 0.84 mg vitamin B_2, 48 mg vitamin C, 12 mg niacinamide) and iron (as ammonium citrate 10% solution, 2 ml/kg/day) was given during the whole period of study.

As criteria for following the response to the treatment, we observed the following items: a) improvement of humour and social contact; b) increase of diuresis or loss of edema; c) improvement of muscle tonus and healing of skin lesions; d) rise of serum albumin level; e) equilibrium or increase of weight.

Data Recorded

- Summary of clinical and biochemical investigation
- Special clinical records
  - Picture: admission-discharge
  - Weight: daily
  - Height: admission-discharge
- RX studies
  - Bone age
- Blood studies
  - Red blood cells, Hemoglobin, Hematocrit — 1-21-46 days after admission
  - Total serum protein, Electrophoresis — 1-11-21-36-48 days after admission
  - Calcium, Phosphorus, Phosphatase, Cholesterol — 1-21-48 days after admission
- Urine studies
  - Creatinine — on balance periods
- Metabolic studies

Nitrogen balances	1st)	7-8-9	days after admission
Calcium balances	2nd)	18-19-20	
Phosphorus balances	3rd)	32-33-34	
Fat absorption	4th)	44-45-46	

Diet

The children were distributed at random in the 3 groups and received the soy or cow's milk diet for 21 days. They received half-strength formula for 10 days, 2/3 formula for 6 days and full-strength formula (3.5 gm % protein) for the last 5 days.

After that period the children were placed on a mixed animal and vegetable protein diet having about the same protein content as was received at the end of the milk period.

The basic portion of the mixed diet (home diet), having 25% of the total protein content, was composed of rice, beans, vegetable pear, pumpkins, tomatoes, ground corn, oil, sugar and banana.

The variable portion, 75% of the total protein content, was composed of animal or vegetable protein in the following proportions:

animal protein:	meat	15%
	eggs	15%
	milk	70%
vegetable protein:	soy flour	15%
	ground beans	15%
	soy milk	70%

Composition of diets. The whole diet was calculated to provide a caloric intake of 80 to 120 cal/kg/day. The composition of the diets follows.

TABLE 3
COMPOSITION OF DIETS

	Mixed Animal Protein Diet*					Mixed Vegetable Protein Diet*				
	Amt gm	Cal-ories	CHO	Protein (in gm)	Fat	Amt gm	Cal-ories	CHO	Protein (in gm)	Fat
BASIC DIET										
Rice	22	77.88	16.83	1.76	0.31	22	77.88	16.83	1.76	0.31
Beans	25	84.00	13.24	5.65	0.61	25	84.00	13.24	5.65	0.61
Vegetable pear	110	41.80	9.35	0.52	0.22	110	41.80	9.35	0.52	0.22
Pumpkin	110	15.07	3.63	0.55	0.11	110	15.07	3.63	0.55	0.11
Tomatoes	54	10.80	1.84	0.54	0.16	54	10.80	1.84	0.54	0.16
Ground corn	11	38.88	8.07	0.86	0.24	11	38.88	8.07	0.86	0.24
Vegetable oil	11	102.30	—	—	11.00	11	102.30	—	—	11.00
Sugar	25	99.45	24.75	—	—	27	107.41	26.73	—	—
Bananas	50	49.50	11.00	0.65	0.30	55	54.45	12.10	0.71	0.33
TOTAL		519.68	88.71	10.53	12.95		532.59	91.79	10.59	12.98
SUPPLEMENT										
Cow's milk powder	60	209.70	28.80	21.60	0.90					
Meat	20.6	23.90	—	4.33	0.62					
Eggs	35	53.69	—	4.33	3.95					
Soy flour						41	179.21	9.34	20.27	6.75
Ground beans						8	26.40	1.79	4.37	0.19
Soy milk powder						19	63.84	10.06	4.29	0.46
TOTAL		287.29	28.80	30.23	5.47		269.45	21.19	28.93	7.40
TOTAL BASIC DIET + TOTAL SUPPLEMENT		806.97	117.51	40.76	18.42		802.04	112.98	39.52	20.38

*Table of G. Franco "Tabela de composição química dos alimentos." Rio de Janeiro

To have a more uniform product to deal with, we prepared a batch of soy milk dry powder in one of the spray dry milk plants near Ribeirao Preto.

We soaked the soybeans in water for 8 to 10 hours to remove the husk, then

ground them in a common electric meat grinder. They were then mixed with water and steam-cooked for 10 to 15 minutes. After squeezing through a cloth filter, the product went through the same process as for cow's milk to obtain the dry powder. It was canned and kept in a refrigerator until needed.

Both the soy milk and the cow's milk were reconstituted to have 3.5 gm of protein per 100 ml. A sample of the milk given to the children during each balance period was kept every day in the freezer and analysed for nitrogen. Its calcium, phosphorus and fat content were calculated on the basis of the nitrogen content and previous analyses of the dry powder.

Metabolic Studies

Balance studies of nitrogen, calcium and phosphorus were performed 4 times on each child, lasting 3 days each period. One was done at the end of the half-strength milk formula, one at the end of the full-strength milk formula, and two during the mixed-diet period, one around the 32nd day and the other on the 44th day.

Fat absorption studies were also made during the same balance periods.

A sample of the milk was kept in the freezer every day during the balance periods of the children. It was analysed for nitrogen and the calcium, phosphorus and fat content were calculated.

A duplication of the food given to each child during the balance period was kept in the refrigerator, homogenized in a Waring Blendor and analysed in duplicate for nitrogen (semi-micro Kjeldahl method), calcium (Clark and Collip method), phosphorus (Fiske and Subbarow method) and fat (van de Kamer method).

Urine was collected every balance day, kept in the refrigerator and acidified with acetic acid. The stools were collected in the same container, kept in the refrigerator, homogenized and then analysed. Nitrogen, calcium, phosphorus in feces and urine and fat in stools were determined, using the same methods as for the food.

RESULTS

Analysis of Food

Analyses of cow's milk and soy milk, soy flour and ground beans were made, using the same methods as for our balance studies.

Response to Treatment

Group I. Vegetable protein. Four children were studied in this group, three cases with kwashiorkor and one case with marasmus. They received soy milk feedings during 21 days and later the mixed vegetable protein diet. Their response to the treatment can be summarized as follows:

a) Disappearance of apathy and more interest in the environment became noticeable in two cases between the 2nd and 3rd weeks and in one case only by the end of the 4th week.

b) Edema disappeared in one case at the end of the 1st week and in two others at the end of 3rd and 4th weeks respectively.

TABLE 4
ANALYSIS OF FOOD

	N gm/100 ml	Fat gm/100 ml	Ca mg %	P mg %	Moisture %	Ash %
Skim milk protein reference	5.65	0.55	1161.1	982.5	2.18	7.92
UNICEF Skim milk	5.31	0.47	1188.5	985.6	3.49	8.11
Brazilian Skim milk "A"	5.23	1.18	1217.6	910.6	3.60	7.64
Brazilian Skim milk "B"	5.61	0.68	1241.8	937.7	4.14	7.78
Soy milk dry powder	7.91	16.47	149.7	681.8	4.67	6.64
	%	%				
Soy flour "incobrasa"	7.53	1.11	178.6	555.8		
Ground beans	4.25	1.44	87.2	533.7		

c) Improvement of muscle tonus was observed from the 5th week.

d) Levels of serum albumin showed a tendency to increase from the 2nd determination (end of half-strength formula period), except for one case in which this happened at the end of the full-strength formula period.

e) Weight curves approximated an equilibrium at the 2nd week.

Group II. Animal protein. Four children were included in this group, three with kwashiorkor and one with marasmus. They received cow's milk formula during the first 21 days and later the mixed animal protein diet. Their response to the treatment can be summarized as follows:

a) Disappearance of apathy and more interest in the environment was noted in two cases by the 3rd week, in two by the beginning of the 4th and 5th weeks, and in the other cases by the 40th day.

b) Edema disappeared between the 1st and 3rd weeks.

c) Skin lesions, shown by one case, started to heal at the end of the 1st week.

d) Serum albumin showed increase at the end of the half-strength formula period, except in one case.

e) Weight curves approximated an equilibrium in three cases from the 2nd week and in the other from the 3rd week of treatment.

Group III. Animal protein-vegetable protein. Six children were included in this group, four with kwashiorkor and two with marasmus. They received skim cow's milk during the first 21 days and later the mixed vegetable protein diet. Their response to the treatment can be summarized as follows:

a) Disappearance of apathy and increased interest in the environment were noticeable in three cases in the 3rd week and in the others before the end of the 4th week.

b) Edema disappeared in the 2nd week in two cases. In two cases this happened at the beginning of the 2nd week.

c) Improvement of muscle tonus was observed between the 3rd and 6th weeks. Two cases having skin lesions started the healing, one at the 1st week and the other between the 4th and 5th weeks.

d) Serum albumin levels were increasing at the end of the half-strength formula period. In one case the initial value was lost.

e) Equilibrium of weight started in the 2nd week in four cases, and in two cases in the 4th week.

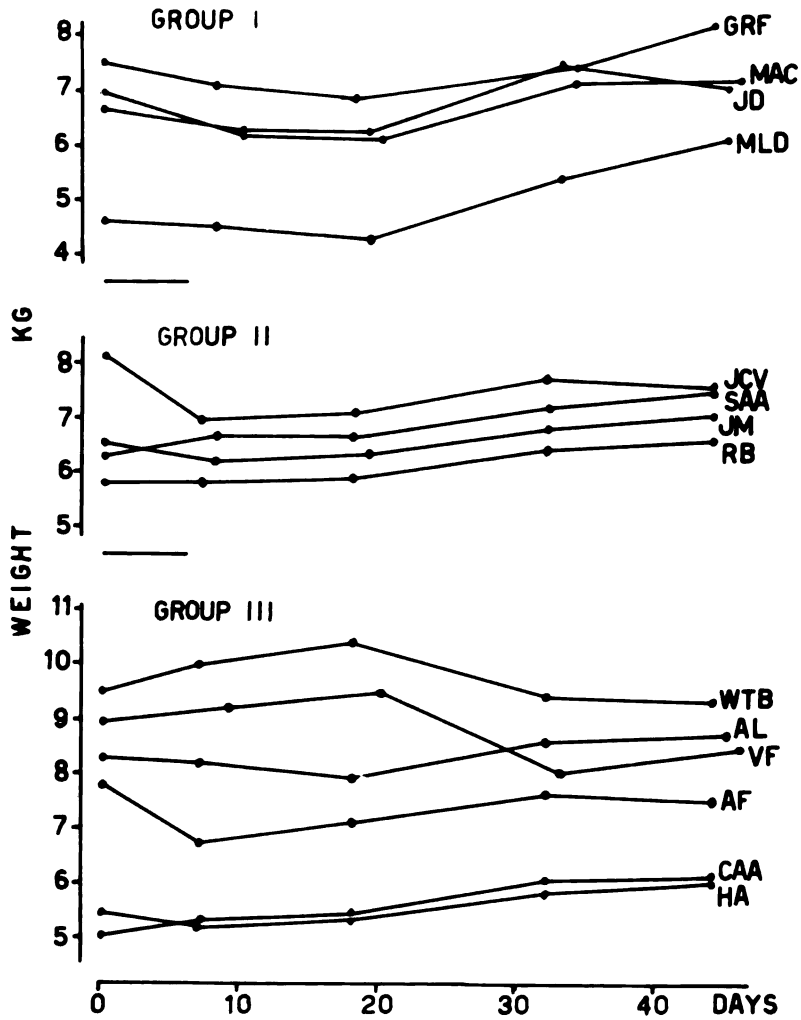

Figure 1—Weight curves. The points represent the weight at admission and just prior to each balance period.

DATA

Weight curves are given in figure 1, electrophoresis studies in figure 2, nitrogen balance results in figure 3, creatinine excretion results in figure 4.

Detailed data, too extensive for publication, have been furnished the Committee on Protein Malnutrition. As indicated earlier, we have studied only 14

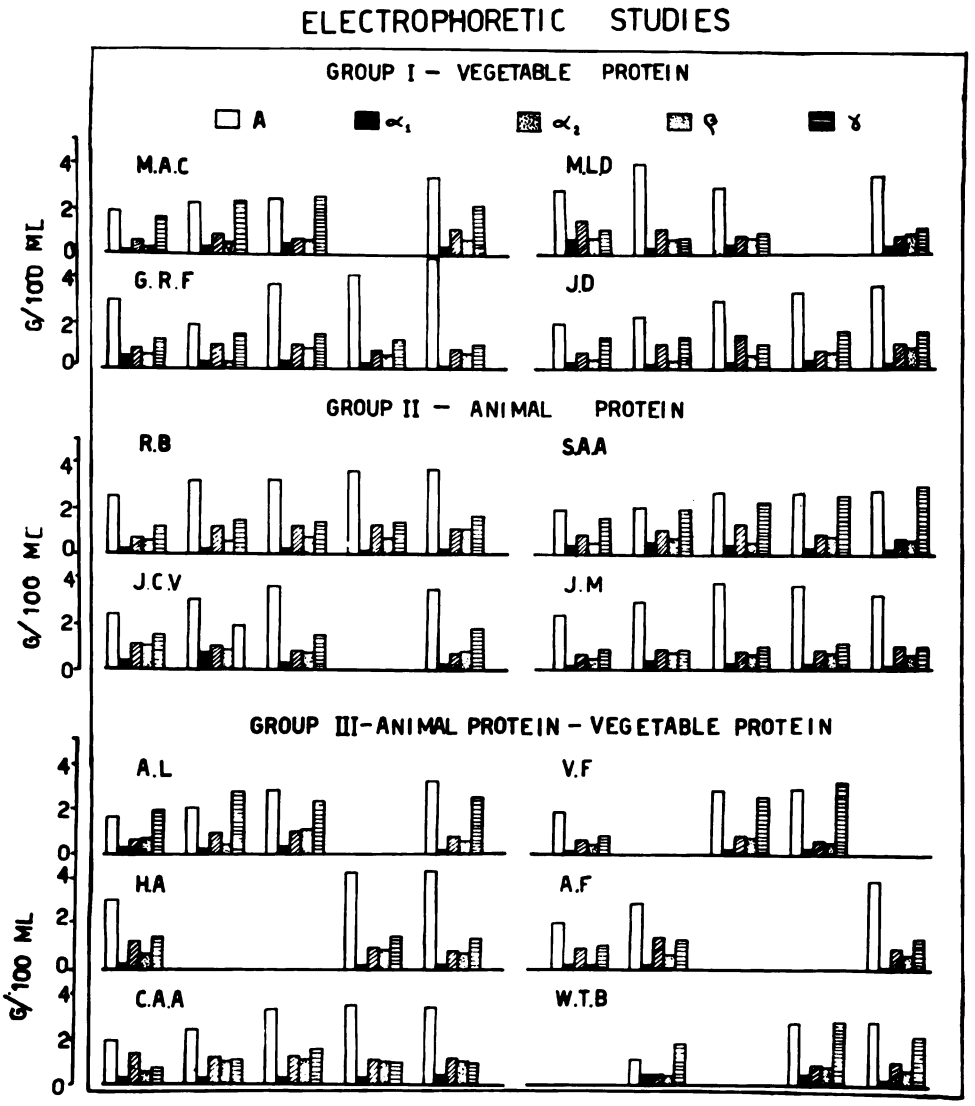

Figure 2—Electrophoretic pattern of the children in the 3 groups. Each child was supposed to have electrophoresis studies at admission and after each one of the 4 balance periods. Clear spaces mean that samples were lost.

children and the results do not lend themselves to interpretation in favor of any of the 3 groups. The results do indicate that combinations of animal and vegetable proteins are practical for the treatment of kwashiorkor and marasmus.

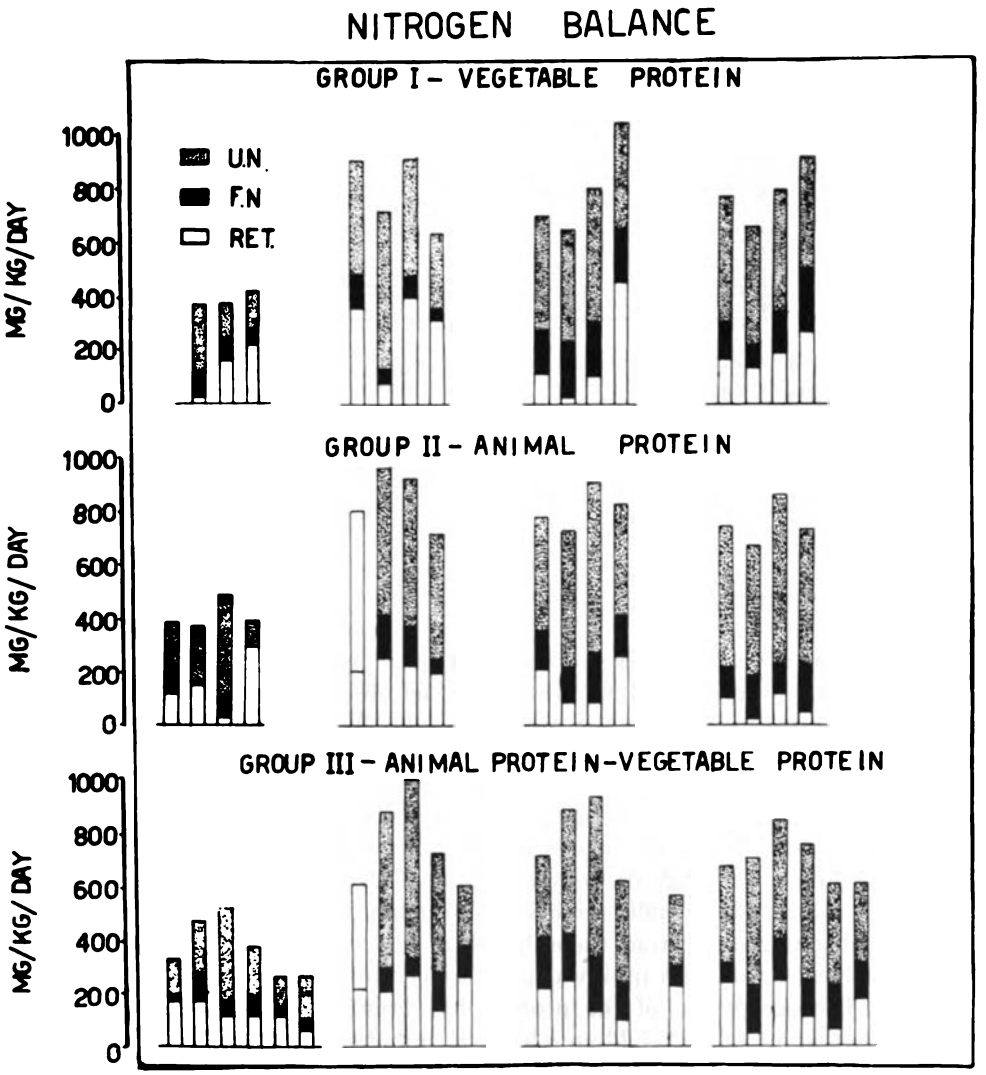

Figure 3—The columns correspond to each child in the same order as they are in table 2. Each group of columns represents one of the 4 successive balance periods. In group 1 the first balance of subject 1 was lost. When the upper part of the column is clear it is because there was some mixture of urine and feces. The height of the column represents the intake of nitrogen.

U.N.—Urinary nitrogen F.N.—Fecal nitrogen Ret.—Nitrogen retention

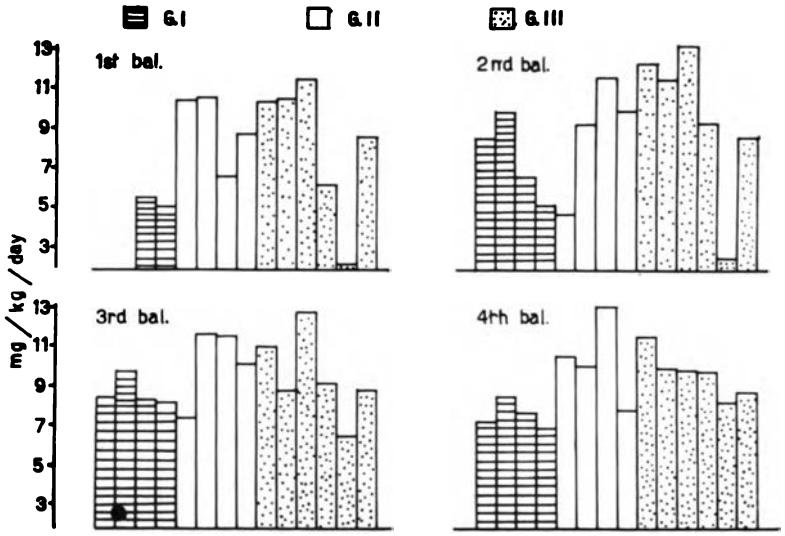

Figure 4—Urinary creatinine excretion. The columns represent the excretion in mg/kg/day of each child.

DISCUSSION

DR. HUNDLEY: I would like to ask Dr. Dutra: What kind of soy milk was it? Was it an enriched preparation? Did he observe any particular difficulties in the acceptability of the product by the children—intolerance, diarrhea, or other manifestations?

DR. DUTRA: We used a soy spray-dried milk that we made in Brazil in one of our cow's milk plants. It was made by the same methods we recommended for homes or hospitals, only on a larger scale. The soybeans were soaked in water and the hulls removed. The dehulled beans were ground, heated and squeezed to extract the milk, which was pasteurized by the same process as cow's milk. This milk was not enriched in any way.

The acceptance of the product has been quite good among children. Even some of our children who were initially treated with cow's milk and subsequently changed to soy milk accepted it in the same way as the cow's milk after drinking it one or two times.

No problems of diarrhea or other intolerance were encountered in the children receiving soy milk. It has also been our impression that the edema in some of these children disappears faster than in children receiving cow's milk, possibly due to the low sodium content of the soy milk.

DR. GOPALAN: Did you find generally that abdominal distention is somewhat greater in cases treated with vegetable diets as against cases being treated with milk?

DR. DUTRA: Concerning the abdominal distention, we did not see much difference between animal and vegetable protein treatment. In general, all of our children have this abdominal distention at the end of the treatment.

DR. RAO: I would like to ask you as a matter of clarification exactly the difference between marasmus and kwashiorkor. This question has existed since 1952. Some of us would be happy to hear your own observations on the clinical difference between marasmus and kwashiorkor.

DR. DUTRA: In Brazil, the terms marasmus and kwashiorkor are not used very much. We believe there is a multiple deficiency in both cases. Kwashiorkor is a protein malnutrition combined with several deficiencies, but mainly protein malnutrition. This is the picture we find in children with edema. Marasmus, which we call a global malnutrition, is also a multiple deficiency, but mainly protein and caloric deficiency. In these cases the children have no edema. We also think it is quite confusing to use ill-defined terms such as marasmic kwashiorkor. Thus we classify this clinical picture in two groups, one considered primarily a protein malnutrition and the other a global malnutrition. Of course, in both groups the malnutrition can be more or less severe.

DR. SREENIVASAN: I did not follow clearly when you made some comment on the extent to which serum albumin is restored in the three groups. Is there any conclusion to suggest?

DR. DUTRA: I do not think we have seen a sufficient number of cases to say that the serum albumin is restored first in the children receiving animal or vegetable protein, as it has been about the same in both groups. In some of them it regenerated faster with animal protein, and in others, more quickly with vegetable protein. There have been some cases where the albumin has never reached a point considered normal, even in children receiving animal protein. Their total serum protein is normal, but there is also an increase of the gamma globulin fraction at the end of the treatment.

Some Plant Proteins Used in the Northeast of Brazil

Nelson Chaves

THE VALUE OF A FOODSTUFF as a source of protein depends upon factors such as the concentration and availability of the protein, its digestibility, its amino acid composition and amino acid correlation and the influence of energy foods—carbohydrates and fats—in the diet.

Besides the chemical and physiological aspects of malnutrition, there are some others like the high cost of animal protein, the increase of population, the rapid industrialization, the abandonment of agriculture in some countries and the difficulty of cattle breeding in the acid soil of the tropical regions. It is not possible to overlook the economic and social point of view in nutrition; it is necessary to consider the foodstuff production, dietary habits, acceptability and the physiological and chemical mechanism together. Nutrition problems are influenced by many factors, including those of environment, and it is not possible to generalize for the whole world from the peculiarities of one region. For example, the milk problem in some countries is due to the absence of significant milk production and in others the major difficulty is transportation.

Dr. Sebrell[18] considering the problem from a general point of view said: "Where milk production and milk conservation are possible and feasible this is a very desirable thing for us to encourage as long as it is possible to do so. Don't forget that there are areas in the world where animals can be raised where there is pasturage, where milk conservation and milk production are poorly managed at the present time. However, there are still other areas where animal grazing and milk production are completely impossible in the foreseeable future."

There is nowadays an increasing interest in the vegetable proteins, not only because of the early knowledge of their chemical and physiological aspects, but also because the economic and social considerations are very close to the nutritional problem. Henry and Kon[9] wrote: "Early concepts of nutrition divided proteins into those of first and those of second class, the former being of animal and the latter of vegetable origin. With increasing knowledge it became evident that the differences between foods as protein sources were reflecting differences in amino acid composition and also that the superiority of 'first class' protein was often

partly due to accompanying vitamins or minerals. We now know that distinction between animal and vegetable protein is neither rigid nor always justified."

In some countries the foodstuff rich in animal protein is still inadequate to the normal requirements, and consequently the use of plant protein must be increased. In Brazil, however, there is a great quantity of animal protein (cattle, milk, eggs and fish) but there is severe protein malnutrition in some areas, like the Northeast. Nowadays cattle breeding is concentrated in South and Central Brazil, where there is no protein malnutrition, but transportation to other regions is very difficult. There are not too many ships and boats, and the roads do not facilitate transportation and distribution. Now, the Brazilian government has developed a plan to improve transportation and communication between the different parts of the country.

The rapidly increasing world population is not in proportion to food production, and this is one of the causes of the increasing undernutrition and famine in the world. Thus, it is necessary to make a great effort to increase the food production in many lands. The following words of Hundley[10] are impressive: "I remind you that the present population of the world is something in the nature of 2.5 billion. It is predicted with considerable certainty that by 1980 its population will increase to 3.5 billion."

Foodstuff production varies in many countries according to different factors, including soil and climate. In general, nutrition problems and malnutrition are less important in cold or temperate climates in comparison with the tropics, where malnutrition is predominant. In many well developed countries with cold and temperate climates there is sometimes another side of the problem—over-nutrition.

There is no agreement on the minimal and physiologically desirable level of protein for human beings. Concerning this problem, King[13] wrote: "We have discussed the protein requirements of age groups in which growth can be used as the criterion of an adequate intake and we must pass on now to consider the requirements of adults, and the criteria by which these requirements should be determined. It may well be that we cannot reach agreement either on a definitive figure for adult requirements, or on the criteria which must form the basis for recommendations put forward. In that case it will be useful to record our differences of opinion as an indication that further work is urgently needed."

One factor that has been well studied is the effect of different carbohydrates on protein metabolism and nitrogen fixation. Harper and Katayama,[8] Womack, Marshall and Parks,[19] Munro[14] and Register and Peterson[17] observed differences in protein metabolism with starches or sugars.

As manioc is one of the most important energy sources in the diet of the Northeast population in Brazil, we undertook a study to investigate the influence of manioc starch on protein metabolism. Manioc is widely cultivated in this region and contributes largely to the caloric intake.

We have observed that at the level of 16% casein (13.5% protein) the protein efficiency has the same value with manioc starch, maize starch or sucrose. With a diet of 16% casein, the liver content of fat and glycogen was the same with the three carbohydrates. However, with a diet of 8% casein (6.5% protein), the fat

infiltration was greater when the manioc starch was the carbohydrate. With the diet of 8% casein, protein efficiency was inferior when manioc was the carbohydrate, in comparison with maize or sucrose.

THE NORTHEAST OF BRAZIL

The Northeast of Brazil is a large region divided according to the climate, soil and flora into forest and "caatinga" regions. The latter are subdivided into "agreste" and "sertao" areas. The forest region is the most populous one, occupying the most extensive part of the arable area. Sugar cane monoculture predominates in this humid and tropical region where malnutrition is most evident. The shortage of milk production is an important feature in the nutrition problem in this region, and it is known that the lack of milk production is one major aspect in the nutrition problem in many countries where people eat principally cereals and other plants. The "caatinga" region is dry, semiarid and reserved to cattle and goat breeding and cereal cultivation (bean, maize, cotton, manioc and some fruits). However, cattle breeding is very difficult in this region, due to water deficiency and periodic droughts. It is known that vast areas of fertile lands in the world are uncultivated because of the lack of water.

The difficulty of breeding cattle and the drought have exerted marked effect on the agriculture and food production in this semiarid region. Due to drought, periodic famines have stimulated emigration to other regions of Brazil (South and Center). Cutting down of trees in all the Northeast had great repercussions on the agriculture and fertility of the soil. We think that this is the principal cause of undernutrition in this part of Brazil. Everybody knows that plants have great importance in the development of agriculture and are the principal protection against erosion and soil exhaustion; on the other hand, photosynthesis is the basis of nutrition for animals and man. Tropical soil, in general acid, must be protected. Decays of civilization have been caused by the destruction of forests. Many lands in the world are infertile, and desert conditions and changes in climate have been brought about by denuding and erosion.

The principal source of protein in the Northeast of Brazil is vegetables, principally beans. Rice, maize, potato and manioc are used, in general, as energy sources.

Several varieties of beans are used, but not the soybean, which is practically unknown to the people in the region. The Mullato bean "feijão mulatinho" (*Phaseolus vulgaris*) and the Macassa "feijão de corda" (*Vigna sinensis*) are the basis of vegetable protein. The bean broth is generally used every day by people of different classes. *Phaseolus vulgaris* is poor in methionine (traces). The acceptability of these beans is excellent and their production is high in Brazil, including the Northeastern region. The black bean is used more in the South and was studied by Luz and Pechnick.[11]

Due to the difficulty of increasing production and distribution of animal protein, it is necessary to investigate the value of many plants as sources of protein

without neglecting the animal sources. We have made in our Institute a few studies on this subject:

1) *Dioclea grandiflora Benth*, used only in very severe droughts and periodic famines. Its protein has a low BV and it is toxic.

2) *Artocarpus intergrifolius*, which is not widely used. The results showed: CD—54.30, BV—92.25, PER—0.97. The composition of the cooked seeds was: protein—14.55%, carbohydrates—57.70%, ash—2.63%, fiber—10.58%.

3) The mixture of salted meat, manioc flour and cooked bean (xarque, farinha e feijão) is a dish peculiar to the poor population in this region, principally in the sugar cane area.

The protein efficiency, digestibility and nitrogen balance of this mixture gave the following results in comparison with industrial casein: CD: mixture—78.0, casein—91.8; BV: mixture—75.8, casein—77.9.

Our principal interest, however, has been the beans that are the chief source of plant protein in Brazil. Coutinho[6] emphasizes that beans are practically the strength of Brazilian people, and Moscoso[13] states that beans are the support of the poor.

Bethlem et al.[1] studied the composition of 50 varieties of beans used in Brazil. In this region the *Phaseolus vulgaris* (feijão mulatinho) and the *Vigna sinensis* (feijão macassa) are the most appreciated.

In order to learn the nutritive value of these beans, we undertook some experimental work with rats. Five experimental studies have been made on *Phaseolus vulgaris*. In one series of experiments, protein efficiency was investigated and the results showed that the proteins of this bean promoted growth in rats but were inferior to industrial casein. Another long-term experiment (6 to 16 months) was undertaken to observe the effect on the liver of a diet with this bean as the sole source of protein. Rats of different ages (21 to 25 days, 49 days and adult) were observed. The results of feeding bean protein at a level of 20% showed liver lesion and light fat infiltration after 6 months in the group aged 21 to 25 days at the beginning of the experiment. In the rats of 49 days, no changes in the liver were observed after 9 months, nor in the adult animals after 16 months of experimentation.

Taking into account the delay of appearance of the liver lesion in the weanling rats, and the life span in comparison with man, we assume that the Mullato bean is not responsible for the great frequency of liver cirrhosis in the region. We repeat, however, that protein deficiency is one very important factor of this disease in the Northeast of Brazil.

We also studied the influence of the protein of this bean upon the hair during three generations. Delay of hair growth was observed in animals fed with the beans as the sole source of protein. With the addition of methionine or casein the hypotrichose disappeared. The effect on the hair was much more intensive in the third generation.

The amino acid content of *Phaseolus vulgaris* is:

	By Paula[15]	By Maximov[12]
Arginine	6.10%	4.89%
Phenylalanine	3.25%	3.25%
Histidine	3.32%	2.62%
Leucine	9.65%	9.65%
Lysine	7.88%	4.58%
Valine	1.04%	

Analysis by paper chromatography in our Institute identified the following amino acids: alanine, arginine, aspartic acid, phenylalanine, glutamic acid, hydroxyproline, histidine, isoleucine, lysine, leucine, serine, proline, tyrosine, threonine, tryptophan, valine.

Another type of bean, the *Vigna sinensis* (macassa, fradinho or feijão de corda) has been studied with rats in our Institute, in comparison with casein at the same level. At the level of 20% this protein supported better growth than the Mullato bean, but was inferior to the casein. The nutritive value was also interesting. Three experiments were undertaken: In the first, the protein efficiency was studied for 35 days; in the second, for 48 days and the animals were observed for 148 days; in the third, the protein efficiency was studied for 59 days and the animals were observed for 104 days. No changes in the hair and no liver injuries were observed. However, the protein efficiency was 82.92% of that of casein. In the second experiment, the gain of weight with Macassa bean was 185.88% and with casein 221.98%. In the experiment with the Mullato bean, the gain of weight was 134% in comparison with 354% for casein. The composition of *Vigna sinensis* as determined (1) by Professor Oswaldo Lima, Escola de Química da Universidade do Receife, and (2) by the Instituto Agronomico de Campinas, Sao Paulo, transcribed by Paula,[16] showed the following percentages:

	(1)	(2)
Moisture	12.252	11.90
Protein	24.500	24.13
Starch	47.200	53.83
Fat	1.777	1.50
Ash	3.594	3.34
Crude fiber and other	10.677	5.30

Analysis by paper chromatography by our Institute showed the following amino acids: Aspartic acid, alanine, arginine, valine, leucine, tyrosine, threonine, histidine, proline, methionine, lysine.

In another very long experiment with Macassa we determined BV of proteins (Mitchell) and the CD.

Twenty Wistar rats (10 male and 10 female) weanling at 25 days were divided into 2 groups of 10 rats each and observed during 37 days. One group was fed a balanced diet with industrial casein at the level of 23.9 (protein 20%) and the other the same diet, but with Macassa proteins instead of casein. All the animals were kept on a nonprotein diet for 8 days in order to determine the endogenous and metabolic nitrogen.

The data showed:

	Casein (10 rats)		Macassa, *Vigna sinensis* (10 rats)	
BV	82.8	± 1.13	82.5	± 1.05
CD	93.3	± 0.35	88.2	± 1.01
PER	2.0	± 0.07	1.3	± 0.07
Plastic value	2.5	± 0.07	1.7	± 0.67
NPU	78.9	± 1.15	72.9	± 1.42

This bean grows well in arid regions and its cycle is about 60 days. It is much used in the dry area (Sertão) and in Salvador, Bahia, where people eat extensively a cake named "*Acaraje*" made with Macassa, oil and pepper. The amino acid composition of "Feijão Macassa" (*Vigna sinensis*) determined by Professor Marcionilo Line, Instituto de Bioquimica da Faculdade de Medicina da Universidade do Recife was (in per cent): leucine 2.40, valine 1.40, methionine 0.60, lysine 1.80, aspartic acid 0.70, threonine 0.22, arginine 0.90, alanine 2.37, histidine 0.71, proline traces.

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Biological Value of Some New Sources of Protein in Mexican Malnourished Children

Silvestre Frenk

THE COMMUNAL EVENTS leading to chronic malnutrition may be well defined in terms of availability, consumption and utilization of nutrients. Whatever procedure is chosen for the study of the nutritional status of the individual or the community, programs should be designed in such a way as to lead to an explanation of the involved factors, which certainly will show particular features varying from one region to another.

Comprehension of protein malnutrition as it occurs in Mexican children may be greatly enhanced by the study of the natural history of this pathological situation. Briefly summarized, this history as observed in the field is as follows.

Newborn babies are equal in height and weight to North American children[1] but, as observed in other developing areas,[2] are precocious in motor development. They maintain this superiority during the first 3 or 4 months of life, during which time they are fed exclusively at the breast.

However, human milk alone seems to be insufficient to maintain an adequate rate of growth beyond this stage;[3] at an age of 4 to 6 months the babies start growing more slowly and to lose their advantage in psychomotor development.[4] Since the phenomenon is general, mothers generally do not pay any attention to it. If told about the need of providing food in addition to breast milk, they will resist. The fear concerning food seems to be a definite cultural characteristic and, among other factors, may have originated as a consequence of a low standard of environmental and personal sanitation; however, because of the prevalence of "premicrobian" concepts about the etiology of disease,[5] this attitude is more often based on the belief that the stomach of the baby cannot digest anything but mother's milk or thin cereal gruels. These same gruels, generally made out of cheap corn or rice flours, are used for weaning, which is generally initiated before the age of one year and completed more or less rapidly, according to the degree of maternal hypogalactia. Also at this stage there is reluctance to use foodstuffs of better nutritional

quality; if cow's milk is used, it generally is provided in minimal amounts and heavily diluted. The same tendency prevails when the child has been completely weaned and immediately starts to partake of the familial diet, in itself of poor composition; in spite of his relatively greater needs, the child is permitted less food than his older siblings and his parents, and this seems to be true for all socio-economic levels. Thus introduced to deficiency, the baby is further deprived of food and his diet reverted to gruels every time he becomes sick; gradually increasing exposure to fecalism, and decreasing resistance against the stress of infection,[6] make episodes of infectious diarrhea more frequent, and with each episode food is drastically reduced or even totally suppressed; in the event of measles infection this regimen may last for 40 days. To "occidental" eyes, it may be inconceivable that, generally, children of one year of age have been subjected to complete starvation for a period equivalent to one month. The results in terms of mortality rates are well known and have been taken as indicators for the assessment of the magnitude of the problem of protein malnutrition in children,[7] although official certifications of deaths still do not show this as the main cause of mortality in small children.[8] It may be foreseen that only patient and continuous hygienic education, by both professional and empiric health workers, and widespread efforts towards sanitation will be able to provide a definite and radical remedy for this situation. In the meantime, emergency measures are urgently needed.

Factors related to the availability of food must also be known in this connection. On one hand, the small-scale peasant of our countries has found the kind of food which is easiest for him to cultivate, in function of climate, quality of the soil, market prices of the seeds, efforts needed for cultivation, time in which the crop is raised, but, above all, its hunger-stilling capacities. Thus, he may have won the "fight against hunger" at the expense of losing the struggle against protein malnutrition. On the other hand, there is a marked tendency of big-scale agriculture in many developing countries to substitute industrially important products for food crops. This phenomenon is illustrated in table 1. It may be seen that cotton, cultivated on 10% of the harvested surface, accounts for 30% of the Mexican

TABLE 1
RELATIVE PROPORTIONS OF CULTIVATED SURFACE AND COMMERCIAL VALUE OF MAIN MEXICAN CROPS
(1955)

	Land harvested (hectares)	%	Market value (US dollars)	%
Total	10,011,324		874,575,400	
Corn	5,371,413	51.25	189,110,161	21.62
Beans	1,187,097	11.33	37,065,373	4.24
Cotton	1,058,990	10.10	263,036,164	30.08
Wheat	799,887	7.63	54,112,787	6.19
Sugar Cane	257,696	2.46	40,195,823	4.59
Other	1,806,170	17.23	291,052,731	33.28

Source: Dirección General de Estadística, Secretaría de Industria y Comercio, República Mexicana.

agricultural income. On the other hand, as suggested by the data in table 2, when industrially important crops decrease, food harvests tend to increase. However,

in a bad year for cotton, production of cottonseed exceeds that of beans, one of the main popular foods in Mexico. So far, no factory exists in this country for the production of cottonseed flour suitable for use by humans.

TABLE 2
PRODUCTION OF CORN, BEANS AND COTTONSEED—MEXICO, 1958-1959
(METRIC TONS)

	1958	1959
Corn	5,276,909	5,563,000*
Beans	509,524	610,000*
Cottonseed	917,082	692,168

* Estimated figures.

Source: Dirección de Economía Agrícola, Secretaría de Agricultura y Ganadería. República Mexicana.

Searching for a solution for the increasing nutritional problems of underprivileged and industrially developing countries, a compromise has to be found by the agencies and investigators involved. New foodstuffs are looked for, which may combine factors related to availability, culture and economy. Accordingly, the problem in the case of the baby before or at the age of weaning may be postulated as follows: 1) While the appropriate re-educational methods are found and put into practice, he will continue to be fed with thin gruels of low nutritional quality; 2) there is a wide-scale potential source of materials like cottonseed flour which, aside from retaining the capability of being used as a gruel, have a good nutritional composition and, being byproducts so far, will possibly be cheap.

Under these premises, assessment of the nutritional efficiency of cottonseed flour was made on suitable children by means of metabolic balance procedures.[9] Nitrogen utilization was studied on 6 children (aged 3 to 5 months), all of them showing the clinical picture of marasmus. They were fed a gruel made from the flour during 16 balance periods of 4 days each. The same children had previously been and were afterwards used for other nitrogen balance studies with different kinds of milk formulas, and were put on the cottonseed gruel at different stages of recovery. The balance technique conformed to accepted procedures; no corrections for fecal metabolic nor endogenous nitrogen were applied.

The cottonseed employed was prepared in Texas and had the following composition, according to the manufacturer: proteins, 57%; lipids, 4.9%; moisture, 4.0%; crude fiber, less than 2.5%; gossypol (free), 0.04%. The gruel was prepared according to the technique used by Mexican mothers, at a concentration of 3% to 6%, in a suspension of 3% corn flour or, in some experiments, of rice flour; sugar was added at a concentration of 15%, and the caloric concentration was 1 cal/cc. A few experiments making up the cottonseed flour in milk were also performed. The average protein intake was 2 to 5 gm of protein/kg/day.

Results of 3 of the individual cases are shown on charts 1 to 9; absorption as per cent of intake and retention as per cent of the intake, as well as the weight curves during the provision of cottonseed flour gruel, may be compared with the periods during which a milk formula was given. Regression lines calculated with all the data and the corresponding equations are shown on charts 10, 11 and 12.

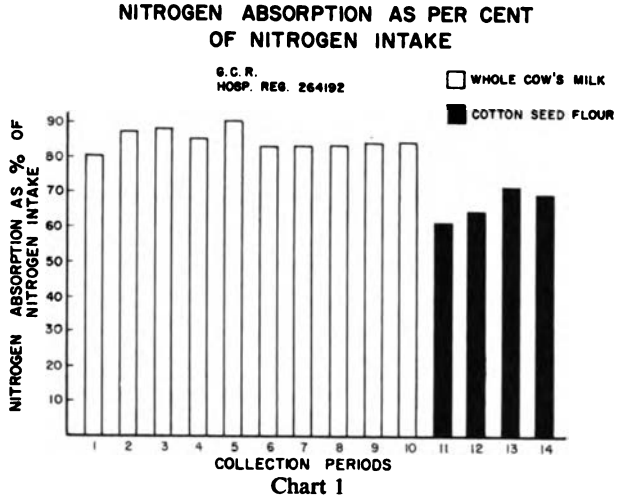


Chart 1

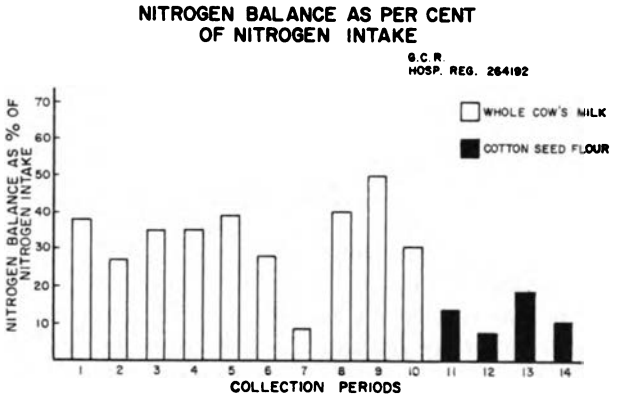


Chart 2

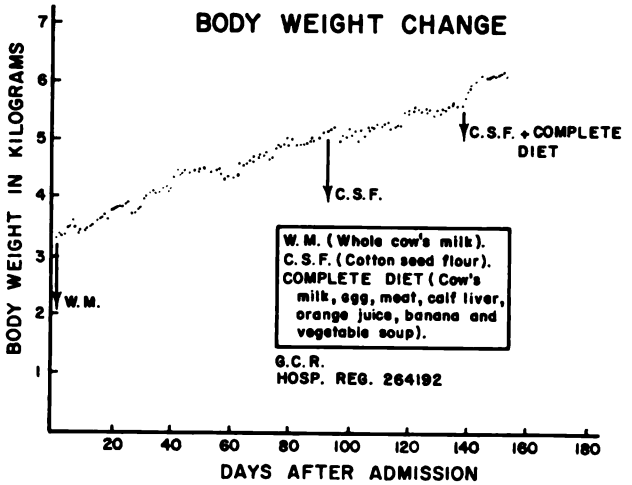
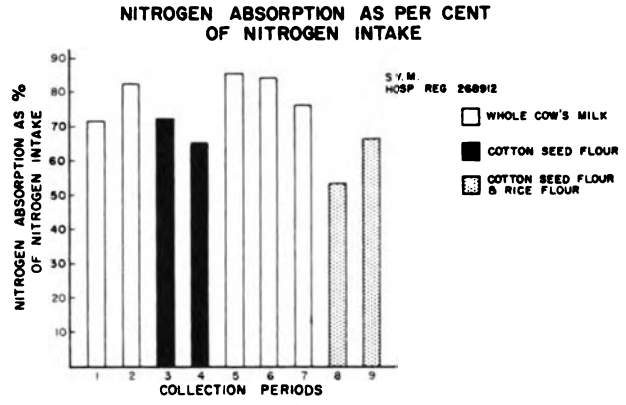


Chart 3



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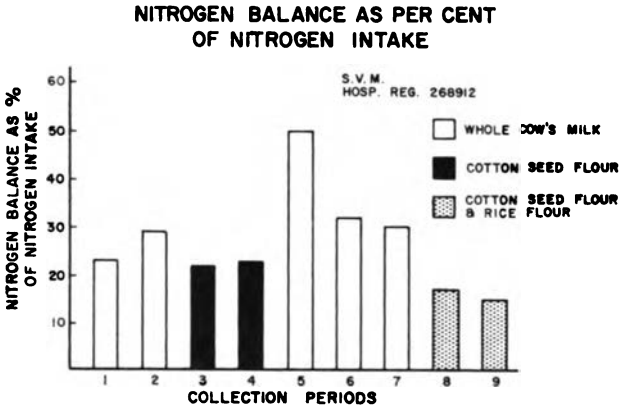

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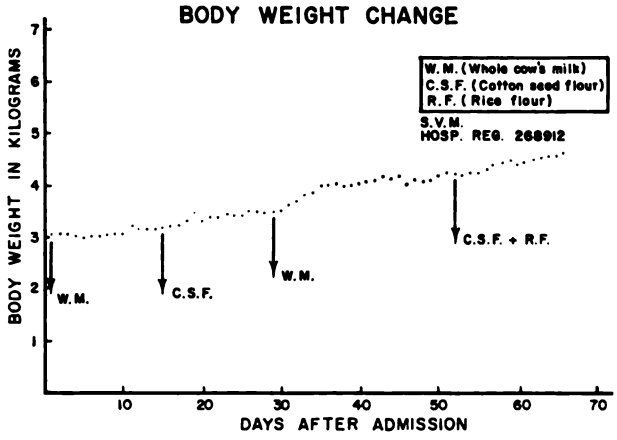

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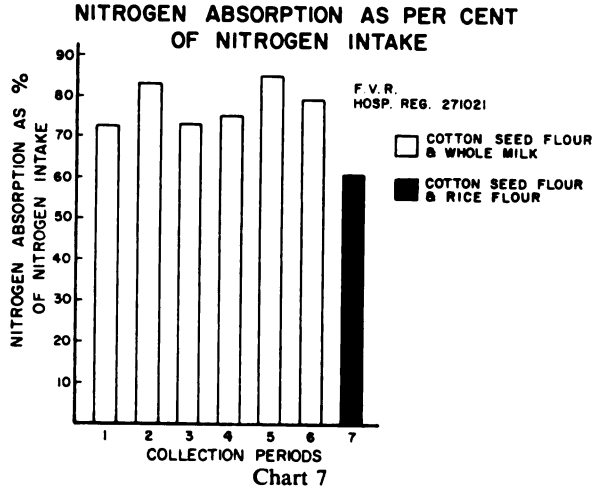

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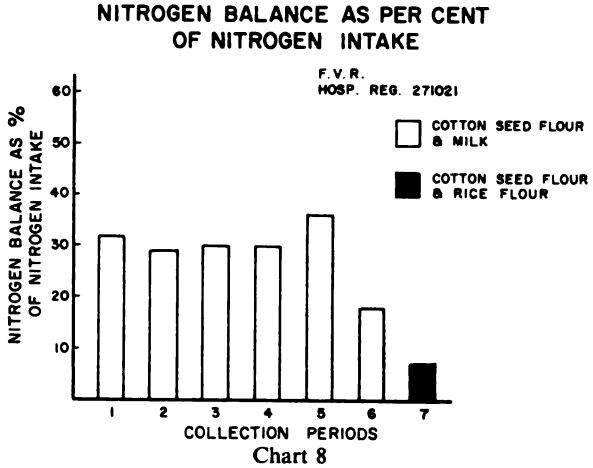

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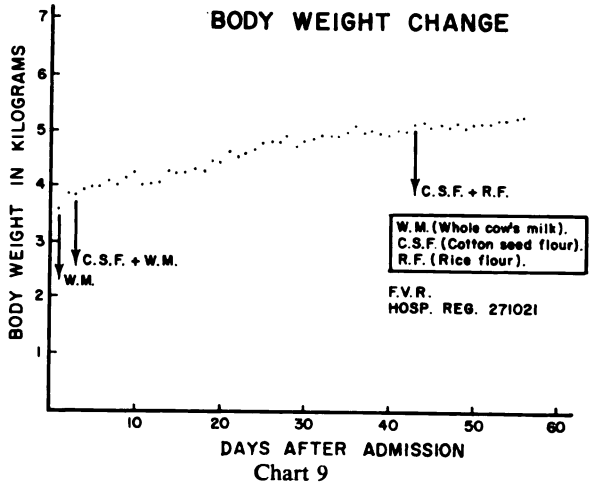

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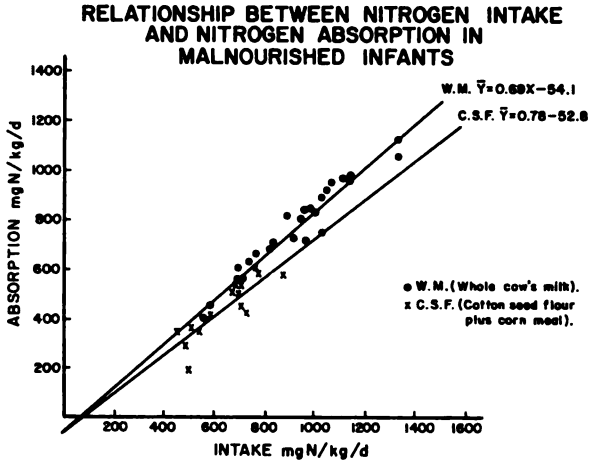

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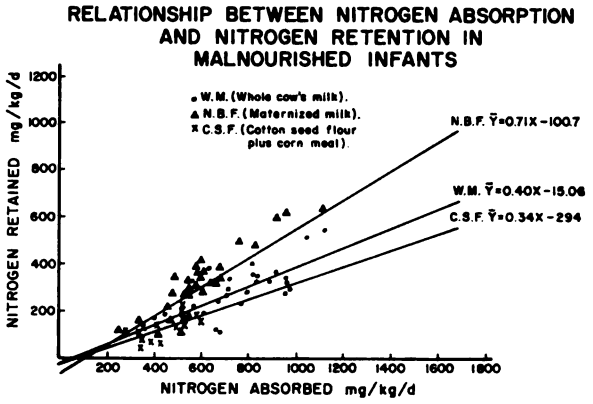

Chart 11

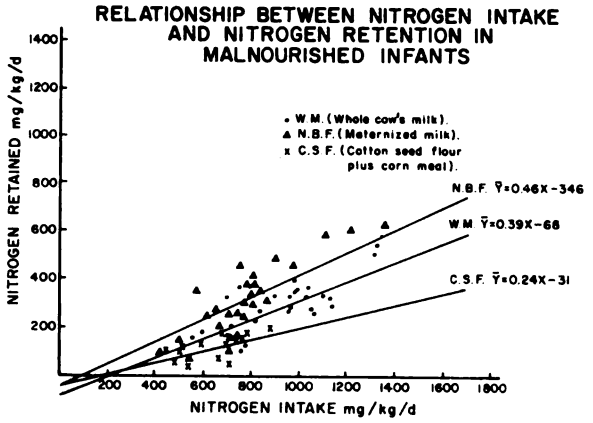

Chart 12

As seen from these data, absorption of nitrogen seems to be equal for the cottonseed and the milk formulas, up to an intake of 800 mg nitrogen/kg/day, although in all individual cases it tended to be lower, especially when made up in rice flour gruel. On the other hand, significant differences were found between the two formulas for the relation of nitrogen retained to nitrogen absorbed, as an index of BV, as well as for the relation of nitrogen retained to nitrogen ingested, considered equivalent to the NPU; differences between the slopes are significant at the 5% level.

Regarding the weight curves, these tend to show a plateau during the time the children received the plant protein. When the regimen was shifted to milk or a complete diet, growth proceeded at the same rate as before the initiation of the study.

Despite the fact that the mixture of cottonseed and corn flour was not good enough for maintenance of the recovery of the children studied, its utilization seems to give results superior to those of cornmeal or rice flour alone, as observed in other studies. On the other hand, since it can be provided as a gruel, it may be hypothesized that it will be accepted, both as part of the normal diet or as the only food during episodes of diarrhea or convalescence from measles. Studies on acceptance at a community level are needed to test this idea and, if it is confirmed, establishment of factories for the production of cottonseed suitable for human use should be promoted.

The problem of the preschool child, partaking of the family diet but in insufficient amounts, requires a solution which permits improvement in the quality of this diet without introducing changes in feeding habits. Previous balance studies in preschool-age children with chronic severe malnutrition[10, 11] have shown that absorption and retention of nitrogen are much poorer with a typical corn-and-beans diet than with milk. However, the corn-and-beans diets show great variations in retention values, which may explain why not all the Mexican children fed this basic diet develop severe protein malnutrition. On the other hand, in one of the series studied, retention of nitrogen from corn and beans as per cent of absorption was quite satisfactory and manifested itself in positive balances when absorption of nitrogen was above 100 mg/kg/day. If, on the other hand, small children systematically receive too little of this diet, no benefit will be derived from its eventual satisfactory BV. While these cultural patterns are modified, the solution of the problem may be sought in a supplement capable of improving the nutritional quality of this relatively poor basic diet. Such a supplement should be easily available and cheap and should permit its incorporation into the usual diet without introducing changes in color, odor, texture or flavor. In a country which, like Mexico, has a huge extension of coasts, with many different water temperatures and increasing potentialities for sea-product industrialization, deodorized, defatted fish flour would seem to be one possible solution. A product with these characteristics has been assayed and proved to be appropriate, without possibility of detection by the consumer, to different foods at the following concentrations:[12]

Cornmeal (for tortillas), 5% to 7%; bread, 10%; beans, 5% to 7%; noodles, 10% to 15%; crackers and cookies, 10%.

In order to assess the nutritional quality of such foods, nitrogen balances were performed on 6 severely malnourished children, aged between 1½ and 3 years, who presented the clinical type known as "marasmic kwashiorkor." Studies were initiated upon admission to the ward; periods lasted 4 days and were alternated with others in which the same diet was supplemented with glycine in isonitrogenous amounts. Nitrogen intake varied between 4 and 6.5 gm of protein/kg/day. Two different sources of fish flour were used.

Data in table 3 show a great variation in the absorption as per cent of the intake, regardless of the addition of supplement; however, both the BV and the

TABLE 3
ABSORPTION AND UTILIZATION OF FISH FLOUR SUPPLEMENTS

Child	Diet	Absorption as % of intake	Retention as % of absorption	Retention as % of intake
	Beans & Tortilla +			
R.G.J.L.	Fish flour	71.9	29.5	21.2
	Glycine	79.7	9.5	7.6
C.S.G.	Fish flour	23.1	37.6	8.7
C.S.V.	Fish flour	32.1	31.6	10.2
C.G.F.	Fish flour	59.8	36.9	22.1
	Glycine	70.4	6.7	4.8
C.H.M.	Fish flour	24.5	33.9	8.3
	Glycine	41.8	—1.9	—0.7
L.P.J.L.	Fish flour	48.1	63.4	30.5

NPU of the diet supplemented with fish flour were significantly better and much less variable than in the control group and in previous series in which corn and beans were fed.

Acceptance studies in these and other groups of severely malnournished children revealed the fish flour to be equally good in supplemented or nonsupplemented diets. On the basis of these studies, a preliminary investigation of the acceptance and intrafamilial distribution of fish-flour-supplemented foods in 94 families of a rural community has been projected. The satisfactory results obtained so far with these studies should encourage further research on the value of this type of fish flour in the improvement of the BV of the poor diets of which small children partake.

However, we would like to emphasize our point of view that the correct solution of the world-wide problem of protein malnutrition does not lie in supplementation of poor foods nor in the provision of substitutes, but in a fair distribution of the opportunities to live a healthy life.

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DISCUSSION

DR. GYÖRGY: What kind of cottonseed flour did you use and do you have gossypol content specifications?

DR. FRENK: The cottonseed was given to us by one of the international agencies. It comes from a factory in Texas which produces cottonseed flour which is very low in gossypol content. I think it was the same one used in Guatemala.

DR. SABRY: What proportion did you add to cottonseed and corn flour as well as the rice flour, and were these proportions the same as given in the local diets of these children?

DR. FRENK: The concentration both of the corn flour, which we added in order to get a better consistency in the mixture of cottonseed and rice flour, and of the cottonseed flour was of 3%, which gives the consistency the mothers like to give their children. They give it in a bottle because it is thin enough to flow through the nipple. We used the same concentration in order to imitate as much as possible the natural conditions.

DR. PATWARDHAN: Studying nitrogen retentions with different dietary regimens, I found the increase came toward the end of the period. In an earlier paper, it has been shown that the retention of nitrogen increased as the experiment progressed. Is it possible that some of the lower retentions which you observed on cottonseed flour mixtures might be the result of such a process? If you had tried these earlier, probably you might have found less difference between the two.

DR. FRENK: That is very true. We always remember the work of Dr. Allison in this respect. Therefore, we tried to switch the periods around. You saw that, in some of the children that we showed, we gave the cottonseed in the third or fourth period. We do not have in this series one which we started with cottonseed flour alone when the child came to the hospital. We have started some of them right at the beginning with cottonseed flour and milk.

It might be a good idea to repeat some of these studies and start the children right away, just as we did with the fish flour experiment when they came in and not after two or three previous periods on milk alone.

DR. HANSEN: The results with the beans are very disappointing. I would like to ask, What was the proportion of beans in the mixture, what percentage of beans and what percentage of cornmeal in the beans-and-cornmeal mixture which had poor nitrogen retention?

DR. FRENK: The data of this study were obtained several years ago, Dr. Hansen. At that time, we used a proportion of cornmeal to beans of 4:5. What characterizes this diet is a very bad nitrogen absorption, since the biological value (that is, retention as percentage of absorption) of this diet is actually very good.

DR. COLLIS: First, what sort of beans did you use and, secondly, I didn't quite get what your final conclusion was. Do I understand you considered that the cottonseed which was imported was not the solution, and that you feel the solution is inside the country itself?

DR. FRENK: Dr. Collis, I like that question very much. Answering the first one, the beans were *Phaseolus vulgaris*, the common beans which we use. We have some other more sophisticated ones, but we don't use them in our balance studies.

To answer the second part, which is very pertinent, don't forget, Dr. Collis, that my country, like many others which are in process of developing industrially, may well have a great interest in the installation of factories for the production of the same kind of flour which is now imported from Texas. At least the stage is now set to take advantage of the great production of cottonseed in Mexico.

DR. DUTRA: I would like to ask if some of your poor results using vegetable proteins could be because the children would not accept the vegetable proteins very well. When we have children with protein malnutrition, we

give them milk and they do quite well. I wonder, if you give the vegetable protein in greater amount than the animal protein, if they would not have given a similar result when given cow's milk.

DR. FRENK: That is quite possible. Nevertheless, I recall that these children who were getting this formula in this instance took it very well, the same as they take very well some other mixtures which contain cottonseed flour, for instance, a mixture which we are going to hear about in the next part of the program, I think. As a general phenomenon, what you say is right—they take milk much better than any other kind of food. But in this series it was not so.

DR. PLATT: Why do you take cow's milk as your standard? Why not take what happens when they get human milk?

DR. FRENK: We have not done this experiment because it is very difficult for us to collect human milk. Nevertheless, the group at Jamaica in collaboration with Dr. György recently published a paper comparing human milk with cow's milk. Perhaps I am not the best person to say what was found. The author is sitting right there in the back.

DR. GYÖRGY: I don't want to comment on that paper. The human milk was found slightly better. I would say human milk would be a good reference protein to use. The difference between cow's milk and human milk in BV is not great.

DR. ROSENBERG: I notice much of the work you have done concerns itself either with cottonseed or cottonseed mixtures with rice or corn. The limiting amino acid in all three of these proteins is, of course, lysine. Therefore, if you mix two of them you would not really expect to get an improvement. On the other hand, if you mixed these with beans—and I know some of that is going on—you would get a more balanced protein. I am wondering to what extent you have information or experience with cottonseed-bean mixtures. I am wondering if you do not agree this would be the primary source of further investigation.

DR. FRENK: The reason that we presented the two groups of this study was that we were thinking, on one hand, of the infants of 4 months of age and up, who are subjected to few other sources of good protein besides the mother's milk and, on the other hand, of the age group which has already been weaned and is taking part of the diet of beans and corn of the parents. Of course, the mixture you suggest would be a very good and perhaps much better combination than the one we tested but, as I said, we were thinking of two different age groups, one which is getting gruels and the other which is getting the diet of the parents. One would be a group of less than one year of age, and the other would be of older children.

DR. ROSENBERG: Do you mean to say it is not practical or possible to feed the very young ones a mixture containing beans?

DR. FRENK: Not before they can eat or before we can convince the mothers to give the babies something different from a gruel, which can be taken out of the bottle. This would be just a matter of re-education. Right now it is being done on a limited scale. At this age the mothers are very reluctant to give any solid food to their children. They obviously would have to give semi-solid food.

The Development of INCAP Vegetable Mixtures

I. Basic Animal Studies[1]

Ricardo Bressani and Nevin S. Scrimshaw

IT IS NOW widely accepted that an important and practical approach to supplying the needed dietary protein in areas where milk and other products of animal origin are costly or in short supply is the development of suitable combinations of vegetable protein sources to supply both essential and nonessential amino acids in the proportions required.

Research efforts to this end are following three slightly different lines. One is to add high-protein foods of vegetable origin as a supplement to the cereal diets of underdeveloped areas. This approach is illustrated by the development of Indian Multipurpose Food.[1] A second means is the development of a protein-rich vegetable mixture which contains in its formula cereal grains commonly consumed in the area where the mixture is to be used. Such a mixture may be essentially a "complete food", even when it is intended for the supplementary and mixed feeding of infants and young children and as a part of adult diets. A third way of making more efficient use of the available vegetable protein supplies of the area is to encourage use of two or three different vegetable foods in proportions which yield protein quality superior to that of any of the components.

In Central America, we have concentrated most of our attention on the last two possibilities. Vegetable protein mixtures of relatively low protein content but of fairly good nutritive value have been studied in the hope of finding ways of improving diets by educational measures alone.[2] For example, the value of mixtures of lime-treated corn (*Zea mays*) and cooked black beans (*Phaseolus vulgaris*) is shown in figure 1. The bars in the lower part of the figure indicate the proportions of protein from lime-treated corn and beans respectively. The curves from top to bottom represent the average gain of rats in 28 days, the protein efficiency ratio (PER) and the liver fat percentage of the rats fed the different combinations. At the same protein level in the diet, the growth obtained with lime-treated

[1] INCAP Publication I—173

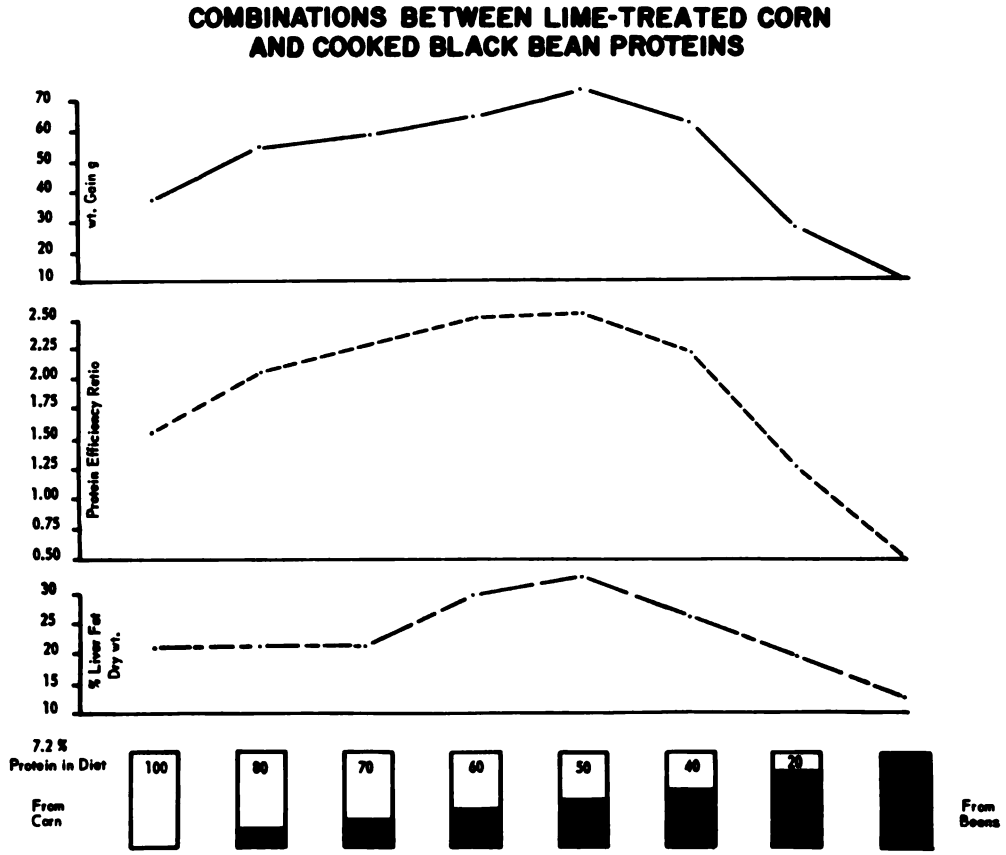

Figure 1—The bars in the lower part of the figure indicate the proportions of protein from lime-treated corn and beans respectively. The curves from top to bottom represent the average gain of rats in 28 days, the protein efficiency ratio (PER) and the liver fat percentage of the rats fed the different combinations.

corn is superior to that obtained with cooked black beans, even though essential amino acid limitations are less in black bean protein than in lime-treated corn. This effect is believed to be due to a lower digestibility of black bean compared with corn protein, and hence a decreased availability of the essential amino acids. The most efficient combination of the two foods is one in which approximately 50% of the protein of the diet comes from each. This finding is very consistent from experiment to experiment with both young and adult protein-depleted rats. The combination obtaining maximum growth produces more fat in the liver. Since lime-treated corn contains about 8.5% protein and black beans approximately 21.0%, the above mixture would have a protein content of 12% with a formula composition by weight of 71% lime-treated corn and 29% cooked black beans.

The amino acid pattern of lime-treated corn and black beans and the optimum combination of the two foods for rat growth is compared in table 1 with that of the FAO reference protein. It is evident that the main amino acid deficiencies

in lime-treated corn have been corrected by the amount of black bean protein added; the mixture is now limiting in total S-amino acids and tryptophan. The higher fat content of the liver of the rats fed the 50-50% protein combination may be the result of a methionine deficiency in the mixture. In other experiments, methionine addition to the 50-50% mixture not only further improved growth and the PER ratio, but also lowered liver fat.[2]

TABLE 1
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Lime-treated Corn	Cooked Beans	50-50 Protein Combination Corn-Beans	FAO Ref. Prot.
		mg/g N		
Arginine	242	388	315	.....
Histidine	249	242	246	.....
Isoleucine	227*	350	288	270
Leucine	576	273*	426	306
Lysine	139*	568	353	270
Methionine + Cystine	195*	125*	161*	270
Phenylalanine	272	339	305	180
Threonine	228	332	280	180
Tryptophan	29*	76*	52*	90
Valine	298	517	408	270

* Limiting Amino Acids.

TABLE 2
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Rice	Cooked Beans	65-35 Protein Combination Rice-Beans	FAO Ref. Prot.
		mg/gm N		
Arginine	519	379	471	.....
Histidine	200	237	213	.....
Isoleucine	329	343	334	270
Leucine	483	269*	408	306
Lysine	258*	556	362	270
Methionine + Cystine	342	123*	230*	270
Phenylalanine	329	331	330	180
Threonine	224	324	260	180
Tryptophan	76*	74*	76*	90
Valine	378	506	423	270

* Limiting Amino Acids.

White rice and black beans are another common and similar type of vegetable mixture.[3] The results of an experiment with these foods are shown in figure 2. The bars in the lower part of the figure again represent the source and distribution of protein in the diet. Rice protein alone produced better growth than did protein from black beans alone. The nutritive value of the two foods combined is better than that of either rice or beans alone; again, the highest amount of liver fat

was found when better growth was obtained. In contrast to the results with lime-treated corn and beans, there was no clearly superior combination observed in this experiment. As shown in figure 2, good combinations were those with rice contributing from 50% to 80% of the protein and black beans 50% to 20%. The most efficient combination was one in which rice contributed 80% of the protein to the diet and the cooked black beans the remaining 20%. The composition by weight of the 80-20% protein mixture is 92% rice and 8% cooked beans, and its protein

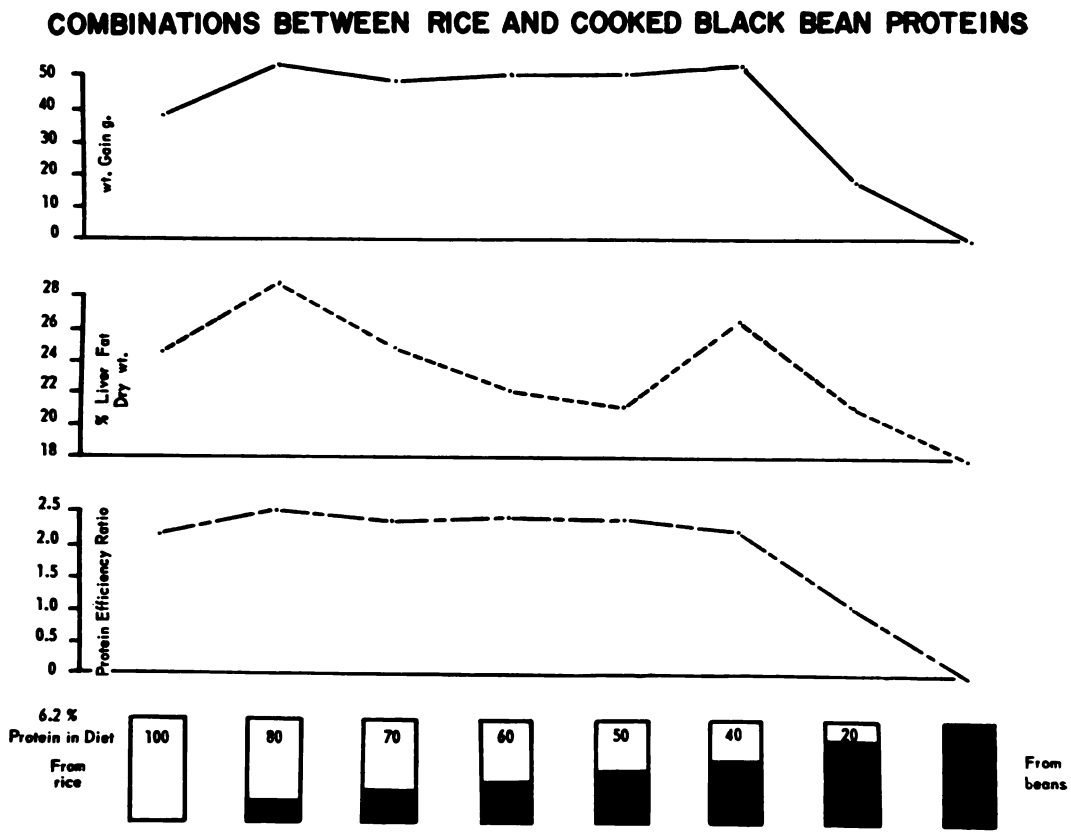

Figure 2—See legend, Figure 1.

content is only about 8%. The "rice-black bean mixture" recommended is the 65-35% protein distribution. It would have a formula composition of 84% white rice and 16% cooked black beans and a protein content of approximately 9.5%.

The amino acid pattern of the two components, the 65-35% mixture and the FAO reference pattern are shown in table 2. The rice-bean mixture appears to be limiting only in total S-amino acids and tryptophan. Methionine and threonine supplementation of the 65-35% mixture resulted in improved rat growth and PER ratio and lowered liver fat.[3] Apparently the threonine deficiency of rice [4, 5] was not completely corrected by the addition of cooked black beans.

Combinations similar to those previously described have also been worked out with corn and cowpea (*Vigna sinensis*).[6] The results of a typical experiment are shown in table 3. These two foods are efficiently combined when corn contributes 25% to 50% of the protein and cowpea from 75% to 50%. A vegetable mixture with 40% of the protein from corn and 60% of the protein from cowpea would have a composition by weight of 64% corn and 36% cowpea, with a total protein concentration of around 15%, which is higher than that of lime-treated corn and of rice and beans. The limiting amino acids in the corn-cowpea mixture are again methionine and tryptophan. All these mixtures share the disadvantage for child feeding of low protein content. The results indicate, however, that the common foods of the rural population of underdeveloped areas can be used more efficiently and supply better protein when combined so that their amino acids complement each other.

TABLE 3
COMBINATIONS BETWEEN CORN AND COWPEA PROTEINS

Protein distribution in Diet				
% From Corn	% From Cowpea	Ave. Wt. Gain, gm	F.E.[1]	P.E.[2]
100	0	54	5.21	1.22
75	25	78	3.96	1.59
50	50	102	3.43	1.84
25	75	104	3.51	1.82
0	100	78	4.41	1.41

[1] Average food consumed per average weight gained.
[2] Average weight gained per average protein consumed.

When compared with the FAO reference protein,[7] the deficient amino acids in all of these mixtures were methionine and tryptophan. In studies of amino acid supplementation of these three mixtures, it was found that, after correcting the first amino acid deficiency, tryptophan addition did not bring about an improvement of the nutritive value of the mixtures. This suggests that the level of tryptophan in the FAO reference protein is probably higher than needed.

Because of the relatively low protein content in these food combinations, vegetable mixtures of higher protein content were sought. The first of these to receive extensive biological testing was known as Vegetable Mixture 8. It consisted of 50% lime-treated corn flour, 35% sesame flour, 9% cottonseed flour, 3% Torula yeast and 3% Kikuyu leaf meal.[8-10] Vegetable Mixture 8 contained 25% crude protein, and the comparison of its essential amino acid pattern with that of the FAO reference protein indicated deficiencies in lysine, methionine and tryptophan. The protein score based on the FAO reference pattern was approximately 67%.

Rat growth experiments indicated that the mixture was palatable and that it gave good growth and efficient feed utilization. Representative results with rats are shown in table 4. The addition of 0.45% lysine or skim milk substituted for part of the corn in the mixture improved the feed efficiencies but not the growth of the rats. When the protein in Vegetable Mixture 8 was diluted to 15% with cornstarch, the addition of lysine improved both the growth and feed efficiencies.

TABLE 4
RAT TRIALS OF VEGETABLE MIXTURE 8
(12 Rats Per Group, 8 Weeks With 25.2% Protein)

	Trial Mixture	Feed Efficiency	Initial Wt. gm	Final Wt. gm
2A	8	2.71	44	223
2B	8 + 0.45% lysine	2.14	44	230
2C	Modified 8 *	2.08	44	233
2D	Modified 8 * + 0.45% lysine	1.90	44	232
2E	Modified 8 * 9% skim milk #	2.29	44	236
2F	Modified 8 * 9% skim milk # + 0.45% lysine	1.98	44	235

* Cottonseed meal replaced by additional sesame.
# Substituted for corn masa.

Representative results with chicks are shown in table 5. Addition of lysine did improve growth and feed utilization by chicks. Further studies with chicks indicated that grain sorghum, buckwheat, rice or whole ground corn could replace lime-treated corn in the mixture without altering its nutritive value.[10]

TABLE 5
CHICK TRIAL OF INCAP VEGETABLE MIXTURE 8
(24 chicks per group, 5 weeks with 25% protein)

Mixture [1]	Feed Efficiency [3]	Initial Wt. gm	Final Wt. gm
8	2.80	42	200
Modified 8 [2]	2.84	42	217
8 + 0.2% Lysine	2.19	42	298
8 + 0.4% Lysine	2.05	42	369

[1] With minerals and vitamins added to meet chick requirements.
[2] Whole ground corn substituted for lime-treated corn (masa).
[3] Grams fed per grams gained.

As a result of the work on Vegetable Mixture 8, several facts became clear. First of all, the work showed that an all-vegetable mixture of good protein quality could be developed; second, that it could cure severe forms of protein malnutrition; and third, that it was possible to achieve good acceptance by the needy populations of technically underdeveloped areas.

Although Vegetable Mixture 8 proved to be good, it was not sufficiently economical for the Central American area because of the short supply of sesame seed, which raised its cost. A less expensive mixture eliminating sesame seed was needed. Cottonseed flour was selected as the sole concentrated protein source for several reasons. During the development of Vegetable Mixture 8, experience was acquired in its use; cottonseed flour, when properly processed, has good protein quality, and cottonseed oil meal is readily available in most of the Central American countries. Although cottonseed flours for human feeding have been known for some time, they have been little used for the purpose. One disadvantage is, of course,

the presence of gossypol pigments and the frequent destruction of protein quality during processing.[11]

The actual research work on Vegetable Mixture 9 was started at INCAP in November 1957. Since then a large amount of information has been accumulated and is being published.[12-14, 16] The first experiments on Vegetable Mixture 9 were designed to learn whether the protein quality of cottonseed flour was similar to the protein quality of sesame meal. The results of one experiment carried out with rats are shown in figure 3. Ten per cent protein was used in the diet of this

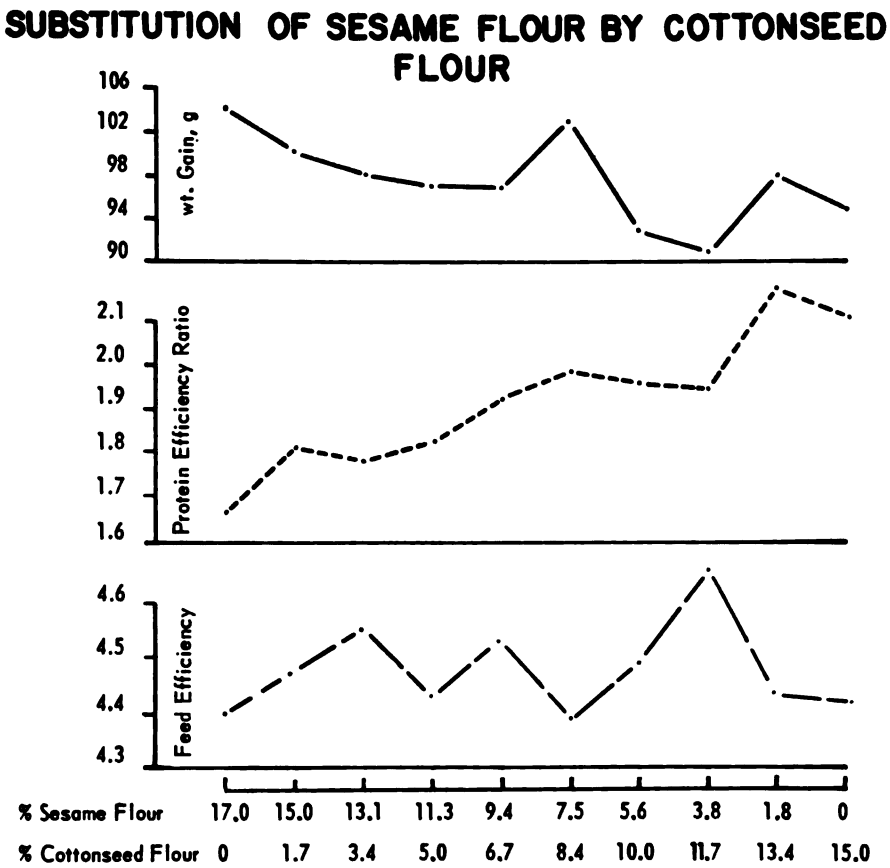

Figure 3—See legend, Figure 1.

experiment. The actual amounts of sesame flour and cottonseed flour used are shown in the lower part of the figure. It is evident that there was little difference in growth and feed efficiency as cottonseed flour replaced sesame flour, although the protein efficiency improved. Experiments carried out with chicks also gave similar results.[13]

The next step was to determine the most efficient combination of corn and cottonseed flour protein. The results of one experiment in rats is shown in figure 4. The bars in the lower part of the figure indicate the protein contribution to the diet

from cottonseed flour and from corn. The upper curve is the weight gain of the rats after 28 days. As the protein contribution from corn increases, there is a decrease in weight gain up to the 10-90% protein proportions between the two ingredients. An increase is found at the 15% to 85% protein ratio. Weight gains then decrease as the protein contribution from corn is increased, and the feed and protein efficiencies behave in a similar manner. It is interesting to note that the 15% to 85% ratio is no better than the 0 to 100% ratio between corn and cottonseed flour. The result was, however, consistent in other rat as well as chick trials.

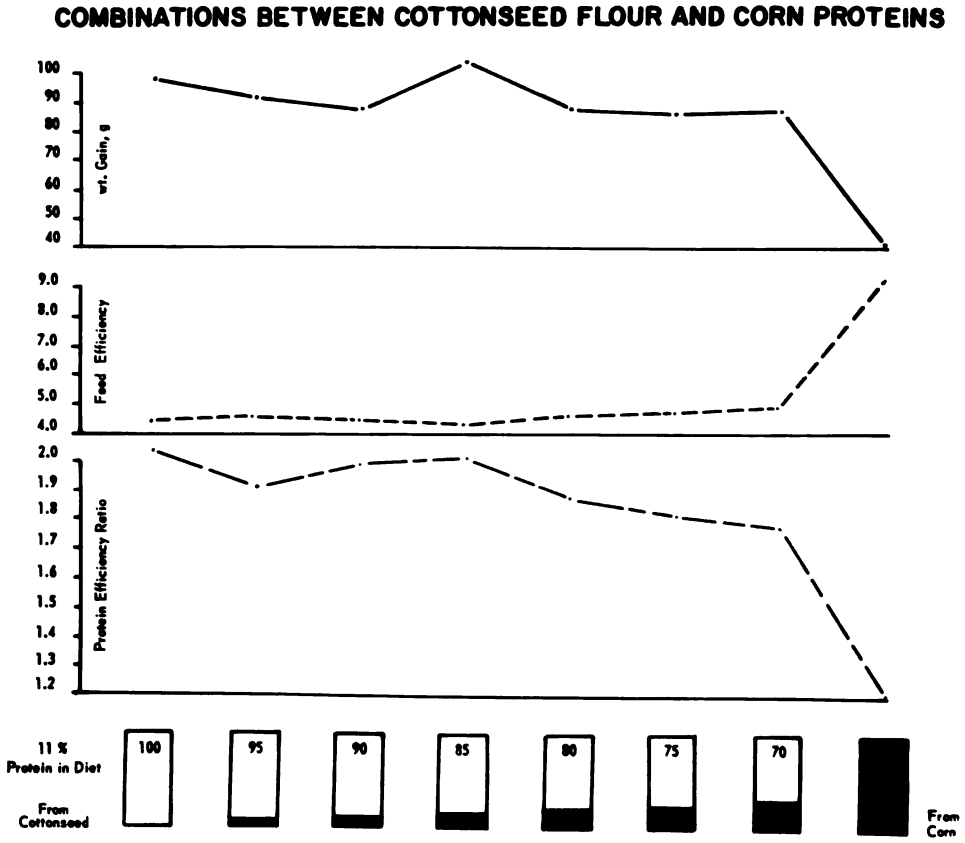

Figure 4—See legend, Figure 1.

The amino acid patterns of corn, cottonseed flour and the 15-to-85% mixture are shown in table 6. Comparison with the FAO pattern shows deficiencies in isoleucine, lysine, sulphur-containing amino acids and tryptophan. Addition of cottonseed flour to corn improves but does not wholly correct the lysine and tryptophan deficiency in the cereal grain. The amino acid proportions in the 15-to-85% mixture, using tryptophan as the base, are similar to those of the FAO pattern. From these experimental results, calculations on protein and amino acid

TABLE 6
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Corn	Cottonseed Flour	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/gm N		
Arginine	262	719	647	.....
Histidine	231	113	132	.....
Isoleucine	213*	231*	228*	270
Leucine	572	413	437	306
Lysine	126*	256*	235*	270
Methionine + Cystine	189*	169*	172*	270
Phenylalanine	276	294	291	180
Threonine	214	294	281	180
Tryptophan	32*	75*	68*	90
Valine	281	331	323	270

* Limiting Amino Acids.

content of corn and cottonseed flour, and calculations on other chemical components in cottonseed flour, Vegetable Mixture 9 was formulated.

The corn-cottonseed protein combination chosen for Vegetable Mixture 9 was the one in which corn provides 20% of the protein and cottonseed flour 80%. This meant that the maximum protein content in the final mixture would be from 26% to 28%. Vegetable Mixture 9 was then formulated as follows: 56% ground corn, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal. Experimental results also indicated that sorghum grain could replace all or part of the corn and, for economic reasons, the experimental formula was made of 28% ground corn, 28% sorghum grain, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal.

The above formula was then subjected to extensive testing in chicks, rats and dogs.[12-15] A representative chick trial is shown in table 7. Chick growth and feed efficiencies are about equal when the mixture is made with 28% corn and

TABLE 7
REPRESENTATIVE CHICK GROWTH TRIAL WITH INCAP VEGETABLE MIXTURE 9
(35 days—20 chicks per group)

Diet	Protein in Diet %	Final Weight gm	Feed Efficiency
V.M. 9	23.5	479 [1]	2.31
V.M. 9 with 56% Corn	23.8	460 [1]	2.25
V.M. 9 with 56% Sorghum	24.1	479 [1]	2.27
"Ace-Hi"	23.9	587 [1]	2.01
V.M. 9	23.0	310 [2]	2.45
" + 0.3% DL-Met	23.0	361 [2]	2.26
" + 0.2% L-Lys HCl	23.0	472 [2]	2.14
" + both A.A.	23.0	490 [2]	2.04

[1] 55 gm initial weight. [2] 45 gm initial weight.

28% sorghum, 56% corn or 56% sorghum. The response is, however, only 82% of that obtained with a commercial chick feed containing animal protein. As shown in the lower part of the table, supplementation with lysine improved growth of the chicks fed Vegetable Mixture 9, and methionine addition had a small effect.

A representative rat repletion study is shown in table 8. Good repletion was observed in the rats fed Vegetable Mixture 9 at the 10% protein level in the diet. This was further improved by lysine addition, but methionine addition had no effect.

TABLE 8
REPRESENTATIVE RAT REPLETION STUDY
AMINO ACID SUPPLEMENTATION OF
INCAP VEGETABLE MIXTURE 9
(10% Protein in diet, 6 rats per group)

A.A. Added	gm%	Repletion Gain in 14 days
None	0	51 gm
L-Lysine HCl	0.1	60
" "	0.2	71
" "	0.3	62
DL-Methionine	0.1	53
" "	0.2	51
" "	0.3	53

The conclusion, based on a large number of experiments with rats and chicks[12-14] as well as supplementary biological studies in dogs and pigs[15] is that Vegetable Mixture 9 has a relatively high nutritive value and is completely free of any adverse effects.

It was stated previously that these protein-rich mixtures are intended for the supplementary and mixed feeding of infants and young children and as a part of an adult diet. It is known from nutritional surveys carried out in Central America[17-19] that the protein intakes of the habitants of rural areas are low in both quality and quantity. It becomes important, therefore, to find the amounts of the vegetable mixtures needed to complement the protein consumed by the rural population of the area. The amount of the vegetable mixture will be dependent upon the quality of the protein consumed, which consists most commonly of rice or of lime-treated corn and cooked black beans. The importance of this problem is evident from the findings of several investigators [20, 21] that amino acid imbalances may result from the addition of proteins which are noncomplementary.

Preliminary experiments along these lines have been initiated at INCAP by comparing the nutritive value of the bean and lime-treated corn mixture with the rice and bean and the corn and cottonseed mixtures. The amino acid patterns of the three mixtures and that of the FAO reference protein are given in table 9. All three mixtures are deficient in methionine and tryptophan as compared with the FAO reference protein. If proportions rather than amounts are used to compare the mixtures, and the proportions are calculated on the basis of the isoleucine content because all three mixtures have a value that is similar to that of the FAO pattern, the main deficiency in the three mixtures is methionine. Theoretically, the

least deficient combination is that with 15% of protein from corn and 85% from cottonseed flour. On the basis of the methionine deficiency, the decreasing order of nutritive value is: Corn and cottonseed, rice and beans and corn and beans.

TABLE 9
AMINO ACID PATTERNS OF VEGETABLE MIXTURES

Amino Acid	50-50 Protein Combination Corn-Beans	65-35 Protein Combination Rice-Beans	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/gm N		
Arginine	315	471	647	.....
Histidine	246	213	132	.....
Isoleucine	288	334	228*	270
Leucine	426	408	437	306
Lysine	353	362	235*	270
Met. + Cys.	161*	230*	172*	270
Phenylalanine	305	330	291	180
Threonine	280	260	281	180
Tryptophan	52*	76*	68*	90
Valine	408	423	323	270

* Limiting Amino Acids.

Table 10 shows the actual nutritive value from an experiment with rats in which the three mixtures were compared with each other. The PER was highest with rice and beans, followed by corn and cottonseed flour and corn and beans in that order. In any case, all three mixtures were of fairly good protein quality as judged by the PER. Experiments are under way to determine the effects of different proportions of the low- and high-protein-containing mixtures, not only the optimum combination of two ingredients, but other combinations as well. The results will provide a basis for recommending the quantity of Vegetable Mixture 9 needed to complement efficiently the common lime-treated corn and bean diet or the rice and bean diet of the rural population of the Central American countries.

The importance of knowing the protein nutritive value of combinations of natural foods is illustrated by the last figures in table 10. The particular mixture was

TABLE 10
COMPARISON OF THE NUTRITIVE VALUE OF FOUR VEGETABLE PROTEIN MIXTURES IN RATS

Vegetable Mixture	Protein Combination Ratio	Ave. wt. Gain 21 Days	F.E.	P.E.R.
Rice + Black Bean	1.85/1.00	95	3.72	2.65
Lime-treated Corn + Black Bean	1.00/1.00	92	3.58	2.19
Lime-treated Corn + Cottonseed Flour	1.00/5.67	94	3.45	2.35
Lime-treated Corn + Cottonseed Flour + Cowpea	1.00/5.67/1.00	97	3.38	2.47

formulated on the basis of results obtained in previous studies of corn and beans, corn and cowpea and corn and cottonseed flour mixtures. It contained the protein from each component in the ratio of 5.67 of cottonseed flour to 1.00 each of corn and cowpea. The results show this formula to have a relatively high PER and feed efficiency. It is being subjected to further biological testing.

It is apparent from these results that consuming two or more selected vegetable foods in such proportions that their amino acid compositions complement each other is one effective way of making more efficient use of the available food supply and improving the nutritional status of the human population of underdeveloped areas. Furthermore, economically feasible vegetable protein mixtures of good nutritive value can be developed to meet the increasing demand for better quality of protein in areas where protein malnutrition is an important problem.

SUMMARY

One approach to the serious problem of protein malnutrition among young children in Central America has been the study of combinations of local vegetable foods in proportions to give a protein quality superior to that of any single ingredient. Such combinations as 84% rice and 16% cooked black beans (65% of protein from rice and 35% from beans), 71% lime-treated corn and 29% cooked black beans (50% of protein from each food), 64% corn and 34% cowpea (mixtures with 40% of the protein from corn and 60% from cowpea) have improved protein quality, but all are somewhat deficient in methionine and are too low in total protein content for effective feeding of young children.

In an attempt to devise a practical mixture with higher protein content, 35% sesame flour and 9% cottonseed flour were combined with 50% lime-treated corn, 3% Torula yeast and 3% Kikuyu leaf meal. This formula, identified as INCAP Vegetable Mixture 8, contains 25% protein and gives good growth and feed utilization in rats and chicks, although it is improved by lysine addition. When cottonseed flour replaced the sesame flour in this formula, there was little difference in growth and feed efficiency and some apparent improvement in protein efficiency. Because of the lower cost and greater availability of cottonseed flour in Central America, biological studies have since been concentrated on INCAP Vegetable Mixture 9 containing 28% corn, 28% sorghum, 38% cottonseed flour, 3% Torula yeast and 3% leaf meal. The sorghum was introduced to lower the cost, but any proportion of corn and sorghum may be used without significantly affecting the nutritive value of the mixture. A large number of experiments with rats and chicks and supplementary studies in dogs and pigs demonstrate that this mixture has a relatively high nutritive value and is completely free of any adverse effects. On the basis of these results both Mixtures 8 and 9 were recommended for experimental feeding trials in human subjects.

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The Development of INCAP Vegetable Mixtures

II. Biochemical Testing[1]

Guillermo Arroyave, Dorothy Wilson, Moisés Béhar and Fernando Viteri

THE RESULTS of basic animal studies leading to the development of vegetable mixtures for human feeding in the Institute of Nutrition of Central America and Panama (INCAP) have been presented by Dr. Bressani.[1] The formulas for three of these, Mixtures 8, 8A and 9, which have been extensively tested in clinical trials in young children, are shown in figure 1. As Dr. Scrimshaw will report,[2] these mixtures have given results similar to those obtained with milk as a protein source in comparative metabolic studies in children who have recovered from severe protein malnutrition, and in therapeutic trials in children with the acute syndrome.

In planning feeding mixtures for human consumption, however, as many criteria as are available should be applied to evaluate the quality and safety of such foods. In addition to the biological studies in experimental animals reported by Dr. Bressani,[1] and to the nitrogen balance and therapeutic trials in children, other biochemical tests to which INCAP Vegetable Mixtures 8, 8A and 9 have been submitted include their effect in promoting the regeneration of serum proteins in the therapeutic trials, and the study of the plasma amino acid pattern before and after a test feeding in children who have been receiving either milk or one of the vegetable mixtures.

REGENERATION OF SERUM TOTAL PROTEINS IN KWASHIORKOR

Serum proteins are a useful and simple biochemical measure of the extent of initial recovery of a child who is being treated for kwashiorkor. For practical purposes, their measurement in these cases has the same significance as that of albumin, and a marked increase in their concentration is a good prognostic sign. The density gradient method of Lowry and Hunter[3] can give the results within less than an hour after the blood sample collection, employing only 5-10 μl of

[1]INCAP Publication I-174

FORMULAS FOR INCAP VEGETABLE MIXTURES FOR HUMAN FEEDING

	8	8A	9	9A	9B
Corn, Lime-treated	50	50	28	---	---
Corn, Cooked	---	---	---	29	---
Corn, Uncooked	---	---	---	---	29
Sesame flour 33% fat	35	---	---	---	---
Sesame flour 18% fat	---	35	---	---	---
Sorghum, Lime-treated	---	---	28	---	---
Sorghum, Cooked	---	---	---	29	---
Sorghum, Uncooked	---	---	---	---	29
Cottonseed flour	9	9	38	38	38
Torula yeast	3	3	3	3	3
Dehydrated leaf meal	3	3	3	---	---
$CaCO_3$	---	---	---	1	1
Vitamin A	---	---	---	4500	4500

Figure 1

serum. Using this procedure, we have compared serum protein regeneration in a number of patients with kwashiorkor who were given therapeutic diets of either Mixture 8, 8A or 9, at levels of nitrogen intake varying in each child from 2 to 5 gm /kg/day. These patients were compared with patients receiving milk at levels of intake not exceeding those of the children given the vegetable mixtures, usually 3 to 4 gm/kg/day. The results of this comparison are represented graphically in figure 2.

The milk treatment resulted in consistent increases in serum total protein (fig. 2A). All three children gained around 3 gm/100 ml in a period of about 4 weeks. The children treated with Mixture 8, 8A or 9 (figs. 2B, 2C and 2D), attained a level of gain around 6 gm/100 ml in 4 to 5 weeks of treatment; it appears that this level was reached regardless of the initial serum protein concentration; PC-67, for example, starting with the highest value, showed the least increment.

From these results, it may be concluded that although higher serum protein values are attained in the same period of treatment with milk, INCAP Vegetable Mixtures 8 and 9 have sufficiently high protein quality to meet the high nitrogen demands of a severely protein-depleted child for plasma protein synthesis.

Plasma Amino Acid Levels After a Protein Test Feeding

The comparative effect of milk, Mixture 9 and corn-bean diets on the plasma amino acid increases of children after a milk feeding is also of interest.

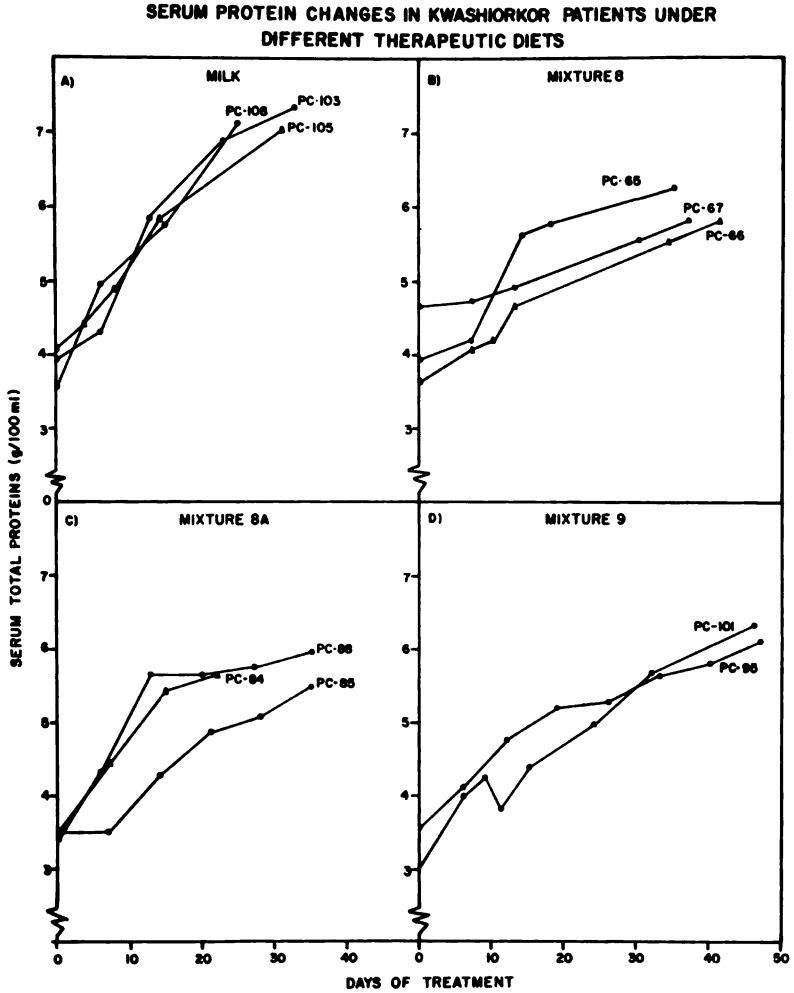

Figure 2

Observations in dogs and children indicate that the plasma amino acid increases after a standard protein feeding are influenced by the physiological state of the subject as determined by the previous diet (INCAP unpublished data). A decreased response seems to occur after a protein of poor nutritional value has been fed for some time before the test.

In view of these preliminary observations, 2 experiments were designed with 3 children each, for the specific objective of comparing the influence of milk, Mixture 9 or a corn-bean combination on the plasma amino acid responses to a milk feeding.

Experiment 1. Child PC-97, a boy 3 years and 8 months old, was maintained on milk at 2 gm protein and 90 cal/kg/day for 6 weeks, followed by 3 days on a nitrogen-free diet immediately preceding the test. Child PC-95, a boy 3

years and 7 months of age, received INCAP Vegetable Mixture 9 at the same protein and calorie intake for 3 months, followed also by 3 days on a nitrogen-free diet just before the test. Child PC-92, a boy 3 years and 6 months old, received a diet in which 50% of the protein was from corn and 50% from beans, also at 2 gm protein and 90 cal/kg/day for 7 weeks, followed by 4 days on a nitrogen-free diet immediately before the test. The period of nitrogen-free diet was included to determine endogenous nitrogen excretion for other purposes.

After a 16-hour overnight fasting a blood sample was taken and the test meal of skim milk at 2 gm protein/kg was administered to the 3 children. A post-prandial blood sample was taken 2½ hours later. The results of plasma amino acid analysis are shown graphically in figure 3. In each block, representing one essential amino acid, the increases observed in the child fed milk are shown in the

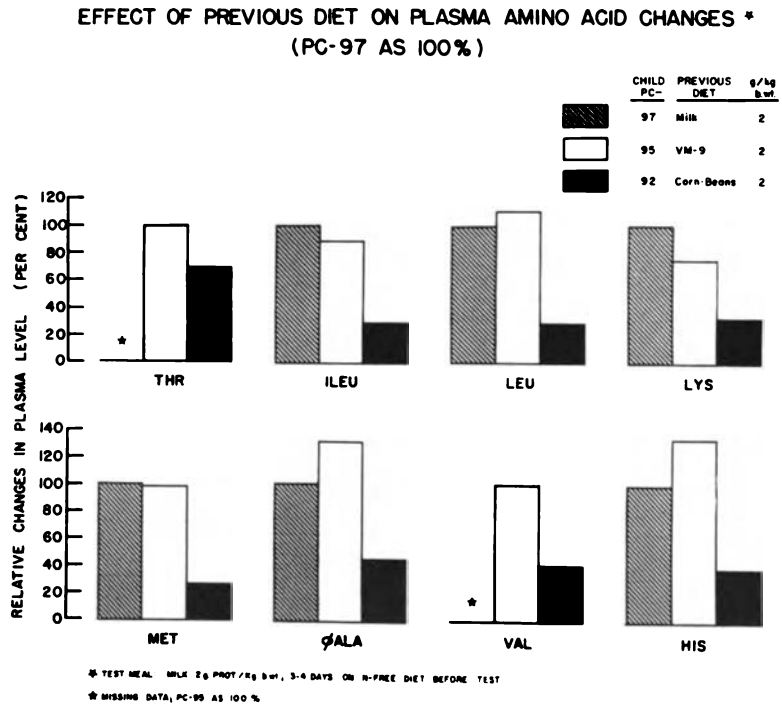

Figure 3

first column as 100%. The second and third columns show the plasma amino acid changes observed in the child fed INCAP Vegetable Mixture 9 and in the child fed corn and beans. The last two are both expressed as percentage of the change observed in the milk-fed subject. Exceptions to this are threonine and valine, for which changes in PC-95 were taken as 100%.

The child fed corn and beans showed consistently lower increases than either the child fed milk or the one fed Vegetable Mixture 9. It is interesting that the children fed milk and Vegetable Mixture 9 responded so similarly.

Experiment 2. In order to confirm the above observations, another group of 3 children was studied according to a very similar plan of investigation, but no period of nitrogen-free diet was included. Figure 4 illustrates the results of this experiment: PC-103, a boy 3 years and 4 months of age, was given a diet in which Mixture 9 was the sole source of protein, for a period of 6 weeks at a level of intake of 2 gm protein and 90 cal/kg/day. PC-101, a boy of 6 years and 2 months, received a milk diet at the same level of protein and calorie intake for 7 weeks, and PC-102, a boy of 5 years and 10 months, was given corn and beans as the only source of protein, which provided 2 gm protein and 100 cal/kg/day.

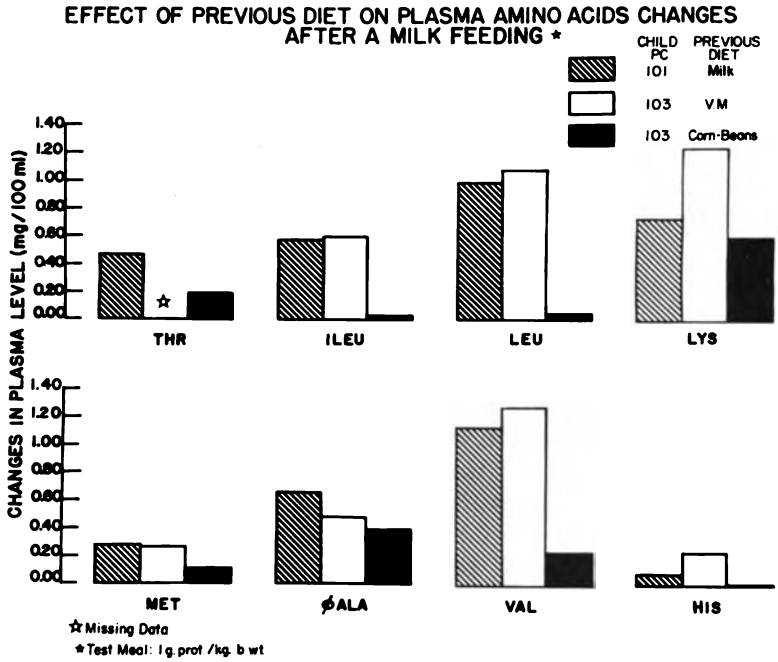

Figure 4

The 3 children received a test meal of milk calculated to provide 1 gm protein/kg/day in one feeding. Child PC-102 refused a small portion of this feeding, so that his actual intake was 0.86 gm protein/kg. The first column represents the plasma amino acid increases in the child whose previous diet was milk, the second represents the Mixture 9-fed child and the third corresponds to the child fed corn and beans. The results clearly confirm those of the previous experiment. Again, the increases in child PC-102 (previous diet of corn-beans) were markedly inferior to both the Mixture 9- and the milk-fed children. The agreement between results of experiments 1 and 2 indicates also that the period of nitrogen-free diet in experiment 1 did not interfere appreciably with the results.

To assist in interpreting the observed differences in the increase of plasma essential amino acids, some known facts must be considered. The 3 diets used, milk, INCAP Vegetable Mixture 9 and corn and beans, have their own character-

istic protein quality as indicated by promotion of growth and nitrogen retention. Milk and Mixture 9 rate as very similar in quality by the above criteria, while the corn-bean diet appears quite inferior. Whether this is due to differences in digestibility, to lower amino acid utilization because of imbalance, or to some other cause, the important fact remains that the relative value of the 3 diets fed to children seems to be well reflected by the magnitude of the plasma amino acid increase after a common test meal of milk protein. Both independent experiments reported here are consistent in giving this result.

In the discussion of the biochemical significance of the relationship between plasma amino acids and the composition of the ingested protein in dogs, Longenecker and Hause[4] stated that one important factor in determining postprandial amino acid levels was the rates at which these compounds were removed from the plasma by the tissues and that these rates were proportional to the specific amino acid requirements. It may be speculated, therefore, that the long-term feeding of corn and beans produces a nutritional stress on the organism which results in an increased tissue demand for essential amino acids.

SUMMARY

The regeneration of total serum proteins was compared in 11 children suffering from kwashiorkor and treated with Vegetable Mixtures 8, 8A, or 9 or with milk. It was apparent that the vegetable mixtures, although not producing as high protein values as milk in the period of treatment, were of adequate quality for satisfactory regeneration of serum proteins. Plasma amino acid changes following a protein test meal were also studied in 6 children who had been given either milk, INCAP Vegetable Mixture 9 or a corn-bean diet for several weeks. The increase in plasma amino acid levels following a test meal of milk was essentially the same when milk or Mixture 9 constituted the previous diet and was much higher than that following the period of corn-bean feeding. Regardless of the specific mechanism or mechanisms responsible, this difference in response to the milk test meal seems to bear a direct relationship to the protein value of the previous diet and illustrates again the similarity of INCAP Vegetable Mixture 9 and milk in this respect.

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The Development of INCAP Vegetable Mixtures

III. Clinical and Field Trials[1]

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THE EXTENSIVE BIOLOGICAL testing which has gone into the development of INCAP Vegetable Mixtures 8 and 9 has been summarized by Dr. Bressani,[1] and the detailed formulas, as well as biochemical results from their use in children, have been discussed by Dr. Arroyave.[2] These studies showed the formulas to have a high nutritive value and to contain protein of good quality. This third presentation in the series will summarize the results, published elsewhere in more detail,[3] of clinical and field trials with these mixtures, particularly with INCAP Vegetable Mixture 9B which has received the most attention.

This formula contains 29% whole ground corn, 29% whole ground sorghum, 38% cottonseed flour, 3% Torula yeast, 1% calcium carbonate and 4,500 IU of vitamin A per 100 gm. Mixture 9A differs only in the cooking of the corn and sorghum prior to grinding. The cottonseed flour must be one of high quality specially manufactured to rigid specifications for human consumption.[4] The mixtures are prepared in the form of a thin gruel, known locally as an "atole," by adding approximately 100 gm of the powder to a liter of water, cooking for about 15 minutes, sweetening to taste with sugar and flavoring with cinnamon, vanilla, anise or chocolate.

As Dr. Bressani pointed out, these mixtures are relatively complete in themselves rather than concentrates to be added to other foods to improve the diet. There are several reasons for adopting this approach. Central American mothers are at present accustomed to give their children "atoles," made from corn or other cereals, which have little nutritive value except for their carbohydrate content. The substitution of a nutritious "atole" seemed practical if it could be kept good tasting and sufficiently inexpensive.

The common animal sources of protein, i.e., milk, cheese, eggs, meat and fish, are also important sources of other essential nutrients. If these vegetable

[1] INCAP Publication I-175.

mixtures were to be successful in reaching families who could not afford, or for other reasons did not consume, sufficient quantities of animal protein, it was highly desirable that they carry the other essential nutrients customarily supplied by foods of animal origin. Ascorbic acid did not fall in this category and was not added because fruits rich in this vitamin are locally available and are usually consumed in adequate quantities. The mixtures are low in calories, but sugar is always added in the preparation of any "atole." Furthermore, calorie-containing staples form the bulk of the diet, and bananas are an inexpensive and readily digested supplementary calorie source in Central America.

Tolerance

When work began with INCAP Vegetable Mixture 8, we had no idea whether mixtures of this sort would be well tolerated. It was conceivable that they would produce loose stools, excessive gas in the intestine, have a disagreeable after-taste or simply be the kind of food that is disliked if consumed too frequently. Fortunately, none of these problems was encountered; Vegetable Mixture 8 was accepted as a daily staple in the same way that milk, rice, bread and tortillas are accepted.[5] Observation of the excellent acceptability of these mixtures, and the continued development of children receiving them as the sole source of protein for many weeks, encouraged us to try them in children partially recovered from kwashiorkor.[4,5] Again, tolerance and acceptability were good and the results excellent. Furthermore, in children with diarrhea the mixture resulted in a more rapid return to normal consistency of the stools than when milk was fed.

The conclusions from these initial acceptability and tolerance trials with Mixture 8 have been amply confirmed by the results of similar studies with Mixture 9[3] and extended field trials described in a later section of this report.

Nitrogen Balance Studies

Eight children, 1 to 3 years of age, in advanced stages of recovery from kwashiorkor were fed alternately INCAP Vegetable Mixture 8 and milk at levels ranging from 2.0 to 3.0 gm of protein/kg.[3,5] Nitrogen intake in food and output in urine and feces were measured by the Kjeldahl procedure. During the periods in which milk was the protein source the children absorbed 81% and retained 20.5% of the ingested nitrogen; the corresponding figures during the vegetable mixture periods were 72% and 19.1%. Thus, despite slightly lower absorption of the nitrogen of the vegetable mixture, the net amount retained did not differ significantly from that retained when milk was fed at the same level of nitrogen intake.

Mixture 9 formulas have been similarly tested in 77 five-day nitrogen balance periods alternating with the same number in which milk was given isoproteically. Table 1, taken from a more detailed article now in press,[3] summarizes the results and indicates that although the nitrogen of Mixtures 9A and 9B, like that of Mixture 8, is not absorbed as well as that of milk, nitrogen retentions are not significantly different at adequate protein intakes. At levels of intake grossly deficient for the physiological needs of the children, retentions are somewhat higher with milk

although the nitrogen balances are still strongly positive with Mixture 9B, an indication of good protein quality.

TABLE 1
COMPARISON OF VEGETABLE MIXTURE 9A OR 9B AND MILK IN YOUNG CHILDREN *

gm prot/kg	No. children	No. balance periods each mixture	MILK			VEGETABLE MIXTURE 9A or 9B		
			Intake gm/kg	Absorption % of intake	Retention % of intake	Intake gm/kg	Absorption % of intake	Retention % of intake
>4.0	1	2	4.0	84.4	22.0	4	66.1	24.1
3.0—3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0—2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0—1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
<1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

*Scrimshaw et al. (Ref. 3)

THERAPEUTIC TRIALS

Five children with acute kwashiorkor have been treated with Mixture 8 as the sole source of protein,[3,4] and five with Mixtures 9A or 9B.[3] In all cases the vegetable mixture was well accepted and recovery was prompt and satisfactory; the results could not be distinguished from those in comparable cases treated with milk except for the slower regeneration of serum protein previously referred to.

FIELD TRIALS

In order to determine the acceptability of INCAP Vegetable Mixtures 9A and 9B among needy families in rural areas, 76 families in four widely separated Guatemalan communities were given sufficient amounts of Mixture 9A for each preschool child in the family to receive 3 glasses daily. The product was packed in plastic bags of 75 gm, using the name "Incaparina," and mothers were carefully instructed in its preparation. The trials varied from 17 to 19 weeks in duration. Initial acceptance was extremely good and tended to improve further during the trial period. Midway through the trial, distribution was changed to formula 9B without causing comment from the recipients. Ninety-nine children out of a total of 129 consumed an average of 2 or more glasses daily throughout the entire period; during the final 2 weeks, 110 children consumed 2 or more glasses daily. The great majority of these children stated that they liked the drink very much and most of the parents said that they would purchase it if it were available at low cost.

A similar trial was carried out among 53 preschool children in a poor district of San Salvador, the capital city of El Salvador.* Eighty-one per cent of the children stated that they liked it at the end of the first week and 88% at the end of the fourth week; every child drank all of the "Incaparina" offered to him. Favorable acceptability information was also received from Honduras and Nicaragua and from other groups in Guatemala.

* Carried out under the supervision of Dra. Amanda Stella de López, Chief of the Nutrition Section of the National Health Department, El Salvador.

Marketing Trials

On the basis of the encouraging acceptability results, an experimental marketing trial was begun in the Guatemalan village of Palin with a predominantly Indian population of about 4,000. When the product was made available in the local stores at 3 cents for a 75-gm bag, sufficient for 3 glasses with a protein content comparable to that of a similar amount of milk, demand early stabilized at 1,200 bags per week and remained at this level during the 5 months of the trial. Promotional efforts were limited to recommendations by personnel of the health center for its use and the distribution of samples to needy families.

The government of Guatemala then arranged for a marketing trial to include 43 widely scattered towns selected because they had functioning health centers which could recommend the use of "Incaparina." Initial production of 8,000 bags per day proved inadequate even before complete distribution to the chosen centers could be effected. Although production was subsequently raised to 12,000 bags per day, demand has been such that distribution has been progressively limited to the two principal cities, thus vitiating the primary objective of the trial. The situation cannot be corrected until the projected completion of more ample production facilities. There has been no opportunity to determine the effect of more intensive promotion on the results of marketing trials.

Additional Comments

In presenting this account of INCAP efforts to develop vegetable protein mixtures for human feeding, we would like to stress several points. These studies and others of a similar nature reported at this conference demonstrate conclusively that it is technically possible and economically feasible to develop acceptable protein-rich food mixtures of vegetable origin which are low in cost and high in nutritive value. For many areas where other good sources of protein are in short supply or beyond the purchasing power of the people most in need, mixtures of this type can provide a valuable means of combating protein malnutrition.

It should be emphasized, however, that the mere availability of such mixtures will not in itself solve the problem any more than the possession of an ax will ensure that the trees in a forest are felled. It may be impossible to cut down the trees without an ax or other suitable tool, but once the tool is obtained, much hard work lies ahead. In the prevention of protein malnutrition, once protein-rich foods are available in adequate quantity at low enough cost, hard work lies ahead in nutrition education. Special efforts are required to bring about an improvement in the methods of feeding young children after weaning.

Furthermore, the limitation of this discussion to protein sources of vegetable origin should not detract from recognition of the need to use all available protein-containing foods of good quality. There are obviously a great many different useful sources of protein for the prevention of protein malnutrition in technically

underdeveloped areas. Milk must still be considered of first importance and, of course, increased production of cheese, eggs and meat should be encouraged. For some regions, fish flour or meat meals may be practical new sources of animal protein. Oil-seed meals such as those from sesame, peanut, sunflower; legumes such as soy, cow pea and chick pea and concentrates of leaf protein may be of major value in one area or another.

Wherever there is sufficient cotton production to support the large-scale industrial processing of the seed into cake and oil, we believe that cottonseed flour will prove to be one of the cheapest and most effective protein concentrates to combine with local cereal staples. For any mixture of cottonseed flour and a cereal grain, the approximate proportion employed in INCAP Vegetable Mixture 9 is likely to prove the most effective.

Summary

Basic studies in animals and biochemical observations on children demonstrate a high nutritional value for INCAP Vegetable Mixtures 8 and 9 and their variants. These mixtures have also proved highly acceptable to young children recovering from protein malnutrition and have been found to be effective protein sources for the treatment of kwashiorkor. When compared with isoproteic amounts of milk in alternate 5-day nitrogen balance periods in young children at adequate levels of intake, the amount of nitrogen retained as per cent of intake is not significantly different. At inadequate levels of intake, retention is still good although somewhat less than with milk.

In field trials in Guatemala, El Salvador, Honduras and Nicaragua, Mixture 9A or 9B was accepted readily in the form of an "atole," a thin cereal gruel cooked for 15 minutes and flavored with sugar and cinnamon, vanilla, anise or chocolate. Packed in 75-gm plastic bags sufficient for mixing with 3 glasses of water, test market trials of Mixture 9B under the name "Incaparina" have been very successful. It is well liked by adults as well as children.

The results of the INCAP work on these mixtures demonstrate that it is possible to develop locally acceptable and low-cost all-vegetable mixtures for human feeding which have a high protein value and which also serve as a balanced source of other essential nutrients. It is believed that, in the world-wide fight against protein malnutrition, such mixtures will be required to supplement the protein from foods of animal origin which are often costly or in short supply in technically underdeveloped areas.

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DISCUSSION[1]

DR. NICOL: How was the amino acid composition of the foods determined?

DR. HOLT: What were the ages of the children in which the comparison was made between milk and the vegetable mixture?

DR. MERTZ: Why is it necessary to use a 3- or 4-day nitrogen-free period before giving this milk tolerance test? Is it possible that with the overnight fast they would not be as deficient?

DR. BENDER: This question has been mentioned, but I don't think answered as completely as I would like to know. How far do the rat and chick laboratory tests agree with the clinical findings? You did mention there was a difference, but can we really forecast for clinical use from the work?

DR. RAO: What cost of transportation is involved in the food mixture which Dr. Scrimshaw has put on the market?

DR. PLATT: I was glad Dr. Scrimshaw asked for a better characterization of the material being used. I wish he would extend it to his colleagues and explain to us what black beans are. I suspect they were *Dolichos lablab*. We can't have proper communication unless we have some standard of reference, and that should be the botanical name of the material. For example, there are at least half a dozen millets. I suspect he was talking about *Pennisetum typhoideum*.

[1]Editor's Note—This discussion covers the three preceding papers. Questions were noted by the authors and discussed as indicated.

In regard to Dr. Arroyave's paper, did you find all of the plasma protein equally affected, or was some less responsive than others?

DR. PICKERING: I should like to ask two questions. One pertains to the mineral content of this preparation. I know the relative proportions of calcium and phosphorus are quite contrary, if I observed the figures properly, to what we normally associate with animal protein, particularly in milk. I would like also to get some information, if possible, relative to the potassium content and the total ash.

Secondly, I would like to inquire about the effectiveness of this compound preparation in terms of the skeletal system. We have had a decided lack of reference to the skeletal system thus far by way of analysis of the tissue, its growth, its composition in various areas. We have not even seen a growth chart from the standpoint of linear growth. Since this has had such an obviously effective acceptance in the laboratory and the field, it would not be fair to leave this undone.

DR. HANSEN: Is there any explanation for the poorer absorption of the vegetable mixtures?

DR. DEAN: I shall be talking this afternoon about a somewhat similar mixture we are using. May I ask the amount of protein per 100 gm and the calories provided by 100 gm of this mixture which, as far as I can tell, is about twice as expensive in terms of American money as the one that we are making.

DR. PATWARDHAN: I would like to ask Dr. Arroyave how far his findings with regard to plasma protein would be affected by changes in plasma volume. In highly protein-malnourished individuals we find there is an absolute lessening. Would these changes in plasma volume be different with different quality proteins, and how far would that affect the figures which he has seen?

The second question which I should like to ask Dr. Arroyave also arises from the paper by Dr. Bressani in which he indicated that the rice-bean mixture gave the best protein ratio. You tried the corn-bean mixture. Do you contemplate comparing these results with children fed on rice-bean mixture which gave the best protein ratio?

DR. ROSE: Dr. Scrimshaw, I wonder if you and your colleagues would like to answer these before you collect any more.

DR. SCRIMSHAW: Perhaps Dr. Bressani should start by commenting on the amino acid analyses, the black beans that we used, and the comparison between the animal experiments and the clinical results.

DR. BRESSANI: The beans were a dark-colored *Phaseolus vulgaris* common in Central America. They are similar to the kidney bean but smaller. The

cowpea was *Vigna sinensis*, and the sorghum was *Sorghum vulgare*. The corn was either white or yellow *Zea mays*.

Their amino acid composition was determined by microbiological methods. Cooked *Phaseola vulgaris* are deficient not only in methionine, but also in isoleucine and valine. In these beans the proportions of isoleucine to leucine and valine are quite different from those in corn.

DR. SCRIMSHAW: A comparison of your biological results with the clinical observations?

DR. BRESSANI: I think they compare quite well. Furthermore, in the studies of Dr. Arroyave on plasma amino acid change in children in relation to composition of the dietary protein, the results reflect the leucine/isoleucine imbalance and the lysine deficiency in corn very well. A high leucine increase was observed in one instance after a test meal of milk when the previous diet given to the child was a corn-bean mixture. In this same child a negative change in plasma lysine occurred. The level of lysine in the corn-bean mixture appears higher than in the amino acid pattern of the FAO reference protein; the latter is 270, and the corn-bean mixture had 330 mg/gm N, but since the beans were cooked, I believe that a binding of the lysine made it partially unavailable to the organism.

If you correct the first limiting-amino-acid deficiency of the corn-bean mixture by adding methionine and then add the lysine, you get a further increase in growth efficiency and a decrease in the amount of liver fat. I think the results of the experiments with chicks, rats and children all tend to agree; the tendencies are always the same.

DR. ARROYAVE: Dr. Mertz asked whether we considered a nitrogen-free diet for 3 to 4 days previous to the milk test necessary. We do not; in the second set of 3 children presented we did not use an initial nitrogen-free period. The children were given their last feeding of the previous diet at about 5:00 p.m. and fasted overnight until the first sample of blood was taken the following morning. In the first experiment, the nitrogen-free diet was really part of another study which we were conducting simultaneously on the same children.

Dr. Platt asked whether all of the serum protein fractions showed changes. In the children presented, only total serum proteins were determined, but in our past experience with many similar children we have followed all of the electrophoretically separable fractions during recovery. Albumin is the fraction which is most responsible for the total serum protein increases. There are some changes in other fractions, but they are of much lesser magnitude.

DR. PLATT: What fractions are responsible for not getting up to the normal, if any?

DR. ARROYAVE: It is the albumin fraction. We have determined both serum albumin and total protein, and whenever our increase in total serum proteins

is unsatisfactory, it is the albumin fraction which lags. We try to stress the value and simplicity of total serum protein determinations, particularly for hospital use in following recovery from kwashiorkor.

Dr. Patwardhan asked about the possibility that plasma volume changes are responsible for some of the differences obtained in the changes in plasma proteins, particularly at the beginning. We have found, as Dr. Patwardhan has reported, that there is an initial hemodilution during the first week or so of recovery, which results in a tendency for the plasma proteins to be low. This tendency is overbalanced by the actual protein synthesis during the initial phase of recovery.

The differences in serum during the first week may be less significant due to this, but I do not believe that these differences in blood volume continue into the third or fourth week of treatment. In other words, except for initial fluctuations in blood volume, the steady increase in serum proteins with treatment is definitely due to plasma protein synthesis.

Dr. Patwardhan, did you ask me why we did not test rice and beans also?

DR. PATWARDHAN: My point was that rice and beans were a better mixture than corn and beans, and I suggested a study of plasma amino acids to follow a diet of rice and beans.

DR. ARROYAVE: In the experiments reported we were interested mainly in trying to find another possible approach to the evaluation of the quality of food proteins. We actually looked for a contrast between a poor protein which would still maintain a child relatively well, and two proteins that from the other tests were known to be of relatively high quality.

DR. SCRIMSHAW: The ages of the children varied from 13 months to 5 years, except for one child who was 7 years of age.

In further comment on Dr. Bender's question as to the agreement between the biological trials in experimental animals and in children. There are two fundamental differences in technique to take into consideration. The human trials were done principally at a relatively high protein level based on the estimated requirements of the child, whereas work with the rats and chicks is conventionally done at a level where the protein level is suboptimal. Under these circumstances deficiencies, particularly the lysine deficiency, are detected more readily than under the conditions in which the mixture is fed to the children.

The second factor is that the mixture is thoroughly cooked before it is given to the children but was not cooked before being given to the experimental animals. If you try to give the cooked mixture to chicks it sticks to their bills and you get poor results.

This has a direct bearing on Dr. Hansen's question as to why we get less absorption but higher retention. Dr. Bressani has a series of studies which definitely show a decrease in digestibility of certain of the corn fractions (R. Bressani and N. S. Scrimshaw, 1958. J. Agr. & Food Chem., 6: 774-778).

It looks as if we have had the good luck with Incaparina to have those protein fractions which are relatively poor in their amino acid distribution become less digestible so that the net result of the cooking is a favorable one. From this point of view the lower absorption may be a very fortunate circumstance.

The problem of costs is an important but very difficult area. The actual ingredient cost in Central America is estimated at 4.18 cents per pound. The production cost, including depreciation of plant and equipment, other materials, return on investment, taxes, and so on, brings the calculated cost to 6.21 cents a pound. On top of that you must allow for a wholesale margin, promotion and distribution costs. The estimated cost, allowing for all of these, comes to 10 to 12 cents a pound for Guatemala.

If you take the figure of 12 cents a pound and six 75-gm packages per pound, the wholesale cost becomes approximately 2 cents for 3 glasses. The small storekeeper must then have a profit for selling it. Since he thinks in terms of whole pennies the retail price in Guatemala was fixed at 3 cents to allow the storekeeper a penny. These costs will obviously vary greatly in different regions, but these data give some idea of the economics involved. It can be made available in bulk to institutions at a much lower cost; probably 12 cents a pound or two-thirds of a cent per glass is a reasonable estimate.

A nonprofit operation with subsidized plant and equipment might have a total ingredient and production cost as low as 6 cents a pound or one-third of a cent per glass, and this is the price which should be taken for comparison with that given by Dr. Dean for his product similarly produced. Price comparisons can be very misleading unless the exact items included in a given price are stipulated.

The effects on the skeletal system, both growth and maturation, are of course very important. I did not have time to present the data along these lines. Dr. Wilson of our Institute will be presenting a 15-minute paper to the International Congress next week on bone maturation in children treated for kwashiorkor. To summarize, there is great variation in recovery, in terms of both growth and bone maturation in children with severe malnutrition. It depends on initial severity, frequency and severity of intercurrent infection, age of the child, etc. One would need a much larger series of cases than we can accumulate to know whether there is any real difference. Suffice it to say, however, that from the approximately 15 cases treated with the various forms of INCAP Vegetable Mixtures 8 and 9, we have not been able to detect differences in growth and bone maturation compared with similar cases treated with milk. Since we are studying individual cases intensively instead of treating a large series, we do not yet have enough children with any one kind of treatment to justify statistical analysis of the growth data.

Mineralization of the bones is entirely satisfactory with any of the diets we have used, and we have no way of distinguishing among them in this respect. The apparent Ca/P imbalance is due to an error in the slide. The

correct figures are, per 100 gm, 656 mg calcium and 698 mg phosphorus, an entirely satisfactory ratio, especially when vitamin D activity from exposure to sunshine is high and some of the phosphorus is bound and unavailable. While the sodium content is low, the mixture is intended as a supplement to a mixed diet to which salt is ordinarily added. For your information the values for sodium and potassium mEq/100 gm are 3.7 and 27.9 respectively.

The use of the mixture as a supplement also applies to Dr. Dean's question as to the protein and calorie content of the mixture. The protein content per 100 gm is 27.5, but neither the content of 370 calories per 100 gm nor the 14 calories per gm of protein in the mixture have much meaning. When the mixture is used, considerable sugar is added and it is consumed along with bananas and other carbohydrate sources. We cannot specify the final protein-calorie ratio, since this depends on the total diet. The concentration of protein in Incaparina is sufficiently high, however, that the mixture makes a useful contribution to the protein content of a mixed diet.

There is one further point I would like to make very clear. There is considerable difference between improving the value of the diet by a favorable combination of corn and beans or by adding a low percentage of something to tortilla flour for a total of only 8% or 10% protein, and starting with a food which can be used for child feeding which contains more than 25% protein. The reason that we specified this higher protein content was that we did not know any other way of getting enough protein into a young child to correct mild protein malnutrition or to prevent protein deficiency from developing under stress conditions. Thus, although all of these efforts to improve the quality of mixtures relatively low in protein content are very useful as one part of the approach to the total problem of protein deficiency, they are not in themselves a solution to it. This is particularly true for protein deficiency in the young child. Even enriched cereals are not a substitute for having available foods of relatively high protein concentration and good quality, whether of animal or vegetable origin.

Preliminary Trials in Refeeding Malnourished Infants with Leaf Protein Concentrates

J. C. Waterlow and E. K. Cruickshank

FOR MANY YEARS it has been possible to prepare from green leaves concentrates high in protein and low in fibre. Many technical difficulties, however, have been encountered and the product as a potential protein supplement for animals and man has been disappointing, although the amino acid pattern closely resembles that of soybean. Duckworth et al. (1961) in an as yet unpublished paper review the various possible causes for the low net protein utilisation (NPU) obtained such as:

(1) Low digestibility of protein through improper processing.

(2) Reduction in the amount or metabolic activity of one or more critical amino acids independent of an effect on digestibility of protein.

(3) Failure to destroy, inactivate or remove physiologically deleterious substances naturally present in the raw material.

(4) Contamination with foreign toxic substances during processing.

(5) Formation of physiologically deleterious substances during storage after processing.

Pirie and his colleagues at Rothamsted Experimental Station have been investigating techniques of extraction which will eliminate as far as possible these factors. Recently they have been able to produce concentrates which in preliminary testing give promising results. Duckworth and Woodham (1961) have carried out biological assays on these products using chicks, rats and pigs. The findings for rats are summarised in tables 1 and 2. The methods of protein extraction and of biological assay are described elsewhere (Duckworth et al. 1961). It is apparent from these results that leaf protein concentrates prepared in this way promise to be a valuable source of supplementary protein.

For this reason use of concentrates prepared in the same manner as supplementary protein in the refeeding of malnourished infants recovering from

TABLE 1
MEAN FOOD INTAKES AND MEAN BODYWEIGHT GAINS OF RATS FED FOUR WEEKS ON DIETS CONTAINING DRIED SKIMMED MILK, LEAF PROTEIN CONCENTRATE, COTTONSEED MEAL OR PEANUT MEAL AS SOURCES OF SUPPLEMENTARY PROTEIN *

Diet	Mean Food Intakes (as dry matter)		Mean Bodyweight Gains	
	Males	Females	Males	Females
	Gm	Gm	Gm	Gm
Dried Skimmed Milk	350 ± 8	304 ± 8	118 ± 2.9	83 ± 2.9
Leaf Protein Concentrate	333 ± 8	299 ± 9	96 ± 2.7	78 ± 3.0
Cottonseed Meal	354 ± 8	318 ± 13	89 ± 2.7	82 ± 3.0
Peanut Meal	333 ± 9	305 ± 10	71 ± 4.7	70 ± 3.6

Statistical Analysis

		Food Intakes	Bodyweight Gains
Treatment x Sex Interaction		N.S.	+++
Treatment Differences	Males	+	+++
	Females	+	+

N.S. = Not Significant
\+ = Not Significant, but P < 0.1
+++ = Significant, P < 0.001

* Duckworth et al. (1961) (Ref. 2)

TABLE 2
PROTEIN EFFICIENCY RATIOS OF DIETS CONTAINING DRIED SKIMMED MILK, LEAF PROTEIN CONCENTRATE, COTTONSEED MEAL OR PEANUT MEAL AS SOURCES OF SUPPLEMENTARY PROTEIN, AFTER 2 WEEKS OR 4 WEEKS ON THE DIETS *

Diet	Gross Protein Value	Protein Efficiency Ratio [1]			
		2 Weeks on Diet		4 Weeks on Diet	
		Males	Females	Males	Females
Dried Skimmed Milk	97	2.70 ± 0.06	2.27 ± 0.06	2.68 ± 0.06	2.17 ± 0.06
Leaf Protein Concentrate	(86) [2]	2.16 ± 0.06	1.99 ± 0.07	2.26 ± 0.05	2.06 ± 0.06
Cottonseed Meal	88	1.96 ± 0.06	1.90 ± 0.07	1.97 ± 0.05	1.94 ± 0.06
Peanut Meal	64	1.68 ± 0.10	1.83 ± 0.08	1.75 ± 0.09	1.81 ± 0.07

Statistical Analysis

		2 Weeks on Diet	*4 Weeks on Diet*
Treatment x Sex Interaction		—	++
Treatment Differences	Males	+++	+++
	Females	+++	+

— = Not Significant, but P < 0.1
\+ = Significant, P < 0.05
++ = Significant, P < 0.01
+++ = Significant, P < 0.001

* Duckworth et al. (1961) (Ref. 2)

[1] Protein efficiency ratio = Gm gain per gm protein eaten

[2] Assumed value

kwashiorkor was considered to be justified. This paper reports the preliminary results obtained at the M.R.C. Tropical Metabolic Research Unit at the University College of the West Indies.

Materials and Methods

Seven infants showing the clinical characteristics of infantile malnutrition in Jamaica (Waterlow 1948, Jelliffe, Bras and Stuart 1954) have been studied in the last 2 years. At the time the measurements were made the average weight was about 60% of the ideal weight for the age (Nelson 1952). Nitrogen balance studies were carried out over a 10-day period. The method used has been described in an earlier paper (Wills and Waterlow 1960). Supplementary feeding was given during the second month in the hospital. The first month of treatment was not used as a test period because during that time gains in tissue mass are masked by losses of water even after the disappearance of clinically detectable oedema (Smith 1960). The babies' weights were measured daily at the same time in relation to feedings.

Results

The results of the nitrogen balance studies are summarised in tables 3 and 4. Figure 1 shows the weight gain in infant DG. The other infants made similar weight gains. Leaf protein data are calculated on the assumption that, when a mixture of milk and leaf protein is fed, the proportion of the milk protein absorbed and retained is the same as in the balance period with milk protein alone. This assumption is in fact untrue, since a very important consideration is the *order* in which the trials are done. It has been shown (Wills and Waterlow 1960) that, in babies recovering from malnutrition, nitrogen retention falls as they become repleted, just as Allison (1951) found in dogs. Therefore, in any consecutive trials, retention tends to be less in the second period than in the first and this must be taken into account in evaluating any comparison. In particular this makes inaccurate the derived retention calculated for leaf protein alone.

The leaf protein concentrate (LPC) mixed in milk was perfectly acceptable to the babies.

It is clear that the nitrogen from the LPC is less well absorbed than that from milk. This is not because of diarrhoea; if anything it causes constipation. Since it is unlikely that poor absorption is caused by the high fibre content of the LPC, there is an interesting problem here for further work.

On intakes of 3.5 to 5 gm protein/kg/day from which at least half the nitrogen was derived from LPC, satisfactory retentions were found in all cases. It is impossible in a few trials to make a precise comparison of the nutritive value of the LPC with that of milk because of the natural variability of the cases and possible variation between leaf protein samples. The results suggest that on the average the LPC has a nutritive value about 75% that of milk.

Case 6, which had the lowest nitrogen retention for LPC, was kept on the mixture for a month and his clinical progress was excellent; his weight gain is shown in figure I. In the 19 days after he came off the balance, when he was no

TABLE 3
LEAF PROTEIN BALANCES ON INFANTS RECOVERING FROM MALNUTRITION

Year	Case No.	Diet	Nature of LP	% of Protein from LP	N-Intake mg/kg/day	% N Absorbed	% N Retained	N Retained % of N Absorbed
1958	1	Milk + LP	Wet Process Block	62	772	77	28	36
		Milk	—	0	770	89	42	47
1958	2	Milk + LP	Wet Process Block	60	951	76	34	45
		Milk	—	0	892	87	46	53
1959	3	Milk + LP	Wet Process Block	71	632	76	29	38
		Milk	—	0	696	88	24	27
1959	4	Milk + LP	Wet Process Block	74	840	84	35	42
		Milk	—	0	685	91	22	25
1960	5	Milk + LP	Freeze Dried Turnip Tops	54	536	92	37	41
		Milk	—	0	470	91	31	34
1960	6	Milk	—	0	483	94	54.5	58
		Milk + LP	Freeze Dried Wheat Leaf	50	476	88	31.5	35.5
1960	7	Milk	—	0	483	93.5	36	38.5
		Milk + LP	Freeze Dried Mustard + Tares	50	510	90	25.5	28

TABLE 4
DERIVED DATA (MILK ALONE AND LP ALONE)

Case No.	% N Absorbed		% N Retained		N Retained % of N Absorbed	
	Milk	LP	Milk	LP	Milk	LP
1	89	69.5	42	19.5	47	28
2	87	69	46	26	53	37.5
3	88	71	24	39	27	54.5
4	91	82	22	32	25	39
5	91	93	31	39.5	37	42.5
6	94	82.5	54.5	8.5	58	10
7	93.5	86.5	36	15	38.5	17
Mean	90.5	79.1	36.5	25.7	43.2	32.7

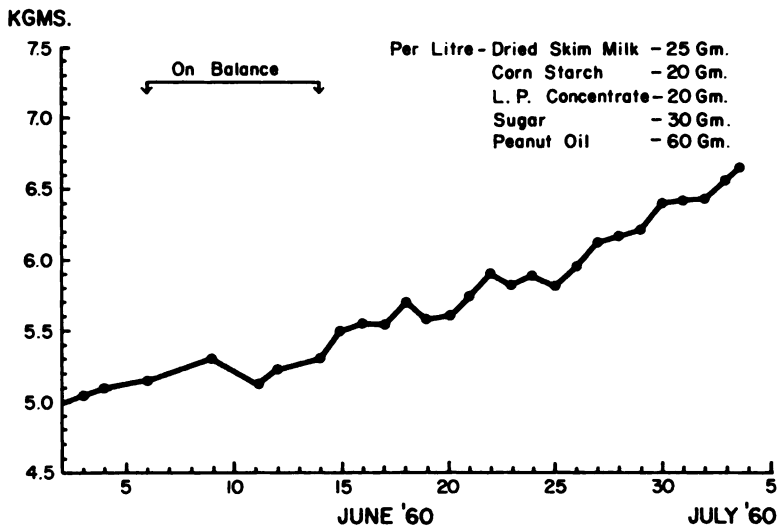

Figure 1

longer immobilised, he gained 1.2 kg. On the basis of the average body weight over the period, the rate of weight gain was 10.6 gm/kg/day on a caloric intake of 156 cal/day and a protein intake of 3.75 gm/kg/day of which half was leaf protein. The expected rate of weight gain on milk alone, calculated from results previously obtained (Waterlow in press), would be 7.2 gm/kg/day. The leaf protein-milk mixture was evidently as effective as a milk mixture of the same protein content.

It is planned to produce LPC in Jamaica. Preliminary studies by Pirie indicate that a number of leaves in Jamaica have a high protein content which can easily be extracted. These include cassava and sugar cane leaves. A pilot plant will be set up shortly and biological assays and trials on infants, similar to those described here, will be carried out. Should this be satisfactory, larger scale production will be contemplated, as it is imperative to find a cheap and effective substitute for dried milk at an early date.

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Africa and the Middle East

Utilization of Indigenous Foods Rich in Protein for the Prevention and Treatment of Malnutrition

R. F. A. Dean

I. THE DEVELOPMENT AND TESTING OF A BISCUIT MEAL

DEVELOPMENT

A biscuit mixture that is intended to supplement the diets of young children has been made, in the form of a dry powder, from cheap locally available ingredients.

Previous experience had shown that, to compete successfully with milk, the mixtures had to include some source of animal protein. The source selected was dried skim milk, and it was necessary to determine a) how the milk could best be added and b) the optimal amount to be used.

The first batches of biscuit were made in a commercial bakery in Kampala. Later, the Unit equipped an experimental kitchen in its own building and made the biscuit there.

In balance experiments on children given first one kind of biscuit and then another, it was found that neither 8% nor 26% dried skim milk produced such good nitrogen retentions as 15% (table 1). By the chemical estimation of ϵ-amino groups of lysine, proof was obtained that, in order to avoid damage to the lysine of the mixtures, the milk should be added uncooked to biscuit made from the other ingredients and powdered (table 2).

Details of the biscuit mixture finally evolved are shown in table 3.

Thus, a food containing 20% protein and providing 492 cal/100 gm has been made from materials locally available, and tested thoroughly.

The cost of the raw materials is 17.8 U.S. cents/kg, and the additional cost of commercial production will probably be 4 U.S. cents/kg.

The biscuit meal is a pale yellowish-grey powder, with a predominant flavour of peanuts. Mixed with a little water, it makes a thick gruel. Mixed with more water, it makes a thin milky drink. It can be incorporated with any staple food and is extremely well accepted by young children. It keeps very well; specimens kept in open containers on the laboratory bench have been edible after one year.

TABLE 1
NITROGEN RETENTION BY CHILDREN GIVEN BISCUIT DIETS CONTAINING 8%, 15%, AND 26% DRIED SKIM MILK

Child No.	Diet	N retention mg/kg/day	Diet	N retention mg/kg/day
14	Biscuit 15U	111	Biscuit 26U	119
15		141	"	133
16		135	"	110
17		94	"	81
18		206	"	124
19		130	"	69
20		184	Biscuit 8U	127
21		200	"	62
22		148	"	109

From Clegg and Dean (1960)

The nomenclature of the biscuits shows the percentage of dried skim milk and the state of the milk (uncooked). From intakes between 550 mg and 900 mg N/kg/day, 6 children on a milk diet (of calcium caseinate, dried skim milk, sugar and cottonseed oil) retained an average of 150 mg N/kg/day, and 10 children on the biscuit diet 15U retained 147 mg N/kg/day.

Advice on commercial manufacture has been obtained from M. Jean Hodeau, a baking expert, sent to Kampala by FAO. The essential points of his report (which is, at the time of writing, not yet in final form) are:

a) Some modifications of the present small-scale method of manufacture are needed so that ordinary machinery can be used.

b) The modifications can be expected to produce a finer and more easily soluble meal.

c) The costs of manufacture should add only about 30 E. African cents per kg (4 U.S. cents) to the cost of the raw materials, provided that no charges on capital expenditure are necessary.

d) The necessary building and machinery would cost about $30,000.

e) Two products could be made from approximately the same raw materials: a biscuit meal for children, that would be packaged as cheaply as possible, and a

TABLE 2
THE LOSS OF "AVAILABLE LYSINE" IN BISCUIT MIXTURES

Mixture	Loss of lysine (% of total)
Peanuts, cereal flour, sucrose, cottonseed oil, cooked with 15% dried skim milk	35
Peanuts, cereal flour, sucrose, cottonseed oil, cooked with 5.9% calcium caseinate (equivalent in protein to 15% dried skim milk)	11
Peanuts, cereal flour, sucrose, cottonseed oil, cooked with 7.5% lactose (equivalent in lactose to 15% dried skim milk)	26
Peanuts, cereal flour, sucrose, cottonseed oil, cooked with 5.9% calcium caseinate and 7.5% lactose	34
Peanuts, cereal flour, cottonseed oil, cooked with 15% dried skim milk	31

The reduction in "available lysine" appeared to be largely due to the lactose of the dried skim milk.

Adapted from Clegg (1960).

TABLE 3
DETAILS OF BISCUIT 15U
For 1 kg biscuit

Ingredient	Cost of 1 kg (E.A. cents)	Amount gm	Cost (E.A. cents)	Calories	Protein gm	Cost of 1 gm protein (E.A. cents)	100 cals.
Peanuts	132.0	410	54.1	2415*	123*	0.44	2.24
				2525*	131*		
Corn meal	33.0	260	8.6	866	22	0.39	0.99
Sucrose	130.0	120	15.6	450	0	—	3.47
Cottonseed oil	263.0	60	15.8	560	0	—	2.82
Dried skim milk	220.0	150	33.0	524	51	0.65	6.30
Total		1000	127.1	4815*	196*		
				4925*	204*	0.65	2.64

* Variations of different batches of peanuts.

The shelled peanuts were ground whole, with their red skins, mixed with the corn meal and sugar and one half of the oil, and baked in a pastry oven in slabs 0.7 cm thick at 200°C for 20 minutes. The biscuit was hammer-milled to a fine powder, into which were mixed the rest of the oil and the dried skim milk.

100 E.A. cents equals 14 U.S. cents.

biscuit of high commercial quality, attractively packaged, that could be sold at a considerable profit and still undercut local prices for similar products.

These estimates have been made on the basis of a production of one ton of both the meal and the biscuits daily.

The modifications necessary consist of changes in the method of preparation of ingredients (a short preliminary toasting of the peanuts and corn flour), a reduction in the time of cooking of the biscuit and the use of a roller mill.

Batches of biscuit made in the way suggested by M. Hodeau are being tested, but no results are yet available.

TESTS

The biscuit mixture was tested in two ways: a) In competition, in the treatment of kwashiorkor in children admitted to the Unit's wards, with a diet of calcium caseinate, dried skim milk, sucrose and cottonseed oil (giving equivalent amounts of protein and calories); b) in competition, as a supplement to the diet of underweight children at the Unit's Child Welfare Clinic, with an equal weight of dried skim milk (giving more protein but fewer calories). The results of the tests are summarized in tables 4 and 5.

One kilogram of one or the other of the supplements was issued fortnightly to children attending the Unit's Child Welfare Clinic. The average weight of the children was 55% of a local standard.

The milk provided about 340 gm protein and 3480 calories, the biscuit about 200 gm protein and 4850 calories.

The weight gains of the children over 3-month periods were used for the comparison. From the point of view of statistical analysis the most satisfactory

TABLE 4
WEIGHT LOSS, WEIGHT GAIN AND ALTERATIONS IN SERUM CONSTITUENTS IN CHILDREN TREATED FOR KWASHIORKOR

	Milk diet	Biscuit 15U
Average weight loss [1] (gm/day)	90 (46)	94 (27)
Average weight increase [1] (gm/day)	64 (44)	68 (34)
Average increase of total serum protein [2] (gm/100 ml/day)	0.188 (58)	0.175 (29)
Average increase of total cholesterol (mg/100 ml/day)	4.5 (49)	3.7 (27)
Average increase of amylase (units/100 ml/day)	4.6 (54)	4.1 (24)
Average increase of pseudo-cholinesterase (units/100 ml)	9.0 (18)	8.6 (32)

(Figures in parentheses refer to number of estimations)

The dietary groups were not entirely comparable because, in a few instances, very ill children were given the milk diet in preference to the biscuit.

[1] Weight loss usually continued for 6 to 9 days. Weight gain was calculated for 8 to 12 days.

[2] Serum changes were calculated only for the first 14 days of treatment. After 14 days, the values were so high that the interpretation of further increases was doubtful.

method, although it greatly reduced the number of data, was by pairs, a child given the milk being compared with a child given the biscuit. The criteria for the pairing were: Tribe, sex, age, weight for age at the beginning of the period, freedom from any recent illness causing drop in weight, attendance for 5 or 6 of the supplements available in the period.

There were 93 pairs. Their weight gains are expressed in table 5. According to the analysis, the gains were the same with either supplement, the advantage apparently held by the milk group not being significant.

The analysis of results of experiments of this kind must be made with extreme care, because there is no doubt that regular clinic attendance by itself is likely to be associated with abnormally high weight gains. The mother who takes the trouble to attend regularly will usually feed her child well.

TABLE 5
PERCENTAGE WEIGHT GAINS OF CHILDREN GIVEN DRIED SKIM MILK OR BISCUIT 15U TO SUPPLEMENT THE HOME DIET

$$\left\{ \frac{\text{expected gain in weight by local standard}}{\text{actual gain in weight}} \times 100 \right\}$$

Age	26 to 52 weeks	53 to 104 weeks	105 to 156 weeks	All ages
No. of pairs	13	47	33	93
Percentage gain				
Milk group	104	150	109	129
Biscuit group	110	130	97	115

None of the differences between the percentages for milk/biscuit is significant at the level of 1:20.

II. STUDIES ON CERTAIN ASPECTS OF KWASHIORKOR

A Study of the Amount of Protein Required for the Treatment of Kwashiorkor

No systematic study of the amount of protein required for the treatment of kwashiorkor has previously been made. The need for knowledge of the amount of protein required arose in our work because it was found that, in sick children, the amount of biscuit meal containing 20% protein that could be taken without difficulty was limited to 160 to 180 gm, that is, to 32 to 36 gm protein (or 4 to 4.5 gm/kg).

A standard diet was used, in which all the protein was derived from calcium caseinate and dried skim milk. The rest of the diet was sucrose and cottonseed oil, in amounts sufficient to ensure that 25 to 30 calories were provided for each gram protein (a ratio of total calories to protein calories of 6 to 7.3).

Two methods of assessment were used. Some children were given an unchanged amount of protein per kilogram, and others were given increasing amounts in the period of weight gain after the loss of oedema. It was found that weight loss and increases in serum total protein, total cholesterol, amylase and pseudocholinesterase were the same when the average amount of protein per kilogram was 3.4 gm or 4.4 gm, but that with the lower protein intake the average daily weight gain was only 23 gm, whereas it was 65 gm with the higher intake.

The results were compared with those obtained some years ago, when diets were used that provided an average of 6.9 gm protein/kg, but only 15 calories/gm protein. On those diets, the average daily gain was 90 gm.

It was considered unlikely that the differences in weight gain were due to any large extent to differences in water retention.

Further work is in hand, intended to explore the possibility that, at different stages of treatment, different amounts of protein are needed for the most rapid and satisfactory results.

A Study of Absorption from the Intestine in Relation to the Activity of Pancreatic Enzymes

It was hoped that this work would show whether the α-amino nitrogen, carbohydrate and fat of the milk and biscuit diets used in treatment were absorbed at the same rate or at different rates, and how absorption was related to the activity of pancreatic enzymes, known to be very low at the beginning of treatment but to increase rapidly.

The work is still in a preliminary stage, in which mixtures of calcium caseinate, d-oxylose and cottonseed oil have been introduced into the duodenum or the stomach, and the changes in α-amino nitrogen, xylose, glucose and total lipid in the blood have been measured.

It is quite clear that there is no simple correlation between the activity of the duodenal enzymes and the amounts of the four substances that appear in the blood. It is necessary to extend the work by using actual diets. Techniques enabling individual amino acids to be traced across the intestinal wall and into newly synthesized protein may be required.

Nitrogenous Constituents of the Urine in Kwashiorkor

In some balance experiments on children during convalescence, measurements of blood and urinary urea were made because it was thought that differences might be found that were dependent on the kind of diet. It became obvious that there were large variations in the amount of the total urine nitrogen appearing as urea nitrogen, and a study of nitrogen partition was begun.

The urinary excretion of nitrogen by severe and less severe cases of kwashiorkor on admission and after treatment is summarized in table 6. There is much of interest, of which the most important may be the large amounts of unaccounted nitrogen in the urine of children before treatment. About one-fourth of this nitrogen has been found to be in the form of substances that appear to be purines; details of four of the substances (referred to as A, B, C, and D) are given in table 7. The substances disappear, or can be found only in very small amounts, in children successfully treated.

Table 6
Average Nitrogen Excretion by Children Before and After Treatment for Kwashiorkor
(mg N/12 hours)

Condition of Children Number	Untreated "Severe" 12	Untreated "Less Severe" 10	Treated 10
Total N	722	404	1235
Urea-N	408	242	1117
α-Amino-N	33	24	27
Ammonia-N	64	33	41
Creatine-N	18	5	9
Creatinine-N	22	15	10
Uric acid-N	9	8	11
Total of the 6 constituents	544	317	1215
N not accounted for	178	87	20

Table 7
Excretion of Uric Acid Nitrogen and Other Purine Nitrogen Before and After Treatment
(mg/12 hours)

		Before treatment						After treatment		
Child No.	Severity	Uric acid	A	B	C	D	Total of A,B,C & D	Uric acid	A,B,C	D
1	Severe	8	5	11	23	13	52	7		2
2	Severe	13	—	8	27	9	44	13		—
3	Severe	8	13	29	—	—	42	8		—
4	Severe	9	10	19	—	14	43	12		—
5	Severe	9	40	16	—	18	74	13	not detected	1
7	Severe	11	3	6	—	16	25	10		3
11	Severe	10	—	1	—	2	3	15		2
12	Severe	10	—	3	—	8	11	13		—
13	Less severe	9	—	17	—	9	26	12		—
14	Less severe	8	2	4	17	12	35	9		—

It is thought that the substances are derived from intracellular ribonucleic acid. Some confirmation has been obtained by the estimation of magnesium and potassium in the urines; the results are consistent with the theory that the substances and the metallic ions have a common intracellular origin.

The help of the National Institute for Medical Research of the Medical Research Council in London has been invoked for the identification of the purines, and work in the Unit is proceeding on various lines, including: a) The correlation of disappearance of the purines with changes in clinical condition and in the serum chemistry; b) a study of the means by which the disappearance of the purines can be accelerated; c) the possibility of the demonstration (in animal experiments) of toxic properties of the purine.

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DISCUSSION

DR. ALLISON: Dr. Dean's observations on urinary N present a very interesting biochemical problem. We are finding protein depletion increases ribonuclease activity with reduced RNA.

DR. PATWARDHAN: I am confining my remarks to the latter part of Dr. Dean's paper, that is, the nitrogen in the urine. It is a fact not wholly appreciated that the percentage of urea nitrogen depends entirely on the absolute amount of total urinary nitrogen. Higher values of urea nitrogen excretion in relation to the total urinary nitrogen are obtained when the latter is high. In Dr. Dean's patients or in kswashiorkor the total urine nitrogen excreted per day is considerably less and one would expect that the urea nitrogen would be relatively less.

A few months ago Dr. Dean and I had some talk about undetermined nitrogen which we find in other studies about which I shall talk tomorrow. What I wish to convey here is that Dr. Dean's findings could be explained on the basis of the very low level of nitrogen metabolism that is occurring in these children; they do not necessarily indicate an abnormality of nitrogen metabolism, because other studies on absorption and nitrogen rctention in kwashiorkor and marasmus tend to make us believe that fundamentally there is no difference in the metabolism of nitrogen in kwashiorkor as compared with that in health. We have observed an appreciable amount of purine compounds in urine. There are some studies to show that as soon as an animal is put on a protein-deficient diet, the first change one observes in the cells of the liver is loss of ribonucleoprotein. The process which starts in the liver is followed by the breakdown of similar compounds in other tissues. With the low nitrogen metabolism in these cases, one finds that the nitrogenous constituents other than urea are present in urine in higher proportions than they normally are. The picture would appear to be abnormal but it reverts to what you call normal on treatment. I maintain that all the time it has been normal and is only an abnormal manifestation of a normal process highlighted simply because the total nitrogen has been less.

DR. DEAN: When these children come in very, very ill, at the beginning of treatment and we put them on 12 hours of glucose, which I told you about, those children are losing 1 gm of nitrogen a day although their intake has been, as far as we can control it, nothing. At that time they are producing large quantities of these purine substances which a week later you cannot detect at all. It isn't as though the amount of purine remains the same. It doesn't. As I told you, they go right on down to nothing. I think there must be something abnormal about it. I admit I may have the wrong interpretation, but I think it must be considered abnormal and not merely an abnormal manifestation of a normal procedure.

DR. PATWARDHAN: May I add, when you feed these patients you arrest the breakdown of tissue protein. When this happens you should expect less and less purine metabolism products in urine.

DR. BENDER: I think I can offer a little assistance, but it may be more confusing, on this particular point. Some years ago we observed if rats were kept on a protein-free diet, after 8 weeks we observed a nitrogen compound in the urine which is unaccounted for. In the rat, by estimating ammonia, urea, creatinine and uric acid, we accounted for 95% to 100% of the total nitrogen. This was true until about the 8th week. Then this unknown compound appeared and amounted to about 30% of the total nitrogen. We did make a concentrate of it, but we never devoted sufficient time to identify it. It was certainly not purine compounds. There was some change in metabolism and the animal was now excreting something it may have been excreting

before at a level of only 3% or 4%. Normally in human and rat urines there is about 5% of unaccounted nitrogen.

In reference to the peanut biscuit, I was rather surprised to hear that, after Dr. Dean had spent so much time finding out that his mixture was a good one, he then toasts his proteins. We have done many assays for UNICEF to try to prevent people from accidentally overheating their proteins. Dr. Dean here apparently deliberately is toasting his peanut and his corn and has admitted no damage is being done.

Have you any evidence from the tests that this is a safe procedure and that you are not reducing the BV?

DR. DEAN: We are hoping to get some assistance from Dr. Bender on the subject. I am not the slightest bit happy about it. I thought I made it perfectly clear that before we go into commercial production we must settle this point. There are two ways by which the point can be settled. One is by making a mixture and feeding it to children which, after all, you probably will agree is enormously important. The other is to ask you to do the tests which you have done before.

Going back to this unaccounted nitrogen, we not only estimate urea nitrogen, but also the free and the combined α-amino nitrogen. As far as I know, nobody has ever estimated the peptide nitrogen.

DR. ARROYAVE: I want to say something about the ratio of urea to total nitrogen. We have the same experience as Dr. Patwardhan in the sense that the ratio is lower in children living under limited nutritional conditions, but not suffering from kwashiorkor, of course. We reach values of about 70% as compared with our well-nourished children of comparable ages, in which it is from 85% to 90%. We have not attempted to interpret this low ratio of 70% as an indication of abnormal metabolism but only, as you said, Dr. Patwardhan, as a function of the total nitrogen excretion.

Another point I want to make is that maybe further informative data could be obtained by observing pentose excretion in conditions similar to or suggesting muscle or tissue deterioration, as, for instance, vitamin deficiency, in which purines, creatine and pentose; the three together would give more information.

I wonder if you have some data on creatine excretion. We do have some, and it seems not to be increased above what it should be. In other words, it is high relative to creatinine because creatinine is so low, but it doesn't decrease with treatment as we would expect if it were an indication of abnormal metabolism.

DR. DEAN: We don't have much information on creatine and creatinine nitrogen, except that in the very severe untreated cases we have 18 mg of creatine nitrogen excreted in 12 hours, and 5 in the less severe, and 9 in the treated children. Creatinine nitrogen is 22, 15 and 10. We have not found the difference in the ratio between creatine and creatinine, the before and after ratio.

DR. ARROYAVE: Would you not expect, if the purine materials are what you think they are, it should be accompanied by a large excretion of creatine?

DR. DEAN: Not necessarily. I would certainly say that, if the purine materials come from where we think they do, they must be accompanied by pyrimidine derivatives and also by sugar. As I say, we are looking for the sugar and are looking for the pyrimidines.

DR. ALTSCHUL: I would like to come back to Dr. Bender's question about the toasting. If I understand correctly, you are toasting the materials at moisture contents of less than 10%. Under those circumstances, I would expect that very little damage would take place.

DR. BENDER: I would like to reserve judgment on that point, because it seems that in various proteins that have been dried under various conditions, there is some peculiar circumstance of moisture and temperature at which the damage is done. It may be that it can happen around 10%. We have had various trials where people have deliberately tried heat damage and have not achieved it, and other trials where they sought to produce a perfect protein and it has been damaged. I think at a low percent moisture it is doubtful. There is some particular series of circumstances under which damage occurs even under relatively mild heating. I think it needs to be tested before we could say we think it is safe.

DR. GOPALAN: You mentioned that you are using whole peanuts, complete with their skin. There is some work implying the presence of goitrogenic agents in the red skin. It has been claimed that feeding this actually retards growth in rats. I do not know whether this work has been confirmed, but it is probable that if you remove the red skin you may have a preparation better than the preparation you now have.

DR. DEAN: Hunter suggests it would be a good thing to remove it. Hunter thinks also it spoils the test. We find people normally eat peanuts with the skins on and are quite happy. So we leave them on because it is one less process.

DR. SCRIMSHAW: I don't want to start another battle of the budget, but it is hard to resist comment on your price discussion, because there is obviously a clear difference in philosophy. We feel strongly that these mixtures must stand on their own feet in terms of commercial production, and that what we are after is the lowest possible price consistent with successful commercial development. This is why we have given the price, not just in terms of raw materials or just in terms of production cost, but in terms of what we can reasonably expect to the consumer. As your clarification pointed out, the production cost of our vegetable mixture was just about what you hope to be your distribution cost. If we discounted the machinery and the

depreciation on machinery, and so forth, then we would come to about half of your figure.

This is reasonable, because an all-vegetable mixture should be cheaper than one which has milk. If we put 15% milk into ours, it is so constituted that it would probably not be improved, but the price would be increased. Whether you use cottonseed flour or whether you use milk or whether you use fish flour or whatever, seems to us to depend on local conditions, but at least the price ought to be on a comparable basis.

DR. DEAN: I quite agree. Mr. Hodeau has suggested we should make two kinds of mixtures, one of them a powder and used for dispensaries in treatment, and so on, the other one using exactly the same formula but kept as a biscuit and made into a sandwich or something like peanut butter. It would be an excellent biscuit, and if we were to charge 100% profit and perhaps another 50% for distribution cost, we could still undercut local biscuit production by about 100%. He says do that and plow the profits back into subsidy, making them a subsidy for your other material. You are absolutely right. A thing like this must be inserted into the economy to be of real use to the country. People must buy it. Free distribution of these things is hopeless as a long-term policy and doesn't work except for a limited purpose.

The Effects of Various Forms of Supplementation on the Nutritive Value of Maize for Children

J. D. L. Hansen

In the research programme on protein foods supported by the Committee on Protein Malnutrition the Cape Town group has concentrated on various ways and means of supplementing maize so as to improve its nutritive value for children.

Maize is the largest cereal crop in the Union of South Africa, and amongst rural populations 52% to 75% of human calorie consumption is derived from it.[1] It is one of the most common foods given to African infants when they are weaned. The association of predominantly maize diets with a high incidence of kwashiorkor is well known both in Africa and in South and Central America. From the economic point of view maize provides more calories per acre than wheat or rice and is resistant to crop diseases. The African is accustomed to eating maize, and even the urbanised African consumes an average of 400 gm of it each day.[2]

As in common with other cereal staples, it is only when maize is used as the sole or main item in diet that its deficiencies from the nutritional point of view become significant. In urban areas in South Africa, maize is frequently the only item in the diet of preschool children because it is cheap and easily available. Supplementation of this diet with animal protein products such as milk, eggs and meat is frequently not within the means of the family income of a vast section of the lower socio-economic group. In large rural areas of Africa, agricultural conditions are such that the raising of livestock is impracticable, and maize and other vegetable crops are the only foods available to young children during most of the year. It is for these reasons that it was deemed advisable to test various ways of supplementing maize protein so as to produce a protein mixture of adequate nutritive value for preschool children.

In this report the nutritive value of maize protein supplemented with lysine and tryptophan, with pea flour, with pea flour and fish flour, or with pea flour and milk will be compared with the nutritive value of diets in which maize or milk are the sole sources of protein. The term "nutritive value" of a protein for children is used in preference to the more exact term "biological value" which has to be

determined experimentally in animals. In this communication the term "biological value" is avoided except where it can be applied in its technical sense.

METHODS

Convalescent cases of kwashiorkor were used as test subjects between 15 and 100 days after their admission to hospital for treatment. Cure had already been initiated in each case and the children were regarded as being in the "consolidation of cure" phase. Care was taken to ensure that there was no clinical or other evidence of infection or diarrhoea at the time of testing. The children were accommodated in a metabolic ward and looked after by a special team of trained nurse-aides.

Nutritive value was assessed by means of 3-day nitrogen balance periods. Weight gain and serum albumin concentration were also observed but periods on the different diets were of insufficient length for statistical analysis of these indices.

Balance technique and chemical estimations were identical to those previously described.[3]

Composition of the Diets

Composition of the diets is shown in table 1 and the amino acid pattern in table 2. It should be noted that corn starch, sugar and oil were added to the basic formulae to provide extra calories and bulk, especially when the various proteins were being tested for nutritive value at lower nitrogen intakes. The synthetic amino acids L-lysine and L-tryptophan were added to the maize to give a mean increase of 176 mg/gm nitrogen of lysine and 47 mg/gm nitrogen of tryptophan to the maize protein. The milk and fish flour supplements to the maize/pea mixture were so designed that they each provided the same proportion of extra protein to the mixture. As the primary object of the investigation was to estimate the nutritive value of the protein of the various diets, a daily vitamin supplement as

Table 1
Diets

Protein Source	% Protein
Maize	9
Maize + lysine + tryptophan	9
Maize + pea flour 66 : 33	14
Maize + pea flour + milk powder 60 : 30 : 20 (18%)	18
Maize + pea flour + fish flour 60 : 30 : 10	20
Milk	35

Calorie additions: Cornstarch (maize), sugar, sunflower seed oil

Vitamin mixture: ABIDEC (Parke Davis Lb. Pty. Ltd.)
Daily dose: vitamin A—5,000 units; vitamin D—1,000 units; vitamin B_1—1 mg; riboflavin—0.4 mg; pyridoxine—0.5 mg; nicotinamide—5 mg; ascorbic acid—25 mg

Mineral mixture: 1.5 gm daily of the following: $CaCO_3$—33%; K_2HPO_4—34%; $CaHPO_4$—7%; NaCl—16%; $MgSO_4$—10%

TABLE 2
AMINO ACID PATTERN
mg/gm nitrogen

Diet	Isoleucine	Leucine	Lysine	Phenyl-alanine	Sulphur Amino Acids	Meth-ionine	Threonine	Trypto-phan	Valine	Protein Score
FAO Prov. Pattern	270	306	270	180	270	144	180	90	270	100
Milk [1]	407	630	496	311	*211*	154	292	90	440	78
Maize [2]	269	800	163		328	175	219		400	
Maize [3]	256	856	175	344	269	150	269	*37.5*	338	42
Maize+Lysine+Tryptophan	256	856	351	344	269	150	269	84.5	338	
Pea Flour [2]	244	413	350	263	125	*56*	150	(74)	294	39
Fish Flour [3]	313	488	488	294	507	194	250	*56*	375	62
Maize/Pea [2] 66 : 33	256	581	269	(291)	212	106	181	(*59*)	344	65
Maize/Pea/Fish 60 : 30 : 10	276	546	349	(293)	319	139	205	(*58*)	352	65
Maize/Pea/Milk 60 : 30 : 20	310	598	352	(300)	*212*	124	221	(70)	376	78

[1] Data from USDA Home Econ. Res. Rpt. No. 4 (1957)
[2] H. E. Bender
[3] Fishing Industries Research Institute

( ) Calculated from tables.

shown in table 1 was given to all children. A mineral supplement was given to all those receiving vegetable formulae.

Preparation of the Diets

The maize and supplemented maize diets were cooked on an open stove for about 20 minutes. Water was added to ensure proper consistency. The 24-hour feed was divided into 5 or 7 portions and administered every 4 hours by spoon.

Special Difficulties when Determining the Nutritive Value of Proteins for Children

The most important of the special difficulties associated with determining the nutritive value of proteins for children is the state of depletion of the test subject. The nutritive value of proteins alters with increasing protein depletion. Allison demonstrated this clearly with his protein-depleted dogs,[4,5] and this has been shown to apply also with protein-depleted infants.[3] Thus kwashiorkor children early in treatment retain nitrogen less avidly but more efficiently than normal children of the same age (chart I). When comparing protein formulae

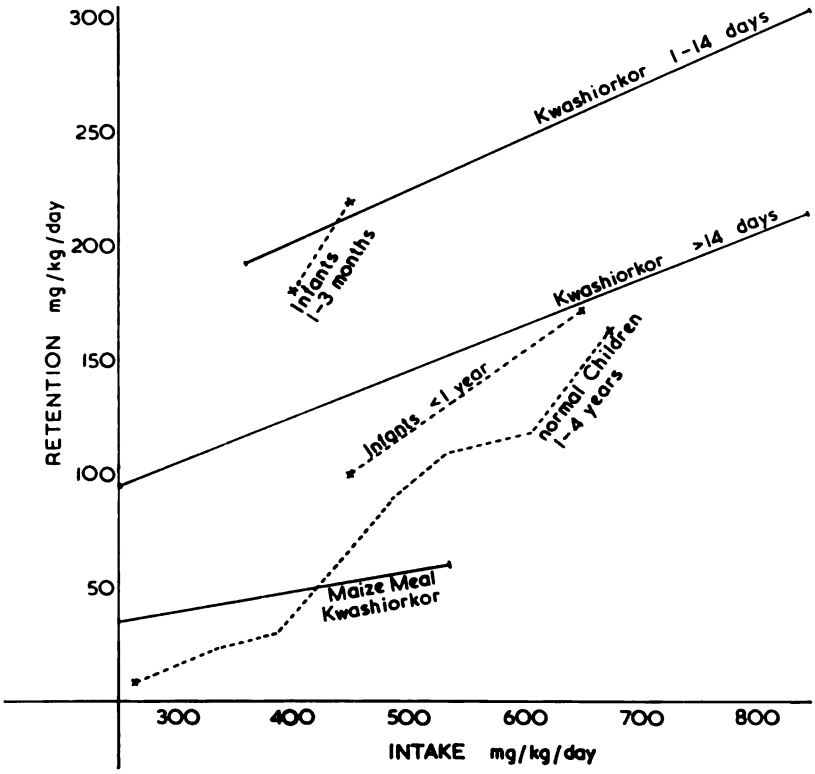

Chart I—Relation of nitrogen retention to nitrogen intake in normal children and in children recovering from kwashiorkor. Data for infants aged 1-3 months are from ref. 10; for infants under 1 year and children 1-4 years, ref. 11.

it is thus desirable to have the test subjects in similar states of nutrition as nearly as it is possible by clinical observation. To do this with accuracy is at present not practicable, but we have found in our experience that 15 to 100 days after admission to the hospital with acute kwashiorkor is a useful period during which metabolic and clinical comparisons can be made. We have been unable to detect significant changes in nitrogen balance on comparable intakes within this time.[3]

Other difficulties include those of keeping nitrogen and calorie intake equal when comparing formulae which differ in bulk and digestibility. In all formulae containing maize, for example, there is decreased digestibility.[3] This means that the absolute absorption of nitrogen is less for any particular intake than it is with diets of better digestibility. This naturally affects nitrogen retention, so care has to be taken to see that absorptions of nitrogen are comparable before conclusions are drawn as to the utilization of a protein with a particular amino acid pattern.

RESULTS

Nitrogen Balance

1. Nitrogen intakes between 300 and 400 mg/kg/day (1.9 and 2.5 gm protein/kg/day) were chosen for detailed comparison of maize with other formulae because with a pure maize meal diet it is seldom possible, on account of the large bulk of the diet, to provide more protein than this. The mean balance data are given in table 3 and the data are summarized in chart II.

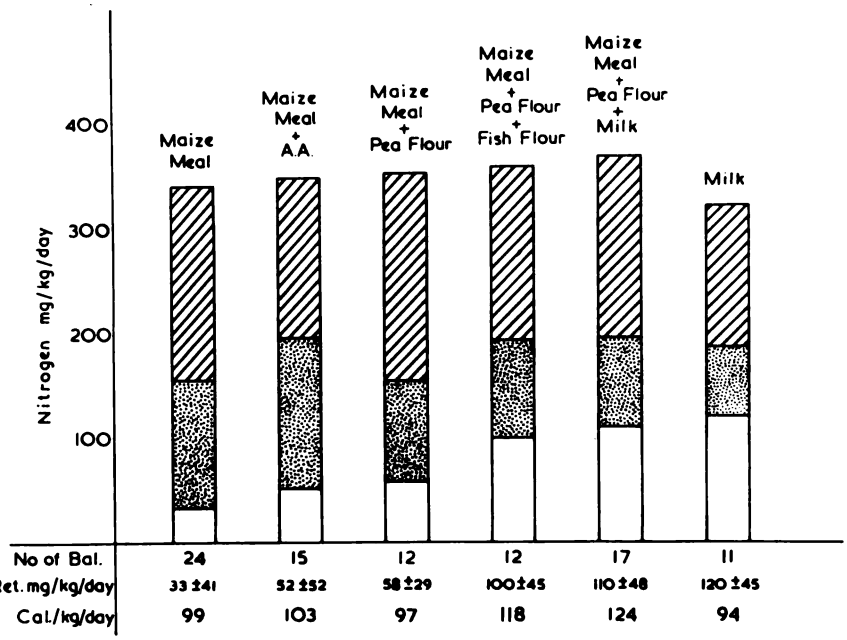

Chart II—Summary of nitrogen balance data. Height of columns represents intake of nitrogen, cross hatching is urine nitrogen, speckled area is stool nitrogen and clear areas represent positive balance.

TABLE 3
NITROGEN BALANCE DATA
mg/kg/day

Diet	No. of Balances	No. of Cases	Calories Total	Calories % from protein	Nitrogen Intake	Nitrogen Urine	Nitrogen Stool	Nitrogen Absorption Total	Nitrogen Absorption % of intake	Retention Total	Retention % of intake	Retention % of absorption
Maize	24	18	99	8.6	341 ± 44	184 ± 35	124 ± 45	217 ± 55	64 ± 14	33 ± 41	9 ± 12	13 ± 20
Maize + Lysine + Tryptophan	15	13	103	8.5	349 ± 50	153 ± 29	144 ± 60	205 ± 59	59 ± 16	52 ± 52	15 ± 13	20 ± 24
Maize+Pea	12	6	97	9.1	353 ± 23	198 ± 21	97 ± 21	256 ± 30	72 ± 6	58 ± 29	16 ± 8	22 ± 10
Maize+Pea+Fish	12	4	118	7.6	359 ± 36	166 ± 24	93 ± 22	264 ± 44	74 ± 7	100 ± 45	27 ± 11	37 ± 14
Maize+Pea+Milk	17	7	124	7.4	368 ± 18	172 ± 35	86 ± 28	282 ± 29	77 ± 7	110 ± 48	30 ± 13	38 ± 15
Milk	11	7	94	8.5	321 ± 37	135 ± 35	66 ± 35	257 ± 43	80 ± 10	120 ± 45	38 ± 14	47 ± 14

a. *The supplementation of maize with the synthetic amino acids, lysine and tryptophan.* The mean retention of nitrogen was increased from 33 ± 41 mg/kg/day to 52 ± 52 mg/kg/day. This increase was not significant. The apparent absorption of maize meal nitrogen was low both with and without the amino acid supplement (59% and 64% respectively) and this obscured the favourable effect of the amino acid supplement on the utilization of maize protein. When the same cases are subjected to alternative periods with and without the supplement, the increase in percentage of nitrogen retained over that absorbed is significant ($p < 0.05$) at all ranges of absorption up to 400 mg/kg/day (chart III). When the nitrogen retention from the amino acid supplemented maize is compared with the retention from the milk diet, it is found to be significantly less ($p < 0.01$).

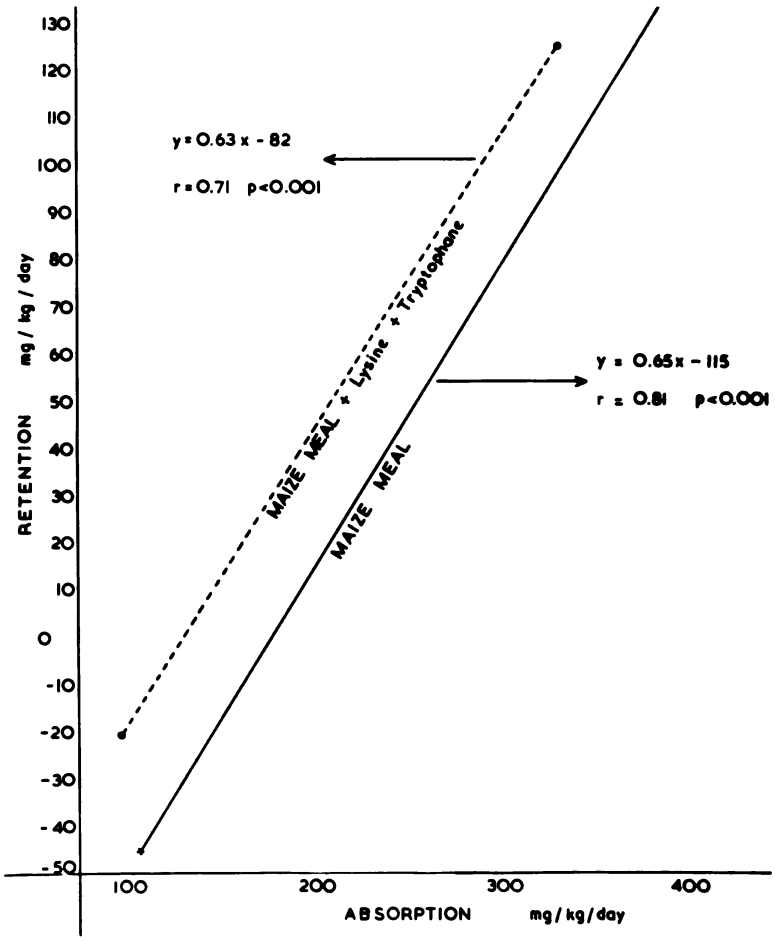

Chart III—Regression lines showing relation of nitrogen retention to absorbed nitrogen on diets of maize meal and maize meal supplemented by lysine and tryptophan.

b. *Maize meal and pea flour.* The nitrogen retention on this diet was 58 ± 28 mg/kg/day. This is not significantly more than that obtained with the maize diet and is certainly less ($p < 0.001$) than the retention from an isonitrogenous milk diet.

c. *Maize meal and pea flour + fish flour.* The nitrogen retention on this diet was 100 ± 45 mg/kg/day which is more than ($p < 0.001$) the retention recorded

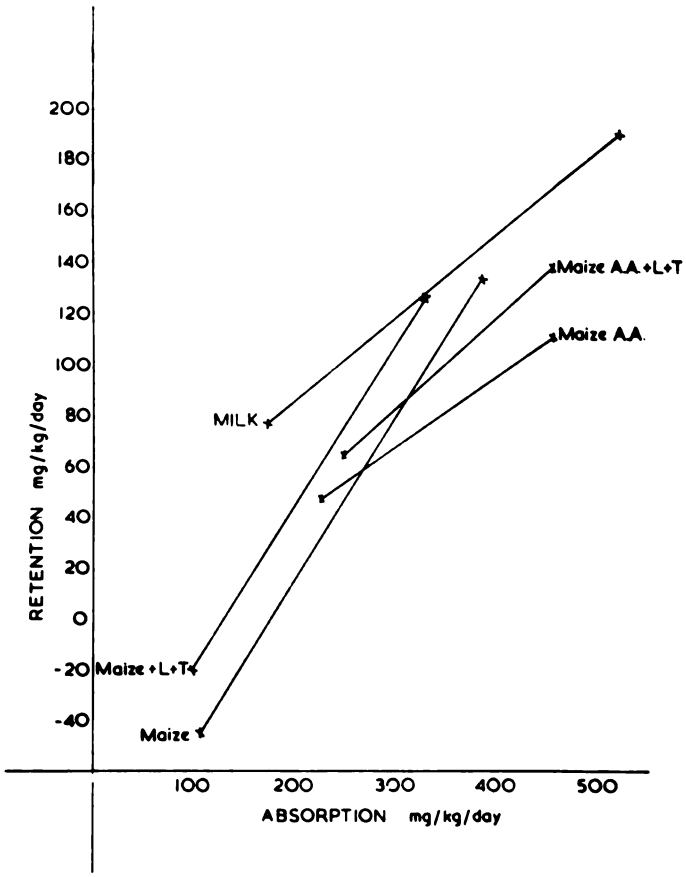

Chart IV—Nitrogen retention from a maize diet compared with nitrogen retention from a synthetic diet based on the amino acid pattern of maize protein (Maize A.A.). The effect on nitrogen retention of lysine and tryptophan supplements to the diets is also shown. At the higher absorption levels, the retention from the supplemented maize diets approaches but does not quite equal the nitrogen retention achieved on a milk diet.

No. of Balances	*Diet*	*Regression equation*	*r*	*p*
23	Maize	y = 0.6455X—114.91	0.8751	< 0.001.
23	Maize + lysine + tryptophan	y = 0.6289X— 81.48	0.8373	< 0.001.
13	Synthetic maize diet	y = 0.2746X— 15.07	0.6359	< 0.02.
13	Synthetic maize + lysine + tryptophan	y = 0.3554X— 23.89	0.7781	< 0.001.

on an unsupplemented maize diet or the maize/pea mixture ($p < 0.02$). It was not different from that of a pure milk diet.

d. *Maize meal + pea flour + skimmed milk.* The nitrogen retention on this diet was 110 ± 48 mg/kg/day which is not different from that of a whole milk diet (120 ± 45 mg/kg/day). It was significantly higher ($p < 0.001$) than the maize diet or the maize-supplemented-by-pea-flour diet.

2. *Nitrogen balance data at all ranges of intake.* These are best illustrated by means of regression lines. It must be remembered that a regression line of retention on absorption or intake is really a tangent to a line that is curvilinear in the upper ranges of the X axis.

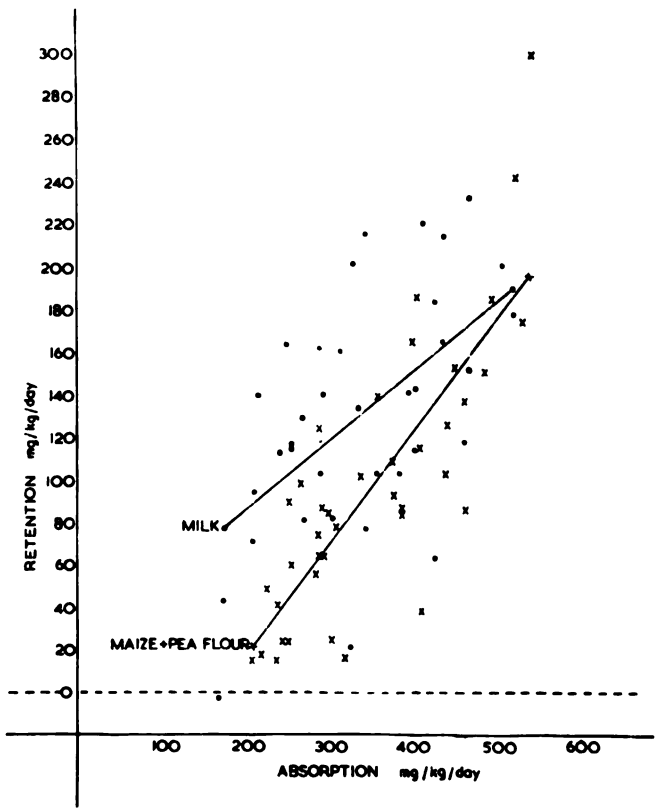

Chart V—Regression lines comparing the retention of nitrogen from the maize/pea mixture with retention of nitrogen from milk. Note how retentions at low intakes are less than milk but that at high intakes there is no difference.

No. of Balances	*Diet*	*Regression equation*	*r*	*p*
36	Milk	$y = 0.3274X + 20.04$	0.5498	< 0.001.
40	Maize/pea	$y = 0.5349X - 90.84$	0.7999	< 0.001.

a. *Comparison of maize, maize plus lysine and tryptophan, and milk (chart IV).* The favourable effect of the amino acid supplementation at all levels of absorption is clearly illustrated by the regression lines. The lines for data obtained from a synthetic replica of a maize diet are also inserted in the diagram so that comparison can be made with a milk diet at higher ranges of absorption. Nitrogen retention from milk protein was superior to that from maize or maize supplemented by lysine and tryptophan even at the higher absorption levels.

b. *Comparison of the supplemented maize mixtures with a milk diet.* In chart V it can be seen that the maize/pea mixture gives nitrogen retentions indistinguishable from those of milk at the higher levels of absorption. This is in contrast to the poor retention in comparison with milk at the lower levels of absorption discussed above. When fish flour (10%) or milk (18%) is added to the maize/pea mixture, however, the retentions of nitrogen are equal to those achieved on a whole milk diet at all ranges of absorption (chart VI).

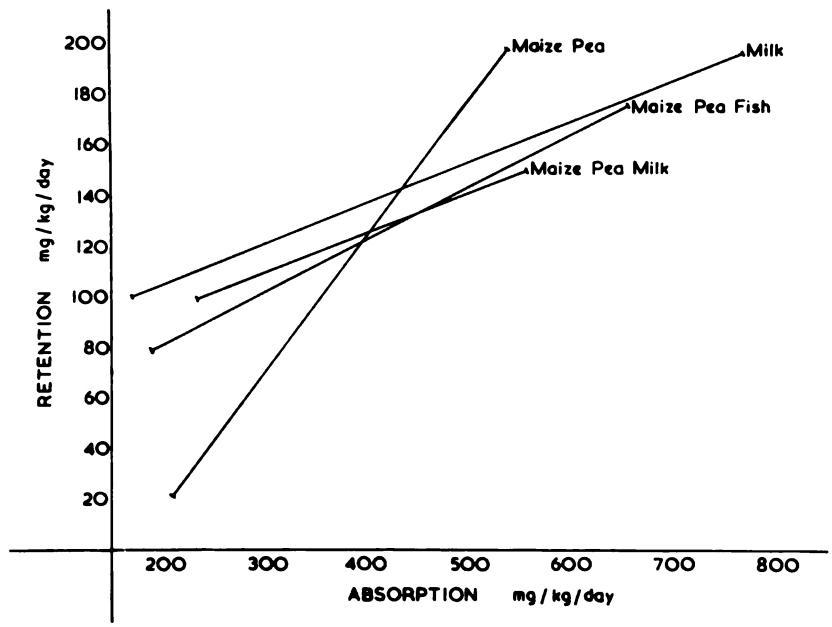

Chart VI—Regression lines comparing retention of nitrogen from milk with that from maize/pea mixture and from the maize/pea mixture supplemented by fish flour or by milk.

No. of Balances	*Diet*	*Regression equation*	*r*	*p*
46	Milk	$y = 0.1595X + 73.14$	0.4135	<0.01.
40	Maize/pea	$y = 0.5349X - 90.84$	0.7999	<0.001.
33	Maize/pea/flour	$y = 0.2062X + 39.97$	0.6002	<0.001.
31	Maize/pea/milk	$y = 0.1560X + 62.32$	0.3868	<0.05.

Digestibility

With all diets containing maize, digestibility is less than that of a milk diet. With a pure maize diet, digestibility is significantly less than that of a milk diet ($p < 0.001$).

Weight Gain

On the maize/pea/fish or maize/pea/milk mixtures, weight gain over a period of a month averaged 28 gm (8 cases) and 39 gm (10 cases) per day, respectively. No direct statistical comparison with milk diets at the same protein intake was possible, but these gains compare favourably with those of a pure maize diet (-1 ± 17 gm/day).

Serum Albumin Concentration

There was no great change in serum albumin concentration during the short period on the diets under test. Cases treated for 3 weeks or more on the maize/pea/fish flour and maize/pea/milk mixtures had the following serum albumin concentrations:

	Maize/Pea/Fish Flour				Maize/Pea/Milk		
	Start	End	Change		Start	End	Change
F.F.	3.65	3.28	−0.37	N.S.	2.20	−3.45	+1.25
M.K.	2.43	3.06	+0.63	R.K.	3.33	−3.22	−0.11
M.P.	3.85	3.42	−0.43	W.A.	2.83	−2.83	0
M.F.	3.65	3.60	−0.05				
D.E.	3.99	3.48	−0.51				

COMMENTS and SUMMARY

The nitrogen balance technique has in this investigation proved very useful in the assessment of the nutritive value of various maize and supplemented maize mixtures. The poor utilization of maize protein as compared with that of milk protein has been a constant finding throughout the studies, and against this background it has been found possible to assess the relative values of synthetic amino acid and animal and vegetable protein supplements.

From the data presented, it is evident that the addition of lysine and tryptophan to maize improves its nutritive value by significantly increasing the utilization of maize nitrogen. From the experience of others,[1,6,7] it would appear that the addition of isoleucine would increase the utilization and therefore the retention of nitrogen still further. The problem of maintaining an adequate total protein intake on a maize diet is not solved by this type of synthetic amino

acid supplementation even if it is found to be possible to bring about retentions of nitrogen equal to those achieved with an isonitrogenous milk diet. The same holds for a wheat diet supplemented by synthetic amino acids.[8] Poor digestibility is a further factor aggravating the quantitative deficiency of maize protein.

The addition of pea flour to maize not only improved the amino acid pattern of the maize protein but made higher intakes of protein possible. The maize/pea mixture proved highly successful in our early experiments where intakes of protein were more than 2.5 gm/kg/day. At intakes below this level, however, the nitrogen retentions were significantly less than those obtained on an isonitrogenous milk diet. From a study of the amino acid pattern of the maize/pea mixture (table 2) it seems probable that at low intakes of protein this vegetable mixture is especially limiting with regard to the sulphur amino acids. It is also marginal with regard to lysine and threonine. Another factor aggravating the deficiency of these essential amino acids is that the digestibility of the vegetable mixture is less than 80%.

In the field, high intakes of protein cannot always be ensured, and improvement of the amino acid pattern of the maize/pea mixture to increase its nutritive value at low protein intakes became necessary. Addition of fish flour (10%) or skimmed milk powder (18%) improved the amino acid pattern, in particular with regard to methionine, lysine and threonine. This improvement appears to have been sufficient to produce protein mixtures the utilization of which was in these studies indistinguishable from that of a milk diet at all levels of nitrogen intake. These animal protein additions have the further advantage of raising the protein content of the maize/pea mixture from 14% to over 20%, which considerably enhances the safety factor with regard to protein deficiency. This is of practical importance in a predominantly maize-eating community.

The balance studies have highlighted the interdependence of qualitative and quantitative requirements of protein in children. The two are really inseparable and in the development of new protein foods must be considered together.

In a recent publication of the Committee on Amino Acids of the NAS-NRC[9] it was stated, on the basis of animal work, that if the biological value of a protein is 60 or more, specific amino acid supplementation is not necessary providing there is a sufficiently high intake of protein. The data reported here from work with children support this concept. The biological value of the maize/pea mixture, as determined by Dr. Bender, is 69 and there is no doubt that it worked very well when sufficient amounts of it were consumed by the children. The biological value of the maize protein was 34 and the children did poorly even under ideal conditions when it was fed as a sole source of protein.

It would seem that it is desirable to produce protein mixtures with biological values of more than 70 to ensure adequate protein nutrition under all environmental conditions. The use of the amino acid pattern to judge protein quality appears to be fully justified if facilities are available to determine amino acid concentrations.

The good results of the maize/pea/fish and maize/pea/milk mixtures have so far only been obtained under metabolic ward conditions. If confirmed by other laboratories and proved successful in the field, they will have important practical

application in the solution of the problem of protein deficiency in the preschool child. Available supplies of animal protein can be stretched much farther if combined with staple cereal or vegetable diets to give mixtures of high biological value. These mixtures could be made commercially available and subsidized where necessary. Economically it is possible in South Africa to produce this type of protein mixture at prices less than that of skimmed milk.

Summary

The nutritive value of maize and of maize supplemented respectively by lysine and tryptophan, pea flour, pea flour with fish flour, and pea flour with milk has been measured by means of nitrogen balance. Nitrogen retention is increased significantly by all the forms of supplementation used. At protein intakes less than 2.5 gm/kg/day the nitrogen retentions achieved with the lysine and tryptophan supplement or with the pea flour supplement were significantly less than those achieved with a milk diet. These differences disappeared with higher intakes of protein. The maize/pea mixture supplemented with milk (18%) or with fish flour (10%) resulted in nitrogen retentions comparable with those of a milk diet at all levels of protein intake. The practical implication of combining small quantities of animal protein with staple cereal or vegetable diets to produce mixtures of high biological value is discussed.

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Protein Malnutrition, Requirement and Supplementation

J. F. Brock

Dr. Hansen's paper[5] illustrates the extent to which the nutritive value of a staple cereal can be enhanced by supplementation with another vegetable and by small additional quantities of skimmed milk or fish meal. In this way the contribution of a given quantity of skimmed milk or fish meal to the nutrition of a community can be increased 6- to 10-fold by dilution with a vegetable combination. In this paper I propose to summarize the results of some tests of the same maize-pea (*Pisum sativum*) vegetable combination, without the milk or fish additions, on older children in an institution for orphans. These tests show, in a different context, the same importance of quantity consumed as is implied in Hansen's figures for intakes of nitrogen less than 400 mg/kg/day. It is at these levels that differences in nutritive value become most apparent.

Terminological Ambiguity and Psychological Resistance

First, however, I want to discuss under this imposing subhead some very important but simple practical problems of administrative procedure in public health nutrition programmes.

It is not easy in the U.S.A. to appreciate the reasons why there exists in underdeveloped countries resistance to the idea that protein malnutrition is widespread even above the age of 5 years and that protein is important for health and longevity. We must face the fact that outside the United States it has been widely assumed that the levels set by the Food and Nutrition Board and other authorities in the U.S.A. for some recommended allowances have been unrealistic and cannot possibly be applied in the greater part of the underdeveloped world. I refer to figures such as 75 mg of ascorbic acid and 0.8 gm of calcium.

If the resistance in underdeveloped countries to these recommended allowances for protein and amino acid is to be overcome it is important to underestimate rather than to overestimate requirements. This is particularly important if, as I believe, protein or some combination of amino acids is likely to be the most limiting foodstuff in the coming decades, during which time mortality rates will fall while family spacing is resisted.

It is also necessary to be very careful in the use of terms since, in the field of protein malnutrition, both confusion and resistance have been created by failure

to define terms accurately. I attempted to promote this accuracy in my Rolleston Lectures (Brock 1959) and have made a further attempt in "Recent Advances in Human Nutrition" (Brock 1961). In the context of the present discussion we must be particularly careful to distinguish between protein and protein foods (foodstuffs). Protein is a chemical concept denoting a variable combination of amino acids. All foods contain protein in significant quantity with the exception of certain highly artificial foods such as refined sugar and butter. In the past we have been accustomed to use the term first-class protein; it is now preferable to speak of protein-rich foods (foodstuffs). The distinction is well expressed quantitatively in terms of grams of protein per 100 calories (Brock and Autret 1952). In practice, if a single food were consumed by a recently weaned child for a sufficient time to run down reserves in the body, cereals would be within the range of real protein requirement; root products would mostly be quite inadequate; the flesh of fish and mammals and skimmed milk would be excessive; and leguminous plants would be slightly to considerably above requirement. These statements apply only to quantity. The concepts have been revised in terms of protein quality and digestibility by Autret and Jacquot (1960) without material change in the general applicability of the principle. Protein malnutrition is another term which has been repeatedly misunderstood and which has therefore led to psychological resistance. Many people who show this resistance appear never to have read the definition of protein malnutrition given by the Joint FAO/WHO Expert Committee on Nutrition in 1953. In my Rolleston Lectures I repeated the definition and drew attention to the fact that after careful reading it remains ambiguous only in one small respect, namely, in the use at one point of the term "protein" when the term "protein food (foodstuff)" should have been used. It is clear from the context that the definition was intended to convey a public health rather than a scientific concept and to denote a state of malnutrition resulting from deficiency of protein-rich foods in conjunction with calories provided in relative excess, or even to optimum level, through starchy foods. The resultant malnutrition will express itself through deficiency of nutrients including many essential amino acids, many vitamins (including especially Vitamin B_{12} and folic acid) and possibly trace mineral elements, ordinarily supplied in the human diet by protein-rich foods. The Central and South American term *sindrome pluricarencial infantil* (SPI) has value in indicating that protein malnutrition is a multinutrient deficiency. On the other hand, the same multiplicity concept was inherent in the original definition of protein malnutrition which was developed out of the consideration of kwashiorkor.

A critical study on initiation of cure in kwashiorkor, including a later report on the reversal of the pellagroid dermatosis on a synthetic formula containing amino acids, glucose, electrolytes and water without any vitamins, has been widely accepted as demonstrating that the most limiting nutrients are a group of amino acids. This seems to imply that the basic cause of kwashiorkor is deficiency of a group of amino acids ordinarily supplied in the diet of developed or privileged communities by protein-rich foods, even though at the same time there is inevitably a deficiency of other nutrients, including vitamins. In other words, to the public health concept that kwashiorkor is due to protein malnutrition can be added that

the most limiting nutrients are a group of amino acids. Failure to distinguish between *protein malnutrition* as a public health concept and *protein or amino acid deficiency* as a scientific concept has led to psychological resistance to the public health importance of protein malnutrition in at least one country.[9] This resistance goes to the heart of the problem involved in the use of the terms *enrichment*, *fortification* and *supplementation*, as applied to foodstuffs and diets. There has been, and still is, ambiguity about the use of these terms.

I would suggest that the term supplementation be used solely in relation to a meal or a diet, whereas the terms fortification and enrichment be used solely in relation to a food (foodstuff) whether in a natural, prepared or processed form. Thus when fish paste or peanut butter is spread on a slice of bread, the nutritive value of the bread is being supplemented. If, on the other hand, fish flour or peanut flour is incorporated in the bread before it is cooked, the bread is being enriched or fortified. There seems here to be no difference in the use of the terms *enrichment** and *fortification* and the latter term will therefore be dropped from the present discussion.

Western mixed diets are based on the principle of enrichment of staple foodstuffs, although this has been worked out over centuries in relation to palatability and only in the modern era has come to have nutritional significance. Originally people spread butter on bread because it was more palatable. Now it is recognized that the butter contains important fat-soluble vitamins and that owing to the high calorie yield per gramme of fat the butter reduces the bulk of the bread which would have had to be consumed to achieve the same calorie intake. Sandwiches have become an important part of the diet for those who take their midday meal away from home. Enrichment of sandwich bread with butter gives not only greater palatability but greater nutritive value. The use of sandwiches in urban communities also accounts for the shift towards wheat as a staple among those who, in their rural life, used other staples such as maize. This is noticeably true of the Bantu in South Africa. Countless other examples could be given of these uses of supplementation in Western diets.

Another method of supplementation has been applied by the gold mining industry of Johannesburg in the Union of South Africa. Owing to Bantu traditional dependence upon maize as the main source of nutrients, "protective" foods have been incorporated in a meat stew and served with maize porridge. This is clearly supplementation of a meal or diet. On the other hand, soybeans and other sources of protein and vitamins have been incorporated in the process of brewing *mahewu*, a nonalcoholic fermented maize gruel to which the Bantu are very partial. These supplements or enrichers have been incorporated in a commercial dehydrated *mahewu* powder. This brings supplementation very close to enrichment, according to the definition given, and illustrates the difficulty of terminology.

For several decades, Western countries have recognized the possible adverse effect of the milling of cereals, e.g. white flour, white rice and white maize. Nutri-

* In the USA the term "enrichment" has a specific legal connotation different from the usage by Dr. Brock in that it is limited to defined levels of thiamine, riboflavin, niacin and iron to be added to enriched cereal products. (Editor's Note).

tionists and dietitians have failed to persuade populations to go back to the obviously more nutritious whole grains; milled products have been preferred because of their palatability, appearance, social status and keeping quality. It became customary, therefore, to enrich white flour with those nutrients which had been removed in the process of milling, e.g. thiamine, niacin and iron. This seems to be an acceptable procedure, although many still complain that not all of the substances removed have been replaced, e.g. cellulose, or that some unknown valuable factor has been removed from the original grain. This latter belief seems to underlie some "back-to-nature" forms of dietary faddism.

A more recent development has been the addition to staple foodstuffs before processing of nutrients judged by health authorities to be relatively deficient in the diet of a population because of too great dependence on the staple foodstuff. This development led to the incorporation of protein-rich foods such as skimmed milk powder, soybean meal and fish meal or fish flour in the Bremer bread of South Africa. When this bread had been found palatable after blind trials on many groups of the population, it was sold as enriched bread at a subsidized price in an attempt to improve the protein intake of the poorer sections of the community. The scheme was, however, criticized on the grounds that: 1) The value of the enrichment had not been demonstrated on rats; 2) the enriched and subsidized bread was consumed not only by the poor but also by the well-to-do; 3) in view of 1) and 2) the cost to the nation was exorbitant; and 4) the money spent on enrichment might better have been expended on bringing food supplements to those groups of the community (pregnant and lactating mothers and preschool children) among whom there was most evidence of protein malnutrition.

This is only one of many examples which could be cited of the difficulties which arise when government departments attempt to apply policies of enrichment, fortification or supplementation.

Recognition of Protein Deficiency

To turn from psychological resistance to scientific observation, it is clear that we still sadly lack objective clinical and biological tests for the demonstration of states of protein deficiency in the human being, at least when the deficiency is mild. My own group has been active in this search for several years, with only partial success. We feel, nevertheless, that progress has been made and that we are within sight of achieving the means for recognizing these milder protein-deficiency states. In a series of papers in preparation,[8] we have summarized the work of several years, which I should like now to discuss.

Biochemical Recognition of Protein Defiency

In comparing formulae for their efficacy in testing for "initiation of cure in kwashiorkor" we have used regeneration of serum albumin as our most sensitive biochemical index. It is not as sensitive an index as was the reticulocyte count in assessing the efficacy of different batches of liver extract in the treatment of pernicious anaemia, but it is certainly as sensitive as the blood haemoglobin which was put forward as a mathematical index. Regression lines for regeneration of serum

albumin constitute a very effective mathematical expression of the comparative efficacy of formulae when used on sufficiently large groups of cases of kwashiorkor.

It appears to follow from this that there must be a marginal range of hypoalbuminaemia which can be used as evidence of impending or early protein deficiency. One can say by analogy that it is now generally accepted that levels of blood haemoglobin between 10 and 12 gm % associated with a low MCH (mean corpuscular haemoglobin) constitute presumptive evidence of iron deficiency. If a group of subjects with haemoglobin within this range is treated therapeutically with iron, and if their mean haemoglobin level rises from the range indicated to a range say between 12 and 14 gm %, most workers will accept this as evidence that the majority of the group, and certainly those whose haemoglobin level rose appreciably, were suffering from a state of iron deficiency which has now been corrected. Applying this analogy to hypoalbuminaemia and protein feeding, we believe that our results in initiation of cure in kwashiorkor establish a range of hypoalbuminaemia which is certain, in the absence of serious complications, to be improved by feeding with a good protein-containing formula. We have examined our records over a period of several years and can define for our own laboratory and environment, and for children between the ages of 2 and 10 years, figures for that range of 2.75 to 3.50 gm %. The figures, of course, apply only to the age and environment indicated and to our own laboratory method (27% sodium sulphate precipitation and Biuret determination). Comparable figures could be established for any other method and any other environment.

Compared with serum albumin, other biochemical indices have been found inferior or less easily applicable. This conclusion applies to serum total cholesterol, serum amylase and serum globulin. Although erythrocyte counts and haemoglobin determinations are almost invariably low in kwashiorkor, we have not found them to constitute sensitive evidence of protein deficiency, probably because of the frequency of associated deficiency of iron, folic acid or even Vitamin B_{12}. The crucial series of experiments on initiation of cure in kwashiorkor with pure protein or synthetic formulae administered only after a control period during which iron, folic acid, Vitamin B_{12} and any other haematinic nutrient were suspected of being deficient, has not to my knowledge been carried out. The conditions for such an experiment seem to me to be too exacting for application to children as ill as the average case of kwashiorkor on admission.

We have devoted several years' work to the examination of nitrogen balance as a method of detecting protein deficiency both in infants and in children. The results of this work have been summarised by J. D. L. Hansen et al.[6] In spite of its admitted defects, this method has given very useful results when applied to large numbers of cases.

Our findings and conclusions on urinary nitrogen partition and body composition are discussed in my communication to the International Congress. They can be summarized here by saying that in the existing state of our knowledge and understanding they are not likely to give us the routine information we want. Low figures for 24-hour urea excretion do, of course, indicate a low intake of protein in the diet, but this information applies at the most to the diet of the pre-

ceding 2 days, and does not indicate anything about the state of protein reserves or protein nutrition.

In summary, therefore, we must conclude that in the present state of our knowledge, the earliest and most sensitive biochemical index of mild or impending protein deficiency is a drop in serum albumin into the marginal range. Causes of abnormal protein loss, such as proteinuria, haemorrhage, or burns, or of failure of protein synthesis (liver failure) must, of course, be eliminated. The significance of the hypoalbuminaemia must be clinched by demonstrating the return of the serum albumin to the normal range under the influence of good protein feeding.

Clinical Recognition of Protein Defiency

It is doubtful whether it is possible at present to identify early protein deficiency by any sort of clinical observation or examination. The approach to this problem must be within the context of the clinical results of malnutrition of any type. I have classified these as follows:

1) Subnutrition (subclinical malnutrition)
    (a) without demonstrable biochemical abnormality
    (b) with demonstrable biochemical abnormality
2) Irreversible structural damage
3) Reversible clinical syndrome of malnutrition
4) Constitutional susceptibility (diathesis) from chronic malnutrition

Testing the Nutritive Value of Protein Supplements Directly on Malnourished Children

In an attempt to study directly the effect of dietary supplementation and enrichment on the health of children, we carried out two controlled experiments in a local orphanage. These should be regarded as pilot studies designed particularly to help in the interpretation of methods. The first objective was to ascertain whether it was possible to achieve the degree of control necessary for a scientific experiment. The second objective was to ascertain whether in a reasonably short time it was possible to demonstrate differences in the effects of control and experimental diets which could be exactly measured and statistically significant. The institution was one used for the temporary housing of children committed by magistrates because of the break-up of the home by death, alcoholism, or imprisonment of the parents. The majority of the children, therefore, were from very poor homes and it must be presumed that they were already malnourished. The basic diet of the institution was satisfactory but certainly not optimum. The ethics of the experiment were carefully considered and it was concluded that the special attention and consideration which would be given to the children by the regular staff and by the staff added for the experiment would compensate for any loss of nutrients which might be suffered as a result of the controlled study and the use of an experimental diet. We were soon satisfied that the standard of care and real consideration for the welfare of the children given by the permanent staff of the institution was exemplary and that the strict but kindly discipline would enable us to achieve adequate control over the experiment.

Because, in effect, the institution was a transit institution aiming to get the children back to a restored home or to permanent foster parents as soon as possible, the period of each controlled experiment had to be short; we had also to be prepared for removal of the children from one group to another at short notice in the interests of their future settlement. The experimental and control groups for the first four to six weeks were selected by matching the children in pairs by age, height, weight and general clinical condition. An additional internal control was applied by reversing the experimental and control groups for a further period of four to six weeks.

The experiments gave surprisingly quick and definite results. This may well have been a result of the poor nutritional status of the children in both groups at the start of the experiment. The most measurable criteria proved to be mean group weights and mean group serum albumin. By grouping these two measurements for each of the 12 or more children respectively in the control and experimental groups, differences came to have statistical significance for the group which would have been negligible for the individual. Had it been possible to get the results for serum albumin immediately, it would have been possible, by relying on the respective group criteria of weight and serum albumin, to predict almost from day to day the progress of the experiment. Actually, circumstances in the laboratory prevented our obtaining the serum albumin figures at less than a 10-day interval. As a result, clinical deterioration in one or more children in an experimental group furnished the first indicators of unsatisfactory performance. Subsequent comparison with the weight and serum albumin figures showed the latter to be more sensitive than the clinical criteria.

In both experiments, which were carried out in the winter, the supervention of epidemic disease in the institution submitted the children to stresses which undoubtedly accelerated and emphasized the differences in nutritive value of the two diets under comparison.

In the first experiment, pea-flour (*Pisum sativum*) was compared, at isonitrogenous levels, with skimmed milk as a supplement to a basic diet of maize meal. A full vitamin supplement was provided to both groups with the object of concentrating the interpretation of differences upon the quality of protein used as a supplement. In previous work on younger infants in a metabolic ward, the nutritive value of the pea-flour supplement has appeared, at high intakes, to be almost as great as that of milk. We were therefore surprised in the orphanage experiment to find its effect to be poor in comparison with milk. Correlation of individual weight gains with quantity of food consumed suggested that the poorer results were in those children who ate least and the better results in those children who ate most. This directed our attention to the fundamental importance of quantity. Hansen's experiments in the metabolism ward have brought out the same important principle and it is now apparent that a diet of poorer nutritive value in respect to protein can give reasonably good results provided it is consumed at a sufficiently high level of nitrogen intake. When the level of nitrogen intake is limited, differences in nutritive value are greatly enhanced in their effects. When the nitrogen content

of a single food is very low it is not possible for a child to consume the quantity necessary for minimum requirements.

This would seem to give the clue to the real nature of potbelly in infants and young children among communities subsisting on diets which lead to protein malnutrition. In order to achieve the nitrogen intake which its system demands, a child is forced, on low-protein staple foods, to consume a quantity which distends its intestines. This effect is aggravated by the poor digestibility of most cereal diets.

In the second orphanage experiment, an attempt was made to compare the supplementary value of isonitrogenous quantities of L-lysine and glycine on a predominantly bread diet. In the final analysis the bread proved to be much lower in nitrogen than had been predicted from food tables and, although the lysine-supplemented group showed distinctly better performance with regard to weight and serum albumin than the glycine-supplemented group, the performance of both groups was poor. This again emphasizes the importance of the quantitative level of nitrogen intake.

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DISCUSSION

DR. PLATT: One comment and one question. The comment is on the terminological ambiguity. Professor Brock talked about the cowpea. He made,

I think, a slip of the tongue. We have had correspondence on this subject. The cowpea is not *Pisum sativum.*

DR. BROCK: I am sorry, Professor Platt, what we identify as *Pisum sativum* in our country has been known as cowpea for the last 70 years—whether it is right or wrong, that is what it is there.

DR. PLATT: The second point was in relation to Dr. Hansen's problem of low nitrogen retention from maize. Are you using whole maize meal for these diets?

DR. HANSEN: Yes. We have used whole maize meal because that is what is used by the people of South Africa.

DR. PLATT: I am not surprised at the results you get, then. You have a lot of indigestible material in them.

DR. GOPALAN: The work of Dr. Hansen and also of Dr. Scrimshaw seems to reveal what I believe to be a rather disturbing trend with regard to some of the conclusions which they have been drawing. They have shown that at low levels of protein intake the differences between the vegetable protein mixtures which they have used and milk are quite considerable with regard to percentage of retention and absorption. In our clinical studies we employ fairly high levels of protein intake, and on the basis of this high intake we conclude that vegetable protein mixtures are nearly as good as milk, and therefore we draw the conclusion that they may be used satisfactorily for preventive purposes. In a preventive campaign we give only low levels of protein.

I would like to know from this group whether the conclusions drawn from our clinical studies, which are based on high levels of intake, are really applicable to preventive campaigns where we are going to use low levels only. It seems to me what we should really strive for is a vegetable protein combination which is nearly as good as milk even at low levels of protein intake.

DR. HANSEN: I think Dr. Gopalan makes a good point, and that is the reason we added animal protein to the vegetable mixture to try to bring the nutritive value at low intakes close to that of milk.

DR. SCRIMSHAW: This is perhaps why both Dr. Hansen and I have been emphasizing this morning that you cannot assume entirely from the clinical performance of vegetable mixtures at high levels that you have an entirely suitable material for prevention. As Dr. Arroyave pointed out, however, in the case of the INCAP mixture we do have independent evidence from the pattern of amino acids, and we also have independent evidence from the biological results. I was trying to emphasize this morning that although from many points of view the mixture seems to be as good as milk, sub-

mitting it to the most extreme test it is not quite so good, but it is quite good enough for the purposes of prevention, as the tests are showing.

We are indebted, as I am sure many other people here are, in each step of our work to these beautifully clear results that come out of South Africa from Dr. Hansen and Dr. Brock. There are a couple of fundamental issues that I think we should face. One, Dr. Hansen in one slide implied that perhaps the child with acute kwashiorkor, because he absorbs more nitrogen at the beginning, even of a relatively poor protein, may be less selective. This is a fundamental question. Is the child with severe protein malnutrition less selective or more selective in terms of quality of protein? Dr. Allison has evidence from dogs on this point.

I would like to ask both Dr. Hansen and Dr. Brock and Dr. Allison to face this issue.

There is another point which Dr. Brock brought out very clearly. That is that the quality of protein for some of these functions may not be the same. Dr. Coachman from our laboratory recently visited Dr. Francisco Vivanco in Spain, who is in the National School or Institute of Nutrition in Madrid. Dr. Vivanco had left over some of the diet which they were feeding to children in some other experiments, and gave it to rats, supplementing part of the group with methionine and part with lysine, because he was not quite sure of the relative deficiencies. If I have it straight, the group with methionine showed very good improvement in growth but relatively poor regeneration of serum albumin, and the group with lysine the reverse: much more rapid regeneration of serum albumin and much slower growth.

So we shall have to take several different functions of protein into consideration in these evaluations.

Another function suggested by one of Dr. Brock's slides which he must take into consideration is the response to stress of infection. Increasingly it is clear that every infection, no matter how mild, imposes a stress on protein metabolism. This stress is extremely important in the development of severe protein malnutrition. Probably very few cases of kwashiorkor would develop without this stress. So when we are developing our mixtures, we want ones which will also help the child to resist the stress of infection on protein metabolism.

DR. HANSEN: I was not quite sure what you meant by selectivity of kwashiorkor children. The point I tried to make is that there is a tremendous difference in nitrogen retention just in those first two weeks of recovery. I think that this is a very inopportune time to test out the nutritive value of a protein.

DR. SCRIMSHAW: Yes. We agree entirely. But the question is: Will a child with kwashiorkor make better use of a poor protein than a child who has partially recovered?

DR. HANSEN: Yes, that is quite true. The nitrogen retentions of maize are much higher, as one slide showed. The nitrogen retention from maize was

higher in the kwashiorkor group than nitrogen retentions from mixed diets of normal children. We are getting nitrogen retentions sometimes of 50 or 60 mg/kg/day. Milk in the same type of child would have a retention of 200, but a normal child receiving a mixed diet, maybe only 30. In other words, maize is giving a nitrogen retention of 60 in the kwashiorkor child as against 30 in the normal child with a mixed diet.

DR. BROCK: I would like to comment on Dr. Scrimshaw's point. I think it is quite clear from animal experiments, particularly Dr. Allison's work, that the animal is more selective when it is nitrogen deficient. I don't believe we have evidence yet on man. What evidence we have certainly points in the same direction. I think we shall finally find the situation is just the same in man.

DR. SCRIMSHAW: The fact that neither you nor ourselves are able successfully to treat most cases of kwashiorkor with corn and beans is proof of this point. So when we do give a vegetable mixture and get good results with kwashiorkor, we are subjecting it to a severe test and not an easy test. That is the point.

DR. BROCK: Dr. Scrimshaw, I would like to suggest it is probably more complicated than that. In the first fortnight when you start to treat the child with kwashiorkor, you have a digestive upset which influences your results in one direction. At the same time the body, being relatively unsaturated with nitrogen, will mop it up more quickly than will a relatively more saturated one. These two things are acting against each other to complicate the results. That is what I suspect, but I am not sure.

DR. SCRIMSHAW: By mopping it up more quickly, it accentuates the imbalances of amino acids in the body, and very soon your cases are clinically deteriorating with corn and beans.

DR. ALLISON: Dr. Hansen, I think you brought out a very important point, that in determining BV in nitrogen balance studies, you are really getting tangents to curves. The results you get have to be interpreted in terms of where that curve is and the part of the tangent that you are drawing to that curve.

It is true that in the depleted animal, both the rat and the dog, and now you have shown it in man, if you feed a poor pattern of amino acids, let's say wheat gluten, it would have a tangent to the lower part of the curve where you have the linear relationship approximately. We say the BV or the nitrogen balance index of wheat gluten is 40, while for cow's milk it would be, say, 75 or 80. But now in the depleted dog, the BV or NBI for wheat gluten will go up to 70. From that viewpoint, of course the retention of nitrogen is much greater in the depleted animal. The animal is using a poor pattern of amino acids much better than would be used by the normal animal.

But that is again misleading in the sense that, as Dr. Brock has pointed out, the animal is soaking up that nitrogen and repleting depleted tissues, but he is developing an imbalanced state. If you keep feeding that poor pattern of amino acids, you make it worse instead of better, in our experience. We can put the plasma albumin back to normal in the depleted animal by feeding certain poor or imbalanced patterns of amino acids. In other words, you do get an animal in imbalance when you feed an imbalanced pattern of amino acids.

We feel very keenly that you should not try to replete a depleted individual by feeding a protein with a BV less than 60.

DR. HANSEN: Are you suggesting that a depleted animal repleted on a poor protein is laying down body protein of unusual composition?

DR. ALLISON: Not of unusual composition, but our data would indicate that you are getting an imbalanced state in that individual. This is a hypothesis, but we have been able to increase the albumin, for example, in a depleted animal back to normal, and the animal still remains very badly depleted. You can produce an imbalance within the tissue proteins in the system, you can produce an imbalance in the system, by feeding an imbalanced protein.

DR. FRENK: I am a little puzzled. Actually, the phenomenon which we have been shown is not a straight line but a tangent from the curve. I was a little amazed to see the curve flattens off at an intake of about ½ gm/kg/day. Am I right? In our cases, in a state of malnutrition the intake goes up to 950 or 1000 mg/kg/day. I do not think one can generalize. Sometimes for a stretch of 10 days you can describe it by a straight line. I think it is a matter of retention of the absorbed amount. This protein was very much depressed by the very low achievement which our cornmeal and bean diet had.

If you see that you have very low retention in terms of intake or very low absorption, this means that, of the amount absorbed, the retention is very good. In other words, the BV is very good. This probably has a purely psychological significance from the standpoint of public health.

I think that the way you express the data depends on what you are trying to demonstrate. It seems a little absurd that corn and beans from the standpoint of retention of the amount absorbed is practically as good as milk. I was afraid this morning to show our data because it seemed a little absurd.

DR. ARROYAVE: I would like to make a short comment on your findings, Dr. Brock, about the relative sensitivity of the serum albumin. You show differences between the two groups that you have. Certainly there are lots of data in the literature which do not agree with your quantitative data in the sense that, at least in our area, serum albumin does not seem to differ between groups of the population on very different nutritional intakes, protein intakes, and nutritional status. It was our impression that it was

not a very good indicator. The definitely lower finding of kwashiorkor seemed to mean an entirely different thing—metabolic impairment of synthesis of albumin, probably. I have not been able to observe this smooth decrease myself. It would be very interesting if it were so, because then we would have an index.

Have you repeated this? Have you had more information about this change of albumin with different nutritional status?

DR. BROCK: We discussed this last year, Dr. Arroyave. The diagram which I showed of marginal hypoalbuminemia represents new data, at least new calculations, since we last discussed this. To me, they are very convincing. I used to say just what you have said, that the fall in serum albumin is a late evidence of declining reserves of protein in the body, but I am beginning to believe that these marginal falls, represented by figures of 3.5 down to 2.75 by our method, are sensitive indicators. Those children in the orphanage experiment—you saw what happened to their serum albumin under unfavorable circumstances. Admittedly, these children were probably quite severely malnourished from the start. I agree with you that serum albumin is by no means a sensitive index. We need to find something much more sensitive.

The point I was making is that I believe it is the most sensitive thing we have at present among a group of very insensitive indicators.

DR. ARROYAVE: Other factors would enter into this. Your experiments were controlled experiments on a specific type of diet. Not the quantity of the protein in the diet, but sometimes maybe the source or the quality of the protein, might determine the final level.

In milk you have to allow for a certain level of protein in the serum differently from what you get with other proteins. The fact that in kwashiorkor in a matter of 2 weeks sometimes you reach levels of 7.5 with milk makes me feel that we would be deceived by taking this level as an indication of complete protein repletion, since we know that at that stage of recovery the children are not repleted.

DR. BROCK: I would agree with you that when the serum albumin is up to 3.5 or even up to 4.0, you cannot conclude you have the body fully repleted with protein.

DR. PATWARDHAN: Dr. Brock, on the same subject of serum albumin, you showed that on controlled diet, placing the children on a vegetable mixture, you found a slight drop in serum albumin. You also showed one graph on which you found if you gave more vegetable protein the children are better in their serum albumin. Have you any idea what their serum albumin was? That might throw light on the point which Dr. Arroyave was making, that possibly the amount of protein intake was not enough, and by increasing it you might also have found a favorable response in serum albumin.

DR. BROCK: The trouble, of course, is that the differences between individuals are not statistically significant. It is only when you compare groups. We will have to look again, Dr. Patwardhan, into whether we could correlate not only weight but also serum albumin with the amount consumed. If we had a big enough series, I suspect strongly we would be able to do so.

DR. DEAN: There is one other point I think is worth considering. In the child recovering from kwashiorkor there is a tremendous debility in water metabolism. It was recently found that well on in treatment the child is still much dehydrated. Children who had been treated for two or three weeks and were getting on extremely well sometimes have to have a blood transfusion because their hemoglobin takes a sudden fall for reasons we are unable to discover.

We have also become very interested in magnesium and potassium excretion because, of course, those are materials which come out of the cells, we think abnormally, with all respect to Dr. Patwardhan, in these abnormal children.

We also have an extraordinary condition in these children, which you may have seen yourselves, in which every now and then a child suddenly becomes dehydrated for no reason we know. We treat this by giving full-strength Hartmann's solution. When the child has been treated for about three weeks, giving a blood transfusion and also full-strength Hartmann's solution, you can get an increase of 3 kg in the weight of an 8-kg child in 10 days. If you then take out the Hartmann's solution and replace it with water, all that excess water, which is what it must be, will be lost in a very short time. We are now wondering whether our weight gains are of any value. We are hoping to do some determinations to settle that.

I have one other comment, if I may take a little more time. As one of the people responsible for these laboratory experiments in which children were given 80% or 90% of their calories in the form of bread for at least a year, we did not see the phenomenon you saw. Those children's bread intakes were almost stationary, but they were malnourished children to begin with. Therefore, it is possible to be interpreted that they were no longer malnourished.

DR. HANSEN: You are quite right, Dr. Dean, about the importance of water metabolism in recovering cases of kwashiorkor. One of the things we have been impressed with, which you mentioned in connection with sudden onset of dehydration, is the large volume of water that is lost in the stools in a recovering case of kwashiorkor. We have found in the first 3 weeks of treatment the average stool weight is 300 to 400 gm per day, whereas the normal stool weight is about 100 gm per day. The child who is recovering from kwashiorkor is losing 3 times the normal amount of water from his bowels. Certain of these cases lose up to 1200 gm per day.

I wondered if the child you mentioned who suddenly becomes dehydrated is not one of those children who are passing out the excessive stool weight.

It may not be a watery stool, but can be semiformed, and nevertheless it weighs that amount.

DR. DEAN: That is not true in our cases. There is no rise in temperature. There is no vomiting.

DR. HANSEN: You have not had stool weights, so you cannot tell?

DR. DEAN: Yes, we have actually gotten stool weights.

DR. HANSEN: You will get an increase of sodium retention in the absence of sufficient potassium intake if you are giving a low-sodium diet. If you used Darrow's solution instead of Hartmann's solution, I suggest this great increase in weight would not have taken place.

DR. DEAN: In the first 11 days we had large amounts of potassium by mouth, as well.

DR. HANSEN: Potassium deficiency can go on for 3 or 4 weeks, according to Dr. Waterlow.

If all the questions are answered, I would like to make one point about the nitrogen retention curves.

The curves of the nitrogen intake and retention that we drew on that slide are theoretical ones. We did not have sufficient data to draw true curves of nitrogen retention on intake. One has to have a very large number of studies to get a significant curve. I am sure it is quite right, Dr. Frenk, that some children go on a linear line up to quite a high intake, but you would have to have a very large number of children to draw that curve so that it is statistically significant.

DR. PICKERING: May I make a brief comment apropos of the mineral metabolism and its relationship to intermediary metabolism. In our laboratories we maintain a rather adequate colony of rhesus monkeys on which we have conducted, over several years, studies particularly oriented to the fetal period and the first year after birth. On the other side, I am a pediatrician and I am interested in intermediary metabolism and have been for some time.

I think we must be very cautious about any dissociation of the role of stress in any form from intermediary metabolism and from the whole responsiveness of the organism to associated disturbances of hydrogen ion concentration.

Certainly the hydrogen ion transport at the cellular level is markedly altered in any state of potassium depletion, and it may be greatly exaggerated when you have an associated protein depletion. This can be further aggravated by stress. I think these are factors which you will ultimately get to in terms of some of the possible misunderstandings concerning the reactions of the patients, particularly along these lines.

Proper attention to the management of the patient from this standpoint, whether it is an experimental animal or a child with acute renal failure, for instance, has permitted us on occasion to manage an acute renal shutdown for as long as 3 weeks in one instance without dialysis.

I think one dare not overlook this as a "must" in the ultimate interpretation of the response of the patient to whatever regimen he might have associated with the primary changes in protein.

DR. SCRIMSHAW: I have been mulling over further Dr. Gopalan's very important question about the relative BV of these mixtures at high and low levels of protein intake. I am not sure this has left us entirely clear. I think it should be made clear that of course we are dealing with a continuous range from poor quality up to good quality in trying to develop these various vegetable mixtures. We are trying to get a mixture which is effective, not necessarily a mixture which is as good as milk. Some of these mixtures may not show up to be quite so good as milk at low levels of protein intake, but a poor mixture would leave a child in strongly negative balance at these levels of protein intake. By and large, a mixture which will give a strongly positive balance in a child, even at relatively low levels, is likely to be a mixture that is good enough for preventive purposes, even though it may not be fully comparable to milk.

Studies on the Use of Peanut Flour In Infant Feeding

Jean Senecal [1]

TESTS MADE IN THE PEDIATRIC SERVICE

I. Use of Peanut Flour with Healthy Infants

Acceptability tests, using the peanut unmixed with the rest of the ration

The children received, at different hours, either feedings of milk or feedings containing peanut flour prepared with water and sugar. Thus the peanut flour was given in replacement of a part of the milk feeding. The quantity of peanut flour given in one meal was established in proportion to the age of the child, ranging from 15 to 25 gm per feeding which included 10 gm of sugar.

Fourteen children between 2 and 13 months of age received, each day for 7 to 15 days, a single feeding of peanut flour, the other meals being those customarily given to a child this age who is deprived of mother's milk; that is, skim milk (usual quantity and dilution) or milk, porridge and vegetables for the oldest children. Since the digestive tolerance was good, for 10 children another milk feeding was replaced with a second peanut flour porridge. The acceptability was satisfactory.

Four children (6, 7, 10 and 15 months old) were then given a peanut flour feeding (25 gm) three times a day, without causing digestive disturbances. These children received 75 gm of peanut flour a day for 17, 23, 27 and 42 days. The test could not be further prolonged because the appetite diminished and three children were even showing a certain distaste for the peanut flour feedings.

A similar study, carried out in a nursery in the city, dealt with 11 children. For three subjects the test of the peanut flour feeding had to be suspended after a few days (3 to 8). One of these children was a month old, another 5 months, the third 24 months. The other eight children, aged 2 to 13 months at the beginning of the test, accepted the peanut flour porridge for several weeks. Four of these (aged 6, 6½, 13 and 16 months) received three feedings, each containing 25 gm of peanut flour, 10 gm of sugar and 180 gm of water, for 3 or 4 weeks; there was interruption for diarrhea in two cases and loss of appetite in two others. The oldest child received peanut flour for 113 days; one and then two feedings a day were tolerated, three feedings (75 gm of the flour) were accepted for 25 days, but the child then showed an elective anorexia for this food.

[1] Paper presented by Madame L. Aubry

This first test may be summarized as follows: Digestive tolerance was good. (We must state, however, that the weight of the fecal excretion was not studied.) Acceptability was rather good on the whole, but most of the children seemed to tire of this food when the diet was maintained more than 6 weeks.

Observations using peanut flour in a mixed ration

The study was done in the Research Unit of the Pediatric Service, on four infants (2 girls, aged 5 and 5½ months; 2 boys, aged 7½ and 8½ months at start), apparently healthy although retarded in height and weight. These four children had lost their mothers at birth and had been raised artificially amidst hospital surroundings. Two had been born prematurely.

The peanut flour was mixed with the other components of the ration (skim milk, millet flour, sugar, water) and this mixture was divided equally among all the meals of the day. The quantity of peanut flour given daily was 60 gm, then 80 gm per day for a period of 3 to 9 weeks. The quantity was increased by diminishing proportionately the other components of the ration, so that the caloric value would remain approximately 100 to 130 calories per kg of body weight. Thus the diet was intentionally very rich in peanut flour—125 or 140 gm a day, which corresponds to 20 or 23 gm/kg; the purpose of this experiment was to study the manifestations of intolerance.

The composition of the diet for 24 hours is shown in table 1.

Table 1
Mixed Ration Using Peanut Flour

Components		Subjects 1 & 2 (girls). Diet B	Subjects 3 & 4 (boys). Diet C
Peanut flour		125 gm	140 gm
Powdered milk, ½ skim		30	35
Millet semolina		10	10
Sucrose		40	45
Water		1,000	1,000
Corresponding to:	Protein	69	77.4
	Fat	8.35	9.5
	Carbohydrate	98	110
	Calories	757	850

It is to be noted that in this type of diet 36% of the total caloric value was of protein origin—obviously an abnormal proportion, and with this amount of protein the amino acid requirements were more than adequately met.

Acceptability and tolerance. Acceptability was improved in relation to the preceding test. The amounts of 60 or 75 gm per day were readily accepted, whereas these amounts could be maintained for only a few days in the preceding test. The quantity of peanut flour could be rapidly increased by diminishing proportionately the other components of the ration (skim milk and sugar). It was possible to reach the daily rate of 125 gm per day for the two girls and 140 gm per day for the two boys in about 2 months.

None of the children showed any anorexia during this period, despite the monotony of the diet.

No acute digestive disturbances (diarrhea, vomiting) were noted. Digestive tolerance was satisfactory during the period of 3 to 5 weeks when the children received 60 and then 80 gm of peanut flour per day. But when these very large amounts were reached, an increase in the volume of the stools was noted. The children who had been receiving 125 or 140 gm of peanut flour a day for 2 months excreted stools of 300 to 350 gm a day, not solid, light brown in color. A carmine study proved that the digestive passage was accelerated.

This part of the experiment may be summarized by saying that very large amounts of peanut flour—125 to 150 gm a day for children weighing approximately 6 kg at the beginning of the test, which represents 20 to 23 gm of the flour per kg of body weight—are accepted without causing acute digestive disturbances, but that these large amounts cause an acceleration in food passage and an increase in the volume of the stools. It was interesting to arrive at this first manifestation of poor tolerance, but it is obvious that it was a matter of an experiment and that, in practice, there is every advantage in using more balanced mixtures.

It was not possible for us to study the digestion by means of coprological examinations. Thinking that the poor digestibility might be due to the very high tamponing power of these porridges, we added citric acid just at the time of giving the feeding; a decrease in the volume of the stools was then noted in three of the four children.

Weight gain. These children, orphans who had spent several months in the creche, had a considerable weight retardation at the beginning of the experiment. The following table indicates the gains in weight recorded, distinguishing two periods: During the first period the children received 60 or 80 gm of peanut flour

TABLE 2
RECORD OF WEIGHT GAIN

Subject (age at start)	Weight (date) at start [1]	Weight (date) at change [2] to higher level	Aver. daily gain	Weight (date) at end	Aver. daily gain
	gm	gm	gm	gm	gm
1. Girl K	4150	4950	23	6350	8
(5½ mo.)	(15 Sept.)	(21 Oct.)		(13 Apr.)	
2. Girl F	5100	6500	20	7750	9.4
(5 mo.)	(18 Sept.)	(27 Nov.)		(12 Apr.)	
3. Boy M	4750	5700	17	7000	9.5
(7½ mo.)	(30 Oct.)	(24 Dec.)		(10 May)	
4. Boy B	5050	5700	17.5	6500	6
(8½ mo.)	(8 Nov.)	(15 Dec.)		(30 Apr.)	

[1] Girls started at 60 gm, boys at 80 gm peanut flour per day (110-130 cal/kg).
[2] Girls changed to 125 gm, boys to 140 gm peanut flour per day (110-126 cal/kg).

daily; during the second period they received 125 gm a day (for the 2 girls, diet B) or 150 gm a day (for the 2 boys, diet C).

In the interpretation of these figures, allowance must be made for the fact that the children had a greater weight retardation to make up during the first

period than during the second. However, it is apparent from the table that the gain in weight was distinctly better when the child received a moderate amount of peanut flour than when he received a large amount (20 gm/kg). This fact is to be compared with the increase in the volume of the stools in the four children when they had been receiving 125 or 140 gm of peanut flour for over 2 months.

Laboratory examinations. The amount of *total serum proteins* always remained normal.

Electrophoretic analysis of the serum proteins also gave normal results; only a very slight decrease (2.65%) in albuminemia was noted in child no. 2 at the end of the experiment.

Tests of *hepatic flocculation* stood at normal values.

Blood urea was high (40 to 50 mg%) but returned to normal with the resumption of a more balanced diet, less rich in protein; this rise in the urea merely shows an important nitrogenous catabolism.

We observed signs of *rickets* in the two boys: In child no. 3 the alkaline phosphatases went from 9.7 to 18, and in child no. 4 from 9.4 to 22, during the third month of the diet and despite the daily administration of a vitamin complex which added 500 units of synthetic vitamin D. In the two girls, both a rise in alkaline phosphatase and the appearance of radiological signs of rickets were noted.

These facts are easy to explain. The diet provides only a very inadequate amount of calcium and the calcium/phosphorus ratio is unbalanced. These established facts led us to enrich the mixture in tricalcic phosphate, which brought about the disappearance of the signs of rickets.

Conclusions

Peanut flour can be used in infant feeding; acceptability is normally satisfactory; digestive tolerance is good as long as the amounts given to the child remain in the neighborhood of 50 to 80 gm per day for an infant 5 to 12 months old.

It was possible to give larger amounts, 125 or 140 gm daily, to four children ranging in age from 7 to 9½ months during a long period (20 weeks). But these large amounts (approximately 20 gm of peanut flour per kg of body weight) represent a very unbalanced diet. No disturbance of the biochemical constants studied was noted except for the rise in blood urea, but the digestive process was accelerated, the volume of the stools was distinctly increased and the gain in weight was slight. This type of diet, very rich in peanut flour, amply covers the requirements for each of the essential amino acids, but the balance of the amino acids is not the best, the amount of S-amino acids being relatively low. This led to a search for other formulas, in particular those combining fish flour with the peanut flour and the basic cereal, millet.

II. Treatment of Malnutrition by Preparations Containing Peanut Flour

The studies made on the use of peanut flour in feeding the normal (healthy) infant were followed by tests on treatment of malnutrition (kwashiorkor). These researches were made at the Maternal and Child Health Center (they will be described in a special section of this report) and at the Pediatric Service. Three types of diet were used successively: 1) Diets including peanut flour and cereals but without the addition of animal protein. These diets showed failures. 2) Millet-peanut-fish mixtures with over 20% of the calories being of protein origin. 3) Millet-peanut-fish mixtures, but with a lower percentage of protein.

Peanut flour and cereals without added protein of animal origin

Eight children suffering from mild kwashiorkor received a diet made up of two daily feedings of 25 gm peanut flour, 10 gm sugar and 180 gm water per feeding, and three meals consisting of rice or potatoes, bananas and jam. The caloric value was approximately 130 calories per kg of body weight, and the protein value was 5 gm of protein (solely of vegetable origin) per kg of body weight.

In the eight observations, this diet had to be suspended six times (three infectious syndromes including one case of measles, three cases of poor tolerance or persistence of signs of malnutrition beyond the 10th day).

In two cases a relatively satisfactory result was recorded (files 1594/57 and 149/58): good acceptability and good digestive tolerance, disappearance of the edema in one of the children, rise in blood proteins, gain in weight. But this improvement was still incomplete. On the 23rd and 25th days of the diet it was necessary to adopt a milk-based diet which rapidly brought about the cure.

Four other children, also suffering from mild malnutrition, were given six feedings per day of a mixture of peanut and millet: 20 gm peanut, 10 gm millet, 15 gm sugar, 180 gm water. In two children this experiment had to be stopped very quickly (1st and 4th days) because of the appearance of an infectious syndrome and poor tolerance. In the two other subjects (malnutrition with distinct clinical and biological signs) acceptability was good. The first child received an average of 1070 gm of porridge per day for 24 days, which corresponds to an average daily value of 1224 calories and 66 gm proteins. The second received an average of 905 gm of porridge per day for 16 days, or an average daily value of 1020 calories and 55 gm proteins. These children showed an increase in appetite, an improvement in behavior, a gain in weight (350 gm in 16 days and 900 gm in 24 days), a rise in the total blood proteins (from 6.50 to 7.60 in the first case and from 6.45 to 8.05 in the second), and finally the regression, in the first case, or the complete disappearance of the periportal hepatic steatosis observed in the biopsy puncture before the initiation of the diet.

The conclusion which may be drawn from these results is that it is difficult to cure kwashiorkor by diets such as those we used, which included no animal protein. It can be done, and the last two observations testify to this, but in uncertain fashion.

MIXTURES OF MILLET FLOUR (OR SEMOLINA), PEANUT FLOUR AND FISH FLOUR

Fish flour was chosen to supplement the peanut rather than another protein of animal origin, milk in particular, because of economic considerations. Senegal will not have a dairy industry for a long time. The production of fish flour, however, is being seriously contemplated, since the Atlantic coast is extremely rich in fish, combining deep-sea fauna in the temperate waters and warm-water fauna at the surface. Since the purpose of this research is to perfect a food for infants that is rich in protein and prepared from local resources, it is preferable to use the millet-peanut-fish mixture rather than the millet-peanut-milk.

Four preparations combining millet-peanut-fish were tested. These four diets (indicated by the letters D,E,F,G) differ slightly in the proportions used. Their composition is indicated in table 3.

TABLE 3
COMPOSITION OF DIETS

Components	Diets					
	D	E	F	G	H	631
Peanut gm	90	90	120	60	70	30
Millet gm	60	60	60	90	20	60
Fish gm	30	30	24	24	10	10
Sugar gm	90	90	60	90	45	35
Peanut oil gm	15	0	0	0	15	15
Water q.s. me	1080	1080	1080	1080	1000	600
Calories		990	954	971	667	623
Carbohydrate gm	156	156	133	169	99	90
Fat gm	22	7	8	7	18	17
Protein gm	74	74	85	58	25	29
Total calories from protein	26	30	35	24	15	18
Calcium mg	858	858	738	687	291	298
Phosphorus mg	1719	1719	1704	1446	683	709
Iron mg	6.6	6.6	7.5	6.1	—	3.8

Acceptability was rather good on the whole.

The *average amount ingested* daily was: 963 ml porridge D, or 1003 calories, 66 gm protein; 972 ml porridge E, or 891 calories, 66 gm protein; 1048 ml porridge G, or 942 calories, 56 gm protein. The average weight of the 26 children was 8310 gm.

These rations seem small for children approximately 2 years old, but the amounts accepted during the first days were generally from 500 to 600 ml of porridge. Then, when appetite returned, the amounts rather often reached figures ranging from 1200 to 1300 ml near the third week.

Digestive tolerance was good. No acute digestive disturbance was caused by the porridge.

Effectiveness was manifested clinically by: improvement in general aspect, return of appetite, and return of a gay and playful disposition in 10 to 25 days, according to the initial degree of malnutrition; disappearance of edema within 10 days in the cases where it was noted; disappearance of lingual lesions and cheilitis in 10 to 15 days generally. The slight skin disturbances at the beginning,

and the brittleness of the hair, often persisted during the time of observation at the hospital.

The *average daily weight gain* was 46.30 gm for the eight children on mixture D, 22.10 gm for the six children on mixture E, and 32.50 gm for the three children on mixture G. The nine children for whom the test could not be continued do not appear in these figures.

This would seem to demonstrate the advantage of a certain amount of fat to enrich the caloric ration; the children subjected to diet D consumed an average of 19.50 gm of fat daily, or 17.5% of the caloric ration, as opposed to 6.3% for the children subjected to diet E and 6% for the children subjected to diet G.

As a parallel to the clinical improvement, a rise in the total blood proteins, and especially the albumin, was noted. It was possible to follow the evolution of the condition of the liver in 11 children, eight on diet D and three on diet E, by two or three hepatic biopsies.

A regression of the hepatic steatosis was noted, but this regression was much slower than that which is observed when kwashiorkor is treated with a mixture of milk and protein hydrolysate.

It appears that all the cases of mild malnutrition were cured, as well as five of six cases of malnutrition type 2; on the other hand, two failures were recorded out of six cases of malnutrition type 3 and six failures out of nine for type 4, the outright form of kwashiorkor. This confirms the difficulty in curing severe kwashiorkor with diets rich in protein but in which the proportion of the various amino acids is imperfect.

Millet-peanut-fish mixture with a smaller percentage of protein

In the tests just reported, the content of the diet was such that over 20% of the calories was supplied by proteins. Another experiment was done with a mixture (diet H, table 3) slightly less rich (15%) in protein. This diet was used with 13 children: one case of very slight malnutrition, type 1; two cases of slight malnutrition, type 2; five cases of malnutrition of moderate intensity, type 3; five cases of malnutrition of marked intensity, type 4.

Acceptability. With four children, the diet had to be suspended between the fourth and eighth days (diet poorly tolerated, or tendency toward accentuation of disturbances). This involved three of the five cases of malnutrition type 4 and one of two cases of type 2. The other nine children accepted the diet rather well, and this could be prolonged for a period varying from 27 to 82 days, averaging 48 days. The amount of porridge taken by the children, who ranged in age from 17 to 24 months, provided them with an average value of 719 calories and 27 gm of protein per day. This average ration was small. In order to increase the caloric value, two solutions are possible: increase the amount of porridge or increase the amount of fat (oil).

Digestive tolerance. We have already indicated that the diet had to be abandoned for four children, including three with severe malnutrition type 4. In the other nine children digestive tolerance was good. In them an improvement in clinical characteristics was noted: an important gain in weight; a rise in blood

protein, with the average going from 6.30 to 7.15 in 13 days; in short, a favorable development. But it must be pointed out that the hepatic steatosis regressed slowly and sometimes incompletely.

III. Use of Millet-Peanut-Fish Mixture with Healthy Children

Diet H was given to four healthy children: two girls aged 13 and 14½ months, and two boys aged 14½ and 15 months at the beginning of the experiment. It was possible to maintain this diet for 122 days. The quantities of porridge taken at the end of this period were: child no. 1, 1300 gm; child no. 2, 1350 gm; the two boys, nos. 3 and 4, 1400 gm.

Acceptability was satisfactory despite the very great monotony of such a diet for children over a year old. Only child no. 4 presented some difficulty. This child was a special case. He gained practically no weight during the whole time of the experiment; from the 3rd month of this diet he showed a slight abdominal distention and had episodes of diarrhea; the stools had an abundance of mucus, bacteriological and parasitological examinations were negative; the disturbances ceased promptly when he returned to a normal varied diet.

Aside from this case, *digestive tolerance* was good; in the other three children the stools had a good appearance but were abundant, averaging 200 to 250 gm per 24 hours.

Weight gain was satisfactory; the average gain during the 4 months of observation was: 17 gm per day for child no. 1; 11.6 gm per day for child no. 2; 10.8 gm per day for child no. 3.

The amounts of *blood proteins* and *electrophoretic fractions* were normal and remained so.

The *blood urea*, determined at the end of the experiment, was normal, ranging between 9 and 18 mg.

The *hemoglobin count* and the *globular value* had a tendency to drop. This confirms what was said earlier: It would be advisable to increase the iron percentage of these preparations.

IV. Use of Hyperprotein Millet-Peanut-Fish Mixture with Healthy Children—Nitrogen Balances

Diet 631 (table 3) was a mixture of millet, peanut and fish with the addition of sugar and oil.

Six orphan boys, aged 6 to 16 months, were put on this diet. We intentionally chose orphans in order to avoid the supplying of other foods by the mother and to reduce, if possible, the psychological influence that would have resulted from the separation of mother and child which our balance studies necessitated.

Clinical examination of these children before the introduction of diet 631 showed that only one of them (A) presented a height-weight, dental and psychomotor development almost normal for his age (13 months, weight 9100). The other five presented a definite, sometimes even considerable, weight retardation and a slight dental and psychomotor retardation: child C (6 months) weighed 4600; child E (9 months) weighed 6100, did not sit up; child D (11 months) weighed

6750, did not sit up; child B (13 months) weighed 7000, did not sit up; child F (16 months) weighed 8100, did not stand up. The clinical examination had shown no disease clinically or radiologically discernible.

Before receiving diet 631, these children had normally balanced and varied nourishment, adapted to their age. They presented a normally and regularly rising weight curve in the preceding months except for child C, whose weight curve, while rising, had presented some phases with stationary weight or a slight drop.

The total absence of weight gain in these six children appears to be unfortunate if one compares their weight at the beginning of the diet and 2 months later. For all, this 2-month period shows a loss of weight: slight (100 gm, child A); moderate (230 gm, child C; 250 gm, child D); large (450 gm, child F); very large (950 gm, child E; 1300 gm, child B). In two cases (child E and child B), the weight distinctly and progressively falls following the beginning of the diet, necessitating resumption of a more varied diet and doubtless expressing these children's intolerance to this mixture, at least when it is given exclusively. In the other four cases, the weight presents two phases: a first, falling phase, immediately following installation of the diet and extending over a period of 14 to 43 days, and a second, rising phase. Weight gain is slow in this latter phase, irregular from one week to the next, sometimes even interrupted by a sudden temporary drop or a standstill. Nevertheless, weight gain in this second period seems certain for these four subjects.

Two children, B and E, did not always consume completely and regularly the specified amounts of the mixture, refusing some feedings and drinking others only partially. These are the children whose weight curves distinctly and progressively fall. The other four, however, accepted the diet rather well and consumed the specified quantities, with a few infrequent exceptions, during the 2 months of observation.

In all these children we noted a constant increase in the volume of the stools, the average being around 200 ml per day. These stools had an unusual appearance, resembling the peanut porridge in color and consistency. As a parallel we noted frequent abdominal distention, which appeared after the introduction of the diet.

We made comparative examinations of the amount of protein, the A/G ratio and the blood urea. Table 4 gives the figures for these examinations for the six children. It will be noted that the amount of blood urea is not much modified, having in every case a tendency to drop, and that the protein amounts are not greatly altered, since the changes noted are not large enough to be significant.

Results of the nitrogen balance studies are presented in table 5. The purpose of these studies was to determine the nitrogen retention in children receiving a millet-peanut-fish diet (631) of a definite composition. It is difficult to regard this study, under the conditions in which we made it, as having a sure value in the eyes of the classical physiologist. Our children were of different ages and presented different weight patterns. We limited ourselves to a single diet, of definite composition, without varying its percentages and without adding other components (milk, meals, pap). We sought the percentage of nitrogen retention in terms of two given facts: The children selected were put on the balance when they were clinically in

perfect health and, during the balances, the children received ingesta identical in kind but differing in their caloric and nitrogenous values.

Finally, let us point out that the quantities of protein furnished each child by our mixture may seem surprising, since the amount of protein consumed per kg per day is high (5 gm). We wished, in fact, to study a mixture susceptible of furnishing caloric value capable of completing a diet poor in protein.

TABLE 4
BLOOD UREA, PROTEIN, AND A/G RATIOS

Child	Days on diet 631	Blood urea mg/100 ml	Proteins mg/100 ml	Electrophoresis A mg/100 ml	Electrophoresis G mg/100 ml	A/G
A	19	24	6.05	3.40	2.65	1.28
	51	11	6.35	3.45	2.90	1.18
B	19	28	6.95	2.90	4.05	0.71
	61	17	7.75	3.50	4.25	0.82
C	33	25	6.50			0.93
	56	13	6.70	3.05	3.65	0.83
D	9	36	7.60	3.65	3.95	0.92
	51	27	7.20	2.85	4.35	0.65
E	9	27	6.35	3.40	2.95	1.15
	41	16	5.85	2.90	2.95	0.98
F	7	27	8.10	2.90	5.20	0.55
	30	23	7.50	3.25	4.25	0.76

Our balances were of 5 days' duration. The children were in complete isolation night and day, in order to avoid addition of other foods to the diet. The apportioning of the nitrogen was carried out in a sample of the powdered mixture, at the beginning of each balance. The prepared bottles were weighed before and after the meals. Special bibs, weighed after steaming, were dried and again weighed in order to determine the amount of regurgitations. The same was done with the vests and harnesses, in case of vomiting. To mark the beginning and the end of the cycle, we added 0.15 gm of carmine to the corresponding bottles.

The children were catheterized at the beginning and at the end of the balance. To collect the urine, we worked only on boys equipped with a nozzle permanently attached and joined to the collecting jar by a rubber tube. The urine, collected in 20 ml of sulfuric acid, diluted and preserved in toluene, was analyzed at the end of the 5 days.

The cycle corresponding to the 5 days was marked with carmine, and the stools collected in receptacles were immediately recovered in jars containing dilute sulfuric acid and toluene. The samplings were homogenized in the mixer and analyzed at the end of the balance. Washing for the total collection of the stools was done with distilled water; the quantity used for the 5 days was noted.

We neglected to recover the cutaneous losses through sweat, for that was impossible for us. Although our balances were carried out at the beginning of the hot season, it does not seem to us that these losses can be important.

TABLE 5
NITROGEN BALANCE STUDIES

Child	Age at start, mo.	Days on diet 631	Dates of balances	Weight		Energy intake	Protein intake	N Balance				
				Start	End	cal/kg/day	gm/kg/day	Intake gm/kg/day	Feces gm/kg/day	Urine gm/kg/day	Bal gm/kg/day	Retention
				gm	gm							%
A 1	13	5	16-21 May	8750	8850	125	5.1	.83	.21	.51	.11	13
2		27	7-12 June	8600	8700	124	5.4	.88	.24	.41	.23	26
3		45	24-29 June	9050	8950	127	6.4	1.02	.23	.43	.36	35
B 1	13	5	16-21 May	6750	6750	120	4.9	.79	.24	.46	.09	11
2		27	7-12 June	6700	6500	115	5.1	.83	.27	.37	.19	23
C 1	6	5	16-21 May	4500	4500	149	6.1	.98	.41	.36	.21	22
2		33	13-18 June	4500	4500	131	5.7	.93	.31	.44	.18	19
D 1	11	9	30 May—4 June	6750	6850	146	6.0	.97	.22	.51	.24	25
2		23	13-18 June	6600	6600	147	6.4	1.04	.39	.32	.33	32
E 1	9	17	7-12 June	5700	5550	173	7.6	1.24	.43	.49	.32	25
2		24	24-29 June	5700	5400	146	7.0	1.19	.33	.42	.44	39
F 1	16	7	8-13 June	7250	6650	116	5.1	.86	.32	.37	.17	20
2		24	24-29 June	6950	6900	129	6.5	1.05	.32	.39	.34	32

ANALYSIS OF RESULTS

These balances permit us to draw a few conclusions, not only by studying the various ratios furnished but by taking into account the time during which these children received the mixture.

The percentage of retention is variable, fluctuating from 11% to 39%. In five subjects the rate of retention improved from the first to the second balance. This may be explained in several ways. First, in almost every case the quantity of nitrogen ingested increased from one balance to the next, either because the quantity of porridge absorbed was greater or because the quantity of nitrogen furnished by 100 gm of porridge was greater in one balance period than in the next. But this does not seem adequate to explain the improvement in the rate of retention. It seems that there may be a second intervening factor, i.e., the number of days during which these children received this mixture. This seems to coincide with the observation of two phases in the weight curve, one falling, the other rising. It would seem, therefore, that the child needs a certain "stage of adaptation" to the proposed diet before retaining a valid amount of nitrogen.

The apparent digestive utilization is good: 60% to 75% for proteins of 75% vegetable origin. We did not make allowance in our computations for endogenous fecal nitrogen. According to physiologists, this would be increased under two conditions: when the total amount of dry matter and the concentration of indigestible material in the ration are high, which is true of our diet, and when the amount of protein in the ration is high, which is also true of our experiments.

We can note a massive elimination of nitrogen in the stools, which we had suspected clinically from their appearance and volume.

Finally, if the net nitrogen balances are compared with the quantities of nitrogen ingested, it seems that *quantity retained*, while proportionate to the quantity absorbed, follows a curve the maximum of which is about 1 gm of nitrogen (or 6.25 gm of protein per kg per day), a figure very high in comparison with normal requirements, which might perhaps be explained by the more or less undernourished condition of five children put on the balance and by the high (75%) vegetable origin of the protein. Percentage of retention improves after a phase of adaptation, as we have seen, but also with the amount of nitrogen ingested.

It does not seem that this mixture should be utilized as an exclusive diet because of the sometimes prolonged adaptation stage, the slowly progressing weight curve, the voluminous stools; however, it may satisfactorily supplement a diet poor in protein. The purpose, in fact, was to perfect a food rich in protein, intended for the weaning period when the child usually receives a diet poor in protein. Preparation of such a food was intended to resemble that of the traditional *rouye*, and its basic ingredients were to be of local origin (millet and peanuts can be produced locally in great quantity, and fish flour is to be produced industrially within a few years). This mixture achieves the goal sought and can supplement regular food by comprising two or three daily meals of the child's diet.

TREATMENT OF MILD MALNUTRITION BY VARIOUS SUPPLEMENTARY DIETS

The purpose of the investigation, undertaken at the Maternal and Child Health Center, was to determine the effectiveness of food supplementation through peanut protein in the treatment of mild malnutrition. We sought to compare the development of symptoms and the weight gain in four groups of children who received, in supplement to their usual food, millet, skim milk, a mixture of millet and peanut flour, or a millet-peanut-fish mixture. The first two types of diet were the control diets, one poor, the other theoretically ideal. The caloric and protein rations—equivalent in the skim milk and the two peanut diets—were set at 250 calories and 16.56 gm of protein per day, which corresponds to 400 ml of reconstituted skim milk with 5% sugar. Caloric equivalence was maintained for the millet control diet, which furnished only 5 gm of protein per day.

Composition of diet supplements (ingredients and protein in gm):

I. Flour 50; sugar 20; protein 5; calories 250
II. Millet 27.6; peanut flour 27.6; sugar 14.8; protein 16.6; calories 250
III. Millet 25; peanut flour 13; fish flour 10; sugar 21; protein 16.6; calories 250
IV. Milk powder 46; sugar 21; protein 16.6; calories 250.

Since effectiveness was to be judged by the cure of the malnutrition syndrome, we chose infants recently weaned who presented: obvious weight hypertrophy or edema with or without weight hypertrophy; mucocutaneous disturbances (loss of pigmentation, cheilitis, smooth tongue); anomalies of the hair (more or less depigmented, uncurled and brittle). Digestive symptoms and psychological disturbances were highly variable, for we systematically eliminated the cases which were too serious and would have required hospitalization or force feeding. Likewise, acute infectious diseases capable of disturbing the symptomatology (stomatitis, measles, etc.) compelled us to reject certain children. We did not allow for dietary inquiry to determine the nutritional etiology of the syndrome, in view of the impossibility of obtaining precise information on the period preceding its appearance.

In order to ensure regular supervision of the subjects of the investigation, only those were retained who lived near the MCH Center, where their mothers could bring them each day, and agreement to these visits was required before registration.

We intended to study 200 cases. We had to stop short of this in our investigation, because it is becoming increasingly difficult to find cases of this kind near the Center. All the mothers capable of accepting the discipline of the daily visit have received dietary advice, and the malnutrition cases seen at the Center are now coming from farther away, or from hostile surroundings which do not lend themselves to the investigation.

In order to eliminate possible seasonal influences, we alternated the four types of diet regularly. Thus each week the diet intended for the new registrants was fixed in advance. A single exception was planned for the children who were

first put on the millet control diet, whose diet had to be modified after the failure of this control diet was noted; if their new registration again put them on millet, they were put on the millet-peanut. However, a stoppage in supplies of fish flour deprived us of the millet-peanut-fish diet for 8 months. Since millet had been lacking for 3 months, we were led to suspend the investigation. This explains the relative inadequacy of the two groups, millet and millet-peanut-fish, which include only 40 cases instead of the 50 planned.

Experimental Procedure

The recently weaned infants, who lived near the MCH Center, were systematically examined by one of us as soon as any symptom whatsoever that might suggest malnutrition was noted by a nurse, a midwife or another doctor at one of the consultations. Thus all the clinical examinations were performed by the same physician.

Some children underwent a blood check (serum protein analysis and electrophoresis) at the same time as the clinical examination. But we generally avoided taking blood, in order not to discourage the mothers as had happened in previous experiments.

Tuberculin tests were systematically checked: two cases of tuberculosis were detected and withdrawn from the investigation.

In the first examination, all children were systematically eliminated who presented a disease not associated with malnutrition (pneumopathy, otitis, angina), as well as all cases of malnutrition which were immediately considered too serious (profound apathy, large hyperpigmented patches or torpid ulcerations indicating outright kwashiorkor). However, diarrhea, even if intense, did not prevent us from treating the malnutrition, by adding adsorbent and anti-infectious therapy if necessary.

After the clinical examination, the children selected for the investigation were immediately sent to the Dietetic Service, where they received the first ration of the fixed diet. A staff member of this service, where a nurse's aide was especially entrusted with the supervision of the children of the investigation, questioned the mother on the habitual diet while giving the practical instructions on the supplementary diet, and then accompanied her to her home. This visit to the home permitted us to have a few details on the manner of family life, to supervise the feeding of the infant and especially to know the exact addresses of the subjects of the investigation in order to be able to find them in case the visits to the Center were interrupted.

Supervision of the children under observation was carried out on the clinical and the dietetic levels. Clinically, the children were re-examined about twice a week during the first month and once a week during the second month. Moreover, any incident, any anxiety on the part of the mother, justified an extra consultation, for the children "in the investigation" had priority in medical consultations, and the cases that were considered alarming were followed daily. This clinical examination, which was always handled by the same doctor, regularly included current entries on the clinical card, with mention of weight, and a check on the

macroscopic appearance of the feces. Such a check appeared necessary in order to determine the condition of the digestion, because the information obtained from questioning the mothers was too vague.

The daily taking of the diet was controlled in every case, either at the Center or, if necessary, at home. Half a ration was served to the child each morning at the Center; the second half was taken away by the mother, who gave it at home in the evening. The nurse or an assistant watched as the child ate at the Center and noted all possible observations. In this way we were able to observe one child's systematic refusal of all porridges and another's selective refusal of the fish porridge. In all the other cases, anorexia, frequent in the beginning, was overcome after 3 or 4 days unless there was aggravation. This daily supervision by the nursing and auxiliary staff enabled us, moreover, to detect immediately the possible signs of aggravation. We were thus able to carry out our observations of the millet control without particular anxiety, since modification of the diet occurred as soon as failure appeared obvious.

Criteria of Effectiveness

We defined two categories of results: satisfactory or unsatisfactory (or failures), the satisfactory being characterized by the disappearance of edema or, in its absence, the resumption of weight gain and the attenuation of clinical signs of malnutrition. The planned period of observation was 60 days.

In order to clarify the differences among the satisfactory results obtained, we also included the daily average weight gains, distinguishing the cases with or without edema at the beginning of the observation.

Table 6 shows the distribution of the results and the averages in each group. The similarity of the groups under consideration is confirmed by the small differences between the age and weight averages.

Table 6
Comparative Effect of the Various Diets

	No.	Average age, mo.	Average wgt., gm	Average duration, days	Weight increase, gm Total	Per day
MILLET ONLY						
Failures	28	21.4	8,020	14.20	0	0
Satisfactory, no edema	6	20.5	8,401	41.66	671.66	16.12
Satisfactory, edema	6	18.5	7,675	35.10	763.30	21.84
MILLET-PEANUT						
Failures	13	21.34	8,227	14.92	0	0
Satisfactory, no edema	7	19.85	8,537	50.71	1,138	22.38
Satisfactory, edema	30	22.41	8,966	53.86	875	16.24
MILLET-PEANUT-FISH						
Failures	9	20.55	8,387	12.66	0	0
Satisfactory, no edema	10	22.00	8,659	38.30	734	19.16
Satisfactory, edema	21	19.76	8,541	39.57	667.10	16.79
SKIM MILK						
Failures	13	21.11	8,153	16.30	0	0
Satisfactory, no edema	16	20.43	8,472	55.93	1,220	21.81
Satisfactory, edema	21	20.90	8,743	50.80	1,309	25.77

The persistence of edema beyond the 15th day, or stationary weight in the absence of edema, led us to class as unsatisfactory certain borderline cases, but most of the failures were revealed by an aggravation of the syndrome (appearance or increase of edema, hyperpigmentation, anorexia and apathy) which necessitated modifying the therapy rapidly.

However, in order to determine the effectiveness of the diet, it seemed to us necessary to require a minimum period of 6 days of observation. The cases of precocious aggravation which necessitated a modification of the diet before the 6th day have therefore been eliminated from the table of results; twenty-six cases were thus removed from our investigations. The same is true of fourteen cases complicated by other diseases noted in the first week of observation. This involved, in addition to the two cases of tuberculosis already mentioned, measles (6 cases), stomatitis (2 cases), otitis, angina, infectious diarrhea and septicemia. Such diseases occurring in subjects under observation for more than 7 days made us suspend these operations. However, we took into account the results already obtained; sixteen observations, rather evenly distributed among the various groups, were thus shortened 7 to 45 days.

Let us note that the pledge of assiduity taken by the mothers at the beginning of each investigation did not protect us from unexpected departures (20) or even refusals by the mother to bring the child to the consultations, despite the insistance of the home visitors (9 cases). Add to this three suspensions for family reasons (illness of the mother) and six cases where shortcomings of the personnel entrusted with the home visits made it impossible to find the child immediately. So, of our 180 subjects, 54 were suspended prematurely, exclusive of the subjects eliminated before the 6th day.

These 180 observations concern 159 children, since 19 had a second diet after failure of the millet diet, and one had three successive diets: millet, millet-peanut, then milk, because he refused the porridge. It is interesting to note that, in order to keep these 159 children, we registered 242, of whom 83 were eliminated before the 6th day. We have already mentioned the 26 aggravations and the 14 diseases associated with malnutrition. In addition, two children refused the fish porridge, 14 mothers refused to come back for the consultations (4 of the latter hospitalized their children directly) and 27 left their homes, which were often only a temporary address. The distribution among diet groups of these early withdrawals is rather peculiar: five on millet, five on millet-peanut, twelve on millet-peanut-fish and nineteen on milk. A significant difference ($P = 0.01$) appears between the millet-peanut and the milk as to the interest shown by the mothers. It was possible to verify this phenomenon when, after 2 months of an experimental diet of porridges, we gave supplementary rations of milk to mothers, along with nutritional advice. Most of the women, who up to that time had been coming very regularly for the distributions of flour, stopped coming to the Center, apparently not attributing the same therapeutic virtues to milk as to the porridge.

Critical Study of Results

The number of failures and of good results is comparable in the three groups who received a hyperprotein supplement. There is no difference between the

millet-peanut group and the milk group, and comparison with the millet-peanut-fish group, although it favors the latter (22.5% failures against 26%), does not show a statistically significant difference.

In contrast, the millet control diet shows 70% failures. One notes a significant difference as to the proportions of success and failure between the group on millet and the other groups ($X^2 = 16$ and 18 - P is less than 0.001).

The value of peanut as a protein supplement is thus demonstrated, but the superiority of the millet-peanut-fish diet to the millet-peanut diet cannot be proved.

The daily average weight gain is definitely higher in the group receiving the skim milk than in the other two (21.62 gm on the one hand, 15.79 gm and 16.03 gm on the other), and this difference is found again in the distribution of the cases.

This study does not allow for the fact that most of the cases in the millet-peanut and millet-peanut-fish groups showed edema. Weight gain is lower, on the whole, in the cases with edema, and the number is insufficient to assign a significant value to the difference noted for the cases without edema among the various groups.

Conclusions

The effectiveness of adding protein, in supplement to the traditional diet, for the treatment of mild malnutrition is demonstrated by the differences—statistically significant as to the number of good results—between the control group on a ration of millet, and the other three groups, where the supplementation provided an additional 16.56 gm of protein per day. The first had only 30% improvement and the other three from 74% to 77%. The value of the millet-peanut mixture is, in this respect, comparable to that of milk, and the addition of fish powder does not change the results in this experiment.

However, the superiority of milk becomes apparent when the weight gain is considered during the period of observation: the daily average is over 21 gm with milk and approximately 16 gm with the millet-peanut and millet-peanut-fish mixtures. The distribution of satisfactory cases according to the weight gain also shows a significant difference between the group receiving milk and the other two, but not between these latter. This advantage of milk should be partly offset by the large number of millet diets which were suspended before 6 days. These disappearances, not so frequent in the millet-peanut-fish group, are definitely more unusual in the millet-peanut group; the mothers seem to prefer the porridges.

Thus, the supplementation of the traditional diet with a mixture of millet and peanut flours is an effective means of treating mild malnutrition.

DISTRIBUTION OF PROTEIN-RICH BISCUITS

The preparation of a high-protein food in the form of biscuits facilitates its distribution through the schools, which is easy to arrange with the cooperation of the teachers.

The biscuit used was prepared for us by a large dietetic products company. It consists of millet flour, peanut-cake flour and fish flour, with the addition of sugar, spices and a substance which gives the compressed biscuit its consistency; this avoids the usual operation of heating at 120°C which, by destroying the lysine, greatly diminishes the protein value of the food.

The chemical analysis of this biscuit is given in table 7 in comparison with that of the biscuit used as a control (biscuit C).

TABLE 7
COMPARATIVE COMPOSITION OF BISCUITS N AND C
(grams per 100 grams)

Ingredient	Biscuit N	Biscuit C
Moisture	5.04	6.70
Protein	30.62	8.75
Fat	10.42	9.37
Carbohydrate	50.35	74.12
Ash	3.57	1.06
Calcium	0.32	0.18
Phosphorus	0.75	0.13
Thiamine (mg)	0.12	—
Calories	418	416

These biscuits were distributed in 1959 in two schools in Dakar and one bush school in Khombole, and in 1960 in 22 classes in Dakar and in five bush schools. Acceptability was excellent in every case. Distribution was carried out by the teachers at recess time and, if they forgot, the children spontaneously demanded their biscuits.

These biscuits, consumed by the children each day throughout the school year (7 months), caused no digestive difficulties.

In order to judge the effectiveness of these biscuits given in supplement to the normal food ration, we compared various groups of children, some receiving biscuits rich in protein (biscuit N), the others ordinary commercial biscuits (biscuit C). The biscuits were distributed in such a way that the supplementary caloric ration was the same and only the percentage of protein differed (table 7).

The results of three studies are reported: A) A preliminary study conducted in Dakar from January to June 1959; B) a study dealing with over 1000 children in 22 primary grades in Dakar, conducted from January to June 1960; C) a comparison study conducted during this period in five bush schools. In the three studies the children were weighed at the beginning and at the end of the distribution period. The scales used were accurate to within a tenth of a gram. The weight gains computed according to these weights were arranged in groups of 200 gm.

A certain number of subjects showed a weight too distinctly divergent from the average for the age, or an aberrant weight variation. These results were due

either to an error or to the operation of an incidental cause (illness during or before the experiment, etc.). They were eliminated from the calculations so as not to falsify the appraisal of an average phenomenon.

Preliminary study (Dakar, January-June 1959)

This study dealt with 1035 children from 23 January 1959 to 30 June 1959; 871 children received the experimental biscuit N; 164 children received the control biscuit C. Their ages varied from 6 to 16 years. In this preliminary study the samples had not been adequately matched beforehand. Consequently, a statistical comparison could bear only on the three samples corresponding to 7 years, 8 years and 9 years. It seemed, in the first analysis, that the regular consumption of protein-rich biscuits resulted in a greater weight gain than the consumption of biscuits poor in protein. However, this experiment could not give us conclusive results because of the small number of subjects in the control sample.

Second study (Dakar, January 1960-June 1960)

Taking into account the lessons learned in the preliminary study, we conducted a second biscuit-distribution experiment in Dakar from January 1960 to June 1960, with 1132 children from two schools in Medina. This time, each of the two samples consisted of eleven mixed classes having equivalent proportions of girls and boys. These classes included four preparatory grades, four middle grades and three elementary grades.

First we eliminated the subjects who showed a weight increase over 7 kg or a weight loss over 3 kg. This eliminated only seventeen subjects. When we established the weight increase distribution, in 200-gm groups, of the children receiving biscuit N and those receiving biscuit C, the distributions appeared perfectly normal, with the following parameters:

Biscuit N: number of subjects, 566; mean, 1108 gm; variance, 1300

Biscuit C: number of subjects, 549; mean, 1320 gm; variance, 1244.

The difference of 212 gm between the means, which corresponds to deviation $C \pm 2.79$, was significant at $P = .006$. Considering that 96% of our population had to come between the limits of two standard deviations either side of the mean, we considered the distribution reduced to the limits of -1.5 kg and $+4$ kg.

On elimination of subjects over 14 or under 8 years of age, the parameters of our two distributions became:

Biscuit N: number of subjects, 553; mean, 1038 gm; variance, 940

Biscuit C: number of subjects, 523; mean, 1184 gm; variance, 980.

This results in a difference of 146 gm, corresponding to a deviation $C = 2.47$, significant only at $P = .015$.

It seems, therefore, in the aggregate, that in two large samples including the same percentages of the various ages between 8 and 14 years, having a comparable social origin and a similar mode of life, a supplement of protein did not bring about a greater average weight gain in the experimental sample than in the control group. We shall see later that the slight difference observed in the opposite direction can doubtless be explained by a factor foreign to the experiment. In order to limit this phenomenon more closely, we then isolated in our two samples

three pairs of equivalent age groups corresponding to the preparatory, middle and elementary grades.

We found again, in all ages, the same phenomenon as was observed in the aggregate—a slightly greater weight gain in group C. We wondered whether we would not find interesting growth differences in terms of the initial weight of the children. We computed the mean initial weight of the subjects of various ages, eliminating the children whose weight varied by more than 5 kg from the mean weight for their age. We observed that, at every age except age 11, the initial average weight for sample N was slightly more than that for sample C.

We then established for each age the weight gain distribution histogram, noting, on the one hand, the subjects with an initial weight lower than the average for their age and, on the other hand, those with an initial weight higher than the average. Regrouping these results, we drew up four distributions having as an average: ± 1032; ± 1052; ± 1228; ± 1426. This indicates that there were no variations connected with the protein supplement, other than those according to the initial weight of the children.

At the conclusion of this second experiment, therefore, we can produce only negative results. At the present stage of experimentation, it seems that the distribution of the protein-rich biscuits does not have a significant effect on the weight variations of children who, in Dakar, doubtless receive a well balanced food ration.

In our experimenting there are still two causes for error which probably explain the slightly greater increase in average weight in sample C: 1) Our samples were not strictly matched from the point of view of age. Sample N may have suffered, in our comparison, from a slightly higher proportion of children aged 9 and 10, whose weight gain is less than at 11 and 12 years. 2) The initial average weights of sample N were slightly higher at each age than those of sample C. Therefore the latter's greater progress corresponded only to a simple fluctuation in the weight variation.

In order to eliminate these two causes for error, we propose to extract from our two samples pairs of children of the same age and weight and to compare the average increases of the reduced samples.

Third study (bush villages, January 1960-June 1960)

The third study was undertaken in five villages—three farming and two fishing villages. In the latter, the children received a diet rich in fish. All the children in these five villages received biscuit N.

We established the weight gain distribution by separating the two fishing villages and the three farming villages. The parameters of these two distributions are:

Fishing: number of subjects, 208; average, 1370 gm; variance, 1108

Farming: number of subjects, 149; average, 746 gm; variance, 636.

The difference is 624 gm significant at $P = .0001$.

Thus, while the children in the fishing villages show a weight gain at least equal to that of the children in Dakar, the weight increase of the children in the farming villages is about one half over the same period of time.

It is interesting to note in comparison that, according to the teachers, the children from the farming villages insistently demanded their biscuits, while in the fishing villages the distribution was merely accepted. Under these conditions, one may wonder whether the bush children's weight increase, which is low in comparison with that of children in the urban and coastal environments, has not already been improved by the distribution of biscuit N. In order to ascertain this, it would be advisable to run a second experiment of similar setup on nine bush villages, with three schools receiving biscuit N, three biscuit C, and three serving as a control group.

PRESERVATION AND STORAGE OF PROTEIN MIXTURES

Beginning this year, and especially in 1961, large quantities of the millet-peanut-fish mixture will be prepared and launched in trade channels or distributed in the MCH Centers or in the communities. Packaging and storage of these preparations pose difficult problems. Because the sale price must be low, within the purchasing power of African families, packing must not be too expensive. But preservation must be satisfactory, avoiding any deterioration in the product despite variations in temperature, dampness, and other difficulties, and also preventing development of parasites, for which these flours have a great appeal.

Preservation: Absence of Change in the Chemical Composition

Deterioration of the flour may be caused by the action of enzymes or the development of germs.

Samples of millet, peanut and fish flours alone, of the mixture of millet (60%), peanut (30%) and fish (10%) and of biscuits were preserved in a sealed metal can, in an open metal can with air holes, and in sealed polyethylene bags. Samplings were taken regularly every other month for chemical analysis, in particular the quantitative analysis of fat, protein, pH, free acidity and moisture, and for bacterial examination.

Packing and Storage

Studies on packing and storage are being carried out in collaboration with the companies who produce or treat the peanut flour and the other components of the mixture utilized. Various solutions are under study.

Packaging in 25-kg bags, intended for communities, youth camps, rural centers. These 25-kg bags might be utilizable for trade channels, but there is a risk that the product may be invaded by insects if the turnover of the 25 kg requires several days.

Packaging in 250-gm or 500-gm boxes of waxed cardboard reinforced with a plastic sack. This would be intended more especially for infant feeding.

August, September and October are the months when climatic conditions—temperature and dampness—are most unfavorable. Consequently, the preservation tests and the studies on storage are to be continued during this period. The product, under various types of packaging, is being stored in a variety of premises—shops, sheds, dispensaries—in the city and in rural areas.

Pollution by Parasites (Worms and Insects)

It is necessary to differentiate between development of parasites from adult insects or eggs present in the flours and invasion by parasites from the outside.

Concerning the first point, it has been proved that the milling processes used (centrifuging through a filter) permit the destruction of the parasites and the crushing of the eggs at the time the flours are prepared. Samples of peanut flour, fish flour, and the millet-peanut-fish mixture have been preserved for as much as 6 months without any development of parasites being observed.

Studies are in progress regarding protection against introduction of parasites during storage, transportation and distribution. Various samples were stored in three places where the risks of pollution were high. Samples were kept in an open metal can or in thin plastic bags; they were contaminated by insects from the outside, chiefly coleopters, identification of which was made by the parasitological laboratory of the Medical School.

This study must make possible either the selection of a packing method which will resist the attempts of these insects to penetrate, or the use of an appropriate insecticide that is nontoxic.

UTILIZATION OF FOOD PREPARATIONS CONTAINING PEANUT FLOUR

The study on the utilization of food preparations containing peanut flour has a twofold purpose: To get as accurate an idea as possible of the size of the market in terms of possible consumers, in order to know what quantities of peanut flour will have to be produced in 1961; and to plan for the organization of distribution channels, obtaining useful information for launching a promotional campaign to encourage the consumption of these protein-rich preparations. The studies are being undertaken in conjunction with commercial companies and Government agencies concerned (Office de Commercialisation Agricole, recently established by the Government of Senegal).

Mixed Flours for Infant Feeding

The mixture of 60% millet, 30% peanut flour and 10% fish flour was satisfactory. The amino acid balance is relatively favorable; the mothers know how to prepare this food, which much resembles the traditional millet porridge. The tests made in the Pediatric Service of the hospital, at the MCH Center and at the ORANA Rural Experimental Center have proved that this mixed meal flour is well adapted to feeding a child from the 6th or 7th month.

Until now, these flours have been distributed free of charge. A trial of the commercial sale of a millet-peanut mixture was made 3 years ago. This year a large amount (4 tons) of the mixture of millet, peanut and fish is to be prepared and a study of commercial possibilities will be conducted during the bad season. (This work has been delayed 4 months because of a delay in the delivery of fish flour.)

Biscuits for Schoolchildren

A large biscuit factory in Dakar has been contacted for studying the possibility of local preparation of protein-rich biscuits containing peanut and fish flours.

Use of Peanut Flour in Family Cooking

Studies on use of peanut flour in family cooking have been made during recent months at the ORANA Experimental Center in Popenguine and some recipes have been perfected and demonstrated to the women of the village of Popenguine. These studies will be carried on during the next 6 months in one urban district and three rural districts, with ORANA assigning nine persons to this investigation. The study will deal with culinary uses of the millet-peanut-fish mixture and of pure peanut flour.

Utilization of Peanut Flour for Feeding Groups

A study on utilization of peanut flour by schools, youth camps, rural centers, the army and similar groups will begin in September 1960. As of now, the support of several ministries (Development, Health, Rural Economy) is assured.

DISCUSSION

DR. BENDER: A question on the first part, on the peanut diets where you were feeding large quantities. You showed on the first slide that in the second period of feeding with 140 gm of peanut flour per day, the growth rate was less than in the first period. How much peanut was fed in the first period?

MADAME AUBRY: Sixty to 80 gm.

DR. BENDER: My second question, then, arises from that. When you had 140 gm you said there was a large fecal excretion of protein. Was that merely twice as much as in the first period, or was it greatly increased? In other words, was the extra excretion merely due to the extra peanut, or was the peanut flour interfering with digestion and absorption?

MADAME AUBRY: It seemed to be both, because fecal volume was increased by about 200 gm. We made several trials to correct this trouble. We found that the digestibility could be lowered by the lower acidity given by peanut flour. We obtained, as a matter of fact, a little lower amount of fecal excretion when we added citric acid. It seems that digestion varies with the amount of protein.

DR. BROCK: You said about your diet no. 631 that the children had appeared to require time to adapt themselves to the diet. Was it your impression

that that was an adaptation of appetite or a physiological adaptation, or have you no evidence on that?

MADAME AUBRY: It is rather difficult to say, because at the time the children were moved from an institution to the pediatric ward, at the same time all the people who took care of them changed. They usually accepted quite well the formulas. They were nice children and not difficult in their behavior. They first lost weight and then were better afterwards.

DR. RAO: I would like to ask what was the maximum proportion of peanut flour in any food preparation you used. I ask this question because we have had some experience with the use of peanut flour, not with a group of children but with older groups. It has been our experience that with any food preparation if you incorporate peanut flour more than 15% to 20%, it becomes unpalatable. We have followed these preparations for three to four weeks, given to children where we can regard their activities. I know in this particular group you are dealing with, the child cannot express himself or herself.

MADAME AUBRY: They received 140 gm of peanut, with 35 gm of milk, 10 gm of millet, 45 gm of sugar, and a liter of water. The peanut provided 490 calories, a little more than half of the total amount which was 850 calories per liter. I think the most we gave was 140 gm/day mixed with milk and millet. With this large amount of peanut flour, they ate fairly well. They accepted it during 4 to 6 months. It was not our purpose to keep them on this diet any longer. In the other trial, which lasted only 3 to 5 weeks and was perfectly accepted, we gave 70 gm of peanut mixed with millet and milk; 240 calories of the 657 per liter were provided by the peanut, more than one-third of the caloric supply. The children seemed quite all right. They were healthy infants of 5 to 9 months of age at the beginning of the experiment.

DR. RAO: Are there any deleterious effects as a result of long-term feeding of the peanut flour?

MADAME AUBRY: We have not had any deleterious effect that we could appreciate up to now. The trials extended no longer than 175 days on the diet with the largest amount of peanut, the most part of the protein being given by peanuts. But other studies were conducted in the pediatric ward and the MCH Center where children received peanut cake flour as a part of the ordinary diet. No trouble was found after 2 to 3 years of such diets—about 10 gm of peanut cake flour per day.

Determination of the Nutritive Value of Different Protein Foods in the Feeding of African Children

E. M. DeMaeyer and H. L. Vanderborght

The incidence of protein malnutrition throughout the world has been widely publicized during the past 15 years. Attention of governments and international agencies has been focused on this important public health problem, and the prevention of malnutrition is being investigated at present in many parts of the world.

Since the supply of animal protein in the form of milk, meat and eggs is limited and expensive, trials have been made on the use of foods rich in protein which are more readily available. Products, abundant in some countries, which could be used are flours made from oil-seed cakes such as cottonseed, peanut and sesame; another product would be fish flour. The determination of the nutritive value of several of these protein-rich foods has been undertaken as part of a study initiated by UNICEF for the purpose of solving at least partly the problem of the eradication of this type of malnutrition. Three other protein foods, i.e. eggs, human milk and cow's milk, were included in the study as reference proteins. Enough information has already been gathered on these, proving their high nutritive value, so that they could be used as indexes of reference.

The digestibility, BV and NPU of whole eggs, cow's milk, human milk, soy milk, fish flour, soybean flour, sesame flour, cottonseed flour, peanut flour and biscuits made of fish, peanut and millet flours have been investigated by the nitrogen balance technique; the results are presented in this paper. Although the nutritive value of most of these foods has already been investigated in animal experimentation (see discussion), their value for human beings is not completely known. Since the problem of prevention of malnutrition is mainly directed towards children, the protein foods above mentioned have been tested on them.

Subjects

Seventeen African children were selected for the experiment. These came from villages located in the neighborhood of the Institute at Lwiro. They belonged to the Bashi tribe which lives on the shores of Lake Kivu in the Kivu Province (Republic of Congo). The group included two children aged 3 years, five 4 years,

six 5 years, three 6 years, and one 7 years; there were a total of 9 boys and 8 girls. All of these children were admitted to the Center for minor parasitic infections, infectious diseases, or mild malnutrition. A few cases were acute kwashiorkor when first admitted; these received a full course of treatment prior to any experimentation. Since all children included in the experimental group had been kept on a good diet for 3 to 4 months, it may be assumed that they were in approximately the same nutritional state when the experiment began. In the interim period, between the admission and the beginning of the experimentation, every child was submitted to a thorough clinical examination including X-ray of the chest and treatment for all parasitic diseases. The children, when the experiment began, were completely free of any intestinal or blood parasites as far as could be determined. During this same period, they were trained until they were disciplined to freely accept the balance techniques. A short description of the children participating in the experiment is given in table 1.

TABLE 1
DATA ON AFRICAN CHILDREN PARTICIPATING IN TESTS

		On admission						Weights (dates)	
						Blood		at start	at end
No.	Sex	Date of birth	Date	Diagnosis	Weight kg	Prot. (1) gm %	Alb. (2) gm %	of tests kg	kg
D 43	F	3/50	11/56	K	15.30	3.90	1.01	17.00(7/57)	21.00(4/58)
D 48	F	2/51	11/56	M	11.60	5.54	2.30	15.25(7/57)	18.35 (4/58)
D 49	F	8/51	11/56	M	11.90	6.61	2.37	14.60(8/57)	19.60(4/58)
D 73	M	11/53	11/58	M	12.40	6.33	2.85	14.90(4/59)	17.00(5/60)
D 75	F	1/53	4/57	K	11.40	5.85	1.60	14.35(7/57)	18.70(4/58)
D 85	M	1/54(3)	6/57	K	12.65	4.41	1.05	14.00(9/57)	18.10(5/58)
D 93	M	1/54	8/57	K	10.40	4.78	1.08	12.75(12/57)	16.50(8/58)
D 95	M	6/51	8/57	P	13.55	7.73	3.87	13.80(9/57)	16.70(5/58)
D 104	F	1/53	10/57	K	13.30	4.21	1.16	14.85(1/58)	16.60(7/58)
D 109	M	7/54	11/58	A	12.10	8.20	3.93	14.45(3/58)	16.30(5/60)
D 132	M	7/54	11/58	I	13.70	7.96	4.68	15.10(3/59)	16.10(7/59)
D 147	F	7/55	3/59	N	12.35	7.86	3.55	14.10(4/59)	14.60(5/60)
D 154	M	9/54	4/59	K	12.30	4.69	1.23	13.45(9/59)	15.30(4/60)
D 161	F	4/55	5/59	K	12.80	4.46	1.58	14.50(9/59)	15.80(4/60)
D 163	M	7/56(3)	6/59	M	10.30	5.03	2.18	12.45(9/59)	13.10(11/59)
D 164	F	4/56	6/59	K	11.85	4.39	1.34	13.25(9/59)	15.70(4/60)
D 176	M	1/55	9/59	K	12.30	4.43	1.15	14.00(2/60)	14.38(4/60)

K—kwashiorkor, M—mild malnutrition, P—minor parasitic ailment, A—ascaridiasis, I—minor infection, N—normal.
(1) Total proteins estimated by the method of Wolfson, Cohn, Calvary and Ichiba (1948).
(2) Albumin fraction estimated by paper electrophoresis, according to Sonnet and Rodhain (1952).
(3) The date of birth is not known exactly.

DIET

A basal diet was devised to which one experimental protein food was added for each experiment. The daily composition of the basal diet, which remained constant throughout the experiment, was as follows: Corn starch, 21 gm; tapioca, 30 gm; butter, 40 gm; maple syrup (artificial), 40 gm; palm oil, 15 gm; jam, 30 gm;

vegetable soup, 100 gm; sugar, 103 to 155 gm; juice of one lemon; salt (NaCl), 3 gm; and a vitamin and mineral mixture.†

In each experiment the amount of sugar was adjusted to provide a uniform daily intake (basal diet + tested protein food) of 1500 calories.

The nitrogen content of the basal diet was low and varied slightly, owing mainly to differences in the composition of the vegetable soup; however, it always ranged between 170 and 220 mg daily.

Experimental Proteins

The following protein foods were used in experimentation.

Eggs. Whole fresh eggs were beaten at low speed in an electric mixer and cooked into omelet.

Milk. A spray skim milk powder, produced by the Land O'Lakes Co., Minneapolis, Minn. by a "low heat" spray dry process, was used. It was distributed by UNICEF under the code "Reference cow's milk". The milk powder was mixed with water in a Waring blendor; the final preparation was not cooked. The composition of the milk powder, according to UNICEF, was: proteins, 36.9%; lactose, 51%; lipids, 0.9%; moisture, 3%; ash, 8.2%.

The nitrogen content of the powder was determined to be 5.363 gm %.

Human milk. The human milk, which was furnished in a lyophilized form by UNICEF, was diluted with water before being served to the children. The flavour of the preparation was quite unpleasant and different trials were made to modify it; the best results were obtained with chocolate. This increased slightly the nitrogen content of the basal diet, but the acceptability was otherwise so poor and vomiting so frequent that it seemed impossible to avoid this modification.

Soy milk. The soy milk was made in Indonesia and provided in a dry form under the name "Saridele Toffaroma" by UNICEF. The product was flavoured. It was diluted with water and mixed in a Waring blendor before serving. The protein composition of the powder averaged 24%.

Fish flour. The fish flour was prepared by OVAPIRU in Usumbura (Ruanda-Urundi) from two fish species (Limnothrissa Miodon and Stolothrissa Tanganicae) which are abundant in Lake Tanganyika. The composition of the fish flour averaged as follows (Roels, 1956): proteins, 71.8%; lipids, 7.9%; ash, 14.2%; moisture, 6.1%.

The nitrogen content as measured during the course of the experiment was 11.07 gm %.

Sufficient water was added to the fish flour to prepare a thick paste, which was cooked for a few minutes before serving; this preparation was readily accepted and well tolerated.

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† Composition of the vitamin and mineral mixture: Aneurin, 5 mg; Lactoflavin, 2 mg; niacin, 20 mg; Adermin, 2 mg; Ca pantothenate. 3 mg; ascorbic acid, 50 mg; vitamin D_2, 330 IU; folic acid, 5 mg; vitamin B_{12}, 10 gammas; Ca gluconate, 500 mg; Ca phosphate, 100 mg; vitamin A acetic, 50,000 IU (once a week in the intervals between the balance period); iron, 50 mg (twice a week in the intervals between the balance period).

Soybean flour. Two different flours were used. The first was purchased from the British Soya Products, London, England. This flour was mixed with a little water and cooked in a pressure cooker for 20 minutes at 15 pounds per sq. inch. The final preparation, which was a thick paste, was not appreciated by the children and some efforts had to be made by the nurses before they would eat it. Different types of flavouring, *i.e.* salt, sugar and anise oil, were tried but with little avail in making the preparation more palatable.

The composition of the flour was determined to be: nitrogen, 6.864 gm %; proteins (N x 6.25), 42.9%; lipids, 9.82%.

The second came from General Mills, Inc., Minneapolis, Minn., U.S.A. and was labeled "Toasted Soy Protein No. 100". The flour was prepared by addition of water and was cooked for a few minutes. Its composition, according to the manufacturer, was: protein, 50%; lipids, 2% (after acid hydrolysis); ash, 6%.

The nitrogen content, measured in the course of the experimentation, was 8.240 mg %.

Peanut flour. The flour used was distributed by UNICEF under the code PF-4. It was prepared in England by UNILEVER from peanuts grown in South Africa. Its composition, according to the manufacturer, was: proteins, 50.2%; lipids, 6.1%; moisture, 3.7%; crude fiber, 3%; ash, 3.7%. The nitrogen content was determined to be 8.043 gm %.

After the addition of a small quantity of water, the flour was cooked for a few minutes until a thick paste was obtained. This preparation was served daily to each child and was readily accepted and well tolerated.

Cottonseed flour. This flour was distributed by UNICEF under the code C.1. and was prepared by the Traders Oil Mill Co., Fort Worth, Texas. Its composition, according to the manufacturer, was: proteins (N x 6.25), 57%; lipids, 4.9%; moisture, 3.99%; crude fiber lower than 2.5%; index of nitrogen solubility 86%; free gossypol, 0.045%. The nitrogen content was determined to be 9.127 gm %. The cottonseed flour was prepared in the same manner as the peanut flour. The taste of this preparation was pleasing to the children and it was well tolerated.

Sesame flour. The product was distributed by UNICEF under the code SF-2 and was prepared by the American Sesame Products Co., Paris, Texas. Its composition, according to the manufacturer, was: proteins (N x 6.25), 54.19%; lipids, 11.85%; glucides, 20.78%; moisture, 7.45%; crude fiber, 4.7%; ash, 5.73%. The nitrogen content was determined to be 8.550 gm %.

The flour was prepared in the same manner as the peanut flour. A few difficulties arose because of the taste of the preparation; however, it was possible to make it palatable enough for the children to accept it.

Biscuits. The biscuits were made by NESTLÉ in Switzerland from fish flour, millet and peanut flour. Their composition averaged as follows: proteins (N x 6.25), 24%; lipids, 11%; glucides, 54%; crude fiber, 20%; ash, 4%; moisture, 5%. The nitrogen content was found to be 4.142 gm %.

The biscuits were eaten as such; they were readily accepted by the children, who appreciated them very much.

Experimental Procedure

The determination of the true BV presupposes many conditions, the first being that the subjects in experimentation are kept either in a negative nitrogen balance or in the region of nitrogen equilibrium (Allison 1955). This is true for adults but, because of the greater requirements of children, the determination may be made while they are in a state of slightly positive nitrogen balance and this is the only condition acceptable if children have to be maintained for long periods of time under experimentation.

We have sought to measure the nutritive value of each protein food at different levels of intake, hoping that the optimum conditions for measurement would be fulfilled at some of the levels selected. Although we were not able to test every food at different levels, we feel that the level selected, when there was only one, met the requirements for the determination of the true BV.

The second requirement for measuring the BV of a protein is to determine the level of endogenous urinary and fecal nitrogen excretion of the subjects under experimentation. This was done by keeping the children for 5 days on the basal diet. The amount of sugar of the diet was adjusted so that each child continued to receive the same caloric intake, *i.e.* 1500 calories. It was observed that the urinary excretion fell for the first 2 days and then remained steady, after which a good estimation of the endogenous nitrogen metabolism could be made during a 3-day balance period. The children were kept on such a basal diet at the beginning and at the end of a complete series of balance studies. Since they were kept on this diet for only a short time, it does not seem that their health could have been affected in any way. This was confirmed by the frequent clinical examinations to which they were subjected.

The method used in the nitrogen balance study has been described previously (DeMaeyer and Vanderborght, 1958). Each balance period lasted 5 days, beginning on a Monday morning and ending on the following Saturday. When the level of nitrogen intake changed, the subjects were first kept for a full week on the new level of nitrogen prior to starting a balance study. When the protein food was changed, but not the nitrogen intake, the interim period lasted only 2 days, from the Saturday until the following Monday when the balance began.

It was also arranged in the plan of experiment that the nitrogen balances used for the determination of the endogenous excretion were always performed after a good-protein, low-fecal-residue (either milk or egg) balance period.

Analyses

Food. A sample of each food was taken every day for analysis. However, when the food was furnished in lots, as in the case of the tested protein foods, the analysis was repeated only twice a week.

Urine and stools. Nitrogen determinations were made separately on each 24-hour sample. The urines were collected under a layer of toluene and kept in the refrigerator until the analysis was completed. A 5- or 10-ml aliquot, according to the expected nitrogen content, was used. The stools, when collected, were immediately mixed with 0.1 N HCl and kept in the refrigerator until the analysis.

They were then homogenized in a Waring blendor in presence of more 0.1 N HCl until a semi-liquid paste had been obtained. A 5-gm sample of the mixture was used for the nitrogen determination.

Ashing of foods, urine and stools was performed in the presence of concentrated sulfuric acid and a catalyst mixture composed of potassium sulphate and mercuric sulfate (Hiller et al., 1948). When it was completed, the acid residues were brought to a predetermined volume (usually 500 ml) of which an aliquot was taken for the determination of the ammonia (Keys, 1940; Ma and Zuazaga, 1942)

Results

The following formulas have been used in the calculation of the data:

$$\text{Absorption } \% = \frac{I-(F-F_n)}{I} \times 100$$

$$\text{Biological Value (BV) } \% = \frac{I-(U+F)+(U_n+F_n)}{I-(F-F_n)} \times 100$$

$$\text{Net Protein Utilization (NPU)} = \frac{I-(U+F)+(U_n+F_n)}{I} \times 100$$

where

I = N ingested
F = Total fecal N
F_n = Endogenous fecal N
U = Total urinary N
U_n = Endogenous urinary N

The entire experiment is described and summarized in table 2; the detailed results have been presented in full to the Committee on Protein Malnutrition.

COMMENTS and CONCLUSIONS

Endogenous Nitrogen in the Urine

The endogenous nitrogen was always determined on a 3-day balance period, following a period where the nitrogen intake was relatively low and was furnished by a good test-protein food such as milk or eggs. Our results indicate that the ratio of endogenous urinary nitrogen in mg per calorie of basal metabolism averages 1.00. This is lower than the ratio of 2.3 found by Terroine and Sorg-Matter (1927) and Smuts (1935) or even than those calculated by Bricker, Mitchell and Kinsman (1945), Murlin, Edwards, Hawley and Clark (1946) (1.34 to 1.48) or Bricker and Mitchell (1947). It is close to the ratio reported later on by Hawley, Murlin,

TABLE 2
EXPERIMENTAL DATA AND SUMMARY OF RESULTS

	Level in gm/day	No. of 5-day balance periods	Average protein consumption in gm/day	Percentage of the daily caloric intake	Absorption in %	SD	BV	SD	NPU	SD
Whole eggs	75	18	10.4	2.8%	96.71	8.02	90.48	10.38	87.33	10.94
Reference cow's milk	20	14	8.09	2.2%	89.56	8.42	90.32	3.68	80.96	13.27
"	40	34	15.56	4.2%	91.65	2.65	85.83	8.89	78.72	11.79
"	60	6	23.16	6.2%	88.45	2.93	91.40	4.49	80.88	5.62
"	80	6	30.55	8.1%	90.73	5.67	82.15	7.19	74.50	7.46
Human milk	Low	10	8.67	2.3%	92.09	14.03	103.03	8.57	94.78	16.16
"	Medium	19	14.35	3.8%	86.96	8.88	98.28	7.56	85.39	10.46
"	High	6	23.26	6.2%	94.57	3.49	99.93	3.03	94.55	5.19
Saridele Toffaroma	30	7	8.23	2.2%	95.29	6.75	81.73	15.11	78.13	17.11
"	60	13	15.93	4.3%	95.51	6.17	79.81	9.98	76.47	12.91
"	90	6	23.01	6.1%	93.48	3.12	80.30	6.30	75.15	7.64
Fish flour	25	6	16.60	4.4%	82.42	4.66	82.90	7.57	68.50	8.62
Soybean flour (Brit. Soya Products)	65	8	26.92	7.2%	87.68	3.15	61.25	6.41	53.60	5.45
Toasted soy protein	15	7	8.67	2.3%	88.31	7.44	81.70	5.97	72.34	9.70
"	30	5	18.24	4.9%	88.40	4.52	90.06	7.08	79.74	8.68
"	45	6	24.71	6.6%	84.02	5.84	79.47	7.04	66.82	8.27
"	60	7	31.83	8.5%	92.47	5.30	76.61	9.66	70.93	10.20
Sesame flour	50	6	26.46	7.1%	85.05	7.33	62.28	12.59	53.57	14.78
"	100	9	50.07	13.3%	89.17	4.72	58.90	7.01	52.55	7.69
Peanut flour	50	6	24.05	6.4%	91.73	5.35	61.23	10.78	56.53	12.52
"	100	9	48.67	13.0%	88.60	6.07	60.37	6.76	53.36	6.34
"	150	7	67.80	18.1%	96.41	2.23	54.11	11.75	52.19	11.53
Cottonseed flour	50	6	26.86	7.2%	81.97	6.43	62.12	7.28	50.83	6.13
"	100	6	54.41	14.5%	87.78	1.80	53.40	4.25	46.90	3.77
"	150	5	79.87	21.3%	91.34	3.96	42.08	7.35	38.50	7.51
Nestlé biscuits	60	21	17.14	4.6%	82.69	12.95	72.79	8.22	60.77	14.27

Nasset and Szymanski (1948), i.e. 1.19 (average on 6 women) and 1.32 (average on 7 men).

Digestibility

It is obvious from the results of the experiment that the level of intake does not affect the nitrogen absorption. For the sake of simplicity, we may recalculate for each protein food the coefficient of nitrogen absorption without taking into account the level of intake. This has been done in table 3.

Table 3
Digestibility of Different Protein Foods

	Absorption in %
Whole Egg	96.71
Reference cow's milk	90.75
Human milk	89.73
Saridele Toffaroma	94.98
Toasted soy protein	88.46
Soybean flour	87.68
Sesame flour	87.52
Peanut flour	91.94
Cottonseed flour	86.78
Fish flour	82.42
Biscuits	82.69

It now appears possible to classify the tested food proteins into four classes, depending on their origin and the results of the experiment.

I Whole eggs

II Reference cow's milk; human milk; Saridele Toffaroma

III Toasted soy protein; soybean flour; sesame flour; peanut flour; cottonseed flour.

IV Fish flour; biscuits.

The average coefficient of nitrogen absorption becomes thus:

I	96.71
II	91.36
III	88.78
IV	82.63

An analysis of variance shows that the variance ratio has a probability lower than 0.001. A t-test indicates, moreover, that the differences of absorption between the four classes are statistically significant in each case:

I — II Difference	: 5.35	P between 0.02 and 0.01
II — III Difference	: 2.58	P between 0.02 and 0.01
III — IV Difference	: 6.15	P lower than 0.001

The differences in digestibility do not seem to be explainable by a change of the endogenous fecal nitrogen. The latter varies with the amount of dry matter in the diet, but the differences in dry matter between the various diets fed do not substantiate the fact that such a factor could be involved. It is possible that the lower digestibility of the protein foods included in class III is due to the presence of other constituents, like cellulose or some carbohydrates, which prevent a more

complete digestion of the proteins by accelerating the intestinal transit or protecting them against the action of the digestive enzymes. It is not possible to answer this question, but the interactions of other food components on the digestibility of the protein seem to be a reasonable explanation. As far as the fish flour is concerned, the low digestibility is probably a reflection of the incorporation into the flour of the whole fish, including indigestible factors such as bones and scales.

Biological Value

The determination of this index is affected by the level of nitrogen intake; when the latter is too high, part of the dietary proteins are catabolized and consequently used by the organism in a very uneconomical way. This results in an apparent decrease of the BV.

It is obvious from table 2 that the figures for the BV are not affected by the nitrogen intake, as long as the latter does not exceed 400 mg/kg/day and in some cases even 600 mg/kg/day. There is no doubt that the figures obtained with levels of nitrogen intake lower than 400 mg/kg/day represent the true BV of the food proteins tested.

Some of these BV's may be compared with those reported previously by other authors. If we refer to table 4, it appears that our results are similar to those of Hawley, Murlin, Nasset and Szymanski (1948) and Bricker, Mitchell and Kinsman (1945). These two groups worked in conditions identical to ours but with adults.

Table 4
Comparison of the Biological Value of Different Proteins, as Determined in this Experiment, With Some Figures From the Literature. The Protein Scores Are Also Indicated

	Authors' data	Literature (8)	(3)	(17)	(16)		Protein score (7)
Whole eggs	90	94		65		43 (12)	100
Milk	84		74	62		51 (14)	78
Fish flour	83					60 (23)	
Peanut flour	61	56			83		56
Sesame flour	62						59
Cottonseed flour	62				91		70
Soybean flour	61		65		81	65 (5)	73

As far as other experimental studies are concerned, the differences are difficult to explain; in some cases the endogenous nitrogen excretion may not have been determined under optimal conditions; in some others, the protein intake might have been too high, resulting in a strongly positive nitrogen balance and a waste of proteins. It is also possible that the processing of the food products was different and resulted in some cases in a definite lowering of the nutritive value. With regard to this, it should be kept in mind that almost all the products that we used were processed very carefully and were, in some instances, of a quite exceptional quality.

As far as the soy milk (*Saridele Toffaroma*) is concerned, the figure 80, found by us, is very good; some reports, however, indicate that the manufacture

of the product is not yet fully standardized and that its quality varies from one batch to the other.

The NPU of the various food proteins tested indicate that the fish flour, the biscuits and the soy milk (with the restriction indicated above) have an excellent nutritive value. Soybean, sesame, cottonseed and peanut flour have a reasonably good nutritive value which may support their use as a supplementary source of dietary proteins.

We have compared our values with those found by Bender (UNICEF study) on rats, using the Miller-Bender Method (1955).

It is interesting to note that in all instances where the same product was used, the figures agree closely. In the case of fish flour, where the products were of different origin, the difference is mainly one of digestibility and is probably related to the nature of the starting material used for the manufacture of the flour.

This last comparison between animal and human experimentations seems to us especially interesting since it confirms the value of the former in the assessment of the nutritive value of proteins for the human being.

When several levels of nitrogen intake were tested, some interesting data were obtained by the use of regression equations. The relation between the nitrogen retained (not corrected for the endogenous excretion) and the nitrogen intake has been calculated in this manner with the following result (table 5):

TABLE 5

	Regression	Value of x for y = o (in mg/kg/24hr)
Human milk	$y = -75 + 0.93\,x$	81 mg
Cow's milk	$y = -70 + 0.75\,x$	94 mg
Saridele Toffaroma	$y = -65 + 0.74\,x$	88 mg
Toasted soy protein	$y = -64 + 0.69\,x$	93 mg
Sesame flour	$y = -91 + 0.50\,x$	181 mg
Peanut flour	$y = -103 + 0.55\,x$	186 mg
Cottonseed flour	$y = -52 + 0.36\,x$	145 mg

These regressions are illustrated in figure 1.

The slope of the regression lines is, in each case, directly proportional to the NPU of the product. An analysis of variance shows that these seven food proteins may be divided into three classes, the regression factor being significantly different between food proteins of different classes.

I	:	Human milk
II	:	Cow's milk Saridele Toffaroma Toasted soy protein
III	:	Sesame flour Peanut flour Cottonseed flour

The same regression equation may be used to calculate the minimum intake required to maintain nitrogen equilibrium (value of x when y = o; see table 5). Thus it appears that, when feeding a child with a protein of high BV such as milk,

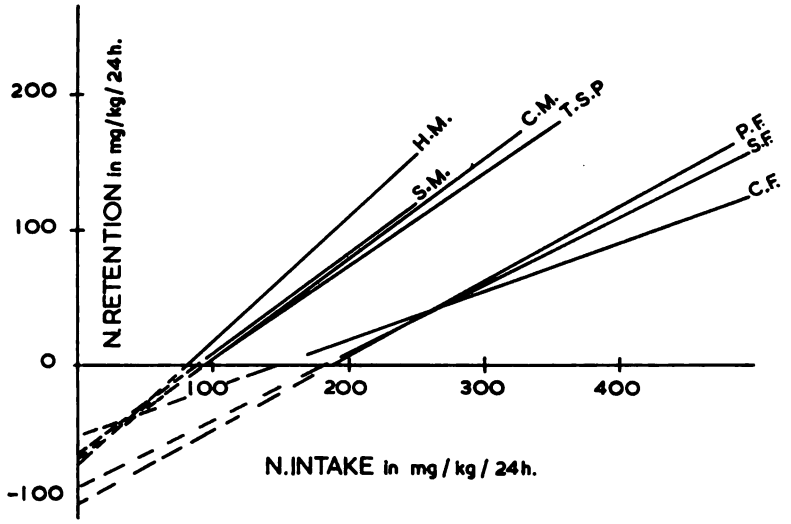

Figure. 1—The regression lines (table 5) for the human milk (HM), soy milk (SM), cow's milk (CM), toasted soy protein (TSP), peanut flour (PF), sesame flour (SF) and cottonseed flour (CF). They show the relation between nitrogen retention and nitrogen intake.

it will be necessary to give him between 0.5 and 0.6 gm of protein per kg and per day to maintain nitrogen equilibrium, no allowance being made in this calculation for the growth requirements. When a protein of lower BV such as sesame or peanut or cottonseed flour is used alone, the intake will have to be increased twofold or even more.

Conclusions

This work shows clearly that the protein-rich foods which have been tested, i.e., cottonseed flour, peanut flour, sesame flour and soya (soybean flour and toasted soy protein) are safe for human consumption and have a reasonably good nutritive value. They constitute a good source of protein for human feeding and their use may be recommended for the prevention of protein malnutrition, when their limiting factors are kept in mind when considering the question of supplementing a diet.

As far as fish flour is concerned, its BV is very good and its manufacture should be encouraged when the supply of fish is abundant and conservation or the cost of transportation prevent its distribution as such. Likewise, the biscuits have a good nutritive value and may constitute a valuable aid in the prevention of protein malnutrition.

The high nutritive value of eggs, cow's milk and human milk has been confirmed in this experiment. An interesting point, although it may have little practical significance, is the fact that this experiment has clearly demonstrated the higher BV of human milk over cow's milk.

SUMMARY

The nutritive value of various food proteins, namely eggs, cow's milk, human milk, soy milk, fish flour, peanut flour, sesame flour, cottonseed flour, two preparations of soybean flour and biscuits made of fish, peanut and millet flour, has been determined in children aged 3 to 7 years. The nitrogen balance technique has been used. The digestibility, the BV and the NPU have been measured.

The results show that:

1. The ratio of endogenous urinary nitrogen excretion per calorie of basal metabolism is 1.00 (SD:0.23).

2. The percentage of absorption, the BV and NPU are respectively: whole eggs 97, 90, 87; cow's milk 91, 87, 79; human milk 90, 100, 90; soy milk 95, 80, 76; fish flour 82, 83, 69; peanut flour 92, 61, 57; cottonseed flour 87, 62, 54; sesame flour 88, 62, 54; soybean flour 88, 61, 54 (British Soya Products) or 88, 85, 75 (toasted soy protein) and Nestlé's Biscuits 83, 73, 60.

3. The protein-rich foods which are the result of the processing of oil-seed cakes, *i.e.* peanut, sesame and cottonseed, are safe and have a relatively good nutritive value which recommends them as a possible source of proteins in the prevention of protein malnutrition. The same is true for the soybean flours tested.

4. A special mention should be made of two products:

a) Some batches of soy milk (*Saridele Toffaroma*) showed a nutritive value similar to that of cow's milk. It seems that, if the product were carefully standardized, it might be useful in the feeding of infants when breast feeding or cow's milk are unavailable.

b) The biscuits have a good nutritive value. Their use can be recommended where prevention of protein malnutrition in children aged 2 to 6 years is needed, since they do not require a special preparation and are thus safe from a microbiological point of view.

5. The experiment has confirmed the high nutritive value of milk and egg proteins. It has also demonstrated the definitely higher BV of human milk compared with cow's milk.

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Reference Groundnut Flour (GNF) and Reference Dried Skimmed Milk (DSM) as Supplements to the Diets of Nigerian Men and Children

B. M. Nicol and P. G. Phillips

The reference GNF (PF 4) and DSM were supplied by the FAO liaison officer with UNICEF, New York, and the ARLAC mixture is of Nigerian origin. These foodstuffs were used in the trials conducted on adult men and schoolboys.

STUDIES ON NIGERIAN MEN

Nigerian men, aged 25 to 40 years and weighing between 50 and 60 kg, were the subjects in the trials reported here. In each trial using the basal diet, or the other diets fed to Nigerian men, 6 subjects took part and ate the trial diet for 4 days before the nitrogen balance period of 6 days. The basal diet consisted of rice, *Cassava gari*, and soup made from Nigerian spinach (*Amaranth* spp.), fresh and dry red peppers (*Capsicum* spp.), melon seed (*Citrullus vulgaris*), red palm oil (*Elaeis guineensis*), a few grams of dried fish, and salt. It was fed at a level of approximately 4 gm nitrogen (24 gm protein) and 42 to 44 cal/kg. The mean daily nitrogen balance of 3 groups, each consisting of 6 Nigerian men, was found to be equilibrium on this basal diet measured over the balance period of 6 days.

It had been decided to measure the effects of supplementing this diet with either reference GNF or reference DSM by estimating the BV of the dietary protein mixtures using the Thomas formula (meeting in Kano 1957, attended by Dr. W. J. Darby, Dr. J. M. Hundley, Dr. D. M. Hegsted, Dr. Martha Trulson and Dr. B. M. Nicol). Therefore it was necessary to estimate the endogenous nitrogen excretion of our subjects. This was done by placing a group of 6 Nigerian men on a very low protein diet (0.77 gm N, approximately 4.5 gm protein) for a balance period of 6 days and then supplementing this diet with whole egg so that the nitrogen intake was around 4 gm, the same level as that of the basal diet. The calories were maintained at the basal level of 42 to 44 cal/kg by withdrawing *Cassava gari*. This foodstuff contains so little protein that it can be used as a source of energy without altering the amino acid pattern of a diet to any significant extent. By extrapolating back to zero nitrogen intake from the mean nitrogen balance recorded from the

6 subjects while they ate the very low protein diet (mean N balance, —2.24 gm) and the same diet supplemented with whole egg (mean N balance + 0.51 gm), values of 1.81 gm and 1.11 gm were obtained as the mean urinary and faecal endogenous nitrogen excretions, a total of 2.92 gm. These values were applied to the average data obtained from each group of 6 subjects when calculating the BV and digestibility (D) of each trial diet. The BV and D of the basal diet varied between 81% to 86% and 86% to 91% respectively, depending upon the group of subjects, the NPU being 74% for each group.

Results

When the basal diet was supplemental with approximately 46 gm reference GNF, to make the total nitrogen intake 7.3 gm, nitrogen retention was 1.4 gm and the NPU of this diet was 37%. The addition of 58 gm reference DSM to the basal diet, which gave a total nitrogen intake of 7.45 gm, resulted in nitrogen retention of 1.92 gm, and NPU of 54%. At higher levels of supplementation, adding 105 gm GNF or 125 gm DSM to the basal diet to raise the nitrogen intake to 11.7 gm and 11.2 gm respectively, nitrogen retention was 2.7 gm on the former and 3.3 gm on the latter supplementary foodstuff, the relative NPU's being 34% and 51%. Protein digestibility was approximately the same, between 84% and 88%, in all these trials.

From these data it has been calculated that approximately the same amount (1.5-1.6 gm) of nitrogen is retained by Nigerian men from 50 gm of GNF as from the same weight of DSM. There is no good reason to suppose that Nigerian women and children would not utilize GNF and DSM in the same way as their husbands and fathers, and it is they who are in greater need of a high-protein dietary supplement than the Nigerian men.

It is very important that Nigerian men retain as much nitrogen from unit weight of GNF as from the same amount of DSM, for the following reasons. Groundnuts are a major Nigerian crop, and GNF is being produced now in Zaria, Northern Region, at a cost of between 6 and 7 cents per pound. This price may be reduced if the demand for GNF justifies operating the mill on a two or three 8-hour shift basis rather than on the present single 8-hour shift. The potential supply of GNF is very large, envisaged in many thousands of tons per annum, whereas the amount of DSM which it will be possible to produce in Nigeria is unlikely to rise above 150 tons per annum for many years, because of the lack of fresh milk available for processing. The DSM now produced at Vom, Northern Region, costs approximately 28 cents per pound at the factory, and imported dried milk costs about 56 cents per pound retail (1960 prices).

SUPPLEMENTING THE DIETS OF SCHOOLBOYS WITH GNF

The pupils of a boys' boarding school in Kaduna were divided into four groups; the first three groups were matched by age within 6 months, by height to within 2 cm, and by weight to within 1 kg; the fourth group was made up of the remainder of the boys. The average age of groups 1, 2 and 3 was 15.5 years at the start of the trial, which lasted for one academic year (9 months). Group 1 was given a daily dietary supplement of 50 mg/reference GNF; group 2 was given

an amount of *Cassava gari* equivalent to 50 gm/GNF plus the major B-complex vitamins and calcium supplied by the GNF; group 3 ate the school diet supplemented by the same amounts of vitamins and calcium as were given to group 2; and group 4, the unmatched remainder, were given a tablet composed of 3 gm lactose flavoured with essence of lemon.

The weight and height of each boy were measured weekly. No other estimate of physical fitness was made, because the staff of the Nutrition Unit, including those recruited and paid for by Grant RF-NRC-8, did not have time to carry out further investigations. Some of the boys liked to mix the GNF into their soup, some added it to the rice, cassava or bean (*Vigna sinensis*) staple, and others made it into a drink with water and sugar. Most boys in group 1 accepted GNF readily, but a few had to be supervised carefully to ensure that they took the daily ration, which was given with the morning meal.

The school diet, calculated to have an FAO protein score of 70, provided slightly more protein than the minimal requirement, assuming that boys of this age need 0.8 gm reference protein/kg/day. This diet supplemented with GNF (group 1) scored 66 by FAO standards and supplied 1.73 gm protein/kg, approximately the same amount as the SPA (1.82 gm) of protein of such quality, this being the FAO minimum requirement multiplied by 1.5.

No significant difference was observed between the gains in weight and height of the three trial groups. Therefore the only positive conclusion which can be drawn from this investigation is that 50 gm of reference GNF, as a supplement to a diet of reasonably good Nigerian pattern, did not cause any harm to those adolescents who consumed it daily for a period of 9 months. The results draw attention also, and in a practical way, to the difference between the minimum requirement for protein and the SPA, as defined by the FAO Committee on Protein Requirements (1957).

TREATMENT OF INFANTS SUFFERING FROM PROTEIN MALNUTRITION WITH A MIXTURE OF NIGERIAN GNF AND NIGERIAN DSM (ARLAC)

A clinic was set up in Tudan Wada, a village near Kaduna, in the same compound with an M.C.H. Centre where Community Nurses are trained, and where the local rather shy peasant farming tribe, the Gwaris, attend for outpatient treatment and confinement, such of them as want European medical treatment and care. They much prefer such "bush" accommodiation to the ritual of the Kaduna General Hospital, and we found that our specially recruited staff could give these mothers and their infants more individual attention than was possible in a hospital ward. A European nursing sister, a Nigerian male nurse and 2 attendants were employed to carry out the treatment of those infants suffering from protein malnutrition who were referred from the M.C.H. Centre. In most instances these babies had been attending the Centre for a few weeks before they were referred to the Nutrition Unit because their mothers knew that they were sick, either with "fever", dysentery, measles, pulmonary and bronchial infections, or some other disease. They had been treated for these specific ailments but were sent to the Nutrition Unit Clinic when

it was realised that they were underweight for age and were not gaining satisfactorily on their present diet, or were obviously suffering from either kwashiorkor or marasmus. If it was considered (by B.M.N.) that such infants were dangerously ill they were sent to the Kaduna General Hospital for admission, because the object of this trial was to determine the effect of supplementing the diet upon which the babies had developed protein malnutrition with ARLAC (3 parts GNF and 1 part DSM) fed at a level of 6 gm protein/kg, approximately the SPA of such quality protein for infants aged 0 to 6 months. At the same time any intercurrent infection was treated in the home or in the Nutrition Unit Clinic at Tudan Wada, where the mothers could, if necessary, be admitted with their children and give them the same food as they had been given at home. Some mothers of children considered to be seriously ill refused admission to the hospital but were ready to be admitted to the Clinic. Thus the series of cases of protein malnutrition, amounting to 145, considered in this report, did not include all the most seriously ill infants who were originally referred to the Nutrition Unit Clinic.

The composition and mortality rate of the whole series was as follows: admitted to hospital, 40, mortality in first 48 hours, 8, in first week 2, in first month 2, after one month's treatment 1, 32.5%; treated in the Clinic, 145, mortality in the first 48 hours 7, in the first week 8, in the first month 2, after one month's treatment 1, 12.4%. The overall mortality for 185 cases was 16.8%. Protein malnutrition was diagnosed if the infant was underweight for age and showed obvious muscular wasting. If any oedema was present the case was classified as kwashiorkor; the remaining cases, all suffering from dehydration of one degree or another, were called marasmus. In all, 76 cases of kwashiorkor and 69 of marasmus were diagnosed. Of these 145 infants, all were followed satisfactorily for 4 weeks, 72 for 3 months, 48 for 6 months and 26 for one year, the word satisfactorily meaning that the Nutrition Unit staff was satisfied that the ARLAC supplement had been ingested by the child in the correct amounts over the period stated. The large difference between the 145 cases of protein malnutrition originally seen at the Clinic and those satisfactorily followed was due either to the mother's inability to follow instructions properly or to migration of the family from one village or compound to another without leaving any indication of its destination.

The mothers were asked to report to the clinic weekly so that their babies' weight could be recorded and haemoglobin determined every 4 weeks (spectrophotometer). In addition the Nursing Sister, a Nigerian nurse and attendants visited the homes to ensure that the supplement was being given to the babies. No other determination of a physical or biochemical nature was made, because the mothers would have been reluctant to submit the babies to the removal of venous blood, and measuring the length of such infants accurately would have taken more time than the limited staff of the Unit could afford.

Figure 1 shows the mean weights of those children originally suffering from protein malnutrition whose diets were supplemented with ARLAC, followed satisfactorily for one year, and compares them with the mean weights of Edinburgh infants and of Nigerian babies who were not suffering from frank protein malnutrition. The mean weight of this last group was approximately the same as that of

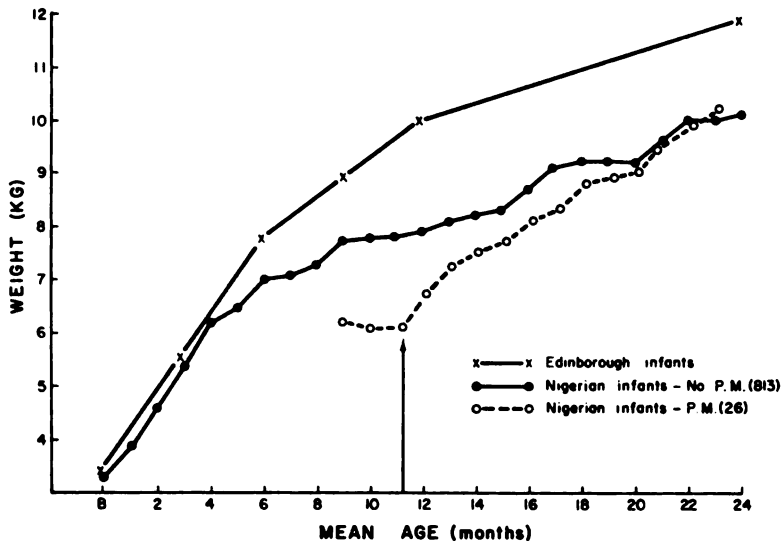

Figure 1—Mean weight of Nigerian Children suffering from protein malnutrition, whose diets were supplemented with a 3:1 mixture of groundnut flour and dried skim milk, compared with weights of Edinburgh children and of Nigerian children not suffering from protein malnutrition.

Edinburgh children until the age of 4 to 6 months, but thereafter the monthly gain in weight was less. The mean weights of Nigerian children originally suffering from protein malnutrition, whose diets were supplemented with ARLAC, were less than those of other Nigerian infants when first seen, but their monthly weight gain was greater. Their average weight was catching up with that of the Edinburgh children in the second year of life, whereas the mean weight of the Nigerian babies consuming an unsupplemented diet ran parallel to, but about 2 kg less, than that of the Scottish infants in this age group.

There was no significant difference in the response to treatment, judged by weight gain, between cases originally diagnosed as kwashiorkor and the other group diagnosed as marasmus. There was a significant difference ($P < 0.05$) between the age incidence of these conditions, the marasmic infants presenting an average age of 10.4 months (SD 2.6) and those suffering from kwashiorkor averaging 13.4 months (SD 3.9).

The monthly weight gain of children originally diagnosed as suffering from protein malnutrition, whose diets were supplemented with ARLAC, was as great in the second year of life as the monthly weight gain of Edinburgh infants aged 8 to 11 months. The mean weight gain of 72 Nigerian infants, divided into 6-month age groups from birth to 35 months, who were initially suffering from protein malnutrition and were followed satisfactorily for 3 months while their diets were supplemented with ARLAC, was at all ages either equal to or greater than that of Edinburgh children.

The mean haemoglobin concentration of the children receiving ARLAC increased from approximately 8.25 gm to 9.0-9.5 gm/100 ml during the first 3

months, but thereafter did not rise higher. Possibly the frequent attacks of malaria, dysentery and other illnesses from which they suffered, and the rate of growth of tissues other than blood taking place at the same time, prevented any further increase in haemoglobin concentration.

A small series of 10 comparable infants aged 5 months to 1 year was treated with supplements of Nigerian DSM at a level of 4 gm protein/kg for 4 to 8 weeks, this quantity of protein being the calculated safe practical allowance for children aged 0-6 months. Their rate of weight gain over this period was the same as that of the children whose diets were supplemented with ARLAC at 6 gm protein/kg of which protein 4.8 gm was derived from GNF and only 1.2 gm from DSM.

The factors considered (by B.M.N.) to be the major cause of the development of frank protein malnutrition in these children follow. Failure of satisfactory lactation from one cause or another, or poor feeding of the infant, were the prime factors in 58% of cases. Infections such as malaria, exanthemata and dysentery were held responsible for 39%. Kwashiorkor apparently was precipitated by breast failure in 34% of cases of this disease, whereas only 23% of cases of marasmus were so caused. Acute infections, including dysentery, were considered to be the precipitating cause of marasmus in 48% of such cases, whereas only 30% of cases of kwashiorkor were held to result from those illnesses. The important point is that both clinical conditions responded equally well to medical care and supplementation of the customary diets of these children with a mixture of groundnut flour and dried skimmed milk. In Nigeria the price at the factory of ARLAC is approximately 13 cents per pound, whereas that of DSM produced in Nigeria, and limited by the availability of fresh milk to about 150 tons per annum, is 28 cents per pound.

OTHER ASPECTS OF HUMAN PROTEIN REQUIREMENTS STUDIED

Our grant was intended for the study of the relative values of GNF and DSM as a supplement to the characteristic diets of Nigerian adults, growing children and infants. At the same time the opportunity was taken to study the effect on nitrogen balance of supplementing the basal diet with synthetic amino acids and nonprotein nitrogen. Also, diets of the same caloric value (42-44 cal/kg) and nitrogen content (4 gm) as the basal diet but having either a low (57) or a high (79) FAO protein score were prepared.

Supplementing the Basal Diet with Methionine, Tryptophan and Urea

A group of 6 Nigerian men ate the basal diet for a prebalance period of 7 days, a balance period of 6 days, and then the same diet supplemented first with methionine, tryptophan and urea, for consecutive balance periods of 4 days each. A period of 4 days on the basal diet was inserted between the last two of these balance periods. The object of this trial was to raise the FAO protein score of the basal diet from 70 to about 100, and to measure any increased nitrogen retention which might result, and also to determine if the addition of nonprotein nitrogen to the basal diet supplemented with methionine and tryptophan increased nitrogen retention and protein synthesis.

The results showed that an addition to the basal diet of 318 mg of DL-methionine, either alone or in combination with 75 mg of L-tryptophan, increased

nitrogen retention from the basal diet by only 0.14 gm, although the score of the diet had been raised from 70 to 97. The mean nitrogen balance, recorded over a 4-day period, returned to equilibrium during the second balance period on the basal diet. The addition of nonprotein nitrogen in the form of urea resulted in a positive nitrogen balance of 0.57 gm. The addition of tryptophan to the basal diet supplemented with methionine, in an amount which increased the protein score from 80 to 97 (isoleucine then becoming the limiting amino acid), did not have any effect on nitrogen retention.

The same subjects on the low-protein-score (57) diet, composed of *Cassava gari*, GNF and a vegetable soup, were in negative nitrogen balance of 0.30 gm, whereas on the same diet supplemented with 500 mg DL-methionine (score 77) they were in positive balance of 0.28 gm. They were in nitrogen equilibrium on the high-protein-score (79) diet, composed of *Cassava gari*, dried fish and vegetable soup.

The data presented above led us to think that it is possible the FAO Committee on Protein Requirements has set the level of S-amino acids and tryptophan too high in the provisional pattern by about 25%. This idea was supported by the fact that all our groups of subjects were in nitrogen equilibrium while eating the basal diet, which provided only 70% of the FAO minimal requirements for S-amino acids and 75% of the same requirement for tryptophan. Further, Rose's minimal requirement for tryptophan and the amount supplied by our egg-supplemented diet, calculated at the level of nitrogen equilibrium, were only 75% of the FAO minimal requirements.

It appeared likely that lack of nonprotein nitrogen, at the levels of nitrogen supplied by the basal diet, with the amounts of methionine and tryptophan added to raise the score to around 100, was possibly a factor which affects the synthesis of protein. However, the fact that the addition of 500 mg DL-methionine to the low-protein-score diet resulted in an increased nitrogen retention of 0.58 gm per day and positive balance of 0.28 gm nitrogen, made the effects of the urea supplement appear less important.

These data suggest to us that raising the score of a diet resulting in nitrogen equilibrium at a level of approximately 4 gm nitrogen intake when eaten by adult men, from around 75 to 100 by the addition of the necessary amounts of methionine and tryptophan, will make little difference to nitrogen retention in such human subjects. On the other hand, raising the score of a diet from the low level of 57 to 77 by the addition of the limiting amino acid produces a significant increase in nitrogen retention. It appears probable that the addition of nonprotein nitrogen to the same basal diet causes a small but significant increase in the retention of nitrogen.

Supplementation of Basal Rice Diet with Leucine

It was shown in earlier studies that a sorghum basal diet at the 4 gm level of nitrogen intake had an FAO protein score of 73, as compared with a score of 70 for the rice basal diet. On the sorghum diet adult Nigerian men were found to be in negative nitrogen balance (—.3 gm) but on the rice diet the same subjects were in nitrogen equilibrium. This was attributed either to an excess of leucine in the sorghum, or to the poor digestibility of the crude fibre in the sorghum. The trial

described here was undertaken to confirm the fact that the sorghum diet fed at the basal level of approximately 4 gm nitrogen was inferior to the rice diet fed at the same level of nitrogen and to ascertain whether (a) an excess of leucine or (b) a low digestibility of sorghum crude fibre was responsible for this difference in nitrogen balance.

Four adult male Nigerian subjects ate the rice basal diet for a period of 2 weeks before the start of the trial. They continued to eat the rice basal diet for a prebalance period of 6 days, then a balance period of 6 days, followed by a further balance period of 6 days during which exactly 1 gm of L-leucine (0.33 gm leucine per meal in aqueous solution) was added to the diet. The amino acid patterns of the sorghum and rice diets are very similar apart from the high amount of leucine in the sorghum diet. The addition of 1 gm of L-leucine to the rice basal diet raised the leucine:isoleucine ratio from 1.5 to 2.4, which is slightly higher than the ratio 2.3 in the sorghum basal diet.

The addition of leucine to the rice basal diet did not result in any significant loss of nitrogen in terms of nitrogen balance. It showed that the BV of the diet was not altered through the addition of L-leucine (BV, 76 in both trials) but there was a small difference in digestibility which reduced NPU. In the rice basal diet BV and NPU were 95 and 72 respectively, and in the rice basal diet supplemented with 1 gm leucine 90 and 68 respectively. The addition of leucine increased both dry faecal weight and faecal nitrogen, the former rising from a mean value of 22.3 gm/day to 25.5 gm/day, and the latter from 1.32 gm/day to 1.50 gm/day. Thus it would appear that the addition of 1 gm leucine to the rice basal diet resulted in impaired protein utilization, but the differences are not statistically significant ($P > 0.05$).

In previous trials on the sorghum diet fed at the basal level of 4 gm nitrogen in which the leucine and isoleucine intakes were approximately the same as those supplied by the basal rice diet supplemented with 1 gm leucine, it was found that the subjects were in a negative nitrogen balance of 0.3 gm/day. The BV of all these diets was approximately the same, namely 76% to 78%.

It appears that the daily addition of 1 gm leucine to the rice diet fed at the basal level of 4 gm nitrogen, which raised the leucine: isoleucine ratio from 1.5 to 2.4, approximately that of the sorghum diet fed at the same level of nitrogen intake, had no significant effect on protein utilization. It is concluded, therefore, that in the sorghum basal trials previously reported, protein digestibility and NPU were lower than those of the rice basal trials due largely to the poor digestibility of the crude fibre fraction.

Changes in the leucine:isoleucine ratio of nearly 100%, at levels of protein intake which result in nitrogen equilibrium, have little effect on nitrogen balance and NPU.

EFFECTS OF FEEDING A HIGH-PROTEIN DIET TO NIGERIAN MEN OVER A PERIOD OF FOUR WEEKS

The Nigerian Nutrition Unit has been interested in the work, directed by Holmes in East Africa, which suggests that African men will retain considerable amounts of nitrogen for several months without translating this apparent gain of

tissue into any orderly gain in weight. A trial has been carried out recently during which 5 Nigerian men aged 25 to 40 years of age, between 50 and 60 kg body weight, consumed the basal diet supplemented by 400 gm of lean beef per day for 28 days. This diet provided 16.8 gm nitrogen, approximately 104 gm protein of which 75% was derived from beef, 42-44 cal/kg and scored 81. Nitrogen balance, total plasma protein, albumin:globulin ratio and haemoglobin levels were determined. The subjects ate the basal diet for 7 days prior to the 28-day period on the beef diet, and nitrogen balances were carried out during days 1 to 4 and days 25 to 28. The subjects were weighed daily, after urinating, before consuming the morning meal. The mean nitrogen balance of the 5 subjects taking part in this trial had been determined earlier to be equilibrium when they ate the basal diet.

The subjects were in positive nitrogen balance of 4.7 gm at the beginning of the month, and in positive nitrogen balance of 4.4 gm at the end of the month. NPU did not vary significantly between the first and second balance periods. The addition of beef to the diet did not make any difference to the plasma cholesterol concentration, haemoglobin rose by 0.8 gm, and total plasma protein also increased a little. The A:G ratio surprisingly fell from a mean of nearly 1 to 0.74, caused by a rise of globulin and slight fall in albumin. The mean weight of the 5 subjects taking part in this trial increased by 1.9 kg during the 28 days. The nitrogen retained averaged 4.5 gm per day, equivalent to approximately 135 gm tissue per day, or 3.8 kg over the 28-day period, and exactly twice the recorded weight gain.

Under these conditions of high protein intake it is obvious that some nitrogen loss was not recorded. Whether this loss was in sweat and exfoliation of skin, in secretions and excretions other than urine and faeces, such as saliva, seminal ejaculations or flatus, cannot be determined. The subjects had been warned about spitting and were mainly sedentary throughout the trial, never showing visible perspiration.

ACKNOWLEDGEMENTS

This report has been written in the absence of P. G. Phillips on leave in the United Kingdom. I hope that he will agree with my conclusions; I take full responsibility for them. Until recently I had the benefit of his critical opinion. He and Mr. Oshinyemi are always meticulous in performing the biochemical work carried out by the Unit.

We acknowledge the sterling qualities of the three nursing sisters, Mrs. J. M. Smith, Mrs. W. Moore and Mrs. D. M. Pearce, who followed up our children with such energy. Without the help of Mallam Abubakar Zukogi Dida, doubly qualified as nurse and laboratory technician, their task would have been much more difficult.

We are very grateful to Mr. Samuel Aigbokhaevbo and his assistant for their work at the typewriters and duplicating machines.

To the driver-mechanic and drivers, who got us without fail to the right place at the right time, often in spite of floods, bush fires and broken bridges, and to the attendants and messengers who helped in innumerable ways, we owe our very sincere thanks.

The Staff of the West African Institute for Trypanosomiasis Research, where the Unit is housed, always have been of the greatest help. We would like to thank particularly Dr. H. J. C. Watson, Major J. G. H. Brotherton, Mr. W. Yates and Mr. W. Petana for their many kindnesses and technical assistance.

DISCUSSION[1]

DR. ALTSCHUL: I would like to offer a possible explanation for some of the interesting results that Dr. DeMaeyer got. There is, I think, a problem of nomenclature here of which we are the innocent victims. Dr. DeMaeyer spoke about two samples of soy products. One was a solvent extracted or an extracted soy flour from England, and the other was toasted soy protein. I suspect that they are the same type of product, that they are both flours containing about 50% protein, and differing in the amount of toasting, which would remove the inhibitor. I think that this might account for the difference, because there has been the habit in some of the literature on American products to call some of these products toasted protein when indeed they are not protein but a concentrate. This is point no. 1.

Point no. 2 is that I believe the discrepancy between the PS and BV of cottonseed flour would be less if one were to use a lysine value that more truly reflected the lysine content of the processed product.

No. 3, I wonder if it is not possible that some of the reduction in the BV of the soy and cottonseed products as you increase the concentration is not owing to incomplete removal of some of the interfering substances which, as you increase the concentration, increase themselves in concentration and affect the BV more.

DR. DeMAEYER: The protein content of the American product was 50%. It was 43% as far as the British product was concerned.

As far as the second point is concerned, I think the protein score of 73 for the cottonseed flour is overestimated, and it should be reduced according to the last availability rating.

As far as the third point is concerned, I may say that we have tested eggs at different levels, and there again we have found a decrease in the BV when the level of nitrogen intake was increased above a certain value. Therefore

[1] Editor's Note—This discussion covers the two preceding papers.

in the case of soy or cottonseed flour, I wonder if the decrease may be caused by a high nitrogen intake or by some other factors interfering as Dr. Altschul suggested.

DR. GYÖRGY: One comment on nomenclature.

Unfortunately, Dr. DeMaeyer tested a Saridele toffaroma which is not used and not prepared for infants. It is a commercial product used in Indonesia with great commercial success for adults. It is an 80% protein, and infant food is 29.5%.

Second, I am unfortunately in disagreement with Dr. DeMaeyer about soy products, not verbal disagreement but factual disagreement. I have used Saridele for over a year on rats and on a large number of premature infants, and I found it far inferior to milk. I cannot even figure out the protein efficiency ratio. There is a 250 or 300% difference. In the food efficiency ratio in infants there is about 200% difference. I have to admit that in rats I used the Saridele not preheated, but for premature infants I had to sterilize it.

I would like to ask you whether you used for infants the Saridele toffaroma which is basically a dilute Saridele, sweetened, and with chocolate or cocoa or coffee added to it because people like it—whether you heated it, boiled it, autoclaved it or not. Obviously, in infants we have to autoclave it. I have used this product on about 50 premature infants, and unfortunately—I am to some extent responsible for this product, not for the preparation of it but for the release of it, so to speak—I would not like to have your 80% value and BV practically identical with skim milk to leave this room without further discussion.

I have one more question to Dr. Nicol.

Dr. Nichol, in your last group of studies where you used 1:3 ratio of milk and peanuts, did you have a control with milk at all?

DR. NICOL: Only the 10 patients reported.

DR. DeMAEYER: We used Saridele toffaroma because it was available. We did not use it in infants but in children between 3 and 6 years old. It was prepared by diluting it with water at a temperature of 45°C. It was not autoclaved.

I may be overconfident, but I would say that we were very careful when we experimented with Saridele. It was a planned experiment. Each child received first the reference cow's milk, then Saridele, then reference cow's milk, and then Saridele. I may say that I was quite astonished by the results, which I did not expect myself.

DR. ALTSCHUL: I must insist on the point that the fact that two products come from the same seed, soybean, has absoultely no meaning about their identity. The fact that you got one set of results and he got another set of results does not necessarily imply any contradiction at all, because it is entirely possible that he had a good product and you had a product that was not the same.

DR. GYÖRGY: I had three different batches of Saridele. Fortunately, the best Saridele, which had a BV and PER in rats just as good as milk, has been used by me in infants. That was a fortunate coincidence. The other two were in rats, with very poor BV. As I stated before, we have to autoclave it, to heat it, and that could make the difference.

In Indonesia, although they boil it, they had to discontinue the use of various batches of Saridele because they got a lot of diarrhea, because the hygienic conditions in Indonesia, even with autoclaving Saridele, are not as good as in Philadelphia.

Prevention of Kwashiorkor in Nigeria

W. R. F. Collis

NIGERIA CONTAINS ONE-FOURTH of the population in Africa south of the Sahara, 35 rising to 40 million.

Ibadan is the largest African town in the same area, having over 500,000 persons living in the old town. Of these, 50% are under 18 years, and 50% of these die before they reach 18. Hence the extreme urgency of the pediatric problem facing the new department of pediatrics at University College Hospital, Ibadan, can be realised.

On taking over this department some 3½ years ago, I became aware that some 75% of the children coming to the hospital with acute conditions were suffering from diseases which should not occur and were preventable. It was also clear that underlying every other condition, from tuberculosis to malaria, was malnutrition. Hence we immediately set up a nutrition clinic.

At the International Conference following this meeting, Dr. Edozian and I are reading papers summing up our findings on 450 cases of kwashiorkor attending this clinic during the last 2½ years. I would like to mention one or two of the findings here. First, 80% of the families from whom the cases came were not poor; in some 20% the cause might be attributed to family troubles, but in only 5% could we say the cause was actual destitution. Hence, if better protein than the people are giving their children can be made available and the mothers taught to give it to their children, the condition is largely preventable.

On the biochemical side our findings confirm those of others, that the blood albumin level is the best single test for protein malnutrition. Though it is not a perfect one, it gives an index of the degree of pre-kwashiorkor protein malnutrition in any given population.

When the results of the above investigation became known to us and we realized the immense amount of malnutrition that it meant among the population of the Western Region of Nigeria, the urgency of attempting to remedy the situation was obvious.

With the help of the Rockefeller Foundation, the following plan has been worked out.

An area of some 10 miles surrounding and including the town of Ilesha, some 60 miles from Ibadan, has been selected for an intensive study divided into 3 phases.

Phase I. This consists of making a demographic map of the area: determining the population and its economic, agricultural, dietetic and medical conditions.

This is being carried out by a team led by a doctor (A. Omolulu) and a fully qualified agricultural nutritionist (I. Dema). Four villages have been selected around the town of Ilesha composed of people of different occupations: cocoa growers, subsistance farmers, gold miners, rice-growing hill villagers; and samples consisting of 300 individuals, taken in family groups, are being obtained from each village and the town of Ilesha.

(All the medical examinations and investigations of the agricultural economy and living conditions are being carried out by the doctors themselves. This will limit the numbers but greatly increase the accuracy of the data obtained.)

When the demographic map has been drawn in this way, we will flag upon it all the cases (some 200 a year) of kwashiorkor coming to the hospital at Ilesha, where Dr. Morley of the West African Medical Research Council is working.

In this way we will be able to know, from every point of view, why these cases got kwashiorkor and, even more important, why other children from the same areas living under the same conditions did not.

Phase II. Having obtained the above information, our next phase will be to teach the mothers how better to feed their children so as to prevent protein malnutrition, and the farmers to grow more and better protein food.

Phase III. This phase will consist in attempting to find out 1) how well we have succeeded in reducing the amount of protein malnutrition, 2) what the mothers have actually learned and 3) how far the farmers have understood the propaganda given them.

We have just completed a pilot scheme, the examination of a small village some 6 miles from Ilesha along the lines described for phase I of the whole scheme. The results obtained so far already pose several problems which deserve consideration.

TABLE 1
PILOT VILLAGE, IJANA, ILESHA

		Ascaris	Malaria	Hookworm	Filaria	Spleen	Heaf
No. of Inhabitants	67						
Persons Dependent	50.8						
Age							
0— 2 yrs.	5	3	2	0	0	2	0
3— 5 yrs.	10	7	1	0	0	2	0
6—14 yrs.	16	15	6	8	1	9	7
15—25 yrs.	4	3	1	1	0	—	2
26—45 yrs.	19	18	4	6	2	—	9
45 yrs.	10	10	2	7	6	—	7

Here some of the results of the medical examination of 67 inhabitants representing a cross section of the village are tabulated. These results have been obtained by single samples only. Hence the tremendous infestation of the people is the first point which becomes obvious; ascaris is almost universal, malaria prevalent at all ages, and hook worm and tuberculosis (positive tuberculin reaction) common in the over-6-year age periods. Filaria was known to be prevalent in the area, and 9 cases were found in this series. If further studies were made, a higher

frequency would probably be found. So far I have not received the values for the blood protein levels or a calculated infant mortality, as both these items can only be obtained after we have won the confidence of the villagers. The hemoglobin levels, though not shown here, average above 70%, a surprising finding considering the amount of parasitic infestation, and one showing that the population has learned to "live with its parasites" to a certain extent.

From the point of view of the sociologist, the health picture of these villagers is obviously a very poor one and presents great problems to the investigator primarily interested in obtaining information on their true nutrition and then improving it.

Table 2 gives the population in age and sex grouping of the whole village, its fertility ratio, and consumer/producer ratio. It will be observed that there are a large number of dependents and very few adolescents. The latter is due to the tendency of the young people to drift away from the land and find employment in the towns. This will mean that the number of farmers, and hence the food production, will fall in the next generation unless steps are taken to overcome this trend or/and increase production per acre.

TABLE 2
IJANA-ITARUA HUMAN POPULATION: AGE AND SEX GROUPING, APRIL, 1960

Group	Age (Years)	Males	Females	Totals	% Totals
Suckling Infants	0—2	9	3	12	11
Toddlers	3—5	9	4	13	12
Elementary School Children	6—14	15	9	24	21
Adolescents	15—21	5	4	9	8
Adults	22—60	21	28	49	43
Old People	60+	2	4	6	5
Total		61	52	113	100

Note: (1) FERTILITY RATIO: $\frac{\text{No. of children under 5 years}}{\text{No. of women aged 16-50 years}} = 78\%$

(2) CONSUMER/PRODUCER RATIO $= \frac{\text{No. of dependents}}{\text{No. of active farm workers aged 15-60 years}} = \frac{113}{58\text{-}15 \text{ in Ilesha Town}}$ i.e., 264%

The small number of adolescents is due largely to mass movement of this age group from the village into Ilesha Town to learn non-agricultural trades and thereby decreasing the numbers of future food producers in a rapidly multipying population.

Table 3 shows an assessment of protein and caloric intakes per head per day in 8 families. These are found to vary considerably, some, as in household 5, being dangerously low and others, as in household 4, reasonably good. The backgrounds of these families are most interesting. In family 5 (1180 calories and protein 13.7 gm) the man appears to sit under a tree all day doing nothing, while his wife does the work. In case 4 (2265 calories, protein 40.7 gm) the man is an excellent farmer. He and his 2 wives cultivate a mixed farm with success. They are well off and well fed. They have had 10 children but *only one has survived.*

TABLE 3
IJANA-ITARUA DIETS
Assessment of protein and calorie intakes per head/day

Total Calories	Protein (Nx6.25) gm	Protein Score (%)	'Reference' Protein Equivalent (gm)	'Ref.' Protein Calories % Total Calories
1658	43.3	51	22.1	5.4
2545	32.9	31	10.3	1.6
1611	28.1	49	13.8	3.5
2265	40.7	47	19.1	3.4
1180	13.7	36	4.9	1.7
2134	38.5	41	15.8	3.0
1033	34.9	57	19.9	7.7
1058	19.4	42	8.0	3.0

Note: The 'reference' protein is one which theoretically should contain 16% nitrogen and be completely utilized.

Table 4 shows the percentage of farm land per crop.

Table 5 shows the areas under farm crops expressed as acres/consumer.

Table 6 shows numbers of livestock.

Table 7 shows sources of dietary protein in diets.

Table 8 shows amino acid content worked out for the same 8 families as in table 3.

This is the general procedure we intend to adopt for the larger survey. I think, if these data are taken together, they should give us a quite complete picture of the health, nutrition and background of the people living in this area.

Clearly, it is unwise to draw any conclusions from such small numbers as are presented in the pilot village scheme, but some of the facts already seen here do begin to pose certain questions in my mind.

TABLE 4
IJANA-ITARUA: 1960 CROPPING
Percentage of farm-land occupied by each crop

Farm No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Total area (acres)	11.20	5.39	6.66	1.90	9.90	9.90	31.10	12.20	6.43	8.12	1.72
Crop area (%)											
Cocoa	85.5	37.7	51.2	67.2	83.3	50.5	77.8	58.0	48.4	68.0	55.9
Cola	—	—	—	—	—	—	0.3	—	9.3	12.6	—
Tobacco	—	—	—	—	—	—	—	1.1	—	—	—
Yams	5.7	9.0	23.4	9.1	4.2	18.8	6.0	15.1	8.6	5.2	13.4
Cocoyams	3.8	4.4	—	9.6	0.5	—	2.1	3.2	2.3	1.5	2.3
Plantains	0.6	5.6	7.4	2.7	—	4.5	—	1.1	3.2	4.5	—
Cassava	—	—	—	—	6.0	—	6.0	2.1	—	—	—
Maize	1.9	12.2	9.5	8.7	6.0	25.2	6.4	10.8	25.0	4.5	15.1
Rice	—	—	—	—	—	—	—	—	—	—	—
Chillies	1.9	26.7	8.0	—	—	—	0.7	3.2	3.2	3.0	11.0
Okra	0.6	1.1	—	—	—	1.0	0.7	1.1	—	0.7	—
Melons	—	3.3	—	2.7	—	—	—	4.3	—	—	2.3
Beans	—	—	—	—	—	—	—	—	—	—	—
Total	100	100	100	100	100	100	100	100	100	100	100

TABLE 5
IJANA-ITARUA: 1960 CROPPING
Areas under farm crops expressed as acres/consumer

Farm No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
No. of Consumers	7	6	6	5	10	5	11	14	5	6	6
Crop area/consumer											
Cocoa	1.34	0.34	0.57	1.48	0.82	1.00	2.20	0.54	0.62	0.92	0.16
Cola	—	—	—	—	—	—	0.01	—	0.12	0.17	—
Tobacco	—	—	—	—	—	—	—	0.01	—	—	—
Yams	0.09	0.08	0.26	0.20	0.04	0.37	0.17	0.14	0.11	0.07	0.04
Cocoyams	0.02	0.04	—	0.21	0.01	—	0.06	0.03	0.03	0.02	0.01
Plantains	0.01	0.05	0.08	0.06	—	0.09	—	0.01	0.04	0.06	—
Cassava	—	—	—	—	0.06	—	0.17	0.02	—	—	—
Maize	0.03	0.11	0.11	0.19	0.06	0.50	0.18	0.10	0.32	0.06	0.04
Rice	—	—	—	—	—	—	—	—	—	—	—
Chillies	0.03	0.24	0.09	—	—	—	0.02	0.03	0.04	0.04	0.03
Okra	0.01	0.01	—	—	—	0.02	0.02	0.01	—	0.01	—
Melons	—	0.03	—	0.06	—	—	—	0.04	—	—	0.01
Beans	—	—	—	—	—	—	—	—	—	—	—
Total/consumer	1.53	0.90	1.11	2.20	0.99	1.98	2.83	0.93	1.28	1.35	0.29

TABLE 6
IJANA-ITARUA
FOOD RESOURCES: LIVESTOCK NUMBERS

Kind	NUMBERS	
	Village Totals	Animal Heads Per 100 persons
Cattle	nil	nil
Goats	43	38
Sheep	16	14
Chicken	78	69
Ducks	1	1
Pigs	nil	nil
Grand Total	138	122

With the amount of infestation with different kinds of parasites seen to be present in this area, will nutritional measures improve the condition of the people by themselves, or will it be necessary first to deal with the parasites? Or would it be better to try to deal with the parasites rather than to try to improve the nutrition?

If we do both at once, how are we to tell which measures have produced the results?

I would like to hear your suggestions on these questions, and to ask if you feel that the blood albumin is the only satisfactory index for demonstrating the level of protein nutrition in a community, for venepuncture as a routine adds greatly to the difficulty of obtaining the cooperation of the villagers.

TABLE 7
IJANA-ITARUA DIETS
Sources of dietary protein: amounts expressed as % protein intake

Foodstuffs	Households							
	Aduroja	Idown	Ige	Oyewole	Ikotun (Ogunldde)	Ogunleye	Oni	Ilori
Starchy roots and fruits								
Yams	13.6	26.3	17.4	5.0	41.0	23.5	7.8	14.0
Cocoyams	11.6	22.0	7.4	—	12.8	18.3	—	14.4
Plantain	—	—	0.2	8.0	0.5	2.3	0.2	0.7
Cassava	1.2	3.6	3.8	6.5	0.9	3.1	7.0	10.1
Cereals								
Maize	0.1	0.3	0.5	4.4	5.0	0.2	2.5	0.2
Rice	—	—	0.2	0.2	—	4.2	0.7	0.7
Wheat (bread)	0.4	2.6	—	—	—	1.3	—	—
Legumes								
Cowpeas	6.7	16.4	6.5	32.5	19.1	6.5	17.8	12.4
Locust Beans	1.7	6.0	2.9	3.4	6.4	2.1	1.4	2.5
Vegetables and Fruits								
Green Leaves	0.4	0.5	1.6	1.2	3.7	0.5	0.6	0.2
Okra	0.3	—	0.2	0.5	—	0.2	—	0.2
Melon Seeds	3.6	3.6	4.2	11.2	7.3	2.0	—	—
Chillies	0.6	0.6	0.5	1.8	0.9	0.2	0.4	0.2
Onions	0.7	0.5	0.2	0.3	0.5	0.2	0.4	0.2
Cola	0.1	0.3	0.5	—	0.5	0.2	—	—
Mushrooms	0.7	—	0.2	0.6	1.4	0.2	—	0.2
Tomato	—	—	—	0.2	—	—	—	—
Animals								
Beef and Bone Scraps	43.4	2.9	53.5	24.2	—	26.2	61.2	39.0
Chicken	13.8	14.4	—	—	—	—	—	—
Fish	—	—	0.2	—	—	5.2	—	—
Snails	1.3	—	—	—	—	4.6	—	4.0
Total (gm Protein per head/day)	43.3	32.9	28.1	40.7	13.7	38.5	34.9	19.4

TABLE 8
IJANA-ITARUA DIETS
Amino acid content of certain Ijana household diets (April, 1960) expressed as a ratio of the F.A.O. (1957) recommended allowance

Iso-leucine	Leucine	Lysine	Methionine & Cystine	Ph-alanine & Tyrosine	Threo-nine	Trypto-phan	Valine	Protein Score
270*	306*	270*	360*	270*	180*	90*	270*	(%)
1.19	1.66	1.60	0.51	1.53	1.46	0.90	1.22	51
0.93	1.34	1.13	0.31	1.09	1.09	0.80	0.93	31
1.18	1.64	1.66	0.49	1.50	1.43	0.93	1.21	49
1.12	1.51	1.41	0.47	1.60	1.27	0.80	1.20	47
1.13	1.71	1.24	0.36	1.52	1.26	1.03	1.22	36
1.16	1.64	1.52	0.41	1.33	1.40	1.00	1.13	41
1.15	1.61	1.73	0.57	1.68	1.40	0.82	1.18	57
1.13	1.58	1.60	0.42	1.45	1.40	0.92	1.22	42

The protein score measures the extent to which the diet supplies the limiting amino acid as compared with the provisional pattern.

* FAO pattern, mg

DISCUSSION

DR. FRENK: This kind of study I believe is very badly needed. You expressed some of the intakes of these families on a per capita basis, but what was actually the familial distribution of these foods? We assume that this village was the same as in other parts of the world, where the father gets the best parts, then the mother, then the oldest child, and so on. Second, in this village you did not find any case of actual kwashiorkor; but what did the other nine children of this man die from, the one who had only one living? Why only rely on albumin levels for the assessment of the nutritional status of the children? How about growth rates, the actual height of the child, not the weight, because he would have too much water.

DR. COLLIS: When you have so much sickness, when you have so much tuberculosis, I must feel that you cannot use the weight as an index for nutrition or, rather, you cannot say that malnutrition is the cause of the lack of weight if they have had, as a good many of them have, this much infestation or infection. That is why I was asking about the matter of the albumin. Of course, we are taking the height and the weight in all cases. That is being done at the same time.

You asked about the distribution in the family. I do not have the figures here. I would like to know exactly the amount of food that each person in the family gets. We shall try to get that. We won't be able to get it in all the cases, quite obviously, because you would have to go into the house and weigh it. When you go into the house and weigh it, they always eat quite differently than when you don't. That is one of the difficulties.

DR. GOPALAN: You spoke of a village where the protein intakes appear to be pretty low but where there was no kwashiorkor. I would like to know what the incidence of infestation and infection was in this village. In our own surveys the thing which interests us is the fact that there is so little protein malnutrition when the diets are so inadequate in protein. We find that probably some of these discrepancies may be explained on the basis of differences in the incidence of infestation of different regions. We find, for example, that the incidence of kwashiorkor is very much greater in certain regions and in certain seasons of the year than in others—during summer, for example, when there is a high incidence of gastrointestinal infection. If, in the village which you talked about, the incidence of parastic infestation was less than in the other villages, this absence of kwashiorkor could be explained.

Professor Platt suggested some years ago that a greater contribution to the eradication of protein malnutrition may be to control infections by malaria, to improve the environmental sanitation, rather than only to correct the protein intake.

DR. SCRIMSHAW: Dr. Gopalan has brought out the role that infection will play in bringing out cases of kwashiorkor. I think at the same time there is still

some tendency to overestimate the effects that the intestinal parasites play. We have kwashiorkor in the presence of severe intestinal parasite infection, and also with relatively little. We have it with great variation in the pattern of intestinal parasites. The absorption and retention of food are not nearly so much altered by intestinal parasites as one would think from the severity.

On the other hand, systemic infections and the systemic part of gastroenteric infections do have a profound effect on protein metabolism. There is a very close correlation between the seasonal fluctuations and enteric disease and the subsequent appearance of kwashiorkor.

On the question as to whether you should attack the parasites and the infections or the nutrition, we are coming to believe that we are dealing with an interrelation between nutrition and infection where, if the child were well enough nourished, there would not be a high mortality as there is from infection, including gastroenteritis. On the other hand, if there were a relatively low prevalence of infection, including gastroenteritis, most of the cases of kwashiorkor might never develop.

We are trying to test this, by taking one village in Guatemala and doing everything we can to improve the nutrition of the preschool child, measuring the effect on morbidity from infection; and in another village doing everything we can to reduce the infection to determine the effect of nutritional status; and in a third village simply keeping track of the severity, duration of illnesses, and so on. It will be another year before we shall be able to answer that. We have been disappointed in our ability to change the situation by trying to control the infection, because we have not been controlling it very well despite all the accepted measures. We have been surprised at the reduction in morbidity in the feeding village.

I don't think one should talk about one or the other, but talk about both together and not give so much attention to intestinal parasites.

DR. COLLIS: We will have to send some pediatricians to Guatemala. I agree entirely with what you say. I think the parasites perhaps prepare the ground, and in our case the things that bring out kwashiorkor more than anything else are measles and high fever, including, of course, the enteric fevers and malaria.

The Ecology of Protein-Calorie Malnutrition of Early Childhood in Three Dissimilar East African Groups

Derrick B. Jelliffe

PROTEIN-CALORIE MALNUTRITION of early childhood is most realistically regarded as the result of ecological imbalance rather than exclusively as a dietary disorder. Responsible ecologic factors will vary from one part of the world to another. However, as a generalization, it is rare for this group of conditions to be due to primary dietary causes alone. They are usually associated with various medical conditioning factors, including intestinal helminthic infections, tuberculosis, diarrhea, respiratory infections (including whooping cough) and probably hyperendemic malignant tertian malaria, as well as various social processes as, for example, the psychological stress of abrupt "weaning", failure of lactation due to mothers working in urban regions and the breakdown of traditional family-spacing techniques.

BASIC PLAN

The basic plan has been to carry out community-based surveys of child health in various contrasting groups in East Africa, with special reference to the prevalence of protein-calorie malnutrition of early childhood, to medical (and, if possible, social) conditioning factors and to qualitative data as to the pattern of infant feeding, especially the ability of mothers to breast-feed their infants.

PURPOSES

These field studies have four purposes.

1. *Public health.* Results can be used to demonstrate the geographic areas where protein-calorie malnutrition is most common, so that the limited staff and budget found in tropical regions can be most advantageously deployed in any ameliorative public health measures that may be planned.

At the same time, by knowing the ecological sequence in various areas, it is possible to define, and so attempt to deal with, the particular ecologic factors operative in the region concerned.

It may be noted that results obtained by field surveys must always be supplemented by information obtained from hospital or dispensary statistics and morbidity figures.

2. *Field assessment.* Surveys of this type provide a method of continuously testing present ideas on suitable "nutritional indicators" for the assessment of protein-calorie malnutrition under field circumstances. Only by this type of "feedback" process can these concepts be continued with, refined or discarded.

3. *Scientific and cross-cultural assessment.* From a general scientific viewpoint, studies under these circumstances can be regarded as assessments of the "experiments in nature" that comprise the adaptation of human communities to their environment. The success or failure of methods of infant feeding, and of adjustments to locally prevalent diseases, can be of great value in a cross-cultural comparative sense. This type of information can be of use in planning methods of infant feeding in other tropical groups and, in addition, may help to clarify, highlight or disprove ideas currently in vogue in the Western world.

4. *Education.* Field studies are carried out by staff otherwise principally engaged in clinical work in the hospital and, should financing permit, by medical students. They have, therefore, an educational value for all concerned in showing nutritional problems of African childhood in their actual ecological setting and in demonstrating the need for preventative measures applied at source.

Techniques

Total child populations, from birth to 3 years of age, are examined in "circumscribed population units" which are judged to be representative and, as far as possible, have been randomly selected.

This age range is used because protein-calorie malnutrition has its main emphasis in this period: kwashiorkor in 1- to 3-year-olds and nutritional marasmus in infants less than 1 year of age.

Problems of logistics and administrative organization with, for example, local chiefs are often considerable because of misunderstanding of motives by rural Africans, distances, communications and weather, and the customary absence of villages in East Africa, necessitating either prolonged, time-consuming home visits or the collecting of populations for examination from a surrounding defined area.

Practice Procedures

Information is collected concerning the prevalence of protein-calorie malnutrition, methods of infant feeding and some of the conditions felt likely to be medical conditioning factors, notably intestinal helminths, malaria and, if possible, tuberculosis.

Other simple information which is not directly relevant such as skin disease and caries is also collected. Treatment, using a limited range of drugs, is available for all sick children at the time.

1. *Social data.* Name, approximate age (checked by local events calendar), sex, number of children alive and dead.

2. *Clinical data:* a) Weight, assessed according to Gomez classification. b) Syndrome: clear-cut kwashiorkor or nutritional marasmus. c) Nutritional indicators: edema; hypochromotrichia: low-arm circumference. d) Disease indicators: splenomegaly; indigenous medical scarification pattern (giving information concerning local symptom complexes).

3. *Laboratory data.* Thick blood film (stained in field with Giesma for malaria); stool (collected from child by glass anal tube technique, preserved in 5% formal-saline for examination later); hemoglobin (collected in Drabkin's solution for examination later).

4. *Infant feeding data.* The unhurried questioning of sample of mothers by medical students, with observation on breast feeding at time of examination.

5. *Miscellaneous.* Other techniques may be used additionally as, for example, the tuberculin test with the Heaf gun (should inspection be possible 2 to 3 days later, which is rarely the case).

Results

Three of the contrasting groups studied in East Africa will be discussed here: Baganda—peri-urban and rural in Central Uganda; Acholi—rural Northern Uganda; Watindiga—a hunting group of North-Central Tanganyika.

Full results have not yet been completely analyzed, but certain generalizations are given in table 1.

Comments

Results of studies in these 3 groups show quite different problems of community child health, and hence suggest difficult public health approaches.

Briefly, there appeared to be little evidence that protein-calorie malnutrition was very prevalent among the children of either the Watindiga hunters or the Acholi millet eaters and, from the purely nutritional point of view, the main concern must be to preserve from cultural erosion the present excellent infant feeding practices. This is, in fact, emphasized by the quite different picture among Acholi children, whose parents have moved to Kampala to work and among whom protein-calorie malnutrition is now quite common, partly as a result of a change from millet to maize meal and plantain.

However, as to medical conditioning factors of potential significance in the nutritional field, plainly malaria is of importance among the Acholi, so that emphasis should in this region be given to malaria control.

The contrasting situation as regards the Baganda is of special interest in that, although they are the most well-to-do and sophisticated of the 3 groups, protein-

TABLE 1
COMPARISON OF ECOLOGY OF PROTEIN-MALNUTRITION IN THREE EAST AFRICAN GROUPS

Group	Location	Main characteristics	Main features of infant feeding: Breast feeding	Main features of infant feeding: Infant foods (first year)	Possible medical conditioning factors (prevalence or incidence): Intestinal Helminths	Possible medical conditioning factors (prevalence or incidence): Malaria	Possible medical conditioning factors (prevalence or incidence): Diarrhea * disease	Evidence of protein-calorie mal-nutrition
Watindiga	North-Central Tanganyika	Hunters-food gatherers. Completely "Unsophisticated." Bush (tsetse)	Uneventful. Prolonged	Bone marrow melted fat (Zebra, warthog) Baobab powder Pre-chewed meat (game) Berries.	Little	Occas.	?	Rare
Acholi	North Uganda	Millet growing. "Unsophisticated." Rural.	Uneventful. Prolonged	Millet gruel Sesame paste Beans (*Ph. Vulg.*) No significant animal protein	Little	Hyper endemic	Uncommon	Unusual
Baganda	Central Uganda	Plantain growing. "Semi-sophisticated." Peri-urban and rural.	1 year with increasing early failures. Abrupt cessation with geograph. separation.	Steamed Plantain (*matoke*) sweet potato.	Hookworm very common.	Quite common.	Increasingly common.	Common

* Based on hospital records.

calorie malnutrition is a major problem, as judged not only by survey work but also by hospital and dispensary statistics.

Factors leading to the development of this situation appeared to include: 1) A shorter period of breast feeding with increasing failure of lactation in the early months and the spread of the "bottle-feeding cult" with its attendant inadequate protein intake and risk of infectious diarrhea. 2) Various social factors, including the psychological trauma following the abrupt cessation of breast feeding and actual geographical separation of mother and child, and possibly the abandonment of family-spacing techniques. 3) Over-emphasis on the protein-poor plantain (matoke) as the culturally dominant food. 4) The blood loss resulting from heavy infection with hookworm.

It would then seem that, for the Baganda, a rather poor traditional dietary is made worse by certain old and also new factors in the cultural pattern. The prevention of protein-calorie malnutrition will have to be based in part on public health planning, especially health education related to the etiologic factors that are locally relevant.

DISCUSSION

DR. BROCK: Dr. Jelliffe's account takes us back to the classical survey of the Kikuyu and Masai people by Orr and Gilks many years ago.

The only point I want to comment on is in regard to the advantages and disadvantages of Dr. Jelliffe's term, "protein-calorie malnutrition." At one time we had considerable discussion as to whether Dr. Gomez' first, second and third degree malnutrition was better than the term "protein malnutrition." Some of you will remember that in 1952, at Gambia, we defined protein malnutrition very carefully indeed. In spite of that definition there is still considerable misunderstanding. I referred in my talk to such terminological ambiguity.

Dr. Jelliffe now has come along with the new term, "protein-calorie malnutrition," and I confess that it is to me quite an attractive term. But as I analyze it carefully, I ask myself, Has it any advantage over the term "protein malnutrition"?

DR. JELLIFFE: I cannot pretend that I would regard this as a final definitive term, Dr. Brock. I think I use it more by contrast to kwashiorkor because it is my feeling that many people in the world believe that protein malnutrition in early childhood is exclusively kwashiorkor. It is my feeling that one should emphasize the fact that there are so many other syndromes from kwashiorkor to nutritional marasmus, with other intermediate groups as well.

You might say that "protein malnutrition in early childhood" would probably cover this equally well. That may well be right. I do think "protein-calorie malnutrition" lays emphasis on the fact that protein is the dominant need, at the same time that calorie needs very much have to come into the picture of infant feeding and the prevention of infant malnutrition in tropical countries. I am quite prepared to bow to other opinion on it.

DR. COLLIS: I must say that I think "protein malnutrition" is better. There is a type of kwashiorkor where the calorie is the least important part of the thing, where it is the protein malnutrition. If we are talking about this form of malnutrition, why not call it malnutrition and protein malnutrition? I think if you simply say "protein-calorie malnutrition," you leave out several other forms of deficiency.

Protein Foods in Middle Eastern Diets

Z. I. Sabry

THE MIDDLE EAST is generally considered to extend from Turkey in the north to the Sudan in the south and from Iran in the east to Egypt in the west, an area with a population of about 70 million. The majority of the people belong to low and lower middle income groups and may be regarded as similar in their dietary habits and cultural pattern.

It was not until recently that the Middle Eastern countries have shown awareness of their nutritional problems. Research and development in fields such as agronomy, animal husbandry, hygiene, food technology and agricultural economics, in addition to nutrition, are greatly needed in order to improve the nutritional status in the area.

NATURE OF MIDDLE EASTERN DIETS

There are few data available on food habits and consumption in Middle Eastern countries. However, some estimates of food supply can be calculated from such sources as the statistical records of FAO and of some Middle Eastern governments. The average calorie supply available per person in Egypt and in Turkey are shown in table 1. These data may be considered to be fairly representative for other Middle Eastern countries as well. It is evident that the greater part of the calories comes from food commodities of plant origin, mostly cereals and pulses.

TABLE 1
ESTIMATE OF FOOD SUPPLY PER PERSON IN THE MIDDLE EAST [1, 2]

Food Group	Calorie Supply	
	calories/person/day	% total calorie supply
Cereals and Cereal Products	1,890	72.5
Starches and Starchy Roots	40	1.5
Sugars and Syrups	145	5.5
Pulses, Nuts and Seeds	113	4.3
Fruits and Vegetables	162	6.2
Meat	59	2.2
Eggs	6	0.2
Fish	10	0.4
Milk and Milk Products	87	3.3
Fats and Oils	103	3.9
Total	2,615	100.0

[1] Data calculated from reference 15.
[2] Values reported are average of those for Egypt and Turkey.

A study of food habits in Lebanon has been undertaken to obtain information on the local diet pattern. The data in table 2 show the frequency of consumption of the various food groups among a sample of 131 rural and 252 urban school children, ranging in age from 6 to 16 years, in general representing low and lower middle income families. Since there is no school feeding program, the food consumed by the children is undoubtedly indicative of the family diet. The study offers further evidence of the importance of cereals and pulses in the diet. The relatively high consumption of fruits may be regarded as characteristic of Lebanon, since it is noted for its fruit production. Lebanon also has one of the highest standards of living among the Middle Eastern countries, which may be reflected in a relatively higher consumption of animal foods, such as meat, egg and milk, than would be generally expected for the region.

TABLE 2
FREQUENCY OF CONSUMPTION OF VARIOUS FOODS AMONG RURAL AND URBAN SCHOOL CHILDREN IN LEBANON

Food Group	FREQUENCY OF CONSUMPTION (servings/person/week)	
	Rural Population	Urban Population
Cereals and Cereal Products	26.8	29.3
Starches and Starchy Roots	2.1	1.7
Sugars and Syrups	3.1	3.9
Pulses, Nuts and Seeds	5.1	6.3
Vegetables	3.8	3.4
Fruits	8.3	9.4
Meat, Poultry and Fish	2.8	3.3
Eggs	1.3	0.7
Milk and Milk Products	3.8	4.4
Fats and Oils	4.0	6.9

SOURCES OF PROTEIN IN MIDDLE EASTERN DIETS

The foregoing would indicate that most of the protein in Middle Eastern diets comes from cereals and pulses. This is substantiated by estimates of the protein supply presented in table 3. These figures are derived from government records of the amount of different foods available for human consumption in Egypt, Iraq, Jordan and Syria. Wheat alone, mostly in the form of bread, accounts for almost two-thirds of the protein available. Maize contributes a considerable amount of the protein, especially in Egypt where its flour is often used in the making of bread. Rice is also consumed in substantial amounts, particularly in Egypt and Iraq. Pulses and seeds come next to cereals as suppliers of protein. Lentils, chickpeas, broad beans and sesame are used in preparing many of the popular local dishes. Less than one tenth of the total protein available is of animal origin. Iraq and Syria produce and consume more meat per caput than either Egypt or Jordan. In Syria a considerable amount of milk is taken in the form of *yoghurt* or *laban*, while pickled cheeses are a popular item in the Egyptian diet. The consumption of eggs is very poor throughout the area.

TABLE 3
ESTIMATE OF PROTEIN SUPPLY PER PERSON IN THE MIDDLE EAST [1, 2]

Protein Source	Protein Supply	
Cereals	gm protein/person/day	% total protein supply
Wheat	44.0	63.6
Maize	5.2	7.5
Barley	3.9	5.6
Rice	2.8	4.0
Millet and Sorghum	1.9	2.7
Pulses, Nuts and Seeds		
Lentils	1.6	2.3
Sesame	1.3	1.9
Beans and Peas	1.1	1.6
Squash Watermelon Seeds	1.1	1.6
Chickpeas	0.4	0.6
Lupine	0.3	0.4
Peanuts	0.2	0.3
Animal foods		
Meat, Poultry and Fish	3.5	5.1
Milk and Milk Products	1.7	2.5
Eggs	0.2	0.3
Total	69.2	100.0

[1] Data calculated from references 3, 11, 12, 13 and 14.
[2] Values reported are average of those for Egypt, Iraq, Jordan and Syria.

The available supply of protein appears to be adequate in quantity. However, the nutritional quality of diets in which such a large proportion of the protein comes from cereals should be a matter of concern. Observations of protein deficiency states have been reported from some Middle Eastern countries. Hanafy[7] reported the common occurrence of "subacute subnutritional syndrome", which was realized later[8] to be identical with kwashiorkor, among the 1- to 3-year-old children of the low-income group in Egypt. A seasonal malnutrition syndrome associated with shortage of animal protein in Sudan has been described by Corkill.[5] Signs of protein malnutrition have been frequently observed in Lebanon.[9]

It is important, when studying the protein sources in a diet, to consider the combinations in which these proteins are eaten, since the supplemental effect of protein is influenced by the timing of ingestion.[4,6,10] Middle Eastern people are great bread consumers. Every meal includes liberal amounts of bread. The local bread is flat and broken into small pieces and is often used instead of a spoon to take up food. In addition to its use in bread, wheat is parboiled and used in preparing many local dishes. It is cooked with lentils to prepare *mejaddarah*, ground with meat to make *kebbeh* and dried with *yoghurt* in the preparation of *kishk*. Parboiled wheat may also be mixed with ground broad beans and chickpeas and fried to make *falafil*. Adolph et al.[2] found that the addition of parboiled wheat to chickpeas, lentils or broad beans caused an increase in their protein efficiency ratio. Rice is often eaten with stew of vegetable and a little meat. It also enters in the preparation of such dishes as *mufattaah*, when it is mixed with sesame and pine nuts, and *sayadieh* in combination with fish.

In addition to the many cereal-pulse mixtures, there are a few dishes where pulses and seeds are combined. For example, cooked chickpeas are mixed with crushed sesame to prepare a popular dish known as *hommos-b-tehineh*. A mixture of roasted chickpea, peanuts and squash and watermelon seeds, known as *makh-louteh*, is a popular pastime food. Germinating lupine and fenugreek seedlings are often consumed together as a between-meals snack. In this case, the state of germination may influence the nutritional value of the protein.

The role of leaf proteins should not be overlooked. In many rural areas throughout the Middle East, wild plants, mostly stems and leaves, are consumed in substantial amount. These are eaten either fresh or cooked with rice or parboiled wheat. Although the quantity of proteins contributed by these leafy plants may be small, it would be of interest to study their nutritional value.

SUMMARY

Cereals and pulses supply most of the protein in Middle Eastern diets. Only small amounts of animal protein are available. Signs of protein deficiency in the area have been reported. It is important in this regard to investigate the nutritional value of the proteins available in the local foods as well as to study the possibility of new combinations for higher nutritional value.

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Kwashiorkor and Marasmus in Turkey

Joe D. Wray

I HAVE COME HERE from a new hospital where all of us are young in experience in dealing with the problem of malnutrition in children.

In our limited experience with this problem we have felt that one of our first responsibilities was to try to define the problem as it is seen in Turkey. I think this will be of interest to you who are concerned with the magnitude of the problem in various parts of the world. We have tried to define our problem both qualitatively and quantitatively. We can dispense with the qualitative aspect of it briefly. What we see in Turkey is almost overwhelmingly the marasmic type of malnutrition, and at this stage of the conference I think all of us have a clear idea of the distinction between marasmus and kwashiorkor. We do occasionally see children who show some of the signs of pre-kwashiorkor, and once in a while, very rarely, a case of frank kwashiorkor. I need not labor you with a description because our children are just like those with whom you are familiar, as are the problems of clinical management.

In trying to define our problem quantitatively in Turkey, that is, to get a reasonably accurate idea of its magnitude, we have encountered another problem which I am sure is familiar to those of you who are working in areas like this. That is the dearth of reliable statistical information.

I can begin by describing the nutrition situation generally in Turkey. There has been only one really adequate nutritional survey in recent years. This was conducted by the Turkish Army with the advice and assistance of ICNND. The survey was limited, of course, to men in the Turkish Army. However, the data gathered from recruits, young adult males arriving fresh from villages for service in the Army, are probably valid for the country as a whole. These young men generally are found to be adequately nourished. The survey bore out the impression held by most of us that Turkish adults in general are reasonably well nourished. Also, it substantiated our impression that the food supply situation in Turkey is not so critical as it is in some other areas of the world; the food is there if only it were used properly.

We must look at the situation in children against this background. When we try to investigate the amount and degree of malnutrition in children we encounter first a lack of data from healthy children for purposes of comparison, and, of course, there are no data from the same group of children over a long period of time. Nor have we any nice percentile tables for height and weight at the various ages.

In seeking standards for comparison, we have felt that it was not quite right to use the regular Western standards. After reviewing the literature we decided, on a very arbitrary basis, to adopt for our own the norms for Mexican children described by Dr. Gomez and his group.[1] We have also accepted his assumption that, for survey and classification purposes, body weight is the best single, objective index of nutritional status, in that it reflects the growth failure always produced by significant degrees of malnutrition.

The upper solid line in figure 1 represents the Mexican "normal" weights plotted against age. The second line represents 85% of that value, the third line 75% and the fourth line 60%. You are all aware that Dr. Gomez and his group feel that children whose weights are below 85% of the expected normal for their age are malnourished. They are considered to have first degree malnutrition if their weight is between 75% and 85% of normal, second degree if it is between 60% and 75%, and third degree if it is below 60%. For reference purposes, the upper dotted line in the figure represents the 50th percentile values from the "Mitchell-Nelson" tables[2] and the lower dotted line represents the third percentile. It is clearly evident that any child who would be classified as malnourished by this system would be well below generally accepted norms for age.

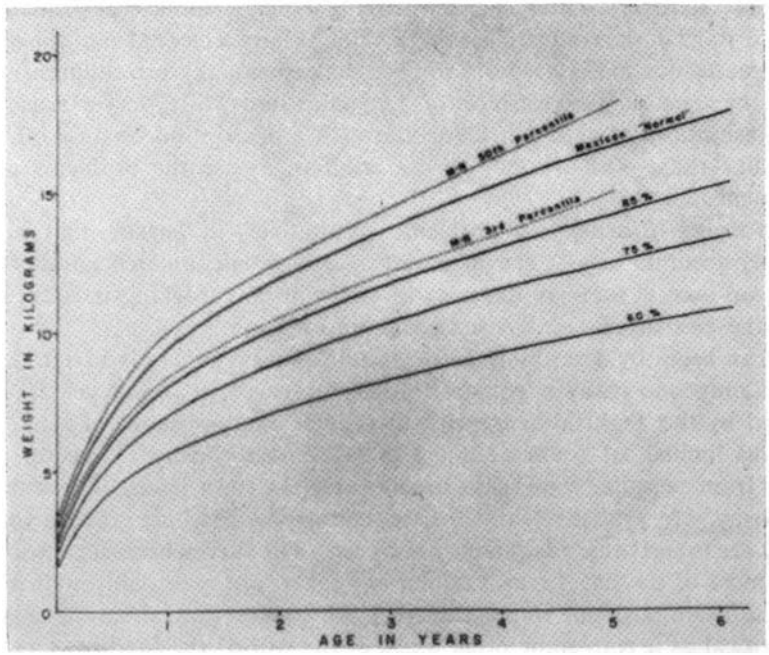

Figure 1—Graph of weight plotted against age.

For our crude survey we took the data from 1,500 consecutive patients registered in our outpatient clinic. These were children ranging in age from a few hours up to 16 or 17 years. We plotted weight against age on a graph similar to that in figure 1. and classified the nutritional status according to the position in which they fell on the chart.

From our 1,500 consecutive clinic visitors, we obtained adequate data on 1,084 children below the age of 6 years. Figure 2 is a spot graph in which weight is plotted against age for each child in this group. The one thing I would like to call to your attention is that in the very early months of life children straddle the mid-line rather well. Then, with increasing age, larger and larger numbers of children fall below the critical level of 85% of body weight.

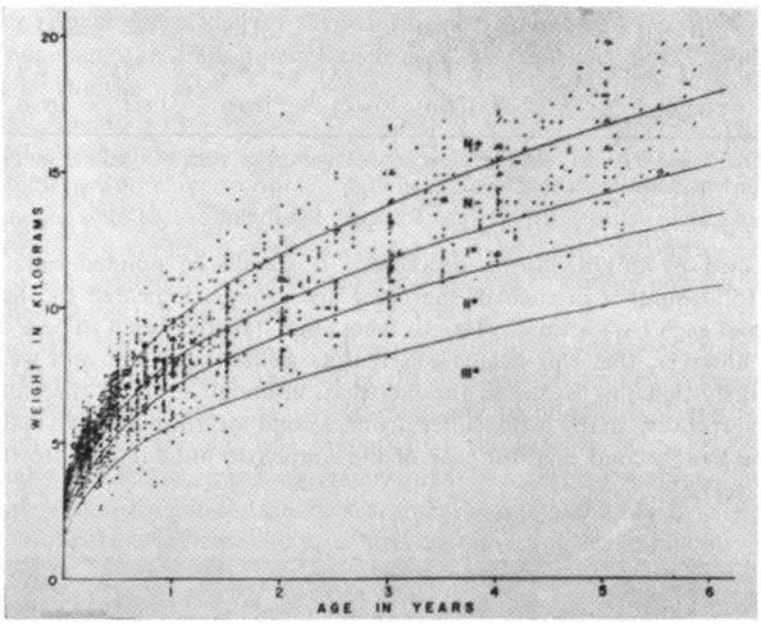

Figure 2—Spot graph of body weight of 1,084 children under 6 years of age who visited the outpatient clinic of Hacettepe Children's Hospital, Ankara.

For classification purposes the children were considered as N-plus if they fell above the normal line, N-minus below the line, first degree malnutrition (I°) if they were between 75% and 85% of expected body weight, second degree (II°) between 60% and 75% and third degree below 60%. Table 1 summarizes grossly the number of children of each sex in each of these categories. It may be seen that in this group of 1,084 children under the age of 6 there were 651 males and 453 females and that in both groups more than a third of our children fell into malnourished categories.

We did not want to overly manipulate our data, but analysis of the incidence of malnutrition in smaller age groups seemed pertinent. Figure 3 illustrates how the percentage of children with malnutrition varies with increasing age. You will notice that even within the first quarter of the first year we have some malnutrition as manifested by body weight deficiency. From then on, the incidence increases very rapidly until within the first half of the second year more than 50% of all the children coming to our outpatient clinic are malnourished by these standards. Then it may be seen that, with increasing age, the proportion of children who are

TABLE 1

	MALES		FEMALES		TOTALS	
CLASS	Number	Percent	Number	Percent	Number	Percent
N+	244	38.8	128	28.2	372	34.3
N−	202	31.9	164	36.2	366	33.7
I°	108	17.1	88	19.5	196	18.2
II°	68	10.8	54	11.9	122	11.2
III°	9	1.4	19	4.2	28	2.6
TOTALS	651	100.0	453	100.0	1084	100.0

Numerical summary of the number of children of each sex falling in the various nutritional categories.

malnourished by this definition decreases. It should be pointed out, however, that the total number in each of the older age groups (indicated by the number at the top of each bar) is small. Here is where our statistics begin to be a bit shaky. We feel, however, that this decrease is real as well as apparent and we consider it quite likely that this is due to the fact that, although some of the children undoubtedly recover, many who suffer from second or third degree malnutrition during the first, second or third year of life simply do not survive into the fourth and fifth years.

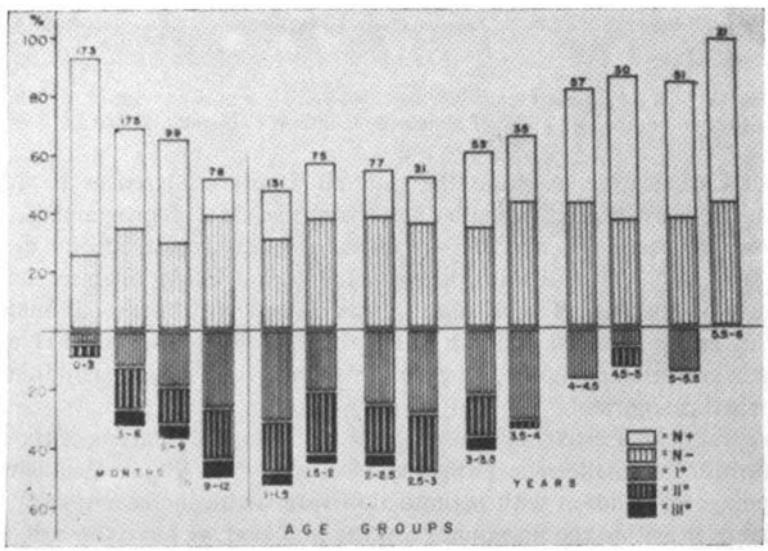

Figure 3—Bar graph illustrating the percentage of children in each age group who fall in each nutritional category. Figures at the bottom of each column indicate the age range, those at the top indicate the total number of children in that age group.

I have indicated earlier a reluctance to manipulate our data too much. As indicated above, the number in each age group varies considerably and there are other deficiencies in our information, such as uncertainty about the exact ages of the children. On the spot graph (fig. 2), for example, it may be seen that there is a heavy concentration of patients at the even ages. This is due to the fact that often the mothers of our patients know neither their exact age nor their birth date, and ages are simply expressed to the nearest year. This leaves much to be desired, but we have to be satisfied with the information available.

Recognising the limitations of such data, we feel that several valid conclusions can be drawn.

First, this does not give us a true picture of the incidence of malnutrition in the Turkish child population at large. These data are from a select group, namely, children who came to our outpatient clinic. However, we feel it does give us a significant clue to the incidence because malnutrition must have contributed to the morbidity that brought these children to the hospital, and certainly the incidence is far greater than one would encounter in a similar population group in the United States, for example.

Secondly, the data substantiate clearly what is well known: that this is a problem which begins toward the end of the first half of the first year of life and becomes increasingly more common through the second half of the first year, and remains serious through the second and third years.

Finally, we feel that this extremely high incidence of malnutrition of the marasmic type, occurring in the youngest segment of a population which is generally adequately nourished, indicates a rather striking failure of the commonly prevalent folk methods of infant feeding and points up dramatically the need for adequate education of mothers.

We consider this sort of data important in Turkey because we need solid information with which to educate doctors and nurses, especially those concerned with maternal and child health, and the public at large. We have, incidentally, found this system of classification very useful in educating our own pediatric residents in training. These doctors have grown up in an area where malnutrition is around them all the time, and they tend to become insensitive to the problem. Yet we feel it very important that they recognize it in the children under their care and that it is especially important that they recognize malnutrition in its early stages. Dr. Gomez and others have pointed out that the first-degree malnourished child is relatively easily treated. It is only when the process has progressed on into the second and third degree stages that the problem is greatly aggravated and requires every bit of effort one can give the child to pull him through the acute superimposed infection, which is almost invariably present, and on into convalescence—a costly and time-consuming procedure.

Our teaching device is a graph, based on the figures already discussed and illustrated in figure 4. The outpatient nurse fills out a graph like this for every child up to the age of 2 years. By having our outpatient clinic doctors plot the weight of each child on this graph at the beginning of the examination, we feel that they become immediately alerted to the nutritional status of the child without

any thumbing through tables, making extrapolations or complex calculations. The weight is simply spotted on the graph and the intern or resident can read off immediately the nutritional classification of the child. In particular, he is alerted to the child who falls in the range between 75% and 85% of the expected norm, who may not look particularly malnourished and yet is well below the third percentile for his age and whose mother most certainly needs education with regard

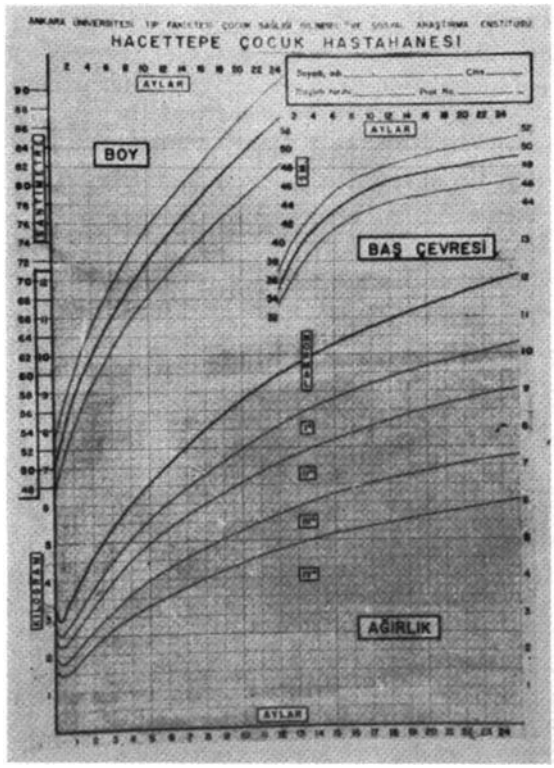

Figure 4—Outpatient clinic weight graph used at Hacettepe Children's Hospital, Ankara. Translation of important terms: "boy": length; "agirlik": weight; "bas cevresi": head circumference; "aylar": months.

to nutrition. Through the use of this device, we feel that our house staff has become more aware of this problem and is giving better care because of this awareness. An added advantage is that our data become comparable with those from other centers where similar standards are used. We are convinced that such a system, which is very simple and perhaps not altogether scientific, might well be equally useful elsewhere.

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DISCUSSION

MADAME AUBRY: I wanted to ask at what age were the children weaned, because we are quite distressed in Dakar to see the period when the weight becomes too low is much before the weaning period because the mother doesn't know how to complete the diet of children. They wean only at 17 months, usually, and are beginning to lower the weight at about six months. That is my first question.

Now I would like to know about the marasmic and kwashiorkor proportion. How much do you see of pre-kwashiorkor? I was extremely astonished and intrigued at the children having no fat in the liver. We had occasion to look at the acute, not pre-marasmic children, who were just fed on breast milk at 15 and 20 months of age, the milk from the mother at about 500 to 300 gm/day and nearly nothing else. So they were underfed but not at all malnourished. Their blood levels were quite normal. There was no fat in the liver by biopsy. Then we tried for a short period to wean them. After two weeks we saw biological changes in the blood, with serum albumin lowering, and biopsy showed the beginning of fatty infiltration of cells and just a few fat cells in the liver. So we had, of course, to stop the diet and give more protein to the children. I would like to know if you see anything of this kind in your children.

DR. WRAY: In answer to the question about weaning, here again we have a very difficult time getting precise data. The Turkish mothers are just not very time-conscious. This sort of retrospective survey is difficult, and one lacks confidence in the information he gets. By and large, Turkish children are breast-fed at least through the first year of life, often through the second year of life, and boys may be breast-fed as long as three or four years. We know that in this group of children the weight loss and growth retardation that we see begins before breast feeding is stopped.

I think it has been pointed out by a number of people here that breast feeding is adequate for full growth perhaps through six months. After that it is not adequate.

In answer to your question about the kwashiorkor-marasmus ratio in Turkey or in our clinic, we have literally lost count of the number of marasmic

children we have seen, but we have seen five in three years who had real, classical, clearcut kwashiorkor with edema and the skin changes which are characteristic.

DR. JACKSON: I just want to compliment Dr. Wray on this type of study, which I think is so very basic. I do not agree with the general concept that weight is a better index than height. However, for his particular reasons I think this is very valid and extremely important. Really to define malnutrition, I think we must not lose sight of the tremendous importance of linear growth.

Just a comment in relationship to weaning. When we attempted to completely breast-feed infants of well-nourished mothers here, we began to get a decline in the normal weight curve of well-nourished American children between the fifth and sixth months. I think the need for additional caloric foods begins to become critical at around the fifth month in a well-nourished mother.

DR. WRAY: This was a rather crude study. I want to emphasize that for our purposes we were almost happy with the bad results, because we need something dramatic to convince people of the importance of this problem, strange as it may seem.

DR. GOPALAN: Just one brief comment. Dr. Wray tells us there is far more marasmus than kwashiorkor in Turkey, and this question as to what produces marasmus and what produces kwashiorkor seems to be a great question. The studies of the type which Dr. Arroyave spoke about are extremely important. He told us of differences between marasmus and kwashiorkor, and I might add one more difference which might interest this group.

We have found in the serum of all cases of kwashiorkor which we have investigated, as also in one case of famine edema in adults, the substance designated VDM by Professor Ephraim Shorr. This substance is identified as ferritin. In cases of marasmic malnutrition we do not find this substance. If you feed a low-protein diet in which calories are moderately restricted, you can induce edematous malnutrition, a kwashiorkor-like syndrome, which is invariably preceded by the appearance of ferritin in the serum. If on the other hand, you give them the same diet but you supplement the diet with aureomycin, quite frequently you fail to produce ferritin and there is marasmic malnutrition.

I do not wish to speculate on the specific meaning of this observation, but it seems to me that what may be happening is that in kwashiorkor the situation is such as to produce a type of liver damage which causes the release of ferritin in circulation leading to edematous malnutrition. In marasmus the situation is such that there is no liver damage which would occasion the liberation of this powerful anti-diuretic substance.

This may be an oversimplification, but this is a factor which must be taken into consideration in considering the marasmic and edematous types of malnutrition.

DR. GARN: I think I can resolve that weight-as-a-measure-of-growth problem. In the lower limits of weight in infants during the first year, you are generally dealing with very little subcutaneous fat. These are children who frequently need some sort of help. Above a given level, however, the relationship between fat-free weight and the weight of the infant literally disappears. So, in American children in general, the fatter children are not necessarily going to grow faster. As a result, the lower limits of weight may be much more meaningful in growth studies, but during the first year the middle and upper limits of weight are of relatively little help because you cannot separate how much of the baby is baby and how much of it is fat.

India and the Far East

Nutritive Value of Cereal and Pulse Proteins

V. N. Patwardhan

A VARIETY OF CEREALS forms the staple diet of the people in the underdeveloped regions of the world. Rice is of course the most important of the cereals used as the staple food by more than half of the world's population. Among the other cereals the more important are wheat and maize, followed by a few other cereals classed as millets and known by several vernacular names depending upon the region.

Pulses and beans constitute important adjuncts to the staple, for they contribute an appreciable proportion of dietary protein in a diet predominantly based, as commonly happens in the economically underdeveloped regions, on vegetable foodstuffs. The contribution made by animal foods to nutrition in these regions is comparatively minor because of their low availability, high cost and the low purchasing power of the majority of the people.

Cereals and grain legumes together may contribute 70% to 90% of the calories in the daily diet and an almost similar proportion of dietary protein; at least, that is the situation in India.[1] In view of this, it becomes necessary to know more about the nutritive value of these foodstuffs. Diet surveys in the tropics have shown that infants during the post-weaning period and young children are given practically the same diets as are taken by adults. It is all the more necessary, therefore, to determine the efficiency of these vegetable proteins for promoting growth.

The results of work on the nutritive value of proteins of cereals and legumes reported from several laboratories in India is summarised in table 1. The information has been collected from the pioneering work of Swaminathan[2] and Basu[3] and that of a few others who were active in the third and fourth decades of this century. The biological value for maintenance of the 7 cereals investigated varied between 60% and 89%, and of 9 legumes between 45% and 74%. In general, the BV of legume proteins is lower than that of cereal proteins. The figures for digestibility coefficients also reflect the same trend. There is little to choose between the values for Protein Efficiency Ratio (PER) of cereal and legume proteins, as seen from table I. Proteins from both seem to be inadequate for normal growth when compared with those of meat, milk and eggs. A survey of the essential amino acid (EAA) composition of cereal and legume proteins reveals the fact that lysine is the limiting amino acid in cereals and methionine, closely followed by tryptophan, is

the limiting EAA in legumes. Reports are available in published literature in which beneficial effects of adding pure EAA to cereals and legumes have been demonstrated.

Protein Efficiency Ratio of Mixed Proteins from Cereals and Pulses

Since neither the cereals nor the legumes alone form the only source of protein in the tropical dietaries, the supplementation effect in a mixture of the two should be considered of practical significance. Swaminathan[4] had attempted but failed unequivocally to demonstrate the supplementary effect of cereal and pulse protein in a 1:1 mixture when studied by the balance sheet method.

TABLE 1
BIOLOGICAL VALUE, DIGESTIBILITY COEFFICIENT AND PROTEIN EFFICIENCY OF COMMON INDIAN DIETARY PROTEINS

Foodstuff	Botanical name	Digestibility coefficient Per cent	Biological value Per cent	Protein efficiency Ratio 8 weeks
CEREALS *				
Rice	*Oryza sativa*	96	80	1.7
Wheat	*Triticum vulgare*	93	66	1.3
Jowar	*Sorghum vulgare*	91	83	0.8
Ragi	*Eleusine coracana*	79	89	0.7
Maize	*Zea mays*	80	60	1.0
Bajra	*Pennisetum typhoideum*	89	83	1.1
Italian millet	*Setaria italica*	91	77	—
PULSES				
Red gram	*Cajanus indicus*	75	72	0.7
Bengal gram	*Cicer arietinum*	82	74	1.1
Green gram	*Phaseolus radiatus*	85	54	0.8
Black gram	*Phaseolus mungo*	80	63	1.0
Lentil	*Lens esculenta*	85	49	0.5
Peas	*Pisum sativum*	90	59	1.1
Cowpea	*Vigna catjang*	78	45	—
Field bean	*Dolichos lablab*	70	49	—
Soybean	*Glycine hispida*	90	60	0.9

* At 5 per cent and the rest at 10 per cent level of protein intake.

The results of diet surveys in India[1] show that, at least so far as the average Indian dietary is concerned, cereal and pulse proteins are more likely to be consumed in a proportion of 2:1 than of 1:1, hence the determination of supplementary effects would be of practical significance at a relatively lower level of intake for pulses than for cereals. We[5] therefore decided to evaluate the PER of cereal proteins singly and in mixture with pulse proteins, keeping as near to the above-mentioned proportions of protein from the two classes of foodstuffs as was feasible. Protein in the diet was adjusted to 10% whether it was from a single cereal or from a mixture of a cereal and a pulse. In the latter case, cereal provided 7% and pulse 3% protein in a total dietary intake of 10% protein. The results of PER determinations in weanling albino rats are summarised in table 2. In a few experiments, dry powder of green leafy vegetable (*Amaranthus gangeticus*) was added. In such a mixture

TABLE 2
SUPPLEMENTARY EFFECT OF PULSES ON PER OF CEREALS
(Eight rats in each group)

	No supplement	Wheat	Bajra (*Pennisetum typhoideum*)	Jowar (*Sorghum vulgare*)
No supplement		1.77	1.60	1.61
Cicer arietinum	1.83	2.18	2.16	1.89
Phaseolus mungo	1.93	2.15	2.10	1.96
Phaseolus aureus	1.52	2.22	2.09	1.80
Cajanus cajan	1.72	2.19	2.05	1.84

10% dietary protein was made up in the following proportions: cereal protein 6, pulse protein 3 and leaf protein 1. The values for PER obtained with such mixtures are compared with the PER of skim milk in table 3.

The results given in tables 2 and 3 demonstrated that in most cases mixtures of cereal and pulse proteins in the proportions in which they were tried had significantly higher PER than that of either the cereal or pulse protein alone. The incorporation of leaf powder yielded a protein mixture which approached skim milk in the PER of its protein. It must be admitted that admixture with leaf powder did not always yield such good results. They were variable to a certain extent, the reason for which is not quite clear. Leaf powder was added to cereal pulse mixture in order to see if the need for the addition of mineral and also extraneous vitamins in the diet could be eliminated. In a typical test carried out with bajra (*pearl millet*), red gram and amaranth, the PER obtained when salts and vitamins were added to the diet was 2.17; when vitamin mixture alone was omitted the PER was 2.11; with salt mixture alone omitted it was 1.94; with both these withheld it was 1.94. The slightly lower values, although not statistically significant, would indicate that under normal circumstances other sources of vitamins and minerals ought to be made available to the animal in addition to the leafy vegetable.

A few other millets which are important from the standpoint of Indian dietary habits were investigated in mixtures with red gram and amaranth. Results are given in table 4. Values for PER of rice and rice-pulse-leaf powder mixture are included for comparison.

It will be noticed that PER of proteins from rice and ragi were not improved significantly in mixture with pulse protein. The PER of these cereal proteins is higher than that of others so far investigated, but the reasons for the lack of supplementary effect of pulses are not clear.

TABLE 3
COMPARISON OF PER OF MIXED VEGETABLE PROTEINS WITH PER OF SKIM MILK

Protein source	No. rats	Wt. gain gm.	PER
Skim milk	8	84	2.57
Rice + *C. cajan* + amaranth	8	72	2.47
Wheat + *C. cajan* + amaranth	7	73	2.35
Bajra + *C. cajan* + amaranth	7	73	2.33
Jowar + *C. cajan* + amaranth	7	69	2.45

Some millets are husked before preparing food from them. The effects of husking are variable.[6] It has a drastic effect on *Setaria italica*, the nutritive value being reduced by over 40%. A similar effect was observed with *Sorghum vulgare* in which a 30% decrease in PER was noted on husking. No such effect was, however, observed in *Pennisetum typhoideum*. On the other hand, in *Panicum miliare* an improvement in PER by 18% was observed on husking. The values with *Setaria italica* given in table 4 show that, in spite of a reduction in PER on husking the grain, supplementation with pulse and amaranth brought about a 100% increase. This observation is interesting in that it shows up to good effect the supplementary value of pulse protein to cereals of low nutritive value.

TABLE 4
PER OF RICE AND OTHER LESS KNOWN MILLETS AND THE EFFECT OF THE ADDITION OF PULSES
(Eight rats in each group)

	Supplement	PER
Setaria italica—Whole grain	nil	0.82
Setaria italica—Husk removed	nil	0.48
Setaria italica—Husk removed	pulse + l.p.	2.22
Panicum miliare—Whole grain	nil	1.09
Panicum miliare—Whole grain	pulse + l.p.	1.83
Paspalum scrobiculatum—Whole grain	nil	0.70
Paspalum scrobiculatum—Whole grain	pulse + l.p.	1.92
Eleusine coracana—Whole grain	nil	1.99
Eleusine coracana—Whole grain	pulse + l.p.	2.07
Rice—Undermilled	nil	2.02
Rice—Undermilled	pulses* + l.p.	2.09 to 2.18

pulse: *Cicer arietinum*
l.p.: dry leaf powder of *Amaranthus gangeticus*
* with different pulse mentioned in table 2.

Regeneration of Haemoglobin and Plasma Protein in Protein-Depleted Rats

The experiences described above indicated that cereal pulse mixtures could be a source of dietary protein of a reasonably high nutritive value capable of promoting growth in normal weanling rats. Their capacity to regenerate body proteins in protein-depleted animals remained to be tested. This was done in adult male rats for reasons of experimental convenience.[7] Adult rats 3 to 4 months old were placed on protein-free diet for 8 weeks. Preliminary experiments had shown that depletion for this period was necessary in order to stabilise the rats with regard to changes in body weight and the blood and plasma volumes. Approximately a 30% reduction in body weight and a 50% reduction in total circulating haemoglobin and plasma protein were observed at this stage. The rats were then fed equal amounts of diets (10% protein level) for 21 days. Periodic determinations of blood volume, plasma volume, haemoglobin and plasma protein were done. The results obtained with rice, wheat, *Pennisetum typhoideum* and *Sorghum vulgare* are illustrated in figure 1. They clearly show the effectiveness of vegetable protein mixtures in the regeneration of plasma protein and haemoglobin. It is true that the rate of regen-

eration was slower with vegetable proteins than with skim milk. However, given sufficient time, return to normal values was assured on vegetable proteins alone.

Metabolism of Vitamin B_{12} on Vegetable Protein Diets

The question of vitamin B_{12} nutrition on diets based entirely on vegetable proteins is a controversial one. Most animal protein foodstuffs are known to contain vitamin B_{12}. On the other hand, the true vitamin B_{12} activity in vegetable foodstuffs is negligible. Hence, the possibility of vitamin B_{12} deficiency in animals maintained on vegetable diets had to be considered. Vitamin B_{12} deficiency has been produced in rats fed soybean diets and its occurrence on other vegetable proteins

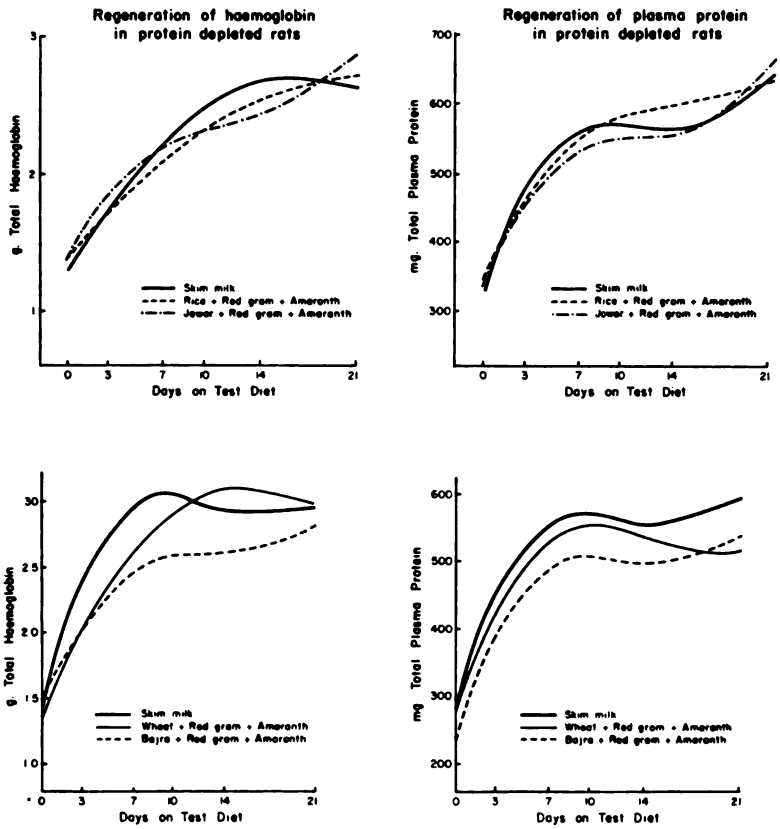

Figure 1

has been reported (Fatterpaker et al.[8]). We[9] therefore utilised the opportunity presented by the investigations on PER of vegetable proteins to determine the vitamin B_{12} status of rats on these experimental diets. Vitamin B_{12} was estimated microbiologically with the use of *L. leichmanii* in food, faeces, serum and liver at the end of the feeding period. The findings are summarised in table 5.

TABLE 5
TRUE VITAMIN B_{12} ACTIVITY IN DIET, FAECES, SERUM AND LIVER OF RATS ON VEGETABLE AND ANIMAL PROTEIN DIETS

Diets	Diet	Faeces		Serum *		Liver	
		Rats		Rats		Rats	μg/whole
	μg/100 g	No.	μg/rat/day	No.	mμg/c.c.	No.	liver
			AT 8 WEEKS				
Colony stock	0.48	7	4.28	10	1.60	8	0.92
Whole egg	1.15	8	1.83	—	—	8	2.62
Skim milk	0.59	10	2.23	8	2.01	8	1.11
Rice + pulse + l.p.	0.06	8	1.69	8	1.77	8	1.08
Wheat + pulse + l.p.	0.13	13	2.62	9	1.87	7	1.27
Bajra + pulse + l.p.	—	9	3.40	—	—	8	0.88
Jowar + pulse + l.p.	0.03	4	3.37	—	—	—	—
			AT 24 WEEKS				
Colony stock diet	0.48	8	3.34	10	1.65	8	1.67
Wheat + pulse + l.p.	0.13	7	3.64	7	1.98	7	1.91

Pulse: *Cajanus cajan* l.p.: leaf powder of *Amaranthus gangeticus*
* not corrected for alkali stable activity which varies from 15 to 20%.

There was only a trace of vitamin B_{12} activity in vegetable protein diets used in our experiments. On the other hand, the colony stock diet (which contains animal protein) and the whole egg and skim milk diets had appreciable vitamin B_{12} activity. The faeces of rats after 8 weeks of feeding showed vitamin B_{12} activity in the animal as well as vegetable protein groups. That some of the vitamin B_{12} synthesized in the intestinal tract was utilised by rats appears probable from the observed values for vitamin B_{12} in serum and liver. These values did not show a fall even after 6 months of continuous feeding with vegetable protein diets. It was possible, as has been suggested by Mickelsen[10] and demonstrated later by Barnes and Fiala,[11] that rats obtained their vitamin B_{12} requirements through coprophagy. Our recent experiments with rats in which coprophagy was prevented seem to indicate that a part of vitamin B_{12} in serum and liver must have been the result of coprophagy, which was not prevented in our earlier experiments. When, in fact, coprophagy was prevented, lower values for vitamin B_{12} in serum and liver were obtained. (Satyanarayana et al.[12]).

It would appear therefore that occurrence of vitamin B_{12} deficiency in man living on purely vegetarian diets is a probability which cannot be ignored. Wokes, Badenoch and Sinclair[13] have found low vitamin B_{12} values in the serum of vegans and have also reported the occurrence of clinical symptoms in a small percentage of British vegans. The authors point out, however, that only slight symptoms appeared after several years of vegan diet. Nevertheless, in any work on vegetable proteins the aspect of vitamin B_{12} nutrition has to be kept in mind.

Feeding Trials with Young Children

In view of the encouraging results on the growth of young rats fed vegetable protein diets and the capacity of these proteins to regenerate and maintain normal haemoglobin and plasma protein values in rats, it was considered worth while to

test whether diets based on cereals and pulses could promote normal growth of young children.

Comparative feeding trials with skim milk and rice and pulse proteins, in which children were given all the meals so as to provide them the full daily quota of proteins and calories, were carried out over a period of 10 months (Ganapati et al.[14]). Three contiguous villages near Trichur town in Kerala State were selected for this experiment. The population in these villages was homogeneous and the agricultural, socio-economic and environmental conditions as well as dietary habits were similar. Children between 1 and 5 years of age from each village were physically examined and their heights and weights recorded before beginning the experimental feeding. Children in one of these villages subsisted on their usual home diets and served as controls. Children in all three villages were given appropriate medical care throughout the experimental period except for mild nutritional deficiencies for which no treatment was given.

The menus for meals in one village were based on rice, pulses and vegetables, whereas in the second village preparations of skim milk replaced the pulse preparations, other foodstuffs remaining the same. The protein and calorie contents of the diets in the two groups were similar (table 6). It was necessary to incorporate additional vegetable oil in the diets of the skim milk group to bring the calorie intake up to the level of the pulse group.

TABLE 6
PROTEIN AND CALORIE INTAKE OF CHILDREN UNDER FEEDING TRIAL AT TRICHUR

Age groups in months	Protein gm	Calories	Number of children		
			Pulse group	Skim milk group	Control
12—24	20 *	650 *	12	14	18
25—36	29	970	8	13	27
37—48	33	1060	10	16	15
49—60	38	1260	14	11	22

* Children at breast in this age group were estimated to receive 5 gm protein and 300 calories from mother's milk. The others were given extra food to bring the total intake to 25 gm protein and 950 calories.

Every child received 3 meals per day except on Sundays. Sweet preparations made from cooked rice and green gram (*Phaseolus aureus*) or cooked rice and reconstituted skim milk sweetened with unrefined sugar were served for breakfast. Cooked rice with lightly spiced soup of pulse (*Cajanus cajan*) and lightly spiced cooked vegetables were served at lunch and the evening meal. Reconstituted skim milk was lactic-fermented, and preparations made from this with cooked vegetables and rice formed the constituents of the menu for the other group. Slight variations in the preparations made from pulse, vegetable and skim milk were adopted to prevent monotony in the diet.

Food was distributed with the help of previously standardized measures. The quantities of food left over by each child were also recorded. Children were fed for a total of 240 days. Measurement of heights and weights and clinical examination of all children were conducted once every 2 months.

Very few children were present regularly at all the meals. Some missed one or two meals occasionally or sometimes all the meals for a few days. The foods as presented were accepted and tolerated well. The wastage of food was greatest in the partially breastfed children. The consumption of food was better on an average in pulse-fed children than in the skim milk group. A varying number of children did receive occasionally additional food in their homes, against which nothing could be done. The average meal attendance throughout the period of feeding was 65.2% and 64.2% in the pulse and skim milk groups respectively.

The average gains in height and weight as calculated from the initial and final observations are given in table 7.

TABLE 7
GAINS IN WEIGHT AND HEIGHT IN CONTROLS AND IN CHILDREN FED AT THE CENTRE
(Trichur, Kerala State, India)

Group	No. of children	Weight (Averages) Initial kg	Final kg	Gain kg	Height (Averages) Initial cm	Final cm	Gain cm
Pulse protein	44	10.40	12.30	1.90* ± 0.10	85.7	91.1	5.4* ± 0.20
Skim milk	54	9.74	11.53	1.79* ± 0.08	82.7	89.1	6.4*α ± 0.19
Control	82	9.79	11.13	1.34 ± 0.07	83.5	88.1	4.6 ± 0.17

* indicates significant difference ($P < 0.01$) from values in control group.
α indicates significant difference ($P < 0.01$) from height gain in skim milk group.

Growth in height and weight was significantly higher in pulse- and skim milk-fed groups than in the control group. Gain in weight in children on rice-pulse diet was not significantly different from that on rice-skim milk diet. Thus it will be seen that pulses can be used in feeding young children with a view to providing the necessary protein in the diet. It must be admitted, however, that the types of preparations used in these trials were not quite suitable for children below 2 years, the main defect being the bulk of food which had to be consumed in order to provide the necessary amount of protein.

Clinical examination at the beginning and at the end of the trial gave equivocal results. The number of children with discolored hair remained almost the same, although those showing sparseness of hair were fewer at the end of the experiment. There were 7 children in pulse group and 10 in skim milk group with enlarged livers at the beginning of the trial; the numbers were 5 and 8 respectively at the end. These were made up of 2 and 4 new cases appearing in the two groups. A somewhat similar situation was found in the control group. It is difficult to explain the significance of these findings. There were a few cases of xerosis conjunctivae, Bitot's spots and angular stomatitis in all the groups; they were not materially affected by feeding. In some of these children the signs regressed, but this was offset by their appearance in children who did not show them at the beginning. Since the diets did not contain any vitamin supplements, this might have been expected. It is therefore important to realise that rice-pulse diets may have to be strengthened with other foodstuffs which will provide adequate amounts of vitamins to prevent these deficiencies from occurring.

SUMMARY

1. The Protein Efficiency Ratio (PER) of a variety of cereals and pulses, the two common classes of foodstuffs in Indian diets, was determined in young albino rats under comparable conditions.

2. A marked beneficial supplementary effect of the incorporation of pulses with cereals in experimental diets so as to provide cereal proteins and pulse proteins in the proportion of 7:3 has been demonstrated in albino rats.

3. Such mixtures were found efficient for the regeneration of blood proteins in protein-depleted rats.

4. A feeding trial in young children 1 to 5 years of age was conducted, using rice and pulse as sources of protein as compared with a diet based on rice and skim milk. Children were fed 3 meals a day for 240 days. Gains in height and weight in both the groups were significantly higher than those in the control groups of children who were not included in the feeding programme.

No significant difference could be detected between the skim milk- and pulse-fed groups so far as growth was concerned.

5. It is concluded that vegetable protein diets could be used in the feeding of young children to prevent growth failure. The need to supplement diets of this type with foods rich in vitamins has been indicated.

Food preparations used in these trials were found not wholly suitable for children below 2 years. Special efforts to solve this problem would be necessary.

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A Report of Some Recent Studies on Protein Malnutrition in India

C. Gopalan

IN THIS COMMUNICATION some observations arising from recent studies on protein malnutrition carried out by the author and his colleagues in the Nutrition Research Laboratories (now in Hyderabad, formerly in Coonoor) are presented.

PROTEIN MALNUTRITION AND VITAMIN A DEFICIENCY

The question of the possible relationship between protein malnutrition and vitamin A deficiency has attracted some attention in recent years. In this connection the results reported below may be interesting.

Serum vitamin A and carotene levels were determined in cases of kwashiorkor with and without clinical signs of vitamin A deficiency, in cases of vitamin A deficiency without kwashiorkor, and in apparently normal children of the same age group belonging to the community. These estimations were repeated after treatment. The different groups of cases on whom these estimations were carried out and the plan of treatment adopted are set out in table 1.

The serum vitamin A and carotene levels in the apparently normal children (group 1) were lower than the average values reported for normal children in other parts of the world. This finding was not unexpected considering that, though the children investigated in this group were free from disease, they were drawn from the poor socio-economic group subsisting on unsatisfactory diets. The cases of vitamin A deficiency (group 3) exhibited considerably lower levels of serum vitamin A and carotene than cases of group 1, as expected. The interesting finding was that cases of kwashiorkor with no signs of vitamin A deficiency (group 2) also showed levels of serum vitamin A and carotene which were significantly lower than those observed in group 1. The lowest levels of serum vitamin A were observed in the group exhibiting signs of both kwashiorkor and vitamin A deficiency (group 4).

On treatment with vitamin A, a marked increase in serum vitamin A was observed in all cases of vitamin A deficiency. The striking observation was that in cases of kwashiorkor exhibiting low levels of serum vitamin A but with no clinical signs of vitamin A deficiency, treatment with high-protein diet containing no vitamin A supplement brought about a significant increase in serum vitamin A levels. It appeared from these observations that protein malnutrition could bring about a

TABLE 1
SERUM CAROTENE AND VITAMIN A IN DIFFERENT GROUPS OF CASES

Group	Clinical condition	Treatment	Serum carotene (mcg/100 ml)		Serum vitamin A (IU/100 ml)			
					Before treatment		After treatment	
			Before treatment	After treatment	No. below 10 IU **	Mean	No. below 10 IU	Mean
1	Apparently normal	—	50 (6) *	—	0	80 (6)	—	—
2	Kwashiorkor only	High protein diet with no vitamin A	22 (13)	19 (7)	5	52 (9)	0	71 (7)
3 a	Vitamin A deficiency only	Vitamin A	18 (13)	26 (11)	6	32 (7)	0	102 (11)
3 b	Vitamin A deficiency only	High protein diet with no vitamin A	12 (4)	11 (3)	2	45 (2)	2	40 (2)
4	Kwashiorkor + vitamin A deficiency	High protein diet + vitamin A	13 (14)	19 (9)	12	17 (2)	0	74 (10)

* The values given are the mean values of the number of samples indicated in brackets. Cases after treatment include some in which initial values were not available.
** The number of samples having a concentration of less than 10 IU have been indicated in this column and the means given in the next column are the average of values above 10 IU.

significant lowering of serum vitamin A and high-protein-diet treatment without vitamin A supplement could correct this.

The findings of a study on nursing mothers just completed are also in line with the above observations. The mean serum vitamin A levels of a series of 12 poor nursing mothers was 73.5 IU/100 ml. After 4 weeks of treatment with a high-protein diet containing no vitamin A supplement, the mean serum vitamin A level in these mothers rose to 103.7 IU/100 ml, a difference which was found to be significant. Though these mothers did not exhibit gross signs of protein malnutrition, their body weights were low, the mean body weight of the group being 41 kg.

The significance of the above observations requires elucidation. Arroyave et al. showed that the absorption of vitamin A was impaired in cases of kwashiorkor. The increase of serum vitamin A brought about in cases of kwashiorkor in the present study by high-protein diet alone would indicate that the effect of protein malnutrition may consist of an impairment of the mobilisation of the hepatic stores of vitamin A.

Contrary to the suggestion that protein depletion may aggravate vitamin A deficiency, the work of Maclaren has indicated that protein depletion may actually delay the development of signs of vitamin A deficiency in rats fed diets deficient in vitamin A. These apparently contradictory observations on the relationship between protein depletion and vitamin A deficiency may perhaps be explained on the basis that protein depletion may on the one hand aggravate vitamin A deficiency by interfering with the absorption and mobilisation of vitamin A and on the other hand may mitigate vitamin A deficiency by sparing the tissue requirements of vitamin A by inducing growth retardation.

Clinical observations would seem to lend support to the above explanation. Over two-thirds of all cases of keratomalacia observed in children less than 5 years of age were found to show clinical evidences of protein malnutrition. The maximal age incidence of kwashiorkor was found to be between 1 and 3 years, while that of vitamin A deficiency was between 3 and 5 years. In spite of this difference in maximal age incidence, the presence of signs of protein malnutrition in over two-thirds of all cases of severe vitamin A deficiency would suggest the possible role of protein malnutrition in aggravating vitamin A deficiency.

On the other hand, the majority of cases of kwashiorkor showed no clinical evidence of vitamin A deficiency. Only one-third of all cases of kwashiorkor investigated showed signs of vitamin A deficiency and in a large number of these the manifestations of vitamin A deficiency were limited to the conjunctiva. In at least 6 cases of kwashiorkor not showing clinical signs of vitamin A deficiency, conjunctival signs of vitamin A deficiency became manifest after 6 weeks of high-protein treatment with skim milk containing no vitamin A.

It would thus appear from these clinical observations that the role of protein malnutrition in vitamin A deficiency may be bidirectional.

A Comparison of the Picture of Protein Malnutrition in Two Regions of India

In the numerous descriptions of the syndrome of protein malnutrition from different parts of the world, some important regional variations in the clinical

picture are noticeable. An examination of these in relation to the diet of the population and the methods of infant feeding in the concerned regions may provide some information regarding the etiology and significance of some of the clinical signs of the syndrome. The shift of the Nutrition Research Laboratories from its erstwhile location in Coonoor to its present site in Hyderabad, 500 miles further north in the country, provided an opportunity for the comparative study of the disease in the two regions. A few salient observations arising from this study are presented below.

Both in Coonoor and in Hyderabad, breast-feeding of infants was continued till late in the second year in the majority of cases. However, an important difference between the children of Coonoor and of Hyderabad was with regard to the age at which supplementary feeding was initiated. While in Coonoor supplementary foods were started in all cases by the sixth month, supplementary feeding was not started till well after the end of the first year in most cases in Hyderabad. The supplementary food itself was generally somewhat lower in calories in Hyderabad than in Coonoor and included jowar (*Sorghum vulgare*) in addition to rice, unlike Coonoor where rice was the sole staple.

The cases of kwashiorkor observed in Hyderabad had lower body weight and higher incidence of associated vitamin deficiency signs such as skin and hair changes and anaemia. The overall mortality in the Hyderabad series was 15% as against 9% in the Coonoor series.

In table 2 the body weights of the cases observed in Hyderabad have been compared with those investigated in Coonoor. The minimum weight reached after the institution of treatment has been taken into consideration. It will be noted that, at all ages, the body weights of the Hyderabad cases were decidedly lower than those of cases in Coonoor. The lower body weight of the Hyderabad cases may be considered to be partly a reflection of the type of supplementary diet fed to these children and partly due to the fact that supplementary feeding in these children was not started till well after the end of the first year.

TABLE 2
BODY WEIGHT OF KWASHIORKOR CHILDREN
(in kg)

Age Group	Hyderabad Series		Coonoor Series	
	Mean	Range	Mean	Range
1—2 years	5.82	4.55— 7.73	7.09	4.55— 8.64
2—3 years	7.00	5.45—11.14	7.50	5.91— 9.09
3—4 years	6.64	5.00— 9.55	7.05	5.45— 9.09
4—5 years	7.00	4.77—10.00	8.41	7.73— 9.09
5—6 years	8.27	5.91—10.91	10.36	10.00—11.36

An interesting finding was that, in the Coonoor as well as in the Hyderabad material, the average weight of patients in the 3-4-year age group was nearly the same as or actually lower than that of cases between the second and third years. This would mean that cases of kwashiorkor seen in the 3-4-year age group were in a more advanced stage of wasting. Information on these lines from other regions may prove useful.

There was a relatively greater incidence of kwashiorkor in children beyond 3 years of age in Hyderabad. A considerable number of these cases in the older age groups showed evidence of pulmonary tuberculosis. It would appear from the experience in Hyderabad and Madras (Dr. Achar—personal communication) that pulmonary tuberculosis may be an important conditioning factor in contributing to protein malnutrition in the older age groups in the ubran centres of India, unlike semi-urban centres like Coonoor.

The Problem of Marasmus and Kwashiorkor

Though a great deal has been written about marasmus and kwashiorkor, we still do not have a clear idea as to the differences in the etiology and pathogenesis of these two clinical states. It is realized that often the two clinical pictures may mingle. A proper understanding of the factors underlying the development of these two different clinical pictures of malnutrition is obviously essential. Some observations bearing on this problem are presented below.

We found two important differences between our cases of marasmus and of kwashiorkor. Firstly, our cases of marasmus did not show the advanced fatty changes in the liver characteristic of kwashiorkor; indeed the liver picture in these cases was either normal or showed only slight degrees of cytoplasmic vacuolation. The second important difference was that all our cases of kwashiorkor which were investigated for this purpose showed in their serum the substance known as VDM, which is now believed to be identical with ferritin and which is detected by a positive rat mesoappendix test. We did not find ferritin in our cases of marasmus. We reported this observation nearly 3 years ago.

On the experimental side, we observed that if monkeys were fed a low-protein diet with moderate caloric restriction, severe oedema could be induced in them (figs. 1 and 2). We studied the serial changes in serum albumin and in the thiocyanate space in these monkeys and noticed that there was no correlation between the fall in the serum albumin and the rise in the thiocyanate space. The most significant and striking observation was the relationship between the appearance of VDM in the serum and the expansion of the thiocyanate space. After a few weeks on the diet, VDM made its appearance in the serum and this was quickly followed by an abrupt and sharp increase in the thiocyanate space. This relation between the appearance of VDM and the increase of thiocyanate space was strikingly observed in every case. On rehabilitation, the disappearance of VDM immediately and invariably preceded the sharp decline in thiocyanate space and disappearance of oedema. It has been shown by other workers that VDM is a powerful antidiuretic agent and that its action is mediated through the posterior pituitary. It has also been shown that VDM could be released from the liver in certain states of hepatic damage. The results of our study would appear to indicate that the damage to the liver in protein malnutrition facilitates the release of VDM, contributing to antidiuresis and oedema.

One interesting point was that if monkeys were fed the same low-protein diet but with aureomycin supplements, VDM did not appear in the serum and there was no increase of thiocyanate space.

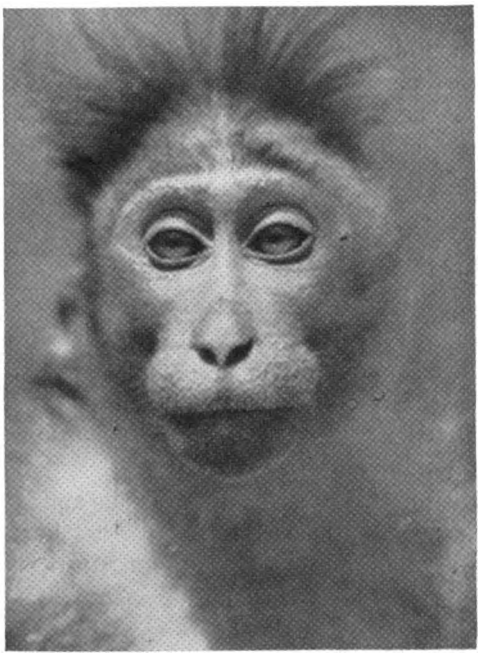


Figure 1—Normal Monkey.

Figure 2—Monkey in which oedema has been induced by protein-deficient diet.

It would appear that in kwashiorkor we are dealing with a dietary situation which results in a type of liver damage leading to the appearance of VDM in the circulation and oedema. In marasmus we have a different dietary situation leading to little or no hepatic damage and consequently no release of VDM and no oedema. This is perhaps an oversimplification, but these observations at least provide a tentative explanation for the occurrence of oedematous malnutrition (kwashiorkor) in certain cases and cachectic malnutrition (marasmus) in certain others. We reported on these observations nearly 2 years ago.

Effect of Excess Leucine on Subjects Receiving Low-Protein Diets

The observations reported here are not based on investigations of protein malnutrition in children, but they have been included because of their possible significance to the problem.

The starting point of this investigation was the finding that, unlike in Coonoor, pellagra was frequently encountered in Hyderabad. Careful examination of the dietaries failed to reveal any striking difference between the two areas with regard to the intake of different nutrients. However, an important difference between the dietaries of the two regions was that, while rice was the sole staple in Coonoor, the dietaries of the poor segments of the population in Hyderabad invariably included varying amounts of the millet *Sorghum vulgare* (jowar). In practically every case of pellagra investigated in Hyderabad, a history of regular consumption of jowar with or without rice was obtained.

A comparison of the chemical composition of rice, jowar and maize (table 3) reveals that the nicotinic acid content of jowar is nearly similar to that of rice. The reported tryptophan contents of different strains of jowar show wide variations, certain strains possessing nearly as high a content of the amino acid as is found in rice while certain others show low values as in maize. Both jowar and maize have, however, one common feature with regard to their amino acid composition, namely a high content of leucine. Elvehjem has reported that the dietary supplementation of leucine at 1% level caused retardation of growth in rats subsisting on low-protein diets (9% casein). The average daily protein intake in the dietaries of the pellagrins investigated here was of the order of 45 gm (9% protein), the protein being mainly derived from cereals including jowar. The possible role of amino acid imbalance resulting from relative excess of leucine in the pathogenesis of pellagra was therefore investigated.

TABLE 3
AMINO ACID COMPOSITION OF MAIZE, JOWAR AND RICE

	gm/100 gm of protein			Nicotinic acid
	Tryptophan	Leucine	Isoleucine	mg/100 gm
* Maize	0.8	14.9	6.4	1.4
** Jowar	1.2	12.9	6.1	1.8
** Rice	1.2	8.0	6.0	1.2

* Baumgarten, W., Mather, A.N. and Stone, L.—Cereal Chemistry, *23*, 135, 1946.
** Balasubramanian, S.C., Ramachandran, M., Viswanathan, T. and De, S.S.—Ind. Jour. Med. Res., *40*, 219, 1952.

The investigation included (i) a study of the effect of the administration of leucine on the urinary excretion of N-methyl nicotinamide (NMN) in normal human volunteers and in pellagrins, and (ii) a study of the effect of isocaloric and isonitrogenous substitutions of jowar for rice on the urinary NMN excretion. The basal diets in these studies provided roughly 10% protein. The basal diet was continued till a fairly stable level of NMN excretion was attained.

In the first study, 5 subjects were given a daily supplement of 5 gm l-leucine for a period of 7 days and their urinary NMN excretion determined daily. In all subjects, there was a rise in the urinary excretion of NMN 50% above the basal level, following on leucine administration. Within a few days after the withdrawal of leucine, the NMN excretion returned to the basal level in all cases (fig. 3). The increase in NMN excretion brought about by 5 gm l-leucine daily did not appear to be influenced by the simultaneous administration of 2 gm of dl-isoleucine (fig. 4). Control studies in the same volunteers, using nicotinic acid administration, indicated that the increase in NMN excretion brought about by the administration of 5 gm l-leucine reflected approximately an additional 3.5 mg of nicotinic acid being metabolised daily.

In the second study the substitution of jowar for rice resulted in a prompt increase in the urinary NMN excretion. This increase was maintained as long as jowar was continued. After the withdrawal of jowar and its resubstitution by rice, the NMN excretion returned to the original low level (fig. 5). Actual analysis of the 2 diets revealed that their nicotinic acid content was nearly identical. It was calculated that the daily amount of leucine contained in the jowar diet was of the order

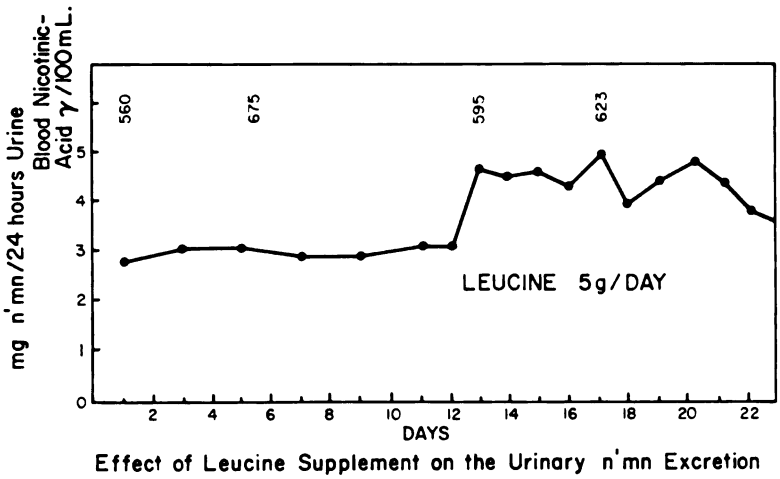


Figure 3

of about 5 gm. The increase in NMN excertion observed was also nearly of the same order as that obtained with 5 gm l-leucine daily in the first study.

The precise significance of the increase in urinary NMN excretion brought about by leucine requires further investigation. This increase is not likely to be a reflection of greater availability of nicotinic acid, considering that both maize and jowar, which are rich sources of leucine, have been associated with increased prevalence of pellagra. It would appear that the amino acid imbalance caused by relative excess of leucine in diets which are marginal with regard to protein may be

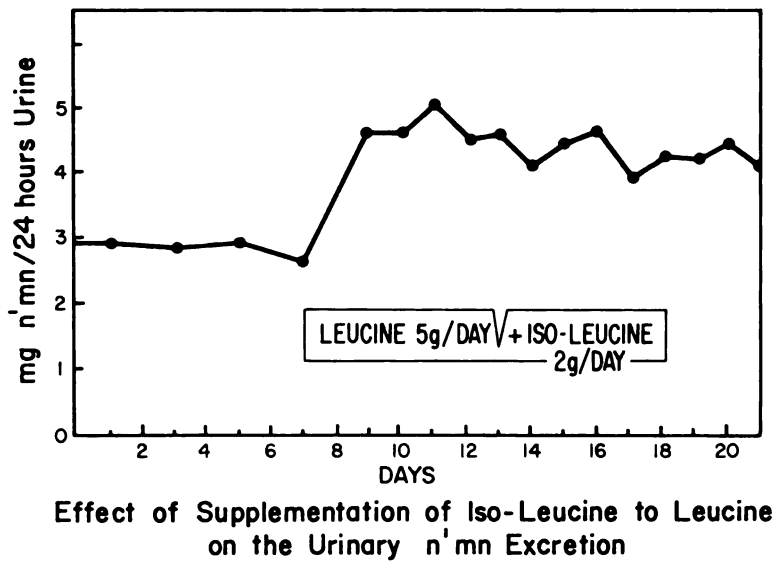
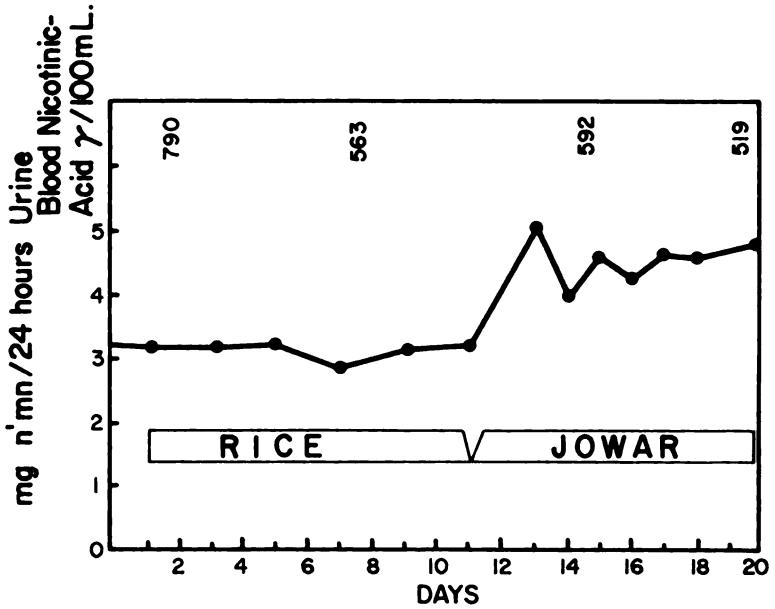


Figure 4

deleterious. This observation may be important while considering the choice of suitable protein-rich foods for the prevention of protein malnutrition.

It was pointed out in the previous section that the cases of kwashiorkor investigated in Hyderabad exhibited more severe signs of protein malnutrition than those seen in Coonoor. The incidence of skin changes in cases of kwashiorkor investigated in Hyderabad was nearly double that of cases observed in Coonoor, and their severity was also greater. An important difference between the diets of these 2 groups was that, while rice was the sole staple in Coonoor, in a majority of the cases in Hyderabad rice and jowar in proportions varying roughly from 2:3 to 1:1 were the main dietary ingredients. How far the inclusion of jowar in the low-protein dietaries of the children in Hyderabad contributed to the severity of the clinical picture of protein malnutrition in this region is, however, a moot question.



Effect of Substituting Rice by Jowar on the Urinary n'mn Excretion

Figure 5

Vegetable Protein Diets in Protein Malnutrition

The efficacy of different vegetable protein foods and combinations thereof in the treatment of kwashiorkor may be a valuable means of assessing their usefulness in the prevention and control of protein malnutrition. In earlier studies, the response obtained in cases of kwashiorkor treated with Bengal gram (chickpea—*Cicer arietinum*) was compared with that obtained in cases treated with skim milk. In recent studies, combinations of vegetable protein foods like Bengal gram, peanut and sesame have been investigated. The results of these studies are briefly reviewed here.

The composition and nutritive value of the diets investigated are shown in table 4. The amino acid composition of the proteins used are set out in table 5, in which the amino acid pattern of the reference protein suggested by FAO is also included.

All cases taken up for investigation were admitted to the hospital and kept under observation throughout the entire period of study. No major difficulty was encountered in administering any of the diets, though the feeding of all the vegetable protein diets in the bulk required to provide the proteins called for more persuasion and vigilance than was the case with skim milk. There was some initial reluctance on the part of the children to take some of the vegetable protein diets, particularly the food containing sesame.

To assess the response to the different lines of treatment, the following criteria were employed: 1) Time in days taken for clinical disappearance of oedema. 2) Time in days taken for reaching minimum body weight (this would be determined partly by the speed with which the oedema cleared). 3) Number of days taken for rise of body weight by one pound (calculated from the date on which minimum weight was reached). (This would be determined partly by the opposing factors—the continuing clearance of occult oedema and the buildup of tissues). 4) Number of days taken for diarrhoea to subside in cases which had noninfective diarrhoea to begin with. (The persistence of diarrhoea with one regimen may not necessarily reflect the continuing effect of protein deficiency but may be a response to the diet). 5) Rise in plasma albumin brought about by about 10 days of treatment.

The results of the study are presented in table 6.

It will be seen from the table that there were no significant differences between the different groups as regards the time of disappearance of oedema and weight changes. It was, however, noticed that the time taken for the increase of body weight by one pound after the attainment of minimum weight was maximal in the cases treated with peanut flour. The improvement of the associated signs of malnutrition was also no different in the different groups. In children who had nonspecific diarrhoea at the time of admission, treatment with vegetable protein diets helped to control the diarrhoea earlier than treatment with skim milk.

The mean rise in serum albumin levels on the 10th and 30th days of treatment was highest with skim milk. Bengal gram ranked next in order of efficacy. The vegetable protein mixtures, viz., Bengal gram-peanut flour and Bengal gram-peanut flour-sesame flour, appeared to be inferior to Bengal gram alone, as was the Bengal gram-rice combination. The least satisfactory response with regard to serum albumin regeneration was obtained with peanut flour used singly.

The possibility of achieving a better level of serum albumin regeneration by prolonging the duration of treatment from 30 to 45 days and by employing higher levels of vegetable protein diets was also investigated. It was noticed that with prolongation of treatment a greater rise in serum albumin could be achieved. It was also observed that, with the increase of level of vegetable protein from 70 gm to 100 gm daily, there was a significantly higher increase in the serum albumin level. On the other hand, increasing the level of skim milk from 70 gm to 100 gm in a

TABLE 4
COMPOSITION AND NUTRITIVE VALUE OF THE DIETS

Diet	Composition	Protein gm	Fat gm	Nutritive value Calories	Calcium mg	Iron mg
1	Roasted Bengal gram—25 parts + Low fat peanut —74 parts + Lucerne powder — 1 part Bread 6 oz. + Jaggery 2 oz.	70	11	1425	1138	23
2	Roasted Bengal gram—25 parts + Low fat peanut —49 parts + Low fat sesame —25 parts + Lucerne powder — 1 part Bread 6 oz. + Jaggery 2.5 oz.	70	23	1430	1625	23
3	Defatted peanut flour—99 parts Lucerne powder — 1 part Bread 7 oz. + Jaggery 2.5 oz.	70	8	1380	1260	
4	Bengal gram	60	15	1475	254	30
5	Bengal gram — 3 parts + Rice — 4 parts + Calcium lactate	60	13	1677	550	15
6	Skim milk	63	2	1100	2000	7

TABLE 5
ESSENTIAL AMINO ACID CONTENT OF THE PROTEINS USED
(gm/100 gm protein)

Amino Acid	FAO reference	Skim milk	Bengal gram	Bengal gram + rice	Peanut	Bengal gram + peanut	Bengal gram + peanut + sesame
Lysine	4.2	8.6	6.4	5.72	3.0	3.35	3.38
Tryptophan	1.4	1.5	0.6	0.76	1.0	1.56	1.79
Phenylalanine	2.8	5.5	5.0	4.89	5.1	5.09	5.69
Methionine	2.2	3.2	1.7	1.82	1.0	2.78	3.51
Threonine	2.8	4.7	4.8	4.40	1.6	1.96	2.51
Leucine	4.8	11.0	8.0	8.00	6.7	6.85	7.03
Isoleucine	4.2	7.5	6.0	6.00	4.6	4.76	4.82
Valine	4.2	7.0	5.4	5.58	4.4	4.51	4.67

group of cases was not attended with a further improvement of serum albumin regeneration.

An examination of the amino acid composition of the different diets used in the study may provide the explanation for some of the findings. The work of Scrimshaw and colleagues has shown that the addition of certain amino acids (e.g., methionine) to diets in which the major amino acid limitations (lysine or tryptophan) have not been corrected may indeed be deleterious. The inferiority of the Bengal gram-rice combination or the Bengal gram-peanut flour combination, which considerably reduced the overall lysine levels, may therefore be expected. The fact that the addition of sesame flour, a rich source of methionine, did not significantly improve the nutritive quality of the Bengal-gram-peanut flour mixture is also in line with the findings of Scrimshaw et al., as this addition left the lysine content unchanged.

The general conclusions that may be drawn from the foregoing studies are that vegetable protein diets of the type used here are nearly as effective in controlling the clinical manifestations of protein malnutrition as those based on skim milk, but are somewhat inferior to skim milk with regard to serum albumin regeneration.

TABLE 6
AVERAGE RESPONSE TO DIFFERENT DIETS

Diet *	No. of cases	Days for disappearance of oedema	Days for minimum weight to be reached	Days for gain in one lb. body weight	Days for control of diarrhoea	Rise in serum calcium on the 30th day mg/100 ml	Rise in serum albumin gm/100 ml 10th day	Rise in serum albumin gm/100 ml 30th day
1	21	12.5	7.1	7.4	7.8	1.1	0.14	0.86
2	31	13.0	8.6	6.1	6.3	1.3	0.12	0.77
3	11	13.0	7.7	14.0	5.9	1.1	—0.28	+0.35
4	56	13.0	5.8	9.5	5.7	—	0.40	1.04
5	19	17.0	14.0	5.2	8.0	—	0.20	0.63
6	49	12.0	5.4	7.3	10.5	—	0.75	1.24

* For details of diet see table 4.

In choosing combinations of vegetable proteins, attempts must be directed towards overcoming the major amino acid deficiencies.

Peanut Protein Isolate in Kwashiorkor

A major difficulty in the use of vegetable protein foods is the fact that they have to be administered in considerable bulk to provide the protein requirement. The use of such vegetable proteins in kwashiorkor therefore called for much persuasion and attention. This difficulty can be overcome by the use of proteins isolated from the vegetable sources. Through the use of such protein isolates, which provide the protein in concentrated form, the bulk of the diet can be considerably reduced. It has been further claimed that the removal of indigestible carbohydrates and some bitter principles from the vegetable protein sources may improve their acceptability and nutritive value. It may also be possible to prepare suitable blends of such isolated proteins of high nutritive value or a mixture of protein isolate with skim milk in desirable proportions, for the prevention and control of protein malnutrition.

A peanut isolate prepared by the Central Food Technological Research Institute, Mysore, is being currently investigated. Three groups of cases, one treated with the peanut protein isolate, the second with skim milk, and the third with a mixture of 2 parts of peanut protein isolate and 1 part of skim milk, are being studied. Forty grams of protein in two divided doses are being administered to each child in the form of 5 ounces of a fluid emulsion suitably sweetened. The children also receive 6 to 8 ounces of bread daily in addition. The product is found acceptable to the children and is easy to administer. So far, nearly 10 cases of kwashiorkor have been investigated in each group. The results indicate that, while peanut protein isolate by itself is unsatisfactory, the combination of peanut protein isolate and skim milk is nearly as satisfactory as skim milk. These studies would thus indicate the possibility of extending the available milk supplies in certain countries through such combinations with peanut protein.

DISCUSSION

DR. ARROYAVE: We are pleased to find how well Dr. Gopalan's data agree with ours. We have in fact found consistently extremely low vitamin A serum levels in severe protein malnutrition. However, upon the administration of skim milk without any source of vitamin A to speak of, we find a marked increase in vitamin A, even to high normal value, within about a week, without any source of dietary vitamin A. But in some instances we did not find that the children responded in this way. However, the other lipid components of the plasma, including cholesterol, phospholipids, neutral fat, free fatty acids, and so forth, which have been previously described very clearly by Dr. Dean's

group and now by others—these parallel the increases in vitamin A very well, both in time and shape of the curve, when the vitamin A increases. But in the children in which the vitamin A did not increase, the other lipids did increase.

We decided it was an opportunity to see whether to go deeper into the question of whether there was a reservoir of the liver. We had the opportunity of doing a few liver biopsy studies in some of these children, and in fact we found that the ones who show the increase in vitamin A had initially significant liver reserves although their serum levels were extremely low. The ones that did not show this increase had a very low content of vitamin A in the liver. When we performed the biopsy at the time of the highest level of vitamin A in the serum, we found the vitamin A reserves or vitamin A content of the hepatic tissue had increased to one-half of the initial value.

In one child who was treated with corn and beans we did not get appreciable recovery for about 3 weeks—we did not get any increase in vitamin A, but when we changed the child to skim milk we did get this very marked increase.

DR. COLLIS: I have two brief comments to make.

First of all, in regard to vitamin A, we have had a somewhat similar experience. Although in the southern part of Nigeria we have palm oil, vitamin A deficiency is not the usual thing. We have had two or three cases showing exactly the same thing that Dr. Arroyave has just said.

The second thing I want to emphasize is, in the treatment of kwashiorkor, tuberculosis from the pediatric point of view is extraordinarily important. We have a lot of tuberculosis, and it accentuates the whole condition. It brings out kwashiorkor, if you like, but protein malnutrition is present in a great many cases of primary tuberculosis. We have a clinic with about 500 cases of primary tuberculosis. Provided you do treat them with a high-protein diet, as Dr. Gopalan said, they do extraordinarily well; the mortality is very small indeed, almost negligible. If you don't, they die.

DR. DEAN: I would like to comment on one point made by Dr. Gopalan which seems to me extremely important. He has said that he can perhaps account for some of the differences in the ratios of marasmus to kwashiorkor and the time of appearance of these diseases by a reference to the diets of the children in the first year. I think Dr. Gopalan said that in one case millet was added, and in another case rice was added. He went on to say that many of his cases were kwashiorkor which occurred in the third to the fourth year.

We have made somewhat similar observations, and we have come to the conclusion—this is nothing new, of course—that the whole of this question must be regarded as an etiological question. The life of the child must be taken into account. When you see a case of kwashiorkor, you must always ask yourself, Why has this particular case of kwashiorkor occurred?

I would imagine that what happens in the first 6 to 12 months is so remote in time that perhaps it doesn't have any relation to the appearance later on

of kwashiorkor. We have found no difference at all in a large study of very carefully observed cases so far as the time of vectation is concerned. We thought the longer you vectated, the longer time you have a chance of avoiding kwashiorkor. I think you have something similar, Dr. Gopalan.

I am absolutely certain that the way to regard these cases is in relation to the whole way of life and the accidents, including tuberculosis, that occur at certain times.

DR. HARPER: I feel I should make one comment about the postulation of a leucine-isoleucine antagonism imbalance being a contributory factor in the development of pellagra.

The concentration of leucine in maize is roughly 20%, and even feeding straight maize with a 10% protein content would not bring the total quantity of leucine in the diet up above 2%. Even with our low-protein diet we get very little growth depression from the quantity of leucine. One percent of leucine in our 9% casein diet really resulted in no growth depression. We have some situations with very low-protein diets. With rice, for example, where the protein content of the diet is about 5%, or with fiber in the diet where the protein content is about 6%, we can show growth depression as a result of a relatively small increment of leucine.

When we get back to a natural protein and the possibility of this effect occurring with natural proteins with the concentrations that are present, we have to keep in mind that even when we use 5% of leucine to induce quite a severe growth depressant in the purified diets, only 0.15% of isoleucine, and the same with valine, is required to reverse this completely. When you increase the concentration of a natural protein in diet, you are increasing isoleucine and valine along with the leucine.

Then the second point is, we know very little about the availability of these in maize. Most of the leucine is in the zein portion of maize. We certainly know that in isolated zein the amino acids are not readily available. So it is quite conceivable that our utilizable leucine concentration is actually considerably lower than we would calculate from amino acid analysis. I don't say it can't happen, but I think we should be very cautious about postulating it.

DR. NICOL: I should also like to comment on Dr. Gopalan's reference cases of pellagra and people eating a diet based on sorghum and maize. These are the staple foods in Northern Nigeria. I happen to have some figures here. In children between 4 and 6 receiving approximately 50 gm of protein and 13 mg of nicotinic acid, the leucine-isoleucine ratio of this dietary mixture having been calculated at 3:1, we see no pellagra at all.

Development and Evaluation of Processed Foods Based on Edible Peanut Flour and Protein

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IT IS NOW GENERALLY RECOGNISED that the diets consumed by a large section of the people in India, Africa, Latin America and several other parts of the world consist predominantly of cereals and tubers and include negligible quantities of protective and protein-rich foods such as milk, eggs, meat, fish etc.[1,2] The consequences of dietary deficiencies in proteins, minerals and vitamins are strikingly seen in the vulnerable groups, viz., weaned infants, young children and expectant and nursing mothers. Protein malnutrition is widely prevalent among children belonging to the low-income groups of the population.[3] There is therefore great need for the large scale production, at reasonable cost, of highly nutritious and acceptable processed foods based on available protein-rich sources and fortified, where necessary, with vitamins and minerals.

The WHO Protein Advisory Group has suggested[4] that, in planning the production of processed protein foods for supplementing human diets, it is necessary to take into consideration: 1) the amino acid content of the individual ingredients and of the final product; 2) the possible presence of toxic or interfering factors; 3) the need for obtaining exact specifications for each of the components; 4) the necessity of avoiding processes that may damage protein quality; 5) the desirability of using products of local origin; 6) the low cost and good keeping quality of the product; 7) the suitability of the product for feeding weaned infants; and 8) the acceptability of the product to the consumers. At the same time the processed protein foods should possess high nutritive value and should have a significant supplementary value to the diets normally consumed by the people in the region.

Although the primary objective should be to provide a supplementary source of protein of good quality, it is desirable that a vegetable protein food should also contain or be fortified with adequate quantities of vitamins and minerals that are likely to be lacking in the diets.

[1] Presented paper.

TABLE 1
ANALYSIS† OF PEANUT FLOUR AND PROCESSED FOODS

1	Protein (Nx6.25) gm 2	Ether extractives gm 3	Carbohydrates gm 4	Calcium gm 5	Phosphorus gm 6	Iron mg 7	Thiamine mg 8	Riboflavin mg 9	Nicotinic acid mg 10	Vitamin A IU 11	Vitamin D IU 12	Calories per 100 gm 13
Low-Fat Peanut Flour	52.7	8.9	21.8	0.07	0.05	2.9	0.95	0.20	19.5	—	—	378
Bulk Foods												
Tapioca macaroni	11.3	1.8	73.6	0.05	0.15	3.0	0.21	0.07	3.6	—	—	356
Mysore flour	13.8	2.7	69.8	0.06	0.18	3.6	0.31	0.07	5.4	—	—	359
Paushtik atta	13.4	2.0	71.8	0.05	0.29	5.0	0.50	0.13	5.6	—	—	359
*Wheat flour, whole	12.1	1.7	72.2	0.04	0.32	7.3	0.54	0.12	5.0	—	—	353
*Rice, raw, milled	6.7	0.3	80.2	0.01	0.13	2.0	0.12	0.04	1.1	—	—	350
Weaning Foods												
Balanced malt food	31.9	4.9	48.0	0.58	0.55	6.7	1.50	3.00	8.5	3000	300	364
Precooked weaning food	31.5	5.2	53.9	0.86	0.60	4.8	1.50	3.00	10.0	3000	300	388
*Milk, cow, whole, dried	25.8	26.7	38.0	0.95	0.73	0.6	0.30	1.46	0.7	1400	17	496
Specialty Foods												
Enriched macaroni	18.0	0.9	66.7	0.49	0.42	3.2	0.68	0.70	5.6	1500	100	347
Nutro macaroni	19.3	2.5	63.6	0.39	0.17	1.4	0.59	1.04	7.6	—	—	354
Nutro biscuit	16.5	16.7	62.5	0.22	0.20	1.2	1.10	2.50	34.0	1520	700	466
Supplementary Foods												
Multipurpose food Formula (A) Seasoned (B) Unseasoned	41.9	8.5	35.8	0.67	0.82	5.1	1.03	3.00	14.0	3000	300	387
Formula (C) Unseasoned with 20% skim milk powder	40.6	7.0	39.0	0.80	0.86	4.2	1.11	2.80	10.4	2400	240	381
*Skim milk powder	35.6	1.0	52.0	1.30	1.03	0.6	0.35	1.96	1.1	—	—	359

† Values are on fresh basis. The moisture content of nutro biscuits and dried, whole or skim milk ranged from 2.0% to 3.5%; other processed foods contained 8% to 12% moisture.

* Included for comparison.

Low-Fat Peanut Flour and its Nutritive Value

The most important and abundant sources of proteins of vegetable origin available in different countries are the low-fat oilseed meals obtained from oilseeds and nuts such as soybean, peanut, sesame, cottonseed, sunflower seed and coconut, and also pulses (legumes) such as grams, beans etc.

Considerable interest has been shown in several countries in the use of the low-fat peanut flour as a protein supplement to human diets.[5] Subrahmanyan et al.[6] have worked out a simple procedure for preparation of edible peanut flour. The flour has a light yellowish-brown colour, a pleasant nutty odour and an acceptable taste. It may contain 5% to 9% residual oil depending on expeller efficiency, about 50% protein and good quantities of certain B vitamins (table 1). The limiting amino acid in peanut proteins (table 2) is methionine; secondary deficiencies are lysine and possibly threonine and isoleucine.[7] The availability of all the essential amino acids from the proteins for the albino rat is uniformly high.[8] At 20% level in the diet, they promote good growth in experimental animals.[9] At lower levels of intake (10%), however, they are inferior to milk and meat proteins.[10] Peanut proteins supplement cereal proteins to a significant extent, but are inferior in this respect to the proteins of skim milk, soybean and meat.[11]

TABLE 2
COMPARISON OF AMINO ACIDS IN PEANUT PROTEIN WITH WHOLE MILK PROTEIN
(gm amino acid/16 gm nitrogen)

Amino acids	Peanut protein	Whole milk protein
Arginine	11.0	4.2
Histidine	2.2	2.6
Lysine	2.9	8.7
Tryptophan	1.3	1.5
Phenylalanine	5.0	5.5
Methionine	0.9	3.2
Threonine	2.4	4.7
Leucine	6.5	11.0
Isoleucine	4.0	7.5
Valine	4.4	7.0
Cystine	1.6	1.0
Tyrosine	4.1	6.0

Low-fat peanut flour has a marked supplementary value when incorporated at 20% level in poor vegetarian diets based on certain tubers and cereals.[12] Feeding trials with school children (6 to 14 years) have shown that supplementing their diets with 1 ounce of peanut flour daily per child results in significant increases in height, weight and haemoglobin content over control groups not receiving the supplement.[13]

Processed Foods with Peanut Flour

During the past 10 years, considerable work has been done at the Central Food Technological Research Institute, Mysore, on the development of certain processed foods containing peanut flour for protein enrichment. A brief account of

this work follows. The products discussed are usable as bulk foods, weaning foods, specialty foods or supplementary foods. Their chemical composition is given in table 1.

Tapioca macaroni. In earlier attempts, composite grains in round shape were developed from blends of tapioca flour and peanut flour in varying proportions (80-20 and 90-10 respectively).[6] Grains with 7% to 11% moisture store well for 8 to 10 months under normal conditions.[14] The nutritive value of these grains when used as replacement for cereals in poor vegetarian diets is significantly higher than that of rice and jowar and is of the same order as that of wheat or ragi.[15]

Although the round grain has general consumer acceptance, the product would obviously have better possibilities of acceptance if the grain could be made in rice shape. A considerable amount of experimental work had to be done to achieve this, and tapioca macaroni as now produced is a blend (60:15:25) of tapioca flour, peanut flour and wheat semolina. It could be made either in rice shape or as short tubes, shells, ringlets, etc., and cooks readily in 5 to 6 minutes. A pilot plant with a capacity of one ton per day has been in operation at the Institute.[16] Feeding experiments[17] with children (6 to 10 years of age) have shown that rice in the diet could be completely replaced by tapioca macaroni without adversely affecting growth, general health and nutritional status (table 3). Complete replacement of rice in a poor vegetarian diet by an equal quantity of tapioca macaroni brings about appreciable though not significant increases in the retention of nitrogen, calcium and phosphorus by children.[18]

TABLE 3
INCREASES IN HEIGHT, WEIGHT, HAEMOGLOBIN AND RED BLOOD CELL COUNT OF CHILDREN FED ON RICE OR TAPIOCA MACARONI DIETS

	Rice diet	Tapioca macaroni diet	Significance of difference
Height (inches)	0.61	0.62	N.S.
Weight (pounds)	1.83	1.94	N.S.
Haemoglobin (gm/100 cc)	—0.17	0.13	Sig. at 1%
Red blood cell (10^6/cu mm)	0.17	0.12	N.S.

32 children, aged 6 to 10 years, were grouped and fed diets containing rice or tapioca macaroni as major components. Duration of experiment was 6 months.

Mysore flour. A blend (75:25) of tapioca flour and peanut flour, called Mysore flour, has been successfully used as a partial substitute for cereals in large-scale feeding experiments in distress areas of Madras State.[19] The gruel prepared from Mysore flour is highly acceptable. From feeding experiments for 6 months with children (6-10 years), it has been shown (table 4) that 50% of cereals in poor vegetarian diets could be replaced by an equal quantity of Mysore flour without affecting growth, general health and nutritional status.[20] These studies have demonstrated that peanut flour could effectively supplement tapioca flour where it is being consumed in large amounts.

Paushtik atta. This is a blend (75:8:17) of wheat flour, peanut flour and tapioca flour and its composition compares well with that of whole wheat flour.

TABLE 4
INCREASE IN HEIGHT, WEIGHT, HAEMOGLOBIN AND RED BLOOD CELL COUNT OF CHILDREN FED ON RICE OR RICE-MYSORE FLOUR DIETS

Character	Rice diet	Rice-Mysore flour diet	Significance of difference
Height (inches)	0.62	0.67	N.S.
Weight (pounds)	1.70	2.61	N.S.
Haemoglobin (gm/100 cc)	0.42	0.66	Sig. at 5%
Red blood cell (10^6/cu mm)	0.20	0.27	N.S.

48 children, aged 6 to 10 years, were fed on a basal rice diet. An equal number of children received rice + Mysore flour (1:1) in place of rice in their diet. The experiment lasted for 6 months.

The flour is acceptable to the consumer and forms good chapathi (unleavened bread) as well as bread. Animal experiments have shown that vegetarian diets mainly based on Paushtik atta promote a slightly better growth (9.6 gm/week) in rats as compared with diets based on wheat flour (8.3 gm/week). Feeding experiments on children age 6 to 10 years, over a 3-month period, have shown that wheat constituting 50% of the cereals in poor vegetarian diets could be replaced by Paushtik atta without affecting growth, general health and nutritional status. No significant difference is observable in nitrogen, calcium and phosphorus retention.

Balanced malt food. This product is a blend of cereal malt (37%), low-fat peanut flour (40%), roasted pulse (Bengal gram) flour (10%) and skim milk powder (10%), fortified with essential vitamins and minerals. Animal experiments have shown that the malt food, when incorporated at 10% level in a poor rice diet, has a supplementary value comparable to that produced by the same level of whole milk powder.[21]

In feeding experiments[22] extending for 9 months, weaned infants (age 9 to 20 months), fed a supplement of 2 ounces daily of the balanced malt food or Indian Multipurpose Food containing skim milk powder (formula C), showed highly significant increases in height, weight, red cell count and haemoglobin levels as well as improvement in nutritional status, as compared with a control group receiving a daily supplement of 2 ounces of rice (table 5).

Precooked weaning food. This has been prepared as flakes from a blend (30:40:30) of refined wheat flour, fortified with vitamins A and D, thiamine, riboflavin and calcium phosphate and carbonate. This product can also be given to children in the form of porridge or pudding with or without added milk. Feeding experiments with albino rats, weaned infants and young children are in progress.

Enriched macaroni. This has been prepared as ringlets from a blend (25:22.5:2.5:50) of low-fat peanut flour, wheat semolina, casein and tapioca flour fortified with thiamine, riboflavin, calcium pantothenate, vitamins A and D and calcium as phosphate and carbonate. It cooks readily in about 5 minutes and the cooked product can be given to weaned infants and young children in place of cereals as pudding or porridge with or without added milk. It contains about two to three times as much protein and three to six times as much calcium and B vitamins as do cereals. Feeding experiments with albino rats have shown that the product,

TABLE 5
INCREASE IN HEIGHT, WEIGHT AND HAEMOGLOBIN OF CHILDREN RECEIVING MALT FOOD AND INDIAN MULTIPURPOSE FOOD (Formula C)

	Control (rice) A	Exptl. (malt food) B	Exptl. (MPF) C	Difference in the increase (Experimental—Control with standard error) B—A	C—A	C—B
Girls						
Height (inches)	1.38	2.43	2.43	1.05 ± 0.25 ***	1.05 ± 0.25 ***	0.00 ± 0.25 N.S.
Weight (pounds)	2.26	5.04	5.18	2.78 ± 0.30 ***	2.92 ± 0.30 ***	0.14 ± 0.30 N.S.
Haemoglobin (gm/100 cc)	0.23	1.22	1.54	0.99 ± 0.32 **	1.31 ± 0.32 **	0.32 ± 0.32 N.S.
Boys						
Height (inches)	1.69	2.15	2.51	0.46 ± 0.14 **	0.82 ± 0.14 ***	0.36 ± 0.14* N.S.
Weight (pounds)	1.63	4.21	4.29	2.58 ± 0.27 ***	2.66 ± 0.27 ***	0.08 ± 0.27 N.S.
Haemoglobin (gm/100 cc)	0.36	1.27	1.21	0.91 ± 0.18 ***	0.85 ± 0.18 ***	−0.06 ± 0.18 N.S.

*** Very highly sig. ($P < 0.001$) ** Highly sig. ($P < 0.01$)
* Significant ($P < 0.05$) N.S. = Not significant

Each group had 18 children (10 boys and 8 girls) aged 9 to 20 months. The basal diet contained mainly rice along with small amounts of pulses and milk. The control group received daily an additional quantity of 2 oz. of rice, while the experimental groups received 2 oz. of balanced malt food or Indian MPF (formula C). Duration of experiment was 9 months.

when fed as the sole source of nutrients, promotes good growth (15 to 17 gm/week) over a period of 8 weeks. Feeding experiments with children are in progress.

Nutro macaroni. This product is prepared, according to a standard process employed for paste goods, from a blend (80:20) of wheat semolina and low-fat peanut flour and is fortified with B vitamins and calcium. It has over 19% protein. Feeding experiments[23] with 36 girls, age 4 to 11 years, have been conducted for a period of 6 months to assess the effect of replacing 50% of rice in their diet by Nutro macaroni. The results show significant improvements in weight, haemoglobin content and nutritional status of children receiving Nutro macaroni (table 6).

TABLE 6
EFFECT OF PARTIAL REPLACEMENT OF RICE BY NUTRO MACARONI IN THE DIET OF CHILDREN

Character	Control group			Experimental group			Difference in the increase (experimental minus control)
	Initial	Final	Increase	Initial	Final	Increase	
Height (inches)	51.02	51.81	0.79	51.07	52.05	0.98	0.19 ± 0.14 *
Weight (pounds)	50.33	54.37	4.04	49.99	56.70	6.71	2.67 ± 0.76 **
Haemoglobin (gm/100 cc)	12.16	11.81	−0.35	11.84	12.32	0.48	0.83 ± 0.17 ***
Red blood cell count (10^6/cu mm)	4.45	4.38	−0.77	4.38	4.46	0.08	0.15 ± 0.10

* Standard error of the mean based on 17 degrees of freedom
** Significant at 1% level
*** Significant at 0.1% level

Each group consisted of 18 girls ranging in age from 4 to 11 years. Fifty percent of rice in the basal diet was replaced by Nutro macaroni (enriched wheat macaroni) in the experimental group. Duration of experiment was 6 months.

Nutro biscuits. Protein-rich biscuits have been prepared by replacing wheat flour to the extent of about 40% with peanut flour.[24] The biscuit formula then consists of peanut flour 25-30, wheat flour 35-40, sugar 18, shortening 15, salt 0.75, calcium carbonate 0.5, glucose 0.5 and added thiamine, riboflavin, niacin and vitamins A and D. The biscuits are baked at 450°F for 4 to 5 minutes. The protein content of such biscuits is 16% to 17%. The biscuits have a shelf life of over one year.

Indian Multipurpose Food (MPF). This is a blend (75:25) of low-fat peanut flour and Bengal gram flour (*Cicer arietinum*) fortified with vitamins A and D, thiamine, riboflavin and calcium carbonate. Three formulations have been developed[25]: (A) seasoned, (B) unseasoned and (C) unseasoned with added skim milk powder (80:20).

Indian Multipurpose Food, when incorporated at 12.5% level in diets of experimental rats based on different cereals and millets, shows significant supplementary effect, and in this respect is comparable to the American MPF based on soybean flour.[26] A diet containing Indian MPF at 40% level as the sole source of protein, vitamins and minerals promotes good growth in rats over a period of 8 weeks.[27] The liver fat content is within normal limits. Thus the MPF proteins at 16% level have proved adequate for promoting normal growth. Different protein foods range in the following descending order in their ability to meet protein requirement in protein-depleted rats: skim milk powder, India MPF and Bengal gram.[28] In feeding experiments with children (table 7), it has been observed that supplementing the diet with 2 ounces per subject daily of MPF produces highly significant improvements in height, weight, RBC count, haemoglobin and nutritional status as compared with control groups not receiving the supplement.[29] The children receiving MPF also retain significantly larger amounts of nitrogen, calcium and phosphorus than the control children.[3]

TABLE 7
INCREASE IN HEIGHT, WEIGHT, HAEMOGLOBIN AND RED BLOOD CELL COUNT OF CHILDREN RECEIVING SUPPLEMENTS OF MULTIPURPOSE FOOD

Character	Control (rice diet)	Experimental (rice + MPF diet)	Difference significant at
Height (inches)	0.52	0.96	1%
Weight (pounds)	1.00	2.61	0.1%
Haemoglobin (gm/100cc)	0.13	1.00	5%
Red blood cell (10^6/cu mm)	0.07	0.33	1%

46 girls, aged 4 to 12 years, were divided into comparable groups and fed on a basal rice diet. The experimental group received a daily supplement of 2 oz. of Indian MPF, while the control group were given 1 oz. of corn starch and 1 oz. of cane sugar to equalise the caloric intake. Duration of experiment was 5 months.

Indian MPF containing 20% skim milk powder (formula C) is effective in the treatment of kwashiorkor in children[31] aged 2 to 3 years. Daily administration of 4 to 5 ounces of the protein food (supplying 40 to 50 gm protein), in the form of a porridge sweetened with sugar, causes marked improvement in the general condition of the subjects within 8 to 10 days. Oedema subsides from the 5th to 7th day and completely disappears in about 3 weeks. Dermatosis and hyper-

TABLE 8
CHANGES IN THE BODY WEIGHT OF CHILDREN SUFFERING FROM KWASHIORKOR TREATED WITH INDIAN MULTIPURPOSE FOOD (Formula C)

Name of patient	J.	G.	P.
Sex	male	male	male
Age (years)	3	3	2
Initial weight on admission (lbs)	26	21	23
Weight at time of clinical disappearance of oedma (lbs)	22	17	20
Time taken for clinical disappearance of oedema (days)	17	21	18
Final weight at time of discharge (lbs)	30	22	27
Total period of treatment (days)	65	39	44

The children were given daily 4 to 5 oz. of Indian MPF (formula C) supplying 40 to 50 gm protein.

pigmentation begin to heal by about the 10th day and are completely cured in 20 to 25 days (table 8). The serum protein level, which is below normal at the beginning, increases steadily, reaching normal values at the end of the treatment period (table 9).

Dried Milk Substitutes from Peanut and Soybean

Considerable attention has been given to the possibility of using nutritious milk substitutes prepared from peanut and soybean as supplements to the diets of infants and children.[32] During the past 10 years, the Institute has produced and distributed substantial quantities of fluid peanut milk fortified with calcium and vitamins to correspond to the composition of cow's milk. One pound of peanut kernel yields about 7 to 8 pounds of the milk substitute and it is generally liked by children, though the older age groups have some objection to the peanut flavour. When the emulsion is subjected to lactic fermentation, however, the peanut flavour is largely masked and the curd obtained from the product has been found to be acceptable to a large section of users.

Milk substitutes will have the advantage of compactness, ease of handling, transportation and storage if made into a dry, soluble powder. At present FAO and UNICEF are helping the Government of Indonesia to produce dried milk substitute from soybean, peanut and sesame for feeding children.[33] A method for the preparation of a spray-dried milk substitute from milk obtained from a blend

TABLE 9
CHANGES IN SERUM PROTEINS, RED BLOOD CELL COUNT AND HAEMOGLOBIN IN SUBJECTS BEFORE AND AFTER TREATMENT WITH INDIAN MULTIPURPOSE FOOD

	J		G		P	
Constituents of blood	Initial	Final	Initial	Final	Initial	Final
Haemoglobin (gm/100 cc blood)	9.42	11.60	6.95	9.42	8.28	10.87
Red blood cell count (10^6/cu mm blood)	3.00	4.20	2.90	3.75	2.98	3.60
Serum						
Total protein %	3.69	7.01	3.52	6.83	3.53	7.20
Albumin %	1.55	4.01	1.53	3.81	1.39	4.03
Globulin %	2.14	3.00	1.99	3.02	2.14	3.17
Nonprotein nitrogen	0.02	0.021	0.018	0.019	0.019	0.021

of soybean (2 parts) and peanut (1 part) has been developed at Mysore. The composition of the vegetable milk powder as compared with that of a modified milk food obtained by partial skimming of buffalo milk and subsequent processing is given in table 10. Animal experiments have shown that the vegetable milk powder promotes good growth (13.3 gm/week) in rats. A mixture of 80 parts of the vegetable milk powder with 20 parts of the modified milk powder promotes even higher growth (16.1 gm/week), the effect being not significantly different from that of the modified milk food (17.1 gm/week).[34] Feeding trials with infants will be undertaken shortly.

TABLE 10
CHEMICAL COMPOSITION OF VEGETABLE MILK POWDER
(values per 100 gm on moisture free basis)

Constituents	Vegetable milk powder	Modified milk food
Protein (gm)	24.7	24.8
Fat (gm)	18.6	18.3
Carbohydrate (by diff) (gm)	51.5	51.4
Ash (gm)	5.2	5.5
Calcium (gm)	1.05	1.04
Phosphorus (gm)	1.13	1.06
Iron (mg)	7.2	6.5
Thiamine (mg)	0.85	0.86
Riboflavin (mg)	1.23	1.24
Nicotinic acid (mg)	8.2	8.3
Vitamin A (IU)	2120	2188
Vitamin D (IU)	400	400

The vegetable milk powder was a spray-dried product from a blend (2:1) of soy and peanut milks.

Peanut Protein Isolate and its Utilisation

There is increasing recognition that isolated proteins from cheap raw materials like oilseed meals offer scope for extensive use in specialised food preparations for feeding infants and children for the treatment of protein malnutrition. Isolated proteins have certain advantages over the parent raw materials in that they are free from 1) insoluble and indigestible carbohydrates which may swell and interfere in the digestion and utilisation of proteins, particularly in children, and 2) odoriferous and bitter principles, growth inhibitors and other interfering materials like phytates which may be present in the natural materials and affect palatability, digestibility and nutritive value. Further, isolated proteins are 2 to 4 times as concentrated as the protein source and possess a bland taste, permitting ready blends with other natural foodstuffs and thus increasing their protein content without affecting palatability.

Among the different oilseeds and oilseed meals, peanut and low-fat peanut meal easily lend themselves to the isolation of the protein. Conditions for the processing of peanut kernel[35] or expeller cake[36] for the separation of protein, oil, starch and fibre fractions have been standardized and improved at the Central Food Technological Research Institute, Mysore. The protein obtained by either process is of a high quality and offers possibilities for use in the preparation of

protein supplements suitable for the treatment and prevention of protein malnutrition. A pilot plant handling 5 cwt of kernels or cake during a 6- to 8-hour working day has been in operation at the Institute for some months.

From experiments with adult rats (150 gm) fed at 10% protein level, it has been ascertained that the isolate possesses a higher digestibility coefficient but slightly lower BV as compared with the total proteins in the cake[37] (table 11). The latter difference could presumably arise from the water-soluble nonprotein fractions which are lost as whey after the precipitation of the protein. Supplementing the isolate with dl-methionine at 0.6% level in the diet enhances the BV to a degree comparable to a blend of peanut protein and casein (1:1) fed at similar levels.[37] However, the values obtained in either case are lower as compared with casein used as the sole source of protein.

TABLE 11
DIGESTIBILITY COEFFICIENT AND BIOLOGICAL VALUE OF PEANUT PROTEIN FROM KERNEL AND EXPELLER CAKE

Source of protein	Digestibility coefficient	Biological value
Peanut protein isolate from kernel	97.9	52.1
Peanut protein isolate from expeller cake	92.2	51.9
Peanut protein isolate + 6.0% dl-methionine	98.2	59.7
Peanut protein isolate + casein (1:1)	96.6	62.3
Peanut cake, whole meal (control)	90.1	56.0
Casein (control)	96.5	73.7

(10% protein level in the diet of experimental rats having 150 gm body weight).

Protein efficiency ratios determined on comparable groups of weanling rats at 10% protein level in adequate diets over an 8-week period indicate that supplementation of peanut protein or a blend of peanut protein and casein (1:1) with l-lysine and dl-methionine results in significant improvement in growth-promoting value (table 12).

TABLE 12
NUTRITIONAL STUDIES ON ISOLATED PEANUT PROTEIN

Source of protein	Protein efficiency ratio 4 weeks	8 weeks
Casein	2.83	2.32
Peanut protein isolate	1.58	1.42
Peanut protein isolate + casein (1:1)	2.22	1.91
Peanut protein isolate + 1.7% lysine + 2.1% methionine	1.92	1.98
Peanut protein isolate + casein (1:1) + 4.25% lysine + 3.90% methionine	3.14	2.53

Equivalent amounts of l-lysine HCl and dl-methionine were used.
12 weanling rats in each group; protein level, 10%; duration, 8 weeks.

Blends of different vegetable protein, viz., peanut, soybean, Bengal gram (*Cicer arietinum*) and sesame (*Sesamum indicum*), with or without casein, and amino acids have been evaluated for their growth-promoting effects in young albino

rats (table 13). These results bring out the possibilities for enhancement of growth value through mutual supplementation of protein and/or fortification with deficient amino acids.

TABLE 13
NUTRITIONAL STUDIES ON BLENDS OF ISOLATED VEGETABLE PROTEINS WITH OR WITHOUT SUPPLEMENTATION WITH CASEIN OR AMINO ACIDS

Source of protein	Level of protein in diet % (N x 6.25)	Average intake		Average gain in body wt. (gm)	Average PER
		Diet (gm)	Protein (gm)		
Skim milk powder	10.6	630.7	66.5	150.0	2.13
Casein	10.9	561.0	60.9	123.0	1.98
Peanut protein	11.0	488.8	54.0	83.2	1.53
Soy protein	10.8	427.3	45.9	72.0	1.56
Peanut + soybeans (1:1)	11.0	518.9	57.0	94.7	1.66
Peanut + soybeans (1:1) + 2.0% lysine + 3.0% methionine	11.3	544.6	61.6	120.6	1.91
Peanut + soybeans + casein (3:3:4)	11.0	545.5	59.9	109.1	1.81
Peanut + soybeans + casein (3:3:4) + 0.75% lysine + 2.4% methionine	11.3	574.8	65.0	137.6	2.07
Soya + casein (1:1)	10.7	540.2	57.6	108.0	1.84
Soya + casein + 1.8% methionine	10.9	571.7	62.4	139.5	2.15
Peanut + bengal gram + sesame (5:3:2)	11.1	533.9	59.2	96.2	1.61

Equivalent amounts of l-lysine HCl and dl-methionine were used; 9 weanling rats per group; duration was 8 weeks.

High-protein blends. Two blends, consisting of 1) peanut protein, soy protein and casein (30:30:40) fortified with l-lysine HCl (0.75%) and dl-methionine (1.95%) and 2) peanut protein and skim milk powder, (58:42—the proportion of proteins being 3:1), have been used in 8 cases of kwashiorkor in children aged 2 to 5 years. The amino acid composition of the 2 blends as compared with that of milk protein and FAO reference protein pattern is given in table 14. The subjects each received 30 gm protein per day in 5 doses in the form of porridge. Supplements of mineral salts and vitamins were also administered. Results, summarised in table 15, show that protein isolates, suitably blended with milk proteins or with required amino acids, can be used for the treatment of protein malnutrition in children. Further trials are in progress.

TABLE 14
AMINO ACID COMPOSITION OF PROTEIN BLENDS
(gm amino acid/16 gm N)

	Protein Blend No. I	Protein Blend No. II	Cow's milk	FAO reference protein pattern
Lysine	7.0	4.4	7.4	4.2
Tryptophan	1.3	1.1	1.4	1.4
Methionine	4.3	1.4	2.8	2.2
Methionine + cystine	5.3	2.8	3.9	4.2
Threonine	3.8	3.3	4.6	2.8
Phenylalanine	5.3	5.3	5.5	2.8
Leucine	8.3	7.9	12.1	4.8
Isoleucine	5.6	4.7	6.7	4.2
Valine	5.9	5.2	7.1	4.2

TABLE 15
CHANGES IN BODY WEIGHT AND SERUM ALBUMIN ANALYSIS OF CASES OF NUTRITIONAL OEDEMA SYNDROME TREATED WITH DIFFERENT PROTEINS OR PROTEIN FOODS

	Blend I (1)		Blend II (2)		Skim milk powder (3)		Casein (4)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Number of cases	3		5		15		2	
Age (range in years)	3		2—5		—		2—4	
Days for disappearance of oedema	7.5	5—10	10.6	8—15	6.7	1—12	7	6—8
Days to reach minimum weight	7.6	6—10	10.6	8—15	7.7	1—19	—	—
Number of days for a 1 lb body gain	4.3	4—5	7.2	6—9	13.25	7—25	5.8	5—7
Days for disappearance of diarrhoea	3	2—4	6	4—7	5.2	2—12	3	2—4
Rise of serum albumin on 10th day	1.02	0.9—1.1	0.91	0.75—1.0	0.76	0.2—2.28	0.96	0.8—1.2
Rise of serum albumin on 30th day	2.18	1.65—2.5	1.89	1.40—2.5	1.77	1.03—3.45	1.7	1.5—1.9

(1) Blend I

Peanut protein isolate (gm)	26.0
Soybean protein (gm)	33.0
Casein (gm)	41.0
L-lysine HCl (gm)	0.75
dl-methionine (gm)	1.96

(2) Blend II

Peanut protein isolate (gm)	52.0
Skim milk powder (gm)	48.0

Essential minerals added per 100 gm of blend

Dipotassium hydrogen phosphate	3.5 gm
Disodium hydrogen phosphate	2.0 gm
Potassium bicarbonate	1.0 gm
Tricalcium phosphate	0.5 gm
Calcium carbonate	0.5 gm

(3) School of Tropical Medicine, Calcutta

(4) Central Food Technological Research Institute, Mysore

Milk foods based on isolated peanut protein. The production of blended and dried vegetable milk products, referred to earlier, has certain drawbacks in that large quantities of unextracted residual solids have to be handled and utilized. Protein isolates have an advantage in this regard since in their production other discrete products including high-grade refined oil are obtained. Methods for preparation of weaning foods using peanut protein isolate have, therefore, been developed. In general, the wet protein isolate, from either the cake or the kernel, is dispersed in water, adjusted to pH 7.3 and mixed with a known amount of milk powder (whole or skim), dextrimaltose or other sugar, buffer salts etc. Vegetable fat is added if necessary to bring up the fat content. The mixture is homogenized and spray dried.

The product is fortified with vitamins and minerals by dry mixing and may be flavoured. The complete product is comparable in composition and nutritive value to milk and has a degree of acceptability. In actual practice, one part of the skim or whole milk powder can in this way be used to prepare 5 parts of the composite milk food. The composition of a product thus obtained is given in table 16. Such a product can also be lactic fermented to make a good curd and buttermilk. Feeding trials with experimental animals and children are in progress with this product.

TABLE 16
CHEMICAL COMPOSITION OF INFANT FOOD FROM ISOLATED PEANUT PROTEIN CONTAINING 20% MILK SOLIDS
(values per 100 gm)

Moisture (gm)	3.0	Niacinamide (mg)	6.0
Protein (gm)	26.0	Pyridoxine (mg)	0.6
Fat (gm)	18.0	Calcium pantothenate (mg)	1.5
Carbohydrate (gm)	48.0	Folic acid (mg)	0.3
Minerals* (gm)	5.0	Vitamin B_{12} (μg)	2.0
Calcium (gm)	1.0	Choline (mg)	100.0
Phosphorus (gm)	0.8	Vitamin C(mg)	30.0
Iron (mg)	4.0	" E(mg)	5.0
Thiamine (mg)	0.6	" K(mg)	0.5
Riboflavin (mg)	1.0	" A(IU)	1500
		" D(IU)	400

Of the total protein 66% is derived from peanut and the rest from milk solids.
* Other essential minerals are present in adequate amounts.

A blend (1:1) of solubilized peanut protein and skim milk powder contains 60% to 61% protein and can be readily fortified with essential vitamins and minerals. Such a food, the chemical composition of which is shown in table 17 reconstitutes readily into a milk in warm water. Hence, it can be easily administered to young children suffering from protein malnutrition and can also be used as an effective supplement to the diets of weaned infants.

Vegetable protein isolates have shown the possibilities for formulating, in forms that are easily acceptable and conform to prevailing dietary habits, various low-cost processed foods of balanced composition and easy digestibility suitable

TABLE 17
CHEMICAL COMPOSITION OF HIGH-PROTEIN FOOD SUITABLE FOR TREATMENT OF PROTEIN MALNUTRITION
(values per 100 gm)

Moisture (gm)	3.0	Niacinamide (mg)	10.0
Protein (gm)	62.0	Pyridoxine (mg)	1.0
Fat (gm)	1.0	Pantothenic acid (mg)	5.0
Minerals (gm)	8.0	Vitamin B_{12} (μg)	2.0
Carbohydrate (gm)		Folic acid (mg)	0.5
(by diff)	26.0	Choline (mg)	100.0
Calcium (gm)	1.0	Vitamin C(mg)	30.0
Phosphorus (gm)	1.0	Vitamin E(mg)	5.0
Iron (mg)	12.0	" A(IU)	6000
Thiamine (mg)	1.0	" D(IU)	400
Riboflavin (mg)	1.8		

The food is made from a 1:1 blend of peanut protein and skim milk powder.

for supplementing the diets of weaned infants, young children and others of different age groups.

Extension and Field Trials

Apart from the work of laboratory and clinical evaluation reported here, it must be stated that considerable amounts of consumer acceptability trials and extension work, so necessary in the popularisation of any new food, have been carried out with most of the products developed. For example, the widespread public acceptance of tapioca macaroni in the populous state of Kerala has been amply demonstrated.[38] Proposals have for some time been under consideration for the setting up of a plant for its production, with an initial capacity of 20 tons a day. Mysore flour has been used with success in distressed areas in certain districts of Madras and Mysore States. Consumer acceptability trials with Paushtik atta in Uttar Pradesh have revealed that the majority of the people generally preferred this product to atta or wheat flour. The production of Nutro biscuit has been taken over by private industry and it is now being routinely manufactured. On occasions, Red Cross and other charitable organizations have been buying and distributing this product in distress feeding programmes.

The Indian MPF is now recognised as among the most effective supplements to dietaries based on cereals and low in proteins. Since October 1957, this Institute has produced some 300 tons of the product. It has been utilised for experimentation and demonstration work as well as for distribution by various agencies, including the American Meals for Millions Foundation, in community and school feeding and other social service programmes. The daily production of MPF at Mysore for such purposes now averages 1500 pounds. Several Municipal and Government schools in the States of Madras and Mysore have already taken or are taking to its use in midday school feeding programmes. The Madras Government is currently committed to a daily off-take of 2 tons of this product which will shortly be produced by industry at one of the cities in the State.

The recognition of the scope that peanut flour and peanut protein offers in the improvement of protein nutrition has resulted in the Indian Government's (Ministry of Food) plans, now under way, for the setting up of two 10-ton-per-day plants for production of edible quality peanut flour. This programme is being implemented with UNICEF help and assistance.

Conclusion

Protein malnutrition has perhaps been the most widely prevalent among nutritional deficiencies in many of the underdeveloped and overpopulated countries of the world. It may range from extremes of protein deprivation to milder degrees, qualitatively and quantitatively, of inadequacy. With infants and weaned and

preschool children, as well as with expectant and nursing mothers, it may assume serious proportions. This has led to an increasing realization of the importance of protein-rich sources that could be successfully used to augment protein supplies taking into account availability of raw materials as well as economic, socio-religious and other factors including consumer acceptability. Our efforts in this direction have been primarily based on the peanut. The annual production of this oilseed is around 5 million tons (in shell), and some 1.5 million tons of peanut cake containing 50% protein is made available every year as a byproduct of the oil milling industry. However, the major part of this product is used as manure and only a relatively small amount goes for animal feeding. With some care and attention to the kernel prior to extraction of oil, an entirely edible quality peanut meal can readily be had while, with the integrated processing technique, high-purity peanut protein can also be obtained economically. With either procedure, a variety of products could be formulated conforming to diverse existing dietary patterns and with good supplementary food value.

The following have been discussed in this report.

1) Basic foods such as tapioca macaroni and Paushtik atta which can augment staple food supplies and which, at the same time, contribute to increased protein intake.

2) High-protein foods such as enriched macaroni which can be used as weaning foods and generally for meeting protein deficiency states.

3) Supplementary foods such as the MPF which in small amounts can add substantially to the protein requirements of preschool and school children as well as of others and which can also be used in the treatment of protein malnutrition.

4) Processed foods based on protein isolates which can be used in a variety of ways such as in the treatment of kwashiorkor, as concentrated protein foods for infants and children and as milk foods.

This report has indicated the many possibilities that these and similar products can offer. While there are data on their experimental and clinical evaluation as well as their feasibility and acceptability, more work remains to be done in order to make them available to large sections of the population. Although our initial efforts have been mainly with utilisation of peanut meal and peanut protein, the processes developed lend themselves with modifications to the utilisation of other protein-rich sources like cottonseed, sesame, coconut, etc. Work along these lines is in progress.

The recognition that suitable blends of vegetable proteins, with or without fortification with amino acids, could compare favourably with animal proteins, together with proven technological possibilities for processed protein-rich foods and protein isolates, would widen the scope for use of vegetable proteins for immediate nutritional improvement and rehabilitation.

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DISCUSSION

DR. DEAN: There is one point I would like to mention because really it will come up on a number of occasions—the necessity to regard speed of recovery from kwashiorkor as one of the factors to take into consideration when evaluating, if you like that word, the various foods that are used.

You used figures that indicate an increase of total serum protein of 0.1 gm per day. Your figures average something like 1.0 gm of increase in total serum protein in 10 days. We regard that as very poor. We say that on the diets we are using, even the best of our casein-dry skim milk-sugar diet or the biscuit mixture, both of which are giving 3.5 gm of protein/kg, you should get an increase of about 0.2 to 0.25 gm of total protein per day.

DR. SCRIMSHAW: I would like to ask you one more question to answer at the same time. That is, the shipment of peanut flour that we received recently in Guatemala for use in developing formulas has not given the results in animal experiments that apparently it gave previously in other trials. Have you any information on the stability of the protein quality of peanut flour?

Then before passing Dr. Dean's question to you, I would like to comment briefly on this. As we pointed out yesterday, there is some dissociation between the rate of recovery of serum protein and recovery from other characteristics. I would disagree with him that we should take the rate of recovery of serum protein or serum albumin as a matter of primary significance.

DR. GYÖRGY: I have a question in the same direction.

You said, "I received three samples of protein isolate, two peanut isolate, one the usual, and then an isolate made from expeller cake, and then an isolate from soybean." I don't know what type of peanut isolate you had, but my protein efficiency ratio is far below 1.5 on one, and the expeller cake protein isolate is less than 1.0.

The same question: Is the stability of the protein good? You used the product at 10% level as usual?

DR. SREENIVASAN: On the recovery of serum protein, in our experiments we get complete recovery of serum proteins, total proteins, in 3 weeks in these studies. It is to be expected that with a protein preparation of this kind, where you substitute other bulk protein for a large part of milk, the rate of recovery will be less. However, our main aim has been to see that there is recovery and that, even if it takes some time, it makes us self-sufficient. We admit there is room for improvement, and we expect that if we were to use blends of proteins, which we expect will be available shortly, this rate of recovery would also be improved.

Regarding the stability of peanut flour, under reasonably good conditions of packaging, peanut flour with 5% to 8% of oil keeps for about one year; but sometimes, due to indifferent storage or other causes, there may be

deterioration during storage even within a year. The protein isolate has no reason to get spoiled at all. I can't tell why the PER was low. I gave the results that we obtained.

DR. ALTSCHUL: I would like to ask Dr. György whether he autoclaved the material prior to testing. If he did, one would expect there would be a difference in the BV of his material as against the other.

DR. GYÖRGY: No.

DR. ALTSCHUL: I doubt very much that there is any deterioration of protein. The point I would like to raise, however, is that, since there is no information on the control, we don't really know that each batch is the same as the next one. Therefore, if there are differences obtained, they may not be intrinsic differences in the protein particularly. They may be due to differences in the day-by-day processing conditions.

DR. HAND: Is there a possible outlet for protein isolates for general feeding in the prevention of protein malnutrition, or are these compounds so expensive that they can be used only in curative studies?

DR. SREENIVASAN: We also have it as our objective to see that protein isolates are used for basic supplementation. At present, with the materials balance and cost figures that we have, we estimate that the price of this isolate would be between 20 and 30 cents per pound. The price of skim milk powder without any subsidy is much more.

DR. MILNER: First of all, I would like to pay tribute, as a representative of the UNICEF staff, to the type of pioneering work in these protein-rich foods which has been going on at the Mysore laboratory. It is only on the basis of this type of work that we are able to step in and extend this area into the commercial aspects, as Dr. Sreenivasan has told you, and I commend their energy to all of you in this regard.

With reference to the peanut flour mentioned by Dr. Scrimshaw, this is not the same type of peanut flour, by any means, that Dr. Sreenivasan is speaking of. The peanut flour Dr. Scrimshaw received was produced in this country in a single special run which was never repeated and under considerably different conditions than the Indian process, as I observed it. Dr. Scrimshaw's statement suggests that this material has indeed deteriorated in storage. The material was roughly 2 years old. While it had been in cold storage, we are not sure of the conditions precisely. I think this should be taken into consideration. Also, the material received may have been heated considerably longer than the other.

Finally, the original values that we had on that flour when it was fresh were considerably better than you have reported to us. I must assume that some deterioration has occurred.

Nitrogen and Water Balance Studies in Infants Fed with Vegetable Protein Foods

Chiung-Fei Chen, Huoyao Wei,[1] *Po-Chao Huang, and Ta-Cheng Tung*

FOUR KINDS OF BABY FOODS of vegetable origin, in flakes, have been developed by Dr. Tung and associates of the Department of Biochemistry of National Taiwan University College of Medicine. The ingredients and composition of these flakes are shown in table 1. In order to test these foods with infants, 4 male orphans were each subjected to 6 balance studies of nitrogen and water. Their ages were: baby C, 1 year and 2 months; babies W and S, 1 year and 4 months; and baby L, 2 years and 3 months. Three of them (W, S and L) were slightly underweight.

TABLE 1
INGREDIENTS AND COMPOSITION OF VEGETABLE PROTEIN FOODS (PER CENT)

Flake	Ingredients					Composition				
No.	Rice	Soybean	Wheat	Sesame	Peanut	Protein	Fat	CHO	H_2O	Cal.
8	20	60	20	—	—	23	4.5	67	5.4	412
9	20	40	20	20	—	24.1	20.5	50	5.4	495
10	20	20	20	—	40	25.3	19.2	49	5.5	483
11	20	40	20	—	20	24.2	11.9	58	5.9	448

They were admitted to the metabolic ward of the Department of Pediatrics. The food formulas and their compositions are shown in table 2. The method of balance studies was as follows. Cow's milk was used as the reference diet. Preparatory period was 3 days after the diet was changed from one flake formula to another and was 1 week after the change from cow's milk to flake formula or vice versa. Collection of stools and urine was carried out for 3 days in each balance. Urine and stools were preserved by the addition of HCl. Carmine was used as the stool marker. Metabolic bed was used for the collection. The food formulas were made to supply approximately 3 to 3.5 gm of protein and 110 to 120 calories/kg/day. As shown in table 2, sugar and/or butter were added to each formula to increase the calories. Vitamins and minerals were used as supplementation daily. The amount of flakes given to each boy was about 125 gm per day.

Beginning on September 11 and concluded on November 6, 6 balances were carried out in a room without temperature and humidity control. The room temperature was between 22° and 31° C and humidity between 75% and 89%

[1] Presented paper

TABLE 2
FOOD FORMULAS AND THEIR COMPOSITION

Balance No.	Formulas		Composition per 100 gm Protein	Fat	CHO	H_2O	Cal.
1	A) Fresh cow's milk + 5% sugar		3.1	3.0	8.2	85.7	74
	B) 10% Sugar + 5% cornstarch				11.0	89.0	45
2	Flake no. 8	25 gm					
	Butter	10					
	Sugar	20					
	Water	200	2.1	3.5	14.7	79.7	102
3	Flake no. 9	25					
	Sugar	25					
	Water	250	1.9	1.8	12.5	83.8	76
4	Flake no. 10	25					
	Sugar	25					
	Water	250	1.8	1.8	12.8	83.6	66
5	Flake no. 11	25					
	Sugar	25					
	Butter	3					
	Water	180	2.4	1.7	8.3	87.6	60
6	Fresh cow's milk	100					
	Sugar	5					
	Cornstarch	10					
	Water	150	2.4	3.4	8.9	85.3	79

throughout the whole period of studies. Because the temperature and humidity were not ideal for balance study, infants were washed before and after the collection periods and each of the second skin washes, in which infants' shirts were soaked, was also analyzed for nitrogen.

The nitrogen retentions, calculated as percentage of nitrogen intake, in 6 balances are shown in table 3. Body weight gain during the 7-week period of studies varied from 0.7 to 1.3 kg. The flake foods were well tolerated and accepted by these boys, at least during this period. Balance 2 feeding with flake no. 8, which consists of 60% soybean and 20% each of rice and wheat, showed a higher percentage of nitrogen retention than the others except balance 6 with cow's milk feeding. It is therefore considered that flake no. 8 can be used for weanling baby feeding.

Water balance studies, done at the same time as nitrogen balances, are summarized in table 4. The table reveals that water balances were negative during the periods of cow's milk feeding and positive, except in one, during those

TABLE 3
NITROGEN RETENTION IN INFANTS

N Balance No.	Formula	N Retention % of Intake
1	Cow's milk	28
2	Flake no. 8	30
3	Flake no. 9	21
4	Flake no. 10	18
5	Flake no. 11	20
6	Cow's milk	45

of flake foods feeding. Because baby L is older than the others, his water balance is shown in a separate column of the table. It shows that water intakes increased as the room temperature rose. It is noted that at a given urine specific gravity the urine volume, expressed as gm per 100 calories metabolized, was larger during the cow's milk feedings than during the flake foods feedings. It is assumed that cow's milk feeding offers much higher osmolar load to the kidneys than does vegetable foods feeding.

TABLE 4
WATER BALANCE
(gm/kg/day)

Food	Vegetable Flake Food								Cow's Milk			
Balance No.	2		3		4		5		1		6	
Av. Room Temp.	26.9		25.4		24.5		22.9		28.4		26.3	
Water Intake	120	110	130	110	130	110	80	70	160	170	110	100
Water Balance												
L (2 yrs 3 mos)		5		—1		1		3		—1		—9
C (1 yr 2 mos)	6		2		5		9		—1		2	
W (1 yr 4 mos)	3		—2		2		3		—7		—3	
S (1 yr 4 mos)	12		0		4		6		1		—2	
Average	7	5	0	—1	4	1	6	3	—2	—1	—1	—9

We have found that the curves of the average body weights of Taiwan infants of both sexes reported by Wu are, compared with those of rather selected infants of Shanghai and Tokyo reported by Gin and Kuriyama respectively, above them during the first 4 months of life and below them after 4 months of age. The common practice of delayed and inadequate weaning in Taiwan is believed to be the main cause of the smaller body weight of infants after 4 months of age.

According to our recent study 4.4% of the in-patients admitted to the Department of Pediatrics of National Taiwan University Hospital during the past 5 years were found to have apparent signs and symptoms of malnutrition; 2.19% of the out-patient cases of 1959 of the same Department were diagnosed as malnutrition. The age incidence of the patients studied shows two peaks, one under 4 months of age and the other between 8 and 16 months of age. Of the in-patients under 4 months of age, 81.1% were fed either artificially or with a mixture of artificial and breast feeding; 61% of those over 8 months of age were found to have had delayed weaning and the other 39% were inadequately fed. Of these in-patients, 57.3% had diarrheal diseases including 10 cases of Shigellosis. The mortality rate of these in-patients under 2 years of age with malnutrition was 25.8% and of those between 2 and 4 years, 48.5%. More detailed observations on these patients will be published elsewhere.

From this work with malnutrition patients in Taipei area it is apparent that delayed and inadequate weaning is one of the main causes of malnutrition in infants and young children in Taiwan, where cow's milk and its products are rather expensive. Flake no. 8 of this study has much higher protein content than rice, which has been commonly used for feeding weanlings in Taiwan, and showed

best nitrogen retention among the 4 kinds of mixture flakes. It was well tolerated and accepted during this study period and seems to be a useful supplementary food for weanling infants. However, experiments of feeding these flakes for longer periods and to younger groups of infants should be undertaken.

SUMMARY

Four male children aged from 1 year and 2 months to 2 years and 3 months were subjected to nitrogen and water balance studies by feeding them 4 kinds of flakes of different mixtures of vegetable protein sources and cow's milk during a period of 7 weeks.

Feeding with the flakes of a mixture of 60% soybean and 20% each of rice and wheat showed the highest nitrogen retention of the four, but lower retention than that of cow's milk feeding. Body weight gain of these 4 boys during this study period varied from 0.7 to 1.3 kg. All of the 4 kinds of flakes were well tolerated and accepted during this period.

Water balances were negative during the periods of cow's milk feeding and positive, except in one balance, during the periods of flake foods feeding. At higher room temperature the water intake increased. At a given urine specific gravity the urine volume, expressed as gm per 100 calories metabolized, was higher during the cow's milk feeding than that during the flake foods feeding.

Experiments of feeding these vegetable protein flakes for longer periods and to younger groups of infants should be undertaken.

Production of High-Protein Food from Fermented Soybean Products*

Yosito Sakurai and Masahiro Nakano

THIS RESEARCH is primarily aimed to produce new types of foods like dehydrated "Natto" or "Kojibean" from soybean, modifying its nutritive value and flavor.

Ultimately, after proper processing, these products, which are of high protein content as well as high digestibility, good palatability and storage life, are expected to be used in diets of weanling infants or small children in malnutrition regions.

"Natto" is one of the soybean food products the origin of which can be traced to very old times in Japan. However, due to relatively high concentration of moisture, "Natto," prepared usually in small-scale plants of poor quality control, is not suitable for long-term storage. In addition, its characteristic odor and viscous sticky substance, which are locally preferred by accustomed consumers, might be considered as unfavorable for general food. Consequently, for this purpose it is necessary to investigate such subjects as selection of the microorganism employed in the fermentation, devising of proper manufacturing equipment, and suitable conditions under which the fermentation process as well as dehydration of the fermented products are run.

After various investigations on these subjects, a suitable manufacturing method was found. In brief, it may be stated as follows: soybean or soybean flake, moistened through either soaking in water or spraying with a certain volume of hot water, is permitted to steam in rotary cooker under pressure, instantly thereafter being cooled in the same cooker under reduced pressure to 45°C. After inoculating at this temperature with starter culture of *Bacillus natto*, the materials are allowed to ferment in special fermenter of which temperature is adjusted to 40 to 43°C for 6 to 8 hours. Thus-fermented soybean products are chopped into paste so as to be spread over metal trays for drying at low temperature, less than 60°C, either in vacuum or aeration, until moisture content is reduced to less than 5%, followed by milling into powder. At the pilot plant furnished with equipment specially designed for this purpose, 50 kg of soybean were treated as one lot in

* Editor's Note—This is a condensed summary of a detailed report prepared for UNICEF.

the way mentioned above in order to provide samples for examination on their nutritive value and acceptability at the National Institute of Nutrition.

With regard to the microorganism employed in the fermentation, *B. natto* SB 3010 has so far seemed to be most suitable for this purpose, because of its vigorous development on steamed soybean resulting in partial degradation of protein to amino acids. In addition, it can be easily multiplied in such artificial nutritive medium as pepton/glucose/yeast extract/KH_2PO_4 at 37°C in preparation of starter culture for bulk fermentation. The optimum concentration of this starter culture appears to be 0.5% to 1.0% by weight of steamed and cooled soybean.

Regarding the treatment of raw materials, recommendable conditions, which might be modified more or less, are: 1) with whole soybean, soaking in water until 110 to 130 parts of water are absorbed, and steaming at 120°C for 30 minutes; 2) with defatted soybean, spraying with 100 to 120 parts of hot water at 90°C, and steaming at 121°C for 30 minutes.

Further, the most favorable conditions for fermentation might be attained as follows. When the temperature of cooked material, either whole or defatted soybean, comes down to 45°C or below, starter culture equivalent to 0.5% to 1% of cooked material is mixed thoroughly with the material as aseptically as possible and allowed to start fermentation at 40 to 43°C. With regard to the fermentation time in this case, as opposed to the conventional method using 16 hours or more, around 6 hours appears to be favorable, from the view points of palatability involving both flavor and taste, digestibility and yield of finished product, as well as simplicity of process. So far as apparent digestibility is concerned, 6 hours of fermentation might not be considered as the best condition, because the extent of protein degradation depends upon the time of fermentation, namely, the longer the time of fermentation, the higher the extent of protein degradation. However, it was shown that when the time was prolonged beyond 10 hours, the ratio of soluble nitrogen and amino nitrogen to total nitrogen was greatly increased, but this fact was counteracted by production of ammonia unfavorable for palatability and probably for nutritive value as high-protein food.

As the result of primary tests of raw materials including whole and defatted soybean, the former was considered superior to the latter, principally in palatability and ammonia content of finished products after 8 hours of fermentation.

With pilot plant including appropriately designed rotary cooker, fermenter and vacuum dryer for this work, 50 kg of soybean was treated as one lot, following the preparation method established in laboratory-scale experiment. In this trial, it was demonstrated that bulk fermentation of at least 50 kg of steamed soybean was possible without any trouble in the process or any difference in the quality of the finished product compared with that of small-scale fermentation, provided the process after cooking and until fermentation was carried out aseptically.

As a practical and technical problem, drying of fermented products is the most important problem to be solved throughout the manufacturing process. Being rather heat sensitive, the fermented product should be dried at as low a temperature as possible. Comparison of vacuum drying with aeration drying

TABLE 1
CHEMICAL COMPOSITION AND MICROBIAL COUNTS OF FINISHED PRODUCTS BEFORE AND AFTER STORAGE

Variety	Storage period (in months)	Moist.	Total N*	Soluble N* (in per cent)	Formol N* (in per cent)	Fat*	Fatty* acid	Soluble N / Total N	Formol N / Total N	Number of bacteria/gm
Whole soy bean	0	4.19	7.25	3.22	0.22	23.9	0.07	44.2	3.15	3.7×10^8
	5	6.89	7.27	2.32	0.11	23.6	0.51	30.7	1.51	5.0×10^7
Crushed whole soybean	0	8.32	7.29	5.01	1.26	24.4	0.07	68.7	17.3	6.5×10^7
	7	9.06	7.06	3.48	0.43	23.5	0.56	49.3	6.1	3.0×10^8
Defatted soybean	0	10.9	9.03	6.78	2.07	1.47	0.0	76.2	23.0	2.7×10^7
	7	12.3	9.00	6.04	1.11	1.66	0.03	67.1	12.3	2.3×10^8

* Data are shown on dry basis

showed that the finished product in vacuum 3 mm Hg was superior in quality to that by aeration at 60°C. However, in pilot plant it took ordinarily more than 10 hours to dry the product until its moisture content was reduced to 5%. In order to apply this method to practical manufacturing, further investigation to shorten the time or simplify the process might be necessary.

A remarkable change in composition caused by fermentation for 8 hours was the increase of the fraction of water-soluble nitrogen up to 38.5% of total nitrogen, and this value was further enhanced up to 65% through autolysis of the powder in water at 40°C for 3 hours. Also during this fermentation, about 5% of total amino acids were liberated, probably with no significant change in their composition.

Microbial counting shows considerable amounts of *B. natto* in the finished products, even when dried at as high as 105°C for 7 hours. Apart from the hygienic problem, the effect of these microorganisms on the property of the finished product during storage or secondary processing has not yet been investigated.

During storage for 5 to 7 months at room temperature some changes were observed in composition, table 1. Water-soluble nitrogen was reduced, though the reason has not been clear, and the acid value of the fat was increased to some extent. Further investigation in this respect may need to be undertaken.

The amino acid compositions are presented in table 2.

There are still problems to be solved in the manufacturing process, particularly in the process of dehydration, as well as in treatment of raw material. With dehydration, another method of much higher efficiency at low cost should be investigated for practical application. Respecting treatment of raw material,

TABLE 2
TOTAL AMINO ACIDS IN SOYBEAN, STEAMED SOYBEAN AND FERMENTED SOYBEAN PRODUCT
(gm of amino acid/100 gm of protein)

Amino acid	Raw soybean	Steamed soybean	Product fermented 8 hrs	Product fermented 16 hrs
Glycine	3.6	3.3	3.4	3.5
Alanine	4.7	4.4	4.5	4.5
Valine	5.8	5.6	5.6	5.7
Isoleucine	5.1	5.2	5.2	5.2
Leucine	8.6	8.6	8.6	8.6
Aspartic acid	11.0	11.0	11.0	11.0
Glutamic acid	18.0	18.0	18.0	18.0
Lysine	6.9	6.8	6.8	6.8
Arginine	6.8	5.6	5.3	5.0
Histidine	3.3	3.3	3.2	3.1
Phenylalanine	4.9	5.1	5.2	5.2
Tyrosine	2.8	2.8	2.8	2.9
Proline	8.5	8.3	8.2	8.0
Tryptophan	1.0	1.0	1.0	1.1
Methionine	1.0	1.1	1.2	1.2
Cystine	0.7	0.7	0.7	0.7
Serine	6.3	6.0	6.3	6.5
Threonine	4.7	4.5	4.6	4.6
Total	103.7	101.4	101.6	101.6

manufacturing trials from dehulled and defatted soybean processed at relatively low temperature might be promising for improving nutritive value as well as storage life of the finished food. Furthermore, in addition to Natto-like products, Kojibean-like products, on which preliminary manufacturing work has already been done, should also be investigated for manufacture in pilot plants.

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DISCUSSION

DR. HUNDLEY: Dr. Nakano may have mentioned, but I missed it if he did, the keeping properties of the Natto.

DR. SCRIMSHAW: I wonder if he could also tell us something of the cost of the new versus the traditional.

DR. SAKURAI: The keeping properties of the new Natto are very good.

DR. NAKANO: In regard to the cost of this product, we cannot make an assumption because it has been produced only in our laboratory, and we have no data to indicate cost. The traditional Natto I would say is nearly three cents per pound.

DR. RAO: Is Natto consumed as such or is it added to any food preparation?

DR. NAKANO: The traditional Natto is eaten with a sauce or with mustard mixed together and eaten with rice.

DR. MILNER: Are there any restrictions as to the technological characteristics and the quality of the raw soybeans that you begin with? Can you make them from any soybeans?

DR. NAKANO: Any soybean can be used.

Feeding Studies With Fermented Soy Products (Natto and Miso)

Tamotsu Sano

Introduction

There are many infants with protein malnutrition in many underdeveloped countries, but it is difficult in these countries to improve the dietary unbalance with animal proteins because of their economic problems.

Increasing attention is being focused on dietary preparations suitable for sucklings and infants, substituting plant protein for animal protein. Among the vegetable protein sources, soybean protein has been advocated by Ruhräh,[22] Rittinger,[20] Takuma,[28,29] and Dean.[4]

Natto and Miso prepared by fermentation of soybeans have been used as foods in our country for many years, and most of the Japanese have maintained their health rather satisfactorily without, or with very slight, animal protein in their diet.

Since 1957 Dr. György has been studying these soybean foods in collaboration with the author for the increasing use of them as diets for infants.

I devised new preparations of Natto powder, salt-poor Miso powder and autoclaved soybean powder without adherence to the previous methods.

Experimental Materials

1) *Dried Natto Powder.* After being sorted and soaked in water at normal temperature for 20 hours, soybeans were steamed at 20 pounds for 20 minutes. After being cooled to about 90°C, the steamed soybeans were sprayed with *Bacillus natto* (subtilis) solution (prepared with 8 gm of dried *Bacillus natto* (subtilis) added to 100 kg of soybeans) and put in the fermentation room. At first the room temperature at 40° to 42°C and 100% humidity were maintained; after 10 to 11 hours the humidity was gradually lowered and fermentation was completed 17 to 20 hours later.

2) *Dried salt-poor Miso powder.* After being soaked in water at normal temperature for 15 hours, 1 kg of soybeans was steamed at 6 to 8 pounds for 2½ hours. Before the temperature dropped below 80°C after steaming, 0.21 gm of vitamin K_3 dissolved in 150 cc alcohol wax was mixed with the beans to prevent sourness, and the mixture was then stirred. A malt, prepared by mixing 1 kg of

rice malt fermented by *Aspergillus oryzae* with 60 gm of salt, was then mixed with the steamed soybeans treated with vitamin K_3, and the mixture was stored in water at normal temperature for 3 weeks as the final process.

Saline content of this product was about 2% (commonly 10-12%) and the quantity of vitamin K_3 used for the prevention of sourness was approximately 0.007% of the total amount.

3) *Dried steamed soybean powder as control.* Soybeans were steamed at 20 pounds for 20 minutes. These soybeans were of the same kind as those used for dried Natto powder and dried salt-poor Miso powder.

For dietary use, all of these soybean preparations were desiccated for 20 hours in the ventilative desiccator.

The composition of these preparations is presented in table 1. There was no distinct difference between Natto (31.71%) and soybean (31.19%) in the protein content, but a reasonable difference between Natto (44.09%) and soybean (13.15%) was observed in the soluble protein content. The most outstanding difference between fermented soybean preparations and unfermented soybean was that vitamins B_1, B_2 and B_{12} were more abundant in the former than in the latter except B_2 in Miso.

TABLE 1
COMPOSITION OF SOYBEAN PREPARATIONS

		Natto powder	Soybean powder	Salt-poor Miso powder
Protein	%	31.71	31.19	24.30
Soluble protein	%	(44.09)	(13.15)	(5.98)
Fat	%	17.9	14.1	10.87
Carbohydrate	%	34.27	36.96	50.96
Calorie	%	425.0	399.5	410.7
Ash	%	4.02	4.32	4.65
Water	%	7.38	8.74	6.47
Fibre	%	4.72	4.69	2.75
	B_1 γ/gm	1.0	0.3	0.6
Vitamin	B_2 γ/gm	8.6	3.0	1.95
	B_{12} γ%	0.16	0.033	0.07

Note: "Soluble Protein %" designates the percentage of "protein %".
protein: N 6.25

Their amino acid content, estimated by microbiological methods, is shown in table 2. Amino acids in skim milk powder were cited from Orr.[18]

EXPERIMENTAL AMIMALS AND COMPOSITION OF EXPERIMENTAL DIETS.

Young rats of pure Wistar line, weighing 30 to 40 gm after being on the standard diet for a period of 10 days, were fed on the above-mentioned soybean preparations and three animal experiments were conducted.

Experiment I.

The influences of different contents of protein of diets on the growth and other physical findings were studied in this experiment. Rats were divided into three main groups (added group, 15% protein group and 30% protein group) by

TABLE 2
AMINO ACID COMPOSITION OF SOYBEAN PREPARATIONS
(gm in 100gm of sources)

	Soybean powder		Natto powder		Salt-poor Miso powder		Skim milk
	Fresh	Preserved	Fresh	Preserved	Fresh	Preserved	powder
ARG	2.90	3.30	2.30	2.50	1.30	1.40	1.30
ASP	4.30	4.50	4.50	4.70	2.80	2.60	2.60
GLU	7.00	7.40	7.90	8.40	3.90	4.00	8.32
HIS	1.20	1.20	1.20	1.30	0.46	0.55	0.94
ISO	2.30	2.30	2.70	2.90	1.70	1.80	2.27
LEU	3.00	3.00	3.20	3.30	2.00	2.10	3.49
LYS	2.10	2.10	2.40	2.50	1.40	1.40	2.77
MET	0.25	0.53	0.59	0.55	0.40	0.26	0.87
PHE	1.50	1.60	2.60	2.70	1.00	1.20	1.72
THR	1.90	2.00	0.88	0.90	1.40	1.50	1.64
TRY	0.43	0.46	0.52	0.58	0.26	0.25	0.50
TYR	1.30	1.46	1.30	1.50	0.63	0.68	1.81
VAL	2.30	2.40	2.40	2.40	1.30	1.30	2.44

the protein content of the diets. Each main group was subdivided into the Natto group, Miso group and soy group, consisting of 5 rats which were fed for 92 days. Composition of the diets is shown in table 3. Diets for both the added and 15% protein groups contained 15% protein source. Diet for the added groups was supplemented with rice flour and the diet for the 15% protein groups was supplemented with starch. Miso used as diets for the 15% and 30% protein groups was raw Miso, not in the form of powder. The diet for the 30% protein group was not supplemented with any carbohydrate sources. The basic diet was No. MC-5 of Oriental Yeast Industry Ltd., Japan. Some vitamins were added to each diet as shown in table 3, but not added to the basic diet.

TABLE 3
COMPOSITION OF DIETS IN EXPERIMENT I

	Diets of Added group				Diets of 15% Protein group			Diets of 30% Protein group		
	Natto group	Soy bean group	Miso group	Con-trol group	Natto group	Soy bean group	Miso group	Natto group	Soy bean group	Miso group
MC-5 diet				100						
Natto powder	36				47			94		
Soybean powder		37				48			96	
Salt-poor Miso powder			50				110			200
Rice flour	61	60	50							
Starch					52	51	40			
Salt	1	1			1	1		1	1	
$CaCO_3$	0.5	0.5	0.5		0.2	0.2	0.2	0.5	0.5	0.5
Calorie	369	360	382	365	373	362	330	399	383	357
Protein	14.9	14.9	15.0	24.7	14.9	14.9	15.0	29.7	29.9	28.5
Carbohydrate	62.7	63.2	65.5	51.3	59.3	60.1	50.8	32.2	35.4	32.0
Fat	6.7	5.5	5.4	5.6	8.4	6.7	6.4	15.8	13.5	11.7

Added Vitamins: A, 5000 IU; D, 500 IU; B_1, 1 mg; B_2, 1 mg; C, 5 mg; E, 3 mg once per week. Cholin, 10 mg per day for first 3 weeks.

Experiment II.

One of the purposes of this experiment was to clarify the value of protein sources of the fermented soy preparations, especially Natto, Miso and soy in comparison with that of skim milk powder.

Rats were divided into five groups consisting of 10 rats which were fed for 93 days on Natto, Miso, soy, skim milk and the basic diet respectively. Each of the diets was prepared to contain 15% protein source supplemented with rice flour as in experiment I., but Ca CO_3 was not used. Vitamins were added to each diet.

Experiment III.

As shown in table 2, methionine and lysine were lowered in Natto. Rats fed on Natto were supplemented with L-lysine and DL-methionine in order to examine the effect of such a mixed diet on their development. The diet containing 15% protein of Natto was supplemented with rice flour. The experimental rats were divided into four groups of 11 and each group was fed one of the following diets for 74 days: 1) Natto (15% protein), 2) Natto + 1% L-lysine, 3) Natto + 0.5% DL-methionine, 4) Natto + 1% L-lysine + 0.5% DL-methionine. The essential amino acids-tryptophane ratio of the diets used in experiments II and III were obtained as shown in table 4. Amino acids required for rats were cited from Rose's[31] and Block's work.[2] Lysine-tryptophane ratios of the diets used in experiment II, especially of Natto and soy, were not sufficient for the requirements of rats generally. The ratio of Natto (4.4) was the lowest, but the ratio of Natto + L-lysine used in experiment III was improved to 5.9. Each of lysine-tryptophane ratios of Miso and skim milk was 5.0. These ratios were sufficient for Rose's requirement for rats.

TABLE 4
THE ESSENTIAL AMINO ACIDS—TRYPTOPHANE RATIO OF THE DIETS USED IN EXPERIMENTS II AND III
TRYPTOPHANE CALCULATED AS UNITY

	Requirement of rat			Diet in experiment II				Diet in experiment III		
	Adult maintenance	Adult repletion	Young	Natto group	Miso group	Soy bean group	Skim milk group	Natto + L-Lys group	Natto + DL-Met group	Natto + L-Lys + DL-Met group
ARG	0.0	1.5	1.0	4.6	5.1	6.4	3.2	4.6	4.6	4.6
HIS	1.1	1.5	2.0	2.1	1.7	2.5	1.8	2.1	2.1	2.1
LYS	2.0	3.7	5.0	4.4	5.0	4.6	5.1	5.9	4.4	5.9
TRY	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PHE	2.2	3.3		4.9	4.0	3.8	3.7	4.9	4.9	4.9
PHE + TRY			4.5	7.7	6.9	7.1	7.5	7.7	7.7	7.7
MET	3.0	2.8	2.0	1.2	1.6	0.8	1.7	1.2	2.0	2.0
MET + CYS			4.0				2.5			
THR	2.8	3.2	2.5	2.1	5.0	4.2	3.4	2.1	2.1	2.1
LEU	2.8	5.2	4.0	6.5	7.8	7.2	7.2	6.5	6.5	6.5
ISO	5.4	4.3	2.5	5.0	6.0	5.1	4.5	5.0	5.0	5.0
VAL	3.7	3.6	3.5	5.0	5.4	5.6	5.2	5.0	5.0	5.0

Methionine-tryptophane ratios of all diets except the diets supplemented with DL-methionine were remarkably low. The ratio of soy (0.8) was the lowest of all. On the contrary, contents of some of amino acids (arginine, leucine, isoleucine, valine etc.) were in excess of Rose's requirement.

Experimental Methods.

Rats were fed ad libitum in separate cages and were weighed three times per week. In the metabolic study, the urine and feces were collected for 48 hours. Rats were sacrificed by bleeding at the end of experiment for the biochemical study of serum. Free amino-N in the urine and feces were assayed by Cocking's[3] ninhydrine method after deammoniation by Maruta's[13] method, total-N in the urine and feces by micro-Kjeldahl method, amino acids in the urine and feces by two-dimensional paper chromatography using Soucheon's[25] Taurin value method, serum total protein by the Hitachi refractometer and serum protein fractions by Tiselius method. The serum sodium and potassium were determined by the flame photometer, chloride by Schales & Schales's method, calcium by Yanagizawa's method, inorganic phosphorus by Tsusky's method, alkaline phosphatase by Fiske-Subberow's method.

Results

Experiment I

Growth and nitrogen metabolism of rats in experiment I were observed by Miura.[15] The results reported were as follows. In all of the three main groups, weight gain of the added group, especially of the Natto group, showed the highest value. In the 15% protein group, the Natto and soybean groups were poor in growth and death sometimes resulted. Dilatation, hyperemia and hemorrhage were observed in the stomach and intestine. Only the Miso group showed a good weight gain. In the 30% protein group, the rats fed on Miso were most excellent in weight gain, and the control had almost the same tendency; the Natto group was poorest.

Experiment II

As shown in figure 1 among the five groups, weight gain of the skim milk group was the highest with significant difference, followed by the Natto group, control group and soy group in order. The increase in body weight of rats per day was 1.82 gm in the Natto group, 0.72 gm in the Miso group, 1.63 gm in the soy group, 2.48 gm in the skim milk group, and 1.71 gm in the control group. Weight gain of the Miso group was the lowest. Most of rats fed on Miso were dystrophic. This was caused by decomposition products of Miso.

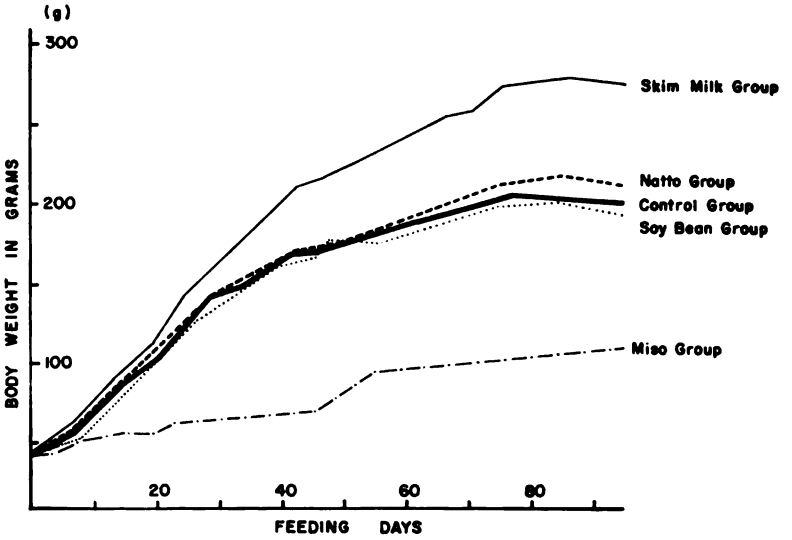

Figure 1—Rat growth, Experiment II.

Experiment III

As shown in figure 2, no difference among the groups could be observed in weight gain through the entire period of experiment, but when the period of observation was divided into three stages by the dates when the mean weight per group attained 100 gm and 150 gm, it was found that in the 1st stage the mean weight of rats

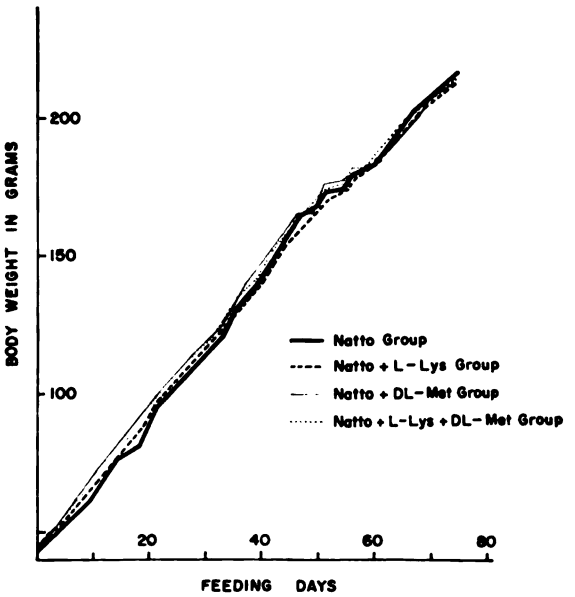

Figure 2—Rat growth, Experiment III.

in the groups fed on the amino acids-supplemented diets, especially with DL-methionine, was the highest, followed by the Natto + L-lysine group and Natto group in order. In the 3rd stage a reversed relationship was observed.

TABLE 5
THE INCREASE OF WEIGHT OF RATS PER DAY IN EACH FEEDING STAGE IN EXPERIMENT III

	Natto group	Natto—L-Lys group	Natto—DL-Met group	Natto—L-Lys—DL-Met group
	(gm)	(gm)	(gm)	(gm)
I Stage	2.34	2.41	2.56	2.48
II Stage	2.72	2.54	2.75	2.71
III Stage	2.11	2.03	1.83	1.89

COMMENTS

Elvehjem and Harper[5] have reported in detail on the importance of the amino acids balance in the diet, and it is well known that the quality of protein in diet is greatly influenced by the quantity and quality of amino acids, especially of the essential amino acids, constituting the proteins. In comparison with animal proteins, vegetable proteins are known to show unbalance of essential amino acids and in particular the shortage of lysine and sulfur-containing amino acids.[17,19] The protein of soybeans is said to be superior in quantity and quality among vegetable proteins. Natto and Miso are made of soybeans by fermentation with *Bacillus natto* and *Aspergillus oryzae*. From the trophological point of view, especially on the metabolism of amino acids, the present author has attempted to examine quality of the protein in these foods.

In Natto the essential amino acids, except threonine, were appreciably increased. Taira[27] said that threonine was decreased but the content of all other amino acids was little changed. In Miso the total protein content was lower than in the other soybean preparations, whereby the content of amino acids was lowered, but the balance closely resembled that in skim milk powder.

The change in the components of these foods after a long preservation was also examined, and it was found that no appreciable change occurred except a very slight decrease in the Vitamin B_{12} content.

Miura[15] contended that the results of György and Wako[6] experiments of feeding rats on Natto powder or Indonesian tempeh—soybeans fermented with *Rhizopus oryzae*—were vitiated by the staleness of the foods used, but I could not confirm such a contention in our analytical examination. It was said that digestive enzymes, amylase, pepsine, trypsine etc. were apparently present

in Natto and Miso, but since I did not conduct any enzymological analysis, I could not definitely affirm their effects.

In experiment I, as already reported by Miura,[15] the increase in body weight of rats was the highest in the added group, in which rats in the Natto and Miso groups grew better than those in the soybean group. In the 30% protein group, rats in the Miso and Natto groups proved also better development than those in the soybean group.

In experiment II, skim milk proved its superiority with a significant difference to others, but in the vegetable protein-fed groups the increase in body weight was better in the rats given Natto than in those given soybean. Saigo[23] made experiments of rats fed on a food containing 18% of protein using Natto as its source, and reported that the results were not different from the cases given soybean protein either in the dietary efficiency or in the protein efficiency.

In our experiment II, rancidity of the Miso sample used debarred us from obtaining satisfactory results, and the increase in body weight was the lowest in the group fed on this Miso which had become rancid during the process of drying and powdering because of a low salt content (2% against 10-12% in market Miso). In the rancid Miso, the total protein content was not reduced at all, but the water-soluble proteins were increased and volatile acids doubled in content. Iwashita,[12] who prepared salt-poor Miso at first, demonstrated the existence of formic acid as one of such volatile acids.

Since the studies of Osborne and Mendel[18] and more recently of Albanese[1] it has been repeatedly claimed that the addition of lysine to a protein-deficient food is very valuable in promoting bodily growth, but after all it seems that the maintenance of good balance in the pattern of amino acid constitution in the total protein content is the primary requirement, and in the cases with polished rice[8,9] and wheat meal[21] in which lysine constitutes the limiting factor in the protein composition, the addition of lysine would be effective if the amino acid pattern is not disarranged. However, in the cases with comparatively small shortage in lysine, as with soybean protein, an extra addition of lysine would be of little avail, and I believe the addition of methionine and such sulfur-containing amino acids would be preferable. In my experiment III, the addition of L-lysine and/or DL-methionine to a Natto diet caused better development of rats at the weanling stage, especially in those fed on Natto with DL-methionine, but the effect was decreased in the advanced stage, showing that bodily growth of rats was worse than that in the controls (Natto). Block[2] also reported that the addition of L-lysine to a soybean protein diet did not improve the nutritional value of the soy proteins as measured by the rate of growth of young rats; this was true when the lysine was added to the soy protein diet alone or combined with cystine and methionine.

On the other hand, Harper et al.[8,9] and Murata[16] demonstrated that the experimental results deviated far from what had been deduced from the calculated analytical values of amino acids. The supplementation of methionine and tryptophane was calculated theoretically as effective but proved fruitless, while the addition of lysine and threonine, deemed sufficiently abundant in soybeans, proved effective. In my experiments, the diets were prepared with the addition of 1%

of L-lysine and/or 0.5% of DL-methionine to Natto so as to approximate the composition of protein in skim milk. The optimum quantity of this addition should be determined in consideration of the amino acid content in the basal diet. The additional quantity of amino acids is thus not uniform among authors and experimental animals.[7,8,10] Block,[2] who investigated rats fed on 10% of soybean protein, has reported that the addition of 0.4% (of the protein content) of methionine enhanced the protein value of soybeans. Wretlind and Rose[31] fed rats on a basal diet containing all amino acids except methionine and citrulline, and reported that both D- and L-methionine proved effective in promoting growth, but without significant difference at the concentration of 0.3—0.8%. The effect was most remarkable at 0.4%, and the addition of 1.4% of methionine brought about obvious inhibition of bodily growth.

The excretion of free amino N in urine was found to be larger with Natto than with soybean or Miso in the added, the 15% and the 30% protein groups in experiment I. In experiment II, too, the excretion was the largest with Natto, followed by the soybean, Miso and skim milk in the descending order. In the added group in experiment I, the ratio of excreted free amino N to the excretion of total N with Natto, soybean, and Miso decreased in that order. The large excretion of free amino N in the urine of the Natto group seemed to be inconsistent with a higher increase in their body weight, but I must also take the following fact into consideration before making such an inference. In Natto, the contents of essential amino acids were larger than in soybeans upon analysis, but if I calculate the ratios of the amino acids content to the tryptophane content, I find the ratios in Natto not to be higher than in soybeans, as shown in table 3. The tryptophane content per 100 gm of food was 0.239 gm in the Natto group, 0.210 gm in the soybean group, 0.171 gm in the Miso group and 0.215 gm in the skim milk group, being the highest in the Natto group. It may be inferred that at elaboration of tissue proteins, the excess of amino acids among the coexisting essential amino acids is eliminated by urinary excretion.

The excretion of free amino N in feces showed similar tendencies to that in urine, but the excretion was far higher in the feces than in the urine, and in the former too the highest in Natto-fed animals. The fecal excretion of free amino acids depended on the quality of protein in the diet and the quantity of the food consumed. Stelgens et al.[24] observed a notable decrease of total N and urea N and a moderate decrease of creatine N, uric acid N and amino acid N in the urine and confirmed decrease of threonine, phenylalanine, lysine, valine, arginine, tryptophane and tyrosine in the urine of a suckling infant fed on a practically protein-free diet. Soucheon[25] and Thurau[30] said that the excretion of amino acids was not uniform according to the difference in alimentation and that the excretion of amino acids in the urine is the lowest in breast-fed infants. They also demonstrated that the excretion of amino acids in feces was affected not only by the kind of ingested protein but also by the intestinal flora.

In experiment II, it was found that total taurine value of amino acids, both essential and nonessential, was relatively high in the Natto-fed cases and the many kinds of amino acids, especially methionine and cystine, were found in the

Miso-fed cases. Generally speaking, the free amino acids in the urine consisted of less of essential and more of nonessential items, but in the feces, more of essential amino acids are detected and the total taurine value is higher. Thurau[30] also found that the excretion of amino acids in feces was large and more of the essential amino acids were excreted than the nonessential, both in breast-fed and artificially-fed sucklings.

It is known that the trophological difference between animal and vegetable foods consists not only in the difference in the amino acid constitution of proteins but also in the difference in their inorganic elements, such as manganese and zinc. The assay has shown a large quantity of manganese and the scarcity of zinc in vegetable food, but Kubo reported the presence of sufficiently large contents of Mn and Zn in soybeans comparable to those in animal food. Besides, vitamins B_1, B_2 and B_{12} (11) were found to increase in fermented food, and it may be said that Natto and Miso present many interesting problems in the field of trophology.

I could understand that Natto, typical of Japanese old soybean preparations, can be useful in the animal experiment, and consequently it is apparent that Natto in the form of powder is useful as the source of proteihs in the diet of suckling and weanling infants.

SUMMARY

1) Dried Natto powder and salt-poor Miso powder were newly prepared for application as a food for sucklings and infants.

2) No appreciable changes occurred in the composition of the soybean preparations after preservation.

Content of essential amino acids in Natto was richer than in soybeans, but the balance of amino acids in Natto was not improved as compared with that of skim milk. On the other hand, the composition of amino acids in Miso containing lowered protein was similar to that of skim milk. It was regretted that Miso was decomposed into a rancid state and the results using it in experiment II were unsatisfactory.

3) The influence of different protein contents in given diets on growth of rats were studied in experiment I. In all of the three main groups (added, 15% protein, and 30% protein groups), the increase in body weight of the added group, especially with Natto, showed the highest value.

4) The value of protein sources of the fermented soybeans in comparison with those of skim milk was examined in experiment II. The increase in body weight of the skim milk group was the highest and that of the Natto group was better than that of the soybean group.

5) No effect of supplementing Natto protein with L-lysine and DL-methionine was observed through the entire period of experiment III.

6) High urinary and fecal excretion levels of free amino N of the soybean preparation groups, especially of the Natto group, were observed through all animal experiments.

7) No marked difference could be found in biochemical findings of the serum of rats through all experiments, except of the Miso group in experiment II.

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Nutritional Research on Fermented Soybean Products

Kinitaro Arimoto

IN JAPAN there are a number of fermented soybean products such as "Miso," "Shoyu," "Tofu" and "Natto." "Natto" is prepared from cooked soybean by fermentation with *Bacillus natto* and is a popular and favored food for Japanese people. In order to get wider utilization of soybean protein, it is necessary to develop soybean products which are digestible, preservable and transportable. Dried "Natto" products have been prepared through the Food Research Institute under different conditions and nutritional investigations carried out in the National Institute of Nutrition. The results reported here are condensed and summarized from a much more detailed report available to the Committee on Protein Malnutrition.

PREVIOUS STUDIES ON NUTRITIVE VALUE OF "NATTO"

There are many reports on the change in constituents of soybean and on the decomposition of soybean protein during "Natto" processing.[1,2,3]

Abe[4] and Honami[5] have recognized that monoamino acid-N and diamino acid-N increase by the fermentation of *Bacillus natto*. Shinozaki[6] has reported the change of free amino acids, and more recently Hayashi[7] reported the increase of water-soluble nitrogen and ammonia nitrogen during the storage of "Natto." The author reported that reducing sugar almost disappears, but contents of fat and fiber do not change significantly by the fermentation, though the change of carbohydrate such as galactan is not clarified.

As regards vitamins, it has been reported that vitamin B_1 contents of soybean decrease by steaming, but increase a little during the fermentation preparation of "Natto".[7,8] This is due to the enzymic action of *Bacillus natto*. It has also been reported that vitamin B_2 contents of "Natto" increase as much as 5 to 10 times as compared with those of soybean.[7]

According to the report of Fujii,[9] protein digestibility of "Natto" is about 10% higher than that of cooked soybean in human experiments, and Hayashi[7] observed that protein digestibility of "Natto" by rats is superior to that of soybean powder (Kinako).

There is no experiment on the BV of "Natto" except that of Hayashi[7] who reported that the BV of "Natto" protein in rats is about 70, a little higher

than that of cooked soybean. He observed the best growth in albino rats by the addition of "Natto" to white rice. Yamazaki[10] reported the effect of protein of soybean cake which was fermented with *Bacillus natto* on growth of chicken and carp. Tadokoro[11] observed that the effect of "Natto" protein on growth is superior to that of cooked soybean in rat experiments with diets adding "Natto" and cooked soybean to brown rice.

Preparation and Composition of Samples

Samples of dried powdered "Natto" were prepared from whole, dehulled and defatted soybeans by Tamgo Natto Company in Tokyo. The raw materials were soaked in water overnight, steamed at 30 lb pressure for 10 minutes, inoculated with *Bacillus natto* and fermented at 40-50°C for 12 hours. The samples were dried at 80-90°C and powdered.

The composition of the raw samples and of the dried samples is shown in table 1. The composition of essential amino acids estimated by microbioassay showed no significant difference between cooked soybean and fermented "Natto."

Table 1
Composition of "Natto"-Like Products

Material	Water %	Protein (Nx5.71) %	Fat %	Carbohydrate %	Ash %
	RAW NATTO				
Whole soybean "Natto"	58.9	18.3	8.2	5.4	2.2
Whole soybean "Natto"	60.8	16.7	8.0	5.7	2.1
Dehulled whole soybean "Natto"	59.6	17.4	8.5	5.2	2.3
Defatted soybean meal "Natto"	60.3	18.9	1.3	6.6	2.7
Defatted soybean flake "Natto"	55.3	22.7	0.7	5.9	3.0
	DRIED NATTO				
Whole soybean (Control)	5.8	38.8	17.2	16.8	5.0
Whole soybean "Natto"	4.9	42.3	19.0	12.9	5.0
Whole soybean "Natto" *	7.0	40.5	18.9	13.5	4.9
Dehulled whole soybean "Natto"	6.9	40.0	19.7	12.0	5.2
Defatted soybean meal (Control)	5.9	44.0	2.6	23.0	6.2
Defatted soybean meal "Natto"	6.5	44.5	3.1	15.3	6.4
Defatted soybean flake (Control)	6.5	46.8	1.6	19.1	6.1
Defatted soybean flake "Natto"	5.6	48.0	1.5	12.3	6.4

* Whole soybean "Natto" was steamed after fermentation.

Results of Experiments

On powdered products of "Natto," protein digestibility *in vitro*, growth rate and BV on rats were examined. The results show that water-soluble nitrogen and digestibility of protein of whole soybean "Natto" *in vitro* are superior to those of cooked soybean as control, particularly of defatted soybean "Natto." But growth rate and protein efficiency of "Natto" were inferior to those of boiled soybean and casein as control.

In animal experiments on diets with different ratios of powdered "Natto" and casein, the group on diet with whole soy "Natto" as the protein source was

most inferior in growth rate and protein efficiency, and the group on diet with 4% "Natto" and 15% casein was most superior in protein efficiency.

Absorption rate and BV of protein of cooked soybean powder and powdered "Natto" prepared from whole soybean and defatted soybean were examined on adult rats, and the results (table 2) indicated that the absorption rate of protein of "Natto" products is inferior to that of cooked soybean.

TABLE 2
NITROGEN ABSORPTION AND BIOLOGICAL VALUE OF "NATTO" PRODUCTS IN ADULT RATS

Protein source	Absorption %	B V
Whole soybean	77.0	52.3
Whole soybean "Natto"	68.4	38.4
Defatted soybean	73.1	56.2
Defatted soybean "Natto"	72.2	54.7
Casein	91.6	75.7

From these experiments, it is considered that the absorption rate of protein by rats and protein digestibility *in vitro* of 6- to 8-hours-fermented "Natto" were superior, but the growth rate of 4-hours-fermented "Natto" was better than that of 8 hours, and also it was observed that the 8-hour product was superior on acceptability trials carried out in the Food Research Institute.

We further carried out digestibility experiments on human subjects with a diet of biscuit containing powdered "Natto" and cooked soybean, and we obtained the result that there was no difference between absorption rate of protein of 8-hours-fermented "Natto" and that of cooked soybean.

Acceptability trials of 8-hours-fermented "Natto" in biscuit, crackers, "Miso" soup and curry soup were conducted. The biscuit containing 15% "Natto" product was preferred to that containing 20% by 68% of the subjects. However, in the cracker test, the crackers containing 20% were better liked than those of 15%. "Miso" soup containing 5 gm of "Natto" products was preferred to that with 10 gm by 79% of the subjects, and curry soup with 5 gm of added "Natto" product was superior in the preference test to that with 10 gm.

In long-term trials with 170 children on acceptability of biscuit containing "Natto" product, children were not observed to tire of the biscuit, and no abnormal symptoms were observed on physical examination.

SUMMARY

Research on "Natto"-like products, a fermented product of soybean, was done to obtain a nutritious, preservable, transportable and low-cost protein food.

In the preliminary experiment on commercial product of "Natto" it was observed that digestibility, growth rate and BV of powdered "Natto" prepared from whole soybeans are inferior to those prepared from defatted soybeans, and these are inferior to cooked soybeans as control.

After the preliminary experiment, we conducted the same experiment on powdered "Natto" prepared in the Food Research Institute, and the following results were obtained.

1. Water-soluble nitrogen of soybeans increased by fermentation with *Bac. natto*.

2. Content of vitamin B_2 of soybeans increased by fermentation, but this was not found for vitamin B_1.

3. "Natto"-like products prepared with 6 to 8 hours of fermentation seem to be superior in protein digestibility *in vitro* and absorption rate on rats to those of products of other fermentation periods.

4. Growth response of 4-hours-fermented "Natto"-like product on rats seems to be superior to that of other products.

5. In protein absorption rate of fermented "Natto" on human subjects, there was no significant difference between 8-hours-fermented "Natto" and cooked soybeans.

6. BV of 8-hours-fermented "Natto"-like product was superior to 4-hours-fermented product and cooked soybean as control.

7. In acceptability trials which were conducted on school children with foods containing powdered "Natto" of 8 hours fermentation, it was observed that addition of 15% of powdered "Natto" in biscuit, 20% in cracker, and 5% in curry-soup is suitable. Children did not tire of biscuit containing 15% "Natto" product in a long-term test of 30 days.

8. In physical examination of school children carried out before and after acceptability trials, no abnormal symptoms were observed.

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Studies on Tempeh--An Indonesian Fermented Soybean Food

Keith H. Steinkraus, Jerome P. Van Buren, David B. Hand.

PROTEIN DEFICIENCY is the most urgent nutritional problem in the world today. Soybeans offer a valuable source of protein. However, there are two objections to the use of dry soybeans for food. One objection is their assumed difficult digestibility by humans. A second objection is the length of time required to cook them. In countries with fuel shortages, the latter can be a very important problem.

The Indonesians have solved both of these problems by fermenting the soybeans with a mold after soaking and partially cooking them. The mold, they believe, makes the soybeans more digestible (van Veen and Schaefer, 1950). This was substantiated during World War II in Japanese prisoner of war camps where even those suffering from gastrointestinal infections were able to tolerate tempeh. The mold also, during the digestion of the soybeans, effects changes comparable to those observed during prolonged (18 hour) cooking. If soybeans are cooked, they become softened. This also follows mold digestion. Microscopic studies reveal that the individual cells of both cooked soybeans and tempeh are released from their intracellular matrix and become resistant to fracture when beaten in a Waring blendor (figs. 1, 2, 3).

The Indonesian process of making tempeh involves wrapping the beans in banana leaves during fermentation. This process is well adapted to the small-scale home production used in Indonesia. However, the dehulling which is accomplished with the bare hands or feet is especially laborious, the temperature of incubation is not standardized and the product, containing a variety of organisms, is wet and of unstable keeping quality. It would be poorly adapted to production of tempeh to alleviate protein malnutrition on a larger scale.

A number of studies have been made on tempeh produced under primitive conditions (van Veen and Schaefer, 1950). In order to expand production and to establish its nutritional quality it will be essential to produce tempeh under standardized, scientifically controlled conditions as a stable, dehydrated product adaptable to commerce.

In our laboratories, the microorganisms involved in the tempeh fermentation have been isolated. The one essential organism is the mold *Rhizopus oryzae*. It is an interesting mold because it grows very rapidly and well at temperatures

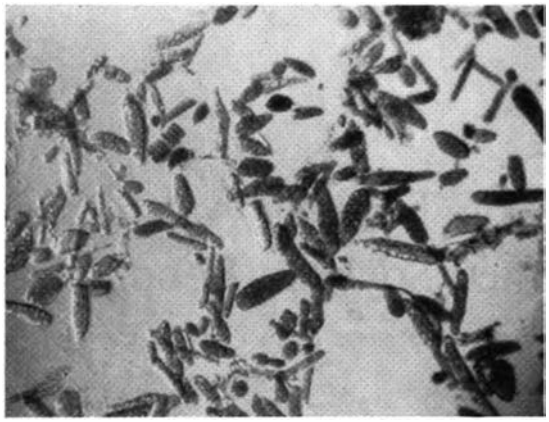

Figure 1—Soybeans autoclaved for 60 minutes and beaten in a Waring blendor for 2 minutes.

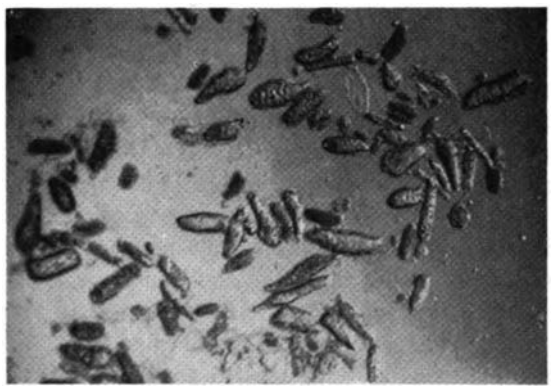

Figure 2—Tempeh, beaten in Waring blendor for 2 minutes (X100, 1/25 sec.)

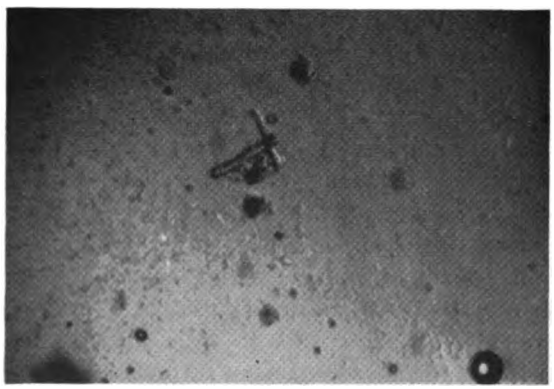

Figure 3—Soybeans, soaked and beaten in Waring blendor for 2 minutes (X100, 1/25 sec.)

of 37°C and even as high as 45°C. While all molds are aerobic, this species does not require an unrestricted exposure to air as do many molds. It will grow well on skinned, soaked, partially cooked soybeans placed in covered containers allowing only slow diffusion of air. It will also grow in rotating drums which permit a slow diffusion of external air. In fact, if too much air is allowed in the atmosphere surrounding the mold, it grows too fast and produces too much heat. The temperature may, under such conditions, rise to 49°C and inhibit further development of the mold. The mold is highly proteolytic and will produce enough ammonia in unbuffered substrates to kill itself.

In the process developed, soybeans are soaked overnight in dilute lactic acid solution. The acid inhibits development of spoilage organisms. The beans are then skinned by passing them through an abrasive peeling machine and floating the skins off in flowing water. The skinned beans are steamed for 90 minutes, cooled to 37°C and inoculated with spores of the mold. Fermentation is carried out in covered stainless pans. Incubated at 37°C, the beans are covered with mold mycelium in 24 hours. As the fermentation progresses, the beans become more tightly knit together by the mycelium, and spores form after 48 to 72 hours (fig. 4).

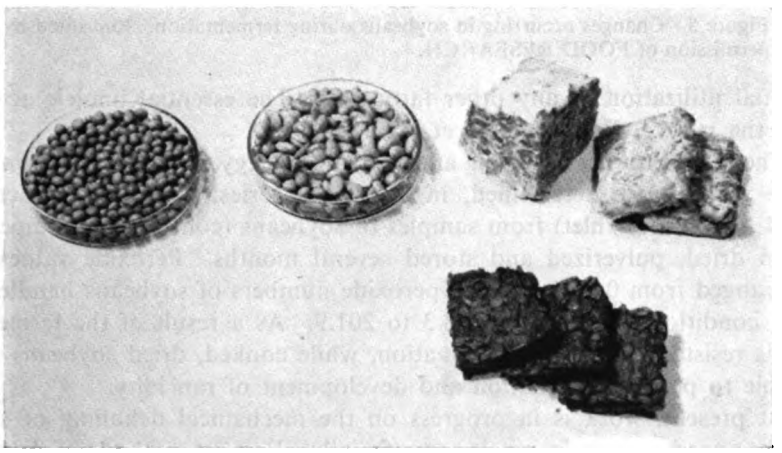

Figure 4—(Left to right) 1. Unsoaked soybeans. 2. Soaked soybeans. 3. Tempeh. 4. Tempeh cooked in fat.

During the fermentation, a number of interesting changes occur in the soybeans. As the mold growth increases, the temperature in the fermenting bean mass rises above incubator temperature. As mold growth subsides, the temperature falls. Soluble solids rise from about 13% to nearly 28%. Soluble nitrogen rises from about 0.5% to nearly 2% while total nitrogen remains about 7.5%. The pH shows a progressive rise during the fermentation until free ammonia is released due to the proteolytic action of the mold (fig. 5).

The mold is highly lipolytic. Approximately one-third of the total ether-extractable soybean lipid is hydrolyzed by the mold after 69 hours incubation. About 40% of the linolenic acid is utilized by the mold, but there is no apparent

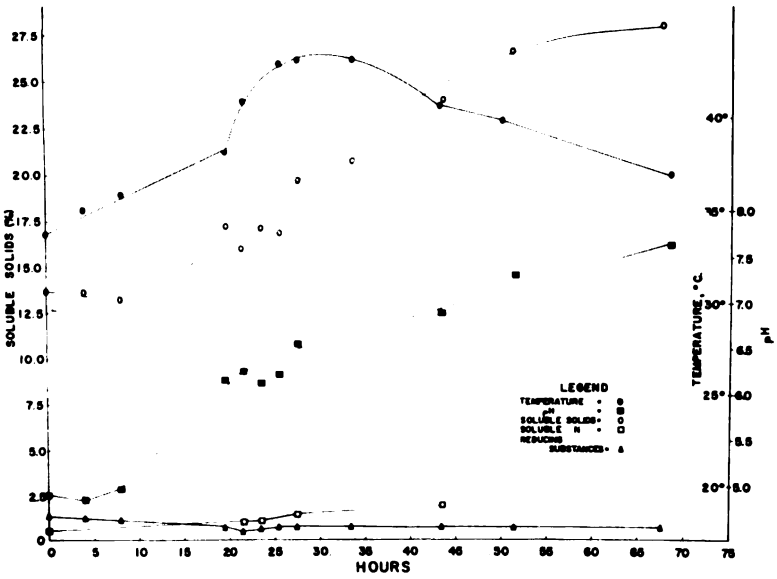

Figure 5—Changes occurring in soybeans during fermentation. Reprinted by permission of FOOD RESEARCH.

preferential utilization of any other fatty acid. The essential linoleic acid is not used by the mold. (Wagenknecht et al., 1960).

The mold produces a strong antioxidant (György, personal communication). Peroxide values were determined, in our laboratories, on lipids extracted with ether (24 hours in Soxhlet) from samples of soybeans (control) and tempeh which had been dried, pulverized and stored several months. Peroxide values on the tempeh ranged from 0 to 1.1, while peroxide numbers of soybeans handled under identical conditions ranged from 18.3 to 201.9. As a result of the fermentation, tempeh is resistant to peroxide formation, while cooked, dried soybeans are very susceptible to peroxide formation and development of rancidity.

At present, work is in progress on the mechanical dehulling of soybeans to facilitate production of tempeh commercially. Various methods of dehydration are being investigated in an effort to preserve the nutritional quality of tempeh. Other substrates, including peanuts, will be fermented with the tempeh mold, to determine whether or not the nutritional quality can be improved by such a process.

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The Nutritive Value of Tempeh

Paul György

SOY PRODUCTS have long been used as food for adults, children and—even as the sole source of nutrients—for young infants. It is not the purpose of this presentation to review the voluminous literature on the subject. It suffices to state that there are very few, if any, nutritional studies available which could be considered sufficiently critical and exact and which were extended over a long period of observation.

Our studies with soy products may be divided into three groups: a) Experimental and clinical observations with Saridele, a soy product manufactured in Indonesia, together with controls, using (in principle) similar soy products in general use for some time in the U.S.; b) investigations on the comparative nutritive value of various soy-protein concentrates and "isolates"; c) experimental studies on the nutritive value of fermented soy products, specifically tempeh, a popular food for centuries in Indonesia.[1]

Only our investigations on tempeh, including chemical studies on changes occurring during the fermentation process, will be discussed here. For all tempeh preparations, controls were provided in the form of corresponding unfermented soybeans.

The first tempeh preparations and control soybeans used in this study were obtained from Indonesia * (1954, 1955) and Southern Rhodesia ** (1955). In the following years attempts, largely futile, were made to produce tempeh in our own laboratory. In 1959 a cooperative arrangement has made it possible to produce tempeh and control soybeans on a larger scale in the Department of Food Science and Technology, New York State Agricultural Station, Cornell University, Geneva, N. Y. under the supervision of Drs. D. B. Hand and K. H. Steinkraus. Under this arrangement, animal studies are carried out independently in our laboratory and in the laboratory of the School of Nutrition (Dr. R. H. Barnes), Cornell University, Ithaca, N. Y. The method of preparation of tempeh is described in the report by Drs. Steinkraus, Van Buren and Hand.

* Through the courtesy of Dr. Poorwo Soedarmo, Institute of Nutrition, University of Djakarta, Indonesia.

** Through the courtesy of the Executive Officer, Nutrition Council, Federal Ministry of Health, Salisbury, S. Rhodesia.

The soybean control preparation was handled the same except for the elimination of the inoculation and fermentation steps. The pH remained lower in the unfermented beans.

In experiment 249, the first batches of tempeh and control soybean flour were tested on rats, in groups of 10 (average starting weight 68 gm). For purposes of comparison, a third group was fed reference skim milk (UNICEF) as the source of protein.

EXPERIMENT 249

Group *	Diet **	Av. Weight *** Gain (gm)	Signif. Diff.	Av. Food *** Intake (gm)	PER	Signif. Diff.
1	Tempeh 20%	203 ± 11.1	No	681.0	1.48 ± 0.04	No
2	Soybeans 20%	189 ± 12.0	No	678.3	1.39 ± 0.045	No
3	Skim milk 20%	201 ± 7.8	Control	680.4	1.48 ± 0.03	Control
4	Tempeh 10%	62 ± 2.7	No	399.5	1.59 ± 0.04	No
5	Soybeans 10%	49 ± 4.7	Yes (2.8)	399.6	1.21 ± 0.10	Yes (3.3)
6	Skim milk 10%	64 ± 2.6	No	399.9	1.59 ± 0.05	Control

* Groups 1-3 and 4-6 were strictly pair-fed.
** Diets 1-3 contained 20%, groups 4-6 only 10% protein. Fat, salt and carbohydrate in diets 1-3 and 4-6 were equalized. Vitamin supplements given.
*** In 10 weeks of observation.

After a feeding period of 10 weeks, identical weight gain and equal protein efficiency were noted for tempeh and skimmed milk when given in rations with 20% or 10% protein. In contrast, statistically significant reduction in weight gain and PER was noticed with 10% (group 5) but not with 20% (group 2) protein in the form of control soybeans in the ration. Thus, after 10 weeks, the superiority in nutritive value of tempeh over the control soybeans was evident only on low-protein intake of 10%. After 4 weeks of observation, the difference between tempeh and control soybeans was noticeable also on higher (20%) protein intake, at least with regard to PER but not to the average weight increment during the same period.

In experiments 248 and 254, the same preparations of tempeh and control soybeans were used as in experiment 249 with 20% of protein in the mixtures, but lard was used instead of peanut oil, for equalizing the fat content in the rations.

After six weeks of observation, when all rats in the various groups were still alive, the weight increment in the groups of rats receiving tempeh was statistically higher than that in the control soybeans.

In experiment 249 three rats in group 5 died after 68, 70 and 77 days, with severe massive hemorrhagic necrosis of the liver. The remaining seven rats in this group and all rats in the other groups survived the total experimental period (16 weeks). At autopsy, six of the seven rats in group 5 showed either cirrhosis of the liver or acute necrotizing nephrosis. The massive hemorrhagic necrosis of the liver indicated severe vitamin E deficiency. This was confirmed by the results of the hemolysis test[2]: all surviving rats in group 5 showed very marked hemolysis (av. 95%). In contrast, rats in group 4 have exhibited average hemolysis

EXPERIMENTS 248 AND 254

Group [a]	Diet	Average initial weight (gm)	Average weight at 6 weeks (gm)	Average weight at 14 weeks (gm)
		EXPERIMENT 248		
1	Tempeh 20%	58 ± 0.8	202 ± 4.5	293 ± 6.9
2	Soybeans 20%	58 ± 0.8	179 ± 3.8	179
3	Tempeh 20%	58 ± 0.8	212 ± 4.4	296 ± 5.2
4	Soybeans 20%	58 ± 0.8	193 ± 3.7 [b]	232
		EXPERIMENT 254		
1	Tempeh 20%	60 ± 1.0	178 ± 4.1	237 ± 4.1 [e]
2	Soybeans 20% [c]	60 ± 1.3	143 ± 4.5	
3	Soybeans 20% [d]	60 ± 1.2	145 ± 3.0	167 ± 3.7 [e,f]

[a] Groups 1 and 2 were pair-fed. All rats in groups 1 and 3 survived and were killed after 14 weeks. In group 2 only two, in group 4 only three rats survived 14 weeks.
[b] Significant difference.
[c] 25 mg methionine daily, added to the vitamin supplements.
[d] 25 mg methionine and 3 mg α-tocopherol 3 times weekly added to the vitamin supplements.
[e] 12 weeks.
[f] Only 3 rats survived.

of only 4%.* Rats in all other groups (groups 1-4 and 6) have shown at autopsy normal liver and kidneys.

In experiments 248 and 254, only very few animals survived in the groups receiving the control soy flour for the total experimental period. Death was caused in all instances by severe massive hemorrhagic necrosis of the liver. Supplement of α-tocopherol in group 3 of experiment 254 prevented this fatal acute hepatic injury and all rats in the group survived. No cirrhosis or nephrosis was observed at autopsy in any of the rats which survived the total period.

The difference between the results in experiment 249 and those obtained in experiments 248 and 254 may be explained by the substitution of lard for peanut oil. Lard is known to aggravate and enhance vitamin E deficiency and, in consequence, hepatic necrosis.[3] The absence of cirrhosis and nephrosis in experiments 248 and 254 is not surprising considering the fact that all rations contained 20% protein. This form of hepatic and renal injury is the result of choline (methionine) deficiency and its occurrence on a low (10%) protein intake (expt. 249) is not surprising.

In addition to the presence of lard in the rations used in experiments 248 and 254, another aggravating factor in the development of the very severe, acute deficiency of vitamin E in the groups receiving the control soy flour was the strong rancidity which developed early after exposure to air at room temperature. In contrast, even after 1½ years, tempeh under identical conditions remained free from organoleptic rancidity. The peroxide readings, performed in Dr. Hand's laboratory, were 201.9 for unfermented soy flour and 1.1 for tempeh, after both had been allowed to stand at room temperature for about 10 months. This combination of lard and rancid soy flour may also explain the occurrence of hemoglobinuria (not hematuria) in several rats, especially in experiment 254. One could

* All hemolysis tests were carried out by Dr. Kiku Murata (Osaka, Japan) in our laboratory.

speculate that the peroxides of the rancid soy fat may have directly hemolyzed (after their absorption from the intestine) the red blood cells, just as H_2O_2 or dialuric acid (in its oxidative transformation, probably with radical formation) act *in vitro* on red blood cells in vitamin E deficiency. It is of special interest that rats on the control soy flour, even after supplements of α-tocopherol, have shown significant retardation of growth. Supplements of α-tocopherol have corrected the direct manifestations of vitamin E deficiency, i.e., massive hemorrhagic necrosis of the liver and the fragility of red blood cells to dialuric acid. It is probable that the nutritive value of unfermented soy flour was impaired by the development of rancidity, probably due to the toxic effect of rancid fat and its oxidation products. In the light of all these observations and findings, it may be assumed that in the course of fermentation a substance with antioxidant properties has developed in tempeh and improved its keeping quality as compared with unfermented soy flour. The importance of this observation for conditions in the tropics is obvious.

The next batch of tempeh and control soy flour was kept in closed cans. When cans were opened to prepare the mixtures they were kept in the refrigerator with their lids tightly closed. No organoleptic rancidity was noticed in these cans or in cans opened later in the course of the experiment.

In order to simplify the procedure in this second batch of tempeh and control soy flour (prepared again by Dr. Steinkraus in Dr. Hand's laboratory), drying by hot air at 150°F was substituted for lyophilization. Tempeh prepared by this technique has shown no improved nutritive value. PER obtained with such preparation was identical with that obtained by feeding unfermented control soy flour which was also dried by hot air (expt. 258).

EXPERIMENT 258

Group	Diet	Av. food intake (gm/day)	Av. weight gain (gm)	PER
1	Tempeh	7.1 ± 0.1	60 ± 2.7	1.22 ± 0.05
2	Soy flour	7.1 ± 0.1	67 ± 3.0	1.35 ± 0.05
3	Tempeh	8.8 ± 0.2	82 ± 4.2	1.34 ± 0.04
4	Soy flour	8.0 ± 0.3	76 ± 5.2	1.35 ± 0.05

Data after 10 weeks of feeding; groups 1-2 pair-fed; groups 3-4 fed ad lib.
All rations contained 10% protein, 4% salts, 12% fat (soy oil).

As a working hypothesis, damage through prolonged heat could be considered as a possible explanation for the difference in nutritive value of tempeh dried through lyophilization as compared with tempeh dried by hot air. During lyophilization, the temperature does not go above 80°F and during most of the process the product remains frozen.

Dr. Steinkraus found significant analytical differences between lyophilized and hot-air-dried tempeh, as demonstrated in the following table:

Tempeh	pH	% Reducing substances	% Soluble solids	% Soluble nitrogen
Fresh	6.3	.71	17.6	2.31
Lyophilized	6.2	.41	19.5	1.19
Hot-air-dried	5.3	.28	13.8	.61

In experiment 258 only one rat in group 2 died from acute hemorrhagic necrosis of the liver before the completion of the experimental period. At the autopsy of the remaining rats in this group, liver and kidneys were found to be normal. On the other hand the average figure for hemolysis was 85% in group 2, and only 25% in group 1. Thus, in spite of the absence of rancidity in the soy flour, rats on this ration developed specific signs of vitamin E deficiency. Rats in the tempeh group were free from manifestations of vitamin E deficiency. Since in experiment 258 care was taken to prevent the development of organoleptic rancidity in the soy flour, the question whether lack of any demonstrable difference in PER of tempeh and control soy flour used in experiment 258 was due to heat damage of tempeh or to absence of organoleptic rancidity (toxicity) of the control soy flour cannot be answered at the present time and requires further exploration.

One conclusion may be drawn with certainty: tempeh is stabilized by virtue of an "antioxidant," produced during the course of the fermentation process. The prevention of rancidity in tempeh and of vitamin E deficiency in rats receiving tempeh can be directly traced to the presence of this "antioxidant." With the effective assistance of Dr. Kiku Murata (Osaka, Japan) we were able to prepare in our laboratory extracts from tempeh with alcohol or ether, using both batches (one lyophilized, the other heat-dried). The alcohol extracts were put through Florisil columns and eluted with petrol ether. Active concentrates were obtained, showing white (light-blue) fluorescence, which gave definite protection in the usual hemolysis test in amounts of 50 gm of dry residue. Such concentrates gave a positive $FeCl_3$, Emmerie-Engel reaction and a distinct peak in UV at 2640°A. The active substance appears to be acidic and is absorbed on Amberlite IR-410 and eluted with formic acid of weak molarity. It has not yet been obtained in crystalline form. Highly purified concentrates were active in the hemolysis test in amounts between 0.1—1.0 mcg (dry residue).

The preparations of tempeh and control soybeans obtained several years ago from Indonesia and Southern Rhodesia arrived as unground beans, sun-dried at the site of production. They were autoclaved and ground in our laboratory before they were used in animal experiments. One of the Indonesian tempeh preparations (expt. 158) and the Rhodesian tempeh (expt.195) have shown significantly improved growth rate when compared with the results observed with unfermented control soy flour. Severe hepatic injury, in the form of cirrhosis

EXPERIMENT 158
TEMPEH AND UNFERMENTED SOYBEANS FROM INDONESIA (1954)

Group	Diet	Duration days	Weight (gm) Start	Weight (gm) End	Liver Cirrhosis +	Liver Cirrhosis ++	Kidney 0	Kidney +
1	Tempeh 30%	125	46 ± 1.3	271 ± 4.3	8	2	6	4
2 *	Soybeans 30%	125	51 ± 4.5	243 ± 5.7	2	8	0	10
3	Tempeh 50%	42	45 ± 0.2	154 ± 2.0				
4	Soybeans 50%	42	45 ± 0.25	128 ± 4.3				

N-content: 63-69 mg/gm in tempeh and soybeans. Diet contained lard and cod liver oil. Vitamin supplements given. Ten rats.
* Two additional rats with "hemorrhagic kidney" after 12 days.

with accompanying regeneration, was significantly more prominent in the groups of rats receiving unfermented soy flour than in the group of rats fed tempeh rations (expt. 158, 159).

EXPERIMENT 159
TEMPEH AND UNFERMENTED SOYBEANS FROM INDONESIA (1955)

Group	Diet	Weight (gm) Start	Weight (gm) End	Liver Cirrhosis +	Liver Cirrhosis +	Regeneration 0	Regeneration +	Kidney 0	Kidney +
1	Tempeh 50%	45 ± 0.8	235 ± 5.2	10	1	10	1	—	11
2	Soybeans 50%	45 ± 0.6	244 ± 5.8	3	8	2	9	—	11

N-content: 68-70 mg/gm in soybeans and tempeh. Rations contained lard and cod liver oil. Vitamin supplements given. Ten rats in each group.

EXPERIMENT 195
TEMPEH AND SOYBEANS FROM S. RHODESIA (1956)

Group	Diet	Duration days	Weight (gm) Start	Weight (gm) End
1	Tempeh	200	50 ± 1.0	349 ± 4.9
2	Soybeans	200	50 ± 1.0	316 ± 14.5
3	Tempeh	100	50 ± 1.0	217 ± 4.4
4	Soybeans	100	50 ± 0.09	188 ± 6.3

(Tempeh—or soybeans 40%, salt mixture 4%, sugar 46%, crisco 10%).
N-content in tempeh 68 mg/gm, in soybeans 71 mg/gm, both autoclaved.
Vitamin supplements given.
Groups 1 and 2 and 3 and 4 were strictly pair fed.
Difference significant only for 3 and 4.

The still unsatisfactory control of essential experimental conditions notwithstanding, it may be stated in conclusion, with fair certainty, that tempeh offers good possibilities for its practical use as a protein-rich food with high BV. However, the final goal is still distant. In order to reach it, several technological problems have to be attacked and solved and a large number of experimental and chemical studies carried out. At present it appears that tempeh produced under favorable conditions contains a stabilizing, not yet fully identified, antioxidant.

Attempts should be made to adapt tempeh, well known for centuries in Indonesia, for production on the "village level," with all essential specifications of a satisfactory, economical, even unique protein-rich food. The possibility of its use as an infant food should also be explored.

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DISCUSSION[1]

DR. PATWARDHAN: The findings on the better nutritive value of some of the fermented food products have enabled us to focus attention on some of the traditional methods for preserving and using food which would otherwise perish on account of the unfavorable circumstances regarding temperature, humidity, etc., which occur in the Tropics; but I think we need to consider the subject of fermented foods in its proper perspective.

You have heard in this conference that, among "Natto," "Miso," and "tempeh," only one of the three gives a higher nutritive value than the other two. It is unfair to say that fermentation would improve the nutritive value of the food which is fermented. There is the type of the food to be considered. There is also to be considered the type of the organism which brings about fermentation. In some of these cases the organism which ferments is under mixed conditions and not a single organism. There usually are mixtures of organisms working together. Sometimes a product may smell, have a flavor of Jamaica rum. Sometimes it may have a flavor and smell which are not so welcome. This only indicates that here you have variables of the particular foodstuff fermented, the variable being the organism which is to be used for fermentation and to get a product resulting in high nutritive value.

Experiments in India in which milk is subjected to lactic fermentation have shown no difference in the nutritive value so far as nitrogen is concerned. We have not carried out any experiments on human subjects. The problem, I admit, is not one which has been extensively investigated. But here is an example where you find that fermentation does not necessarily improve the nutritive value of the foodstuff.

I am not comparing the lactic fermentation with yoghurt, which is prepared by fermentation with *Bacillus bulgaricus*. The domestic culture has been identified as consisting of nearly 30 different organisms under different conditions which exist in homes. The different temperatures the year round under which the fermentation occurs in the home give you a product which cannot be reproduced. It is here that studies of food technology to give a standardized product would be more helpful.

Let us not consider fermented food as a catch word, that everything which is fermented is likely to give us improved nutritive quality, because I think that is the sort of idea which is gaining ground. We have to be more discriminating in the choice of fermented foods and subject them to a proper investigation, as is being done by Dr. György and Dr. Hand in the case of tempeh.

DR. HAND: Dr. György has suggested that I did not point out the degree of heat to which this tempeh was subjected. The amount of heat that soybeans are subjected to of course, as you all know, determines the BV of the protein. There are materials, antienzymes, in the soybean that are inactivated, so

[1] *Editor's Note*—This discussion covers the two preceding papers.

you get an improved BV with a certain degree of heat, and then if too much heat is applied you get a diminished BV. So this is a critical thing. We have not worked it out at all ourselves.

We heated this product before fermentation for 90 minutes in live steam. So it would be 90 minutes at 100° C. Following the fermentation we dried the first product sent to Dr. György in a lyophilizer, and there was essentially no heat damage there. The product was dried at pretty close to freezing temperature. The other product we dried in a small laboratory dehydrator on shallow trays, and the temperature rose to 150° C. It took a matter of 2 hours to complete the drying at that temperature.

There may have been some damage due to the heat during that drying. There was also a difference in the two products in the amount of soluble nitrogen on analysis. This is just a clue. We are frankly puzzled by the difference in the two products. It will require considerably more experimentation.

DR. WILLIAMS: You had your first tempeh in which you got an improved growth rate; then your second one, which you think may have been heated during the drying process, you said did not show any improvement over the control soy product. How much difference was there between the growth rate of the first tempeh and the second?

DR. GYÖRGY: The PER in the second tempeh was 1.34, and in the first 1.59 (always pair-fed at 10% level).

DR. ALTSCHUL: Do you have any information on the comparison between the PER of tempeh and of, let us say, toasted soy grits?

DR. GYÖRGY: No, because I don't have any more of the good tempeh. The toasted soy grits have not been tested, but all toasted soy grits are inferior to the reference milk. The original tempeh tested against the reference milk was equal. Therefore, the tempeh was better than any of the toasted soy grits because we have the comparison with the same reference, skim milk.

DR. HUNDLEY: How did you control the problem of heat applied in the manufacture of your control soybeans and your Saridele, for example?

DR. GYÖRGY: I don't know anything about production. In my rats, all these preparations were not heated. They were given unheated. They were heated by Dr. Hand in his laboratory originally. Saridele and the soy milk were heated in production. That was given to rats without heating.

DR. HAND: When Dr. Steinkraus visited the Saridele plant, he was somewhat disturbed by the possibility that the product was being overheated some days, that an irregular degree of heat was being applied, and that this failure to control the heat treatment would cause big differences between batches of the Saridele. So we really do not know how to compare the degree of heat between our samples and the Saridele samples.

DR. DAVIS: I asked the question if you considered an antibiotic being formed, since your results with rats are very much like the results that are obtained with animals where an antibiotic is added to the protein source. In other words, a lower protein does as good a job as a higher protein if you add antibiotic.

DR. GYÖRGY: I have not tested for antibiotics, but that is a good possibility. Just one comment on Dr. Patwardhan's very apropos remarks. I fully agree with him that we should not speak of fermented foods as a catch word. I have done it, and I repent. As far as tempeh is concerned, that is the real stuff, probably, which in my opinion is worth exploring.

Some Observations on Fermented Foods

M. V. Radhakrishna Rao

THE WORK presented here was mainly carried out by Miss Panna K. Khandwala, under my direction, in collaboration with Dr. S. M. Patel and Dr. S. D. Ambegaokar.

I had the privilege of attending, as a member, the meeting of the FAO/WHO Expert Committee on Nutrition held in Gambia, West Africa, in 1952 and, later, the Conference on Protein Malnutrition, held jointly under the auspices of the FAO, WHO and Josiah Macy Foundation in Kingston, Jamaica, in 1953. During these meetings the importance of protein malnutrition as a global problem was fully discussed. The members had ample opportunities to see cases of kwashiorkor in its various phases, to study environmental conditions and to get acquainted with local foods and traditional food habits.

During one of the informal talks in Kingston, Prof. György had referred to his preliminary observations on rats fed fermented foods. Rats fed on fermented diets (fermented accidentally during transportation) showed a beneficial effect on experimental liver injury.

Soon after my return home, we started collecting information on the traditional foods prepared by fermentation with a view to studying the beneficial effects, if any. It was anticipated that this study might also contribute to our search for protein-rich foods of vegetable origin in our combat with the problem of protein malnutrition.

Fermented foods are prepared from either cereals, pulses or legumes, fruits, milk or fish. However, there is no adequate information either on their chemical composition or on the changes which take place during the process of fermentation.

In Burma, fermented fish ("Nappi") is used as a supplement to a staple rice diet. In Japan, fermented soy products ("Natto" and "Miso") are used. A mixture of black gram and rice is allowed to ferment by air-borne microorganisms in South India in the preparation of "Idli" and "Dosai." Similarly, Bengal gram is used in the preparation of "Dhokla."

For over 25 years I have been actively interested in the study of the relative role of dietary factors in the causation of liver disease in India, both in children and in adults. In general, a low intake of proteins and deficiency of several factors of the Vitamin B complex are commonly encountered in such patients. It has been found that, compared with the widespread protein deficiency in the community, especially in South India, the incidence of liver disease is not very high. A study

of the dietary habits revealed that food preparations, fermented before they are cooked, are consumed in one form or another. One such preparation is "Idli."

"Idli" was prepared as follows for the experimental work: Four parts of black gram (*Phaseolous mungo*) and one part of rice are soaked separately in water for about 2 to 3 hours and then ground into fine batter and mixed. This mixture is allowed to ferment at room temperature overnight and steam cooked into a pudding next morning.

Experiments were carried out on rats, using fermented and unfermented preparations. It has been established in this laboratory that rats fed on a high-fat low-protein diet showed liver damage. This was manifested by an accumulation of fat in the liver, demonstrable both microscopically and biochemically.

Feeding "Idli" to the experimental animal at 50% replacement of dietary casein lowered the fat percentage of the liver. This was quite significant as compared with the feeding of the same preparation before fermentation.

Chemical and microbiological studies on the fermented and the unfermented preparations have shown that, as a result of fermentation, there is an increase in choline, methionine and folic acid values. Fermentation, however, does not influence the B_{12} content of the mixture of black gram and rice. On the other hand, the fermented food preparation "Idli" possesses a better regenerating capacity for RBC as compared with the unfermented preparation.

These findings are summarised in the following table.

COMPARISON OF "IDLI" * WITH THE UNFERMENTED PREPARATION

	Unfermented preparation	"Idli"
1. Fatty change on high-fat, low-protein diet		
a) histological	+++	±
b) biochemical	fatty liver	fat is reduced
2. Lipotropic factors, %		
a) choline gm	0.324	0.434
b) methionine gm	0.129	0.153
c) folic acid mcg	42.38	67.34
d) vitamin B_{12} mmcg	3.93	3.74
3. Protein efficiency ratio	2.28	2.55
4. RBC regeneration	no improvement	improvement
5. Hb regeneration	no difference	
6. Serum protein regeneration	no difference	
7. Liver protein regeneration	no difference	

*See text for preparation procedure.

In view of the widespread protein deficiency, especially in children in many parts of the world, further studies in such fermented food preparations which have a beneficial effect appear necessary.

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DISCUSSION

DR. PLATT: Dr. Rao, from your table, do we understand that there is an increase in the methionine in the fermented? If you are synthesizing methionine, it is a most valuable discovery.

DR. RAO: Choline, methione, and folic acid were increased, but not B_{12}.

Relevant Research in the United States

Nitrogen Balance Studies of Plant Proteins in Infants

Robert Kaye, Lewis A. Barness, Aree Valyasevi and John Knapp

PLAN OF STUDY: The subjects were male, underweight Latin American infants aged 4 to 10 months, who had been admitted to the Driscoll Foundation Children's Hospital because of severe diarrhea. The diarrhea was brought under control by the usual methods. All infants had been well and taking milk and other good quality proteins for not less than 10 days preceding the start of the experimental diet. The infants were transferred to an air-conditioned metabolic unit with special nursing care "around the clock." Five to seven days after starting the experimental diet, and also after 2 days of the experience of being in restraint, the balance study was begun.

Balances for each experimental diet were carried out for 9 days. The duration of study for each subject ranged from 3 periods (27 days) to 6 periods (54 days). Body weights were recorded daily. Blood chemistry and hematologic studies were done at the beginning and the end of each period. Urine and feces were collected separately and analyzed. Chemical determinations were made on pooled 72-hour specimens of both urine and stool.

Two types of data were sought in these studies. First, when one protein source was mixed with another, a positive effect of the supplemental protein was considered to be present if the increase in nitrogen balance during the supplemented period exceeded that of the most positive unsupplemented period by an amount greater than 10% of the nitrogen intake. A negative effect was considered to be present if the control period exceeded the supplemented period by more than 10% of the intake, and no effect if the balances were between the 10% limits.

Second, balance data were obtained with the vegetable proteins compared with milk protein fed in approximately the same amount, thus obtaining an indication of the relative value of a particular vegetable protein compared with milk protein.

The results of our studies involving 22 infants fed wheat alone, wheat-milk mixtures and wheat supplemented with lysine and potassium have been previously presented to some members of this group and will be published in The American Journal of Clinical Nutrition (*9*:331, 1961).

These data indicate that lysine and potassium supplementation of wheat diets increase the nutritional value as indicated by nitrogen retention. The favorable

effects of supplementation were most clearly shown in 9 of 11 balance periods in 7 infants fed wheat alone at a level not exceeding 2.1 gm protein/kg/day and 75 to 100 cal/kg/day.

A representative experiment is illustrated in chart 1. The nitrogen intake in mg/kg/day is indicated by the height of the vertical bar above the base line. Urinary excretion of nitrogen is represented by the clear area and fecal nitrogen by the cross-hatched area. The solid black area represents the nitrogen retention.

The data are presented for the 3 subperiods of 3 days each which make up the 9-day experimental periods. The periods of supplementation with lysine and potassium are indicated by the horizontal lines.

In this subject, the second period serves as the control period, and a positive effect of combined lysine and potassium supplementation is seen in both periods in which the supplements were administered together and is absent in the periods of supplementation with lysine alone and with potassium alone.

We are reporting here on additional studies in 20 infants fed diets of rice, cottonseed and peanut flour at a caloric level of approximately 100 cal/kg/day. In all experiments, comparison has been made of the protein under study with milk and, in most experiments, with mixtures of an equal quantity of other plant proteins. The effect of supplementation of rice with lysine and threonine has also been evaluated.

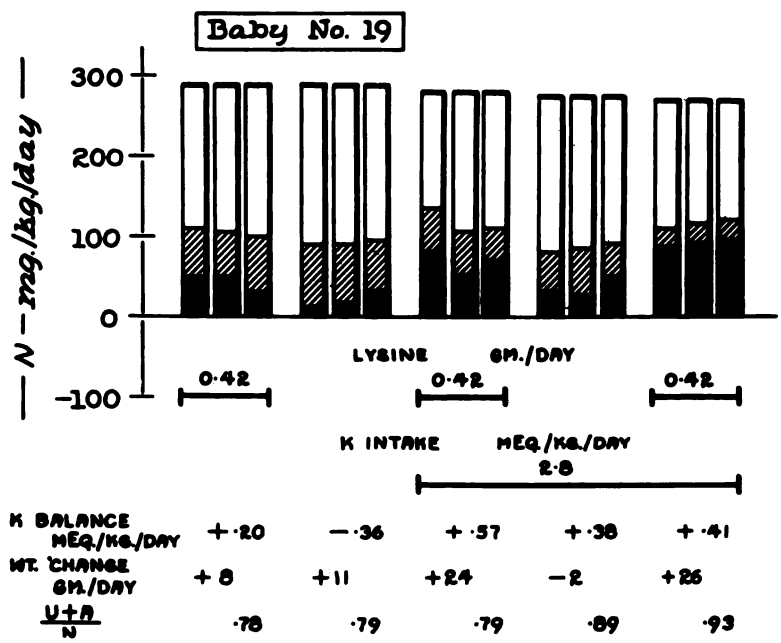


Chart 1—Effect of lysine and potassium supplementation of wheat. Clear area—urinary N, Cross-hatched area—fecal N, Black area—N retained.

Amino Acid and Potassium Supplementation of Rice

Chart 2 and table 1 show the results in 3 babies fed rice and rice supplemented with lysine, threonine and potassium. In the chart, the balance data are charted for the 9-day experimental periods as a whole. At the lower part of the chart, supplementation with lysine, threonine or potassium and control periods of milk or rice plus glycine feedings are indicated by the plus signs. In 2 of the 3 babies, R.J. and M.C., retentions following the supplementations were significantly greater than during the control periods of supplementation with glycine, while no significant effect of supplementation was noted in A.T. In R.J. the positive effect was noted with lysine and potassium but not with lysine, threonine and potassium. In M.C. the combination of all 3 supplements was associated with a positive effect which was absent when threonine was omitted.

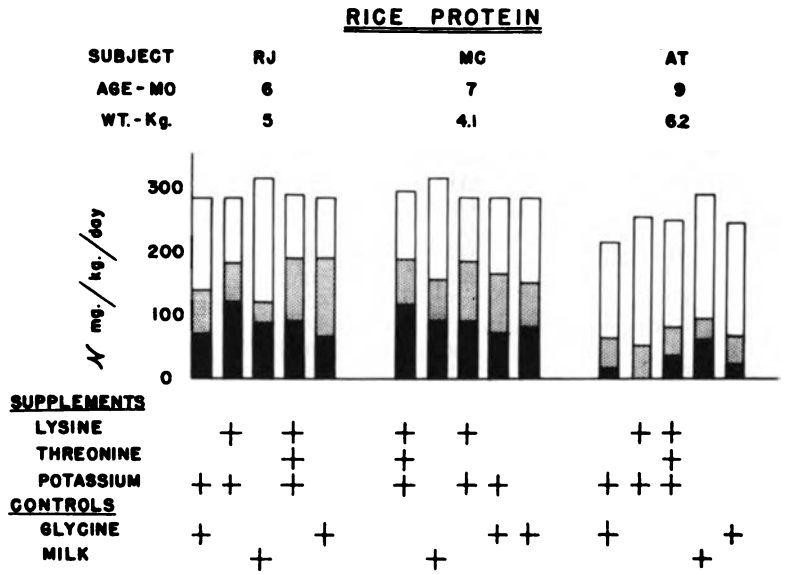


Chart 2—Balance data presented for the 9-day period as a whole. Plus signs indicate the administration of the designated supplement or control.

As noted above, periods of milk feeding were included as a standard of reference for the experimental diets and the individual subjects.

Rice supplemented with lysine and potassium in subject A.T. was a poor source of protein compared with milk. The addition of threonine increased the nitrogen balance to a level which was lower than that of milk, but not significantly so. In M.C. the combined amino acid supplements significantly increased nitrogen retention above that of milk, and in R.J. lysine and potassium supplementation also resulted in a significant increase in nitrogen retention over that of milk.

TABLE 1
NITROGEN BALANCE STUDIES

Subject	Regimen	Intake (mg N/kg/day)	Balance	Percent retained	Wt gain (gm/day)
AMINO ACID AND POTASSIUM SUPPLEMENTATION OF RICE					
A.T. #32	RGK	260	19	7.3	5
9 mos.	RLK	258	+0.2	0.0	5
6.2 kg	RLTK	254	+34	13.4	10
	M	290	+63	21.7	24
	RG	246	+21	8.5	0
R.J. #30	RGK	285	72	25.3	13
6 mos.	RLK	285	125 (1)	43.8	6
5 kg	M	317	82	25.9	13
	RLTK	294	88	29.9	3
	RG	285	67	23.5	—2
M.C. #31	RLTK	293	116 (1)	39.6	13
7 mos.	M	317	90	28.4	3
4 kg	RLK	283	89	31.4	6
	RGK	283	72	25.4	0
	RG	283	80	28.3	0
COTTONSEED PROTEIN					
R.H. #39	C	483	97	20.1	—13
7 mos.	CW	450	110	24.4	22
6.5 kg	CR	411	98	23.8	3.2
	M	470	125	26.6	3.2
P.R. #33	C	298	43	14.4	8
6 mos.	M	312	68	21.8	22
6 kg	CR	292	80 (2)	27.4	18
	CW	267	43	16.1	14
R.V. #34	CW	290	47	16.2	11
10 mos.	M	304	79 (2)	26.0	27
7.5 kg	CR	263	56	21.3	13
	C	274	45	16.4	26
R.A. #21	M	635	142	22.4	14
4 mos.	C	635	125	19.7	5
4.7 kg	M	618	166	26.9	10
A.V. #20	C	445	39	8.8	23.9
10 mos.	M	449	108 (2)	24.0	4.8
6.0 kg	C	445	58	13.0	9.6
J.K. #44	C	485	7	2	14
6 mos.	M	382	53 (2)	16	16
5.2 kg	C	460	21	5	14
R.O. #45	C	470	120	32	—10
7 mos.	M	440	110	28	+10
7.3 kg	C	451	78	21	+18
PEANUT PROTEIN					
H.R. #38	P	545	87	16.0	6.4
10 mos.	PR	470	141 (3)	30.0	9.6
7.5 kg	PW	470	110	23.4	12.8
	M	452	110	24.3	9.6
	P	525	79	15.0	0
P.F. #36	P	482	80	16.6	8
10 mos.	M	334	97 (3)	29.0	14.3
6 kg	PW	269	110 (3)	29.8	20.8
	PR	370	92	24.9	4.8

TABLE 1—Continued
NITROGEN BALANCE STUDIES

Subject	Regimen	Intake (mg N/kg/day)	Balance (mg N/kg/day)	Percent retained	Wt gain (gm/day)
		PEANUT PROTEIN—Continued			
A.M. #37	P	415	81	19.5	4.8
5 mos.	PW	330	84	25.4	44.6
4.6 kg	PR	323	111 (3)	34.4	0
	M	283	91 (3)	32.1	14.4
D.E. #41	P	424	34	8.0	14.3
5 mos.	PR	362	69 (3)	19.1	8.0
4.5 kg	PC	414	64	15.4	3.2
	PW	364	43.5	11.8	9.6
	M	357	99 (3)	27.7	6.3
R.P. #46	P	332	22	7	6
7 mos.	PR	314	70 (3)	22	14
6.5 kg	PC	327	47	14	—5
	PW	324	72 (3)	22	10
	M	296	57 (3)	19	24
E.N. #42	P	608	125	21	26
10 mos.	M	452	86	19	26
7 kg	P	575	91	16	8
	P	450	61	14	14
	M	436	96	22	19
	P	438	106	24	10
M.R. #43	P	538	45	12	—21
12 mos.	P	426	40	9	6
8 kg	M	414	80	19	8
	P	416	70	17	6
		PEANUT—RICE—MILK COMPARATIVE STUDY			
F.M. #48	P	270	6	2.2	0
6.78 kg	PR	290	64 (3)	22.1	10
	R	286	64 (3)	22.4	12
	M	297	78 (3)	26.3	28
J.S. #49	P	245	20	8.2	13
5.7 kg	R	260	58 (3)	22.3	6
	M	274	45	16.4	6
	PR	428	17	6.8	13
E.S. #50	R	303	102 (3)	33.7	21
7.2 kg	PR	278	73	26.2	28
	M	296	94 (3)	31.7	21
	P	250	50	20.0	18

C = Cottonseed L = Lysine R = Rice
G = Glycine M = Milk T = Threonine
K = Potassium P = Peanut W = Wheat
(1) Significant increase in nitrogen balance compared to rice and glycine
(2) Significant positive change in nitrogen balance compared to cottonseed alone
(3) Significant increase in nitrogen retention compared to peanut alone

COTTONSEED PROTEIN CF 1

Three babies were fed cottonseed alone and cottonseed mixtures with rice or wheat proteins, so combined that a supplemented period contained 50% of the protein from the cottonseed and 50% of the protein from the other vegetable

source (chart 3 and table 1). Retention of nitrogen with milk feeding was significantly greater than with cottonseed in only one of the subjects, R.V. In only one subject, P.R., was the cottonseed-rice mixture superior to cottonseed alone, and in no instance did mixtures with wheat lead to significant improvement in nitrogen balance.

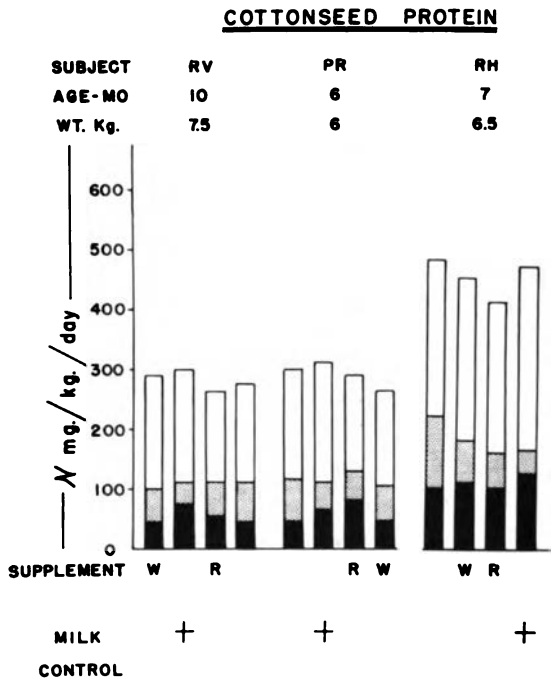


Chart 3—N-balance data are presented for 9-day period as a whole in this and subsequent charts. Supplements consisted of a 50% mixture of cottonseed flour and W = wheat, or R = rice.

Four subjects were studied with alternate milk and cottonseed feeding periods (chart 4 and table 1). In two subjects, A.V. and J.K., milk yielded significantly greater nitrogen retention. Combining the experience of the subjects fed cottonseed, only 3 of 8 milk feeding periods exhibited a significantly increased nitrogen retention over that obtained with cottonseed.

Peanut Flour PF 4

In the experiments illustrated in chart 5 and table 1, the peanut flour was supplemented with an equal quantity of protein from rice, wheat or cottonseed in 5 infants. Because of an error in the initial analyses of the nitrogen content of the peanut flour, the actual quantities of nitrogen fed in some of the periods in which peanut flour was the sole source of nitrogen were higher than planned.

TABLE 2
RATIO OF PERCENT OF INTAKE RETAINED ON INDIVIDUAL REGIMENS TO THAT OBTAINED WITH MILK

Regimen	No. Periods	Ret/Int x 100 Ret/Int (milk) Range	Mean
	RICE		
RGK	3	34-97	74
RLK	3	0-170	93
RLTK	3	62-139	105
RG	3	39-100	76
R	3	85-136	109
	COTTONSEED		
C Int N > 425	8	13-144	60
C Int N < 425	2	63-66	64
CW	3	62-92	76
CR	3	82-126	99
CP	2	56-74	65
	PEANUT		
P Int N > 425	10	47-117	75
P Int N < 425	6	8-63	41
PR	8	41-123	89
PW	5	43-116	87
PC	2	56-74	65

R = Rice L = Lysine W = Wheat
G = Glycine T = Threonine P = Peanut
K = Potassium C = Cottonseed

Fortunately, in spite of the resulting defect in experimental design, certain tentative conclusions can be drawn from the data.

Nitrogen retentions were generally less on peanut flour as compared with milk and significantly lower in P.F., A.M., D.E. and R.P. Peanut-rice mixtures yielded greater retention than peanut alone in 4 of 5 experimental periods (A.M., D.E., R.P. and H.R.). The mixture with wheat was superior to peanut alone in only 2 of 5 periods (P.F. and R.P.). Addition of cottonseed to peanut in 2 periods did not significantly affect nitrogen retention.

In chart 6 and table 1 are the data from 3 experiments in which a comparison is made of nitrogen retentions during periods of feeding peanut, rice or milk alone, and a mixture of equal parts of the two plant proteins. In all instances rice yielded significantly greater retentions than did peanut, but in only one subject (F.M.) did the addition of rice significantly increase nitrogen retention over that obtained with peanut alone. This was in contrast to the results in the preceding group of subjects in whom the addition of rice resulted in significant increases in nitrogen retention in 4 of 5 subjects studied. In accord with results in the previous group of subjects, retentions with milk were significantly greater than with peanut flour in 2 of 3 subjects (F.M. and E.S.). As noted before, retentions with rice were equivalent to those obtained with milk.

In the two additional subjects (E.N. and M.R.) illustrated in chart 7 multiple periods of peanut feeding were compared with milk feeding periods and, in contrast

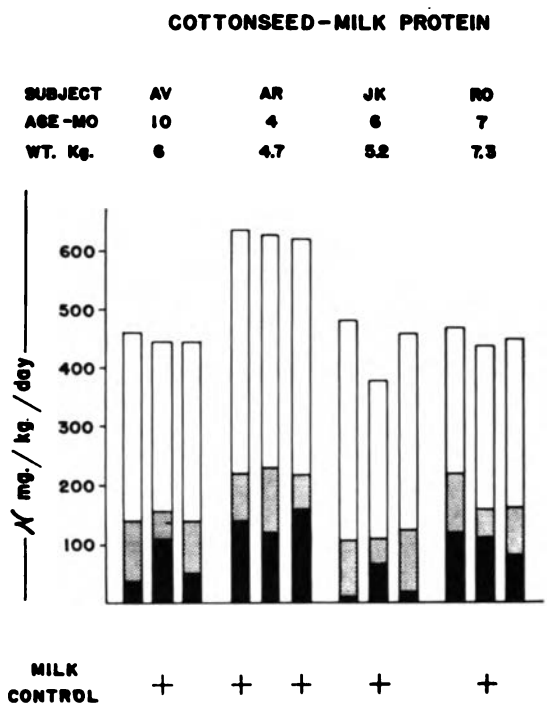


Chart 4—Comparison of diets of milk and cottonseed. Plus sign designates milk control.

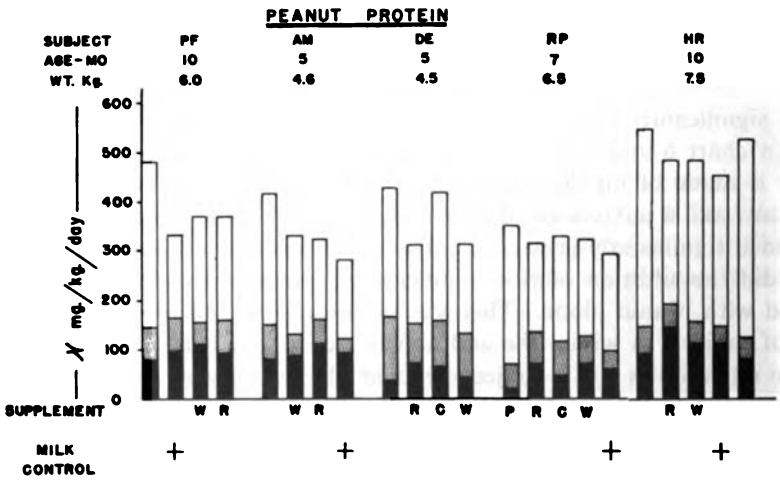


Chart 5—Supplements consisted of a 50% mixture of peanut flour and W = wheat, R = rice, and C = cottonseed.

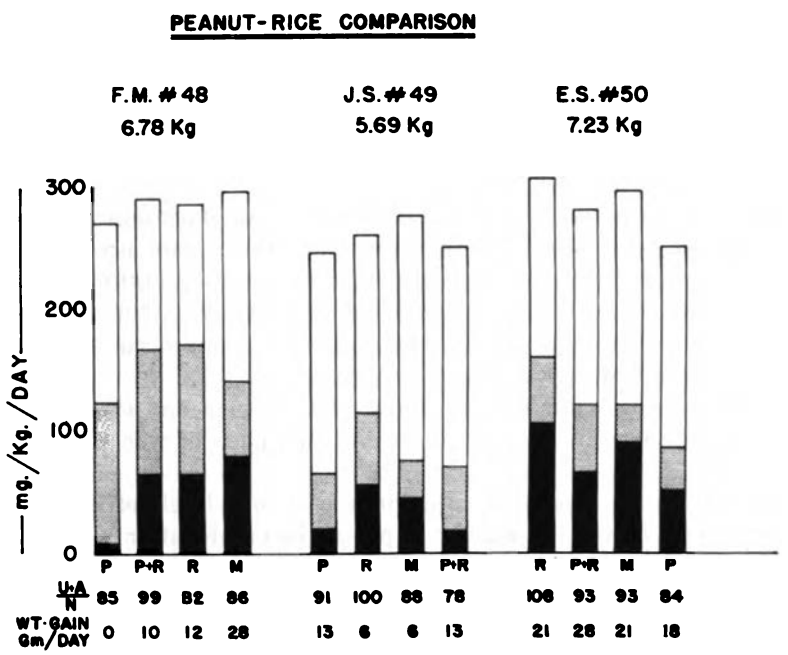


Chart 6—Diets: P = peanut alone, P + R = mixture of equal parts of peanut and rice, R = rice alone and M = milk alone.

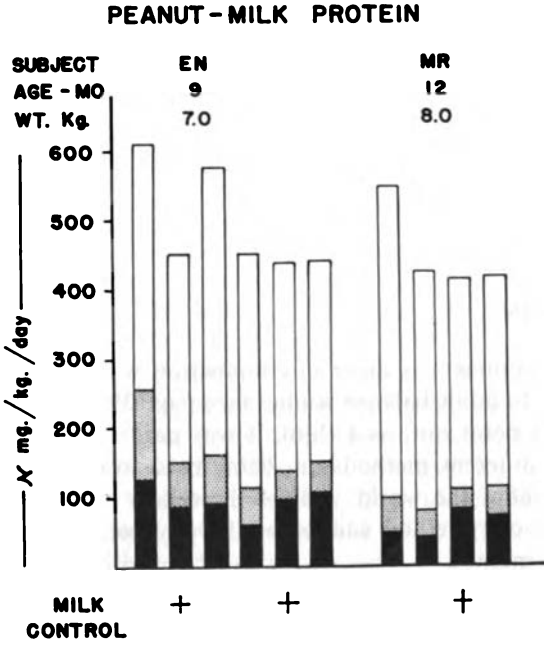


Chart 7—Comparison of diets of milk and peanut. Plus sign designates milk control.

to our previous experience, no significant differences in nitrogen retention were noted between the two protein sources. It should be pointed out, however, that in these two individuals and in H.R. in the preceding chart, who also failed to show a significantly lower nitrogen retention on peanut as compared with milk, the nitrogen intake was about 20% higher than in those studies in which milk yielded significantly higher nitrogen retentions.

At the present stage of our experience using short-term balance studies, certain tentative conclusions may be drawn. In table 2 are presented the ratios of nitrogen intake: retention obtained on the various rice, cottonseed and peanut regimens to that found in the same subject on milk. Rice appears to be a good protein source and may, as we have seen above, be improved in some instances by supplementation with lysine and threonine.

Cottonseed is a fair protein source at both high and low levels of intake and may be improved by combination with equal parts of wheat and rice but not with peanut.

Peanut flour is a fair source of protein at high levels of intake and rather poor at low intake levels. It may be improved by combination with rice or wheat and perhaps by cottonseed.

Rice appears to improve the nutritional quality of peanut and cottonseed flours.

Further studies are planned along the lines of those shown in chart 6 and table 1, in which two plant proteins are compared with each other by feeding them singly and in a mixture of equal parts of each, and with milk fed at equal nitrogen intake. The data on amino acid supplementation of rice will also be extended.

Supported by the National Research Council, Du Pont Company, Mead Johnson Company and Merck-Sharpe and Dohme.

DISCUSSION

DR. RICE: This comment is more in conjunction with Dr. Patwardhan's paper, in reference to adult balance studies (see page 393).

I want to point out, as I think it was pointed out by Dr. Kaye, that a number of different methods are being used to collect and record balance data throughout the world. All of these have their advantages, but often the data by one method cannot be directly compared with data obtained by another method.

In our experience, when working with adults having good protein stores, it is not often possible to get significant results unless you work with a group. We attempted to narrow the limits of error in some balance studies of this kind sponsored by du Pont in 1958 and 1959. We contracted to have the

work done by an independent laboratory with the help of Purdue University faculty advisers. Our emphasis was to try to get statistically significant balance results by minimizing the number of variables. In most of the balance studies that we could find in the literature, several things were being changed at the same time, and as a result statistical evaluation was difficult or impossible.

In feeding our college student subjects, we attempted to approximate the precision of the formula diets used for laboratory animals. We gave them a constant, almost single-food diet consisting essentially of bread. This we found to be quite acceptable to them.

I show you a chart here which is a summation of about 66 balance days with 12 students. This experiment was run between January and the end of May. Each one of the bars represents a 3-day balance period. We had a rest period of about a week between experiments of 18-21 days' duration. We ran control groups parallel to the experimental groups. Thus, we had a control group receiving one gm of protein/kg, another obtaining 0.72 to 0.77 gm of protein/kg/day, alongside experimental groups on the same protein intakes.

STATISTICAL ANALYSIS
SHUMAN CHEMICAL LABORATORY 1959

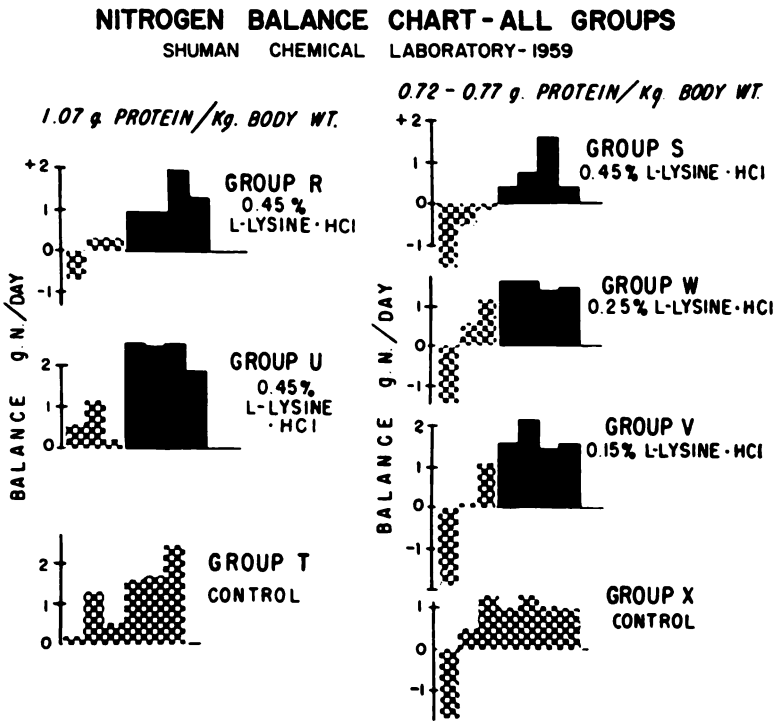
	Observed N Balance variation	Std. deviation
Effect of Protein Level	+ .63	0.24
Effect of L-lysine Supplementation	+ .46	0.20
Interaction Between Protein & Lysine	.15	0.47
Effect of Season on N Balance		
$T_3 + U_3$ vs. R_1 / U_4 vs. R_2 } Average	0.90	.22
$V_5 + W_5 + X_5$ vs. S_1 / $V_6 + W_6$ vs. S_2 } Average	0.80	.19
Effect of Lysine on Creatinine	—	No correlation
Effect of Protein on Creatinine	—	No correlation

As you can see, we have the same finding that Dr. Patwardhan mentioned this morning. All groups tend to come into positive nitrogen balance. Even after 8 balance periods, these students, healthy and with normal protein stores, appear to be retaining better than a gram of nitrogen per day on intakes of 0.7 gm protein/kg, roughly two-thirds to three-quarters of the NRC recommended allowance. We did not want to deplete the subjects on low-protein diets, as is often done to standardize them, because in this study we wished to determine the effect of lysine supplementation in adult subjects with good protein stores.

One of the most important findings was that there is a "seasonal" effect on nitrogen balance, visible here going from Groups R and S to T and U through to W, V and X. As we progressed through the series from January through May, we found increasing apparent retentions of nitrogen in

subjects believed to be actually in equilibrium between intake and outgo. This was an outstanding finding as far as we were concerned.

The tabulation below shows what the statistician found for us on this. We have a significant difference between the protein levels, as I think everyone would expect. Our standard deviation on the experiment was 0.24



gm of nitrogen daily. To have significance, differences of more than two standard deviations must exist in the data. The effect of lysine supplementation was significant—again we have more than two standard deviations. There was no interaction between protein and lysine. They were independent, as shown by this comparison.

What we interpret as an effect of season on nitrogen balance is shown in detail. We believe it to be important to make data comparisons using data collected at the same time and at the same protein level. You will notice there are more than four standard deviations of separation in these figures. We checked for an effect of lysine on creatinine and of protein on creatinine. Seeing no effect, we felt assured we had a good sample collection and a well executed experiment.

The point to make, I think, is that if we are going to run balance experiments on healthy ambulatory adults, we would always prefer to run them on groups. We would avoid studying one individual at a time, because we do not believe you can get significant results unless you deplete

the subject's protein stores rather extensively. If we were going to run balance studies on children in a ward, we might consider individuals, because you have a continual tendency to retain nitrogen that is with you all the time that the child is growing.

DR. PLATT: How do you differentiate the seasons? What are the characters of the seasons, and why do you get this seasonal effect?

DR. RICE: There was no other visible factor that we could correlate with the observed change in balance going from group to group at a given protein level as we progressed in time. That is, if our conditions were truly constant, we would not expect the retention of well nourished adult subjects to increase continually over this period of time from Groups R and S where you have a one-gram retention, to the next group where you have 1.2, to the final group where you have 1.5. Obviously, we had to look around in the environment for some variable or variables that changed with time. These men were not hospitalized. They were engineering students, some were graduate students. At Purdue in January they probably were fairly inactive, while in April and May they were probably fairly active even though they were not athletes. We have assumed that the activities in a university, which normally do increase in this period of warming weather, are responsible for this increased apparent nitrogen retention. We consider this only an apparent retention.

DR. HOLT: I should like to ask Dr. Rice about his controls. When he gave the lysine, was the control a non-supplement control or was it some other form of nitrogen than lysine?

DR. RICE: Ninety-five per cent or more of the nitrogen in the diet came from standard enriched white bread, containing 4% milk solids, baked by the American Institute of Baking in Chicago. The students did not know which group had the lysine supplement. This was essentially a double blind experiment. Glycine was used as control source of non-lysine nitrogen. We had isonitrogenous experimental and control diets in all cases.

DR. BENDER: How does the protein intake in your experiment compare with the protein intake before the experiment started?

DR. RICE: I would assume that the recommended allowances, which are roughly one gram of protein/kg, were usually met. This would be about 70 gm/day. I do not believe many of our college students are living on much less than that. As evidence of this, when we moved from the normal college diet to our study period at one gram of protein/kg, there was very little change in nitrogen retention. So perhaps they normally were eating somewhere between the 0.7 gm/kg level, where they lost some nitrogen at first and then recovered, and the 1.0 gm/kg level. From our experiment, the effect of protein level on nitrogen balance is a real effect, so we conclude they must have

been consuming in their normal diet somewhere between 0.7 and 1.0 gm of protein/kg/day.

DR. SCRIMSHAW: I am glad that Dr. Rice qualified his remarks by saying that perhaps this conclusion does not entirely apply to children. Without in any way questioning the desirability of having groups on balance if possible, I think that Dr. Kaye's very useful data, to us at least, the results which we have obtained with children in supplementing corn and wheat, and so forth, and Dr. Holt's studies, all indicate very clearly that you can use individual children, and the consistency of results in several individual children makes a very good guide.

Dr. Kaye, I note that, as usual, it was not possible to maintain the intake entirely constant from period to period, and yet you expressed your nitrogen retentions in absolute terms. Does it help your comparisons if you express them in terms of percentage of nitrogen intake, or does it obscure the difference that you report?

DR. KAYE: As we considered changes in nitrogen balance to be significant only when they exceeded 10% of the nitrogen intake, we were able to make comparisons between periods when the nitrogen intake was not held as constant as we would have liked.

DR. BROCK: I would just like to say in defense of individual nitrogen balance tests on adults that, provided you use your patient in a metabolism ward, I am sure you can get significant results on individual adults. Dr. Truswell has done work in our department in this direction.

I should perhaps make one other qualification. Our subjects perhaps were nitrogen-depleted compared with the Purdue students. Under ordinary conditions, one would expect them to be. We found it quite impossible to measure. We are still trying to find means of measuring the extent of nitrogen depletion in people. I think this is one of the biggest gaps in our knowledge: How does one know whether a person is nitrogen-depleted or not?

DR. PATWARDHAN: I was particularly pleased to see the results which Dr. Kaye has obtained with rice. I do not think that you need much more evidence to vindicate the rice eater and the rice on which he subsists. Rice has been maligned too much in the past merely because the total amount of protein in rice was less than you find in wheat. Time and again, experiments with animals have shown the high quality of protein in rice. Now you have evidence from experiments in humans and children to show that the retention of nitrogen over absorbed nitrogen is comparable to that obtained with milk.

Of course, it is true that the low protein content of rice has to be made up with supplementation with other protein-rich food. But what rice lacks in quantity it makes up in quality, which is a fact I hope people will remember in discussing rice vis-a-vis other cereals.

The second observation which I would like to make here is apropos of what Dr. Rice stated about supplementation. When I spoke this morning, I knew that the question of techniques and variables was bound to come.

When we start experimentation on subjects, particularly humans, we are aware of variables, the limitations of our knowledge and our experience, and the facilities we possess. Taking all these into consideration, I do not say it casts doubt on the results. All I say is that when you get results which are unusual, the first thing an investigator does is to ask himself whether he has taken due cognizance of all the variables which are liable to vitiate the significance of the results. When you see that the results which you obtain are also comparable with the results obtained in reputable laboratories with investigators who have been in the field for years, then you gain some confidence in the reliability of the results which you obtain.

Interpreting your findings in view of past experience is a different matter. I fully agree with Dr. Brock that you can get consistent results which can be interpreted with a certain amount of certainty even on individual subjects, not necessarily to be hospitalized or put in a metabolic ward. If you have subjects who are themselves workers in the field, as happens with most of the subjects we use in our investigations, who realize the responsibility and who know what is at stake, I think you are pretty sure to get results even more reliable, I would say, than when you are dealing with students who sometimes do things which should not be allowed.

DR. PIRIE: People seem to be overlooking the fact that, besides passing out urine and feces, persons also get rid of hair, skin, sweat and mucus, and it isn't certain that the sum of all these various forms of nitrogen will not come to as much as the 1 gm or 1½ gm a day that is now being looked on as protein absorption by the person being fed.

DR. PICKERING: Dr. Kaye, by what manner did you control the fat source.

DR. KAYE: We matched the fat content of the milk control with lipomul, using none when skim milk was the control diet.

DR. FRENK: What was the objective of the supplemented diet which you were analyzing? Why was potassium put in?

DR. KAYE: The answer to the potassium question first. When we used very low protein intakes in the experiments with wheat, several of our babies were rather weak and hypotonic. We measured the serum potassium levels and found them low. Two or three babies at the low level of protein showed negative potassium balances. These babies were getting something on the order of 1½ milli-equivalents of potassium/kg/day. We had previously done studies with low protein intakes derived from milk and found an intake of one milli-equivalent of potassium/kg/day was adequate, but with wheat this was not adequate, so the supplementation with potassium was carried out.

The reason for supplementation with lysine is the relative deficiency of wheat protein in lysine. We knew of the possible displacement of potassium by lysine in potassium deficiency.

DR. ALLISON: I just want to emphasize the fact that in nitrogen balances the gains and losses of nitrogen from all the various tissues of the body are under the influence of many metabolic changes. So with full protein reserves in the individual such as Dr. Rice was reporting to us, you would expect quite a bit of variation, increase in excretion, in fact temperature changes and things of that type. I disagree with Dr. Rice that you need to have a group, because you can learn a lot from nitrogen balance in the individual. I think one of the main things you can learn is from the direction which the nitrogen balance is taking.

Naturally, if the individual is depleted a bit or if the baby is growing, you can get much better data on individuals, because in the case of the baby, for example, at least in our work in animals, if you have a good mixture of amino acids you can put that individual in positive balance and he will hold it. If it is a poor mixture of amino acids, he may be temporarily in positive balance and always drift down.

So I think you can learn a lot from nitrogen balance, if you interpret the data thoughtfully, and actually use individuals.

DR. JACKSON: I would not agree that Dr. Kaye's babies were in good nutritional status, which I think is also pertinent. The babies I saw that they studied out at the Driscoll Hospital were certainly suboptimal as far as good nutrition standards are concerned. However, they were not severely depleted.

Protein Malnutrition in Mentally Retarded Children

E. T. Mertz, W. J. Culley, D. H. Jolly and J. Calandro

ALTHOUGH NO STATISTICS are available on the relative incidence of protein malnutrition in normal and mentally retarded children in the United States, it is our opinion that the incidence is much higher in retarded children. This applies to the children both outside and inside institutions. At the present time, more than one-fourth of the admissions to the State School nursery have an iron-deficiency anemia. They are placed on iron medication, vitamin supplements and a high-protein diet to correct this condition.

There is a general tendency for parents to treat the mentally retarded child as a younger child. As a result, he is maintained on a predominantly soft-food, milk-type diet, which is low in protein-rich foods requiring chewing. A mentally retarded child maintained on this regimen at home was referred to one of us with a clinical diagnosis of nephrosis. The child had a generalized edema which did appear to be of the type seen in nephrosis. However, laboratory findings, including a low cholesterol, did not confirm the nephrotic syndrome. All symptoms disappeared after the child was placed on a high-protein diet.

About one-third of the children in our nursery are feeding problems because of the impaired motor ability which characterizes their cerebral palsy. These children are fed a special gruel prepared by homogenizing a balanced diet of cooked vegetables, meat and milk. Friendly, attentive, unhurried feeding is required to insure adequate intake of calories and protein. Wolfson et al.[1] and Berman and Noe[2] have also had success with a similar group by careful feeding of a dry preparation (Dietall) as a gruel.

A small proportion (about 5%) of our nursery population are chronic regurgitators. They also need special attention, such as restraining the forearm at the elbow to prevent use of fingers for gagging.

A major breakthrough in the treatment of mental disease with diet has been the successful use of a low-phenylalanine diet for the treatment of phenylketonuria (Phenylpyruvic Oligophrenia). Of our nursery population of 250, 20 are diagnosed as phenylketonurics.

If the low-phenylalanine diet is started shortly after birth of the phenylketonuric child, mentation appears to proceed normally.[3,4,5] The results with older children are not encouraging.[4] Since most food proteins contain about 5%

phenylalanine, protein intake must be drastically curtailed or serum phenylalanine levels will rise from a desirable value of 2 to 5 mg% to the pretreatment level of 20 to 50 mg%. The nutritionist is thus confronted with the problem of supplying the minimum level of protein to permit growth and prevent hypoproteinosis, without increasing serum phenylalanine levels. Meyer, Mertz et al.[6] fed a diet containing 30 gm of crude protein daily, of which 13 gm was high-quality animal protein, 5 gm plant protein and the remainder free (mainly nonessential) amino acids. NRC recommended allowances[7] were satisfied for all nutrients with the exception of good-quality protein, which was fed at a level of 0.6—1.0 gm per kg daily.

Three phenylketonuric children, aged 4, 10 and 12 years, consumed the low-protein diet for 6 months. At the end of the first month, subject 4 (10 yrs., 20 kg, female) had a total serum protein of 4.8 gm% with an albumin-to-globulin ratio of 0.5 to 1.0. At this time she had a rash on her shoulders, elbows and forearms, and her face and hands were edematous. The diet was continued, however, and the rash and edema disappeared; the total serum protein rose to 5.6% at the end of the third month, then leveled off at 5.1% at the end of the fourth and fifth months on the diet.[8] Subject 2 (12 yrs., 30 kg, male) had serum protein levels of 6.5% and 6.3% respectively[8] at the end of the third and fourth months on the diet. At the end of the fifth month, this had dropped to 4.7% and pretibial edema was observed, with swollen red feet. The diet was continued, however, and the serum protein rose to 5.2% in 4 days, with disappearance of the edema. The serum protein level was also 5.2% at the end of the sixth month on the diet. Two and one-half months after subject 2 was returned to the normal nursery diet, his serum protein had risen to 6.9%.[8] Subject 3 (4 yrs., 14 kg, female) had a serum protein level of 6.8% at the end of 4½ months on the diet and never showed signs of protein malnutrition. Her intake of total protein was about three-fourths that of the other two subjects.[6] Hsia et al.[4] also reported difficulties in maintaining adequate intakes of calories and protein for 30 phenylketonuric patients receiving a diet based on Ketonil (low-phenylalanine acid hydrolyzate of casein-Merck).

Dodge et al.[9] reported, not only hypoproteinemia, but also hypoglycemia as a complication of feeding a low-protein, low-phenylalanine diet. Their subjects received diets based on Ketonil. A boy, aged 3 years and 8 months, with a serum protein level of 6.7% (albumin-to-globulin ratio of 3.2 to 1) received 30 gm of Ketonil daily in a diet containing 1,540 calories. In 7 weeks he was admitted to the hospital with hypoglycemic convulsions. Serum protein was 5.6% on the day of admission and 4.8% on the second day. He died on the second day. Autopsy showed severe fatty infiltration of the liver.

An 11-month-old boy had received 32 gm of Ketonil daily for 5 months, at a daily calorie level of 1,060. He was admitted to the hospital with hypoglycemic convulsions which were corrected with intravenous glucose. Two months later (still on the diet) he was admitted for pneumonia. At this time he was found to have hepatomegaly, severe hypoproteinemia and anemia. Liver biopsy showed extensive fatty infiltration.

A third infant, a 13-month-old girl, received a phenylalanine-low diet for 7 months. At the end of this time she had a serum protein level of 4.82 gm with an

albumin-to-globulin ratio of 1.27 to 1. This patient showed no symptoms of hypoglycemia.

Dodge et al.[9] concluded that, in susceptible children, "refusal to take an adequate amount of an unpalatable diet over a period of weeks or months resulted in a state of undernutrition accompanied by fatty metamorphosis of the liver. On this background, a relatively short period of fasting was responsible for severe hypoglycemia, with convulsions and coma."

Protein malnutrition in the types of mentally retarded children discussed above has a clear etiology. The children fail to ingest adequate calories, or adequate amounts of good quality protein, or both. Symptoms of protein malnutrition most frequently observed in such patients are iron-deficiency anemia, serum protein levels below 5 gm %, albumin-to-globulin ratios below 1.5 to 1, edema and emaciation.

We recently[10,11] had an opportunity to study a group of 22 emaciated children in our nursery who were consuming amounts of calories and protein close to NRC allowances. Their daily intake of good-quality protein averaged 3.6 gm/kg for a 28-day observation period, with individual average daily intakes ranging from 2.4 to 6.1 gm. Each child weighed at least 35% less than the mean for his particular age. Seven were found to have caloric intakes at least 11% above the estimated[10] NRC allowances, 12 had caloric intakes that were plus or minus 10% of the NRC allowances, and 3 had intakes less than 90% of the NRC allowances.

Table 1 shows the age, height and weight of 6 children between 4 and 11 years of age who were chosen for intensive study. They were 21% to 40% below

TABLE 1
DATA ON EMACIATED CHILDREN

Subject	Age (yrs.)	Height (in.)	Height % below av. [1]	Weight (lbs.)	Weight % below av. [1]	Intake Calories % above NRC-RDA [2]	Intake Protein gm/kg	Serum protein (gm %)	Albumin/ globulin
J.R.	4	31	24	19	47	11	4.4	7.0	1.4
T.E.	8	35	30	25	58	18	3.7	8.6	0.9
L.B.	11	45	21	41	48	26	4.4	6.7	1.1
C.F.	5	32	26	20	50	41	4.8	6.5	1.2
T.S.	6	28	40	18	63	56	5.3	6.9	1.2
K.M.	5	34	22	17	58	62	6.1	6.9	1.8

[1] See ref. 13.
[2] RDA: Recommended Dietary Allowances; see ref. 10.

average for height and 47% to 63% below average for weight, and their caloric intakes exceeded NAS-NRC allowances by 11% to 62% with the luxury consumption of good quality * protein (3.7—6.1 gm/kg daily). Analysis of their blood sera showed normal levels of total protein (6.5—8.6 gm %). However, the albumin-to-globulin quotient was depressed (0.9—1.8), in the majority of the subjects. Total serum cholesterol was somewhat high, ranging from 112 to 256 mg %.

In all 22 patients, the emaciation occurred in spite of high intakes of good-quality protein, and we considered the following factors as possible causes: a) Poor

* Sixty-five per cent of the protein was derived from liver, milk and eggs.

absorption of nutrients from the gastro-intestinal tract; b) incomplete utilization of absorbed nutrients, with subsequent loss of metabolites in the urine; c) unusually high energy requirements.

With one exception, the fecal nitrogen values of the 22 emaciated children were within normal limits. The fecal fat values were high in several cases, but this did not account for a significant loss of calories, except for the patient (K. M., table 1), with high fecal nitrogen, where fecal fat accounted for 48% of the dry weight of the feces. This patient had a deficiency of pancreatic enzymes, as shown by duodenal intubation. None of the 22 children excreted abnormally high amounts of protein, glucose or other carbon compounds in the urine, and urine volumes were normal.

After elimination of poor absorption and poor utilization as possible causes of emaciation, the energy metabolism was studied. For this purpose, an insensible weight loss (IWL) apparatus designed by Dr. G. W. Guest et al.[12] was employed. This apparatus consists of a Toledo scale and attached recorder, which continuously records loss in body weight of the patient by means of a scale pointer making a tracing on a kymograph. With the room temperature at 28°C to 30°C, and the humidity between 35% and 70%, triplicate IWL determinations were made on 12 of the 22 emaciated patients, (including those in table 1), and on 11 patients of normal weight (between the 25th and 75th percentiles[13] for their ages). The average IWL value for the 12 emaciated children was 1.82 gm/kg/hour, and for the 11 nonemaciated children, 1.31 gm/kg/hour. The difference between the IWL values of these two groups is significant at the 5% level of probability. The IWL values of the 6 children whose caloric intakes exceeded the estimated NRC allowances by 11% to 60% (table 1) averaged 2.06 gm/kg/hour.

Since the IWL data indicated an abnormally high energy consumption in the majority of the emaciated patients, protein-bound iodine (PBI) determinations (modified Barker Method) * were made on samples of serum from the 22 emaciated children and 30 control patients (25th to 75th percentile for their ages). The medical records showed that none had received iodine-containing medication at or near the time of venipuncture. Among the 22 emaciated children, 12 values were above 7 μgm %, with 8 values above 8 μgm %. Among the 30 control retardates of normal weight, only 4 values were above 7 μgm %, with one above 8 μgm %. The number of values exceeding either 7 or 8 μgm % in the emaciated group is significantly higher than in the control group ($P < 0.01$).

It thus appears that a high rate of energy expenditure accounts for the emaciation in these mentally retarded children who consumed a normal diet. In many of the children there is evidence of thyroid dysfunction but, in some, increased energy expenditure does not appear to be associated with a high PBI value. The increased demand for calories in these subjects apparently causes a large part of the protein to be burned, leading to a depressed albumin-to-globulin ratio in the serum. Either the high rate of energy expenditure must be reduced, or caloric intakes considerably greater than NRC allowances must be fed, to eliminate this unusual type of malnutrition.

* Performed by Biochemical Procedures Laboratory, Hollywood, California.

SUMMARY

Protein malnutrition is a continuous problem in the treatment and maintenance of mentally retarded children. Inability to consume NRC allowances of calories and/or protein may be the result of physical handicaps or of special diets. This applies especially to children with cerebral palsy, the chronic regurgitator and the treated phenylketonuric. Recently, we have found another type of mentally retarded child who is emaciated in spite of high caloric and protein intakes. Our studies suggest that his emaciation and malnutrition are caused by an unusually high rate of energy expenditure.

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DISCUSSION

DR. HOLT: I would like to echo some of the thoughts which Dr. Mertz has expressed, that the reason some of these children are not well nourished is that the appetite center does not seem to function properly. They have very poor appetites as a group, some much more than others.

I would like to mention one more thing in connection with the treatment of phenylketonuria. We have had experiences very similar to those of Dr. Mertz. I would also like to call to your attention that there are now 6 and possibly 7 other congenital disorders of amino acid metabolism which are potentially correctable by diet. We have had experience with one other variety, namely, the so-called "maple sugar" urine disease, characterized by excretion of abnormally high levels of methionine and three branched-chain amino acids. By reducing the intake of these 4 amino acids we are able to preserve the brain.

DR. RAMALINGASWAMI: Do the children with high PBI levels show high I-131 uptake?

DR. MERTZ: First, I would like to thank Dr. Holt for his remarks and agree with him that we have other conditions where diet may be of value. Here again, I think we will be walking a tightrope as far as adequate nutrition is concerned.

In answer to Dr. Ramalingaswami's question, we plan to do I-131 uptakes. We have not had an opportunity to do it as yet. We plan to continue the study of the thyroids of those children who seem to have thyroid dysfunction.

Adolescent Nutrition in Relation to Tuberculosis

J. A. Johnston

THE LITERATURE ON THE relation of nutrition to tuberculosis prior to 1950 is reviewed by Keys et al.[1] A bibliography of pertinent contributions between then and 1958 is listed by Dubos and Schaedler[2] in reporting their own study. They were able to demonstrate a clear-cut increase in susceptibility to tuberculosis in a group of mice receiving a diet containing 8% casein as compared with groups receiving 20% casein. The defect could be corrected by the addition of 12% amino acids. It is notable that gain in weight on the diet defective in protein was as adequate as on the diets containing the larger amounts. This paper summarizes material from 2 studies reported in detail elsewhere.[3]

The history, followed for over 20 years, of 932 children who reacted to tuberculin and who had been removed from their source of infection to foster homes, confirmed the observation that reinfection occurred with significant frequency only when adolescence was reached. Of the 29 cases observed to develop the reinfection type after removal from their sources of infection, 20 were girls; the average age was 15 years, and the average age at which they had experienced the menarche was 12 years.

On an active tuberculosis service for children it was possible to conduct nitrogen balance studies, in many instances for periods longer than a year without interruption. A good correlation was established between the development of the reinfection type of tuberculosis, its course when once developed, and the nitrogen metabolism. Evidence of previous depletion, not appreciated when estimates of nutrition were based on weight, but inferred from abnormally high nitrogen retentions on adequate intakes with flat or declining weight curves, was recorded frequently. The typical metabolic picture of recovery from depletion was a high nitrogen storage with a plateau in the weight curve, following which, nitrogen retention fell to the level appropriate for the expected gain in weight. Negative nitrogen balances were associated with spread of the disease process; regression of the pulmonary lesion was associated with normal or high retentions.

Wallace[4] points out that there are sources of error in the determination of the nitrogen balance of the magnitude of 20% to 30% in the direction of being too high, and with this we would agree if we are to understand the composition of the tissue that results in recovery from malnutrition. Nevertheless, in the case of the

long balances we have reported, the error can be presumed to be the same throughout and the child serve as his own control.

Among the things which affect the retention of nitrogen in adolescence is the nature of the growth impulse itself. Growth at puberty follows a sigmoid curve, the moment of deceleration occurring in the girl at about the time of the menarche and terminating about 3 years later. The percentage of a given intake of nitrogen retained will be a function of this curve, declining as the rate of growth declines (fig. 1). With marginal intakes one may anticipate positive nitrogen balances in the highly anabolic accelerative phase that precedes the menarche, but negative balances in the postpuberty phase. Whether this in inherent in the growth process itself or resulting secondarily from the action of the sex hormones is not clear.

Next to the influence of the phase of growth on nitrogen is the adequacy of the intake. Studies were done on 29 children for an average of 10 months each. The caloric intake was varied from an admittedly low level to a point at which the child was satisfied. With calories constant the percentage derived from protein was varied from 10% to 25%. Consistently positive balances were obtained at the 15% level, although usually 13% sufficed. At this level also, the basal metabolism, which declined to abnormally low levels on the inadequate diets, became normal. Amounts greater than 20% tended to be refused or to provoke nausea. A single example of the study is reproduced in figure 2.

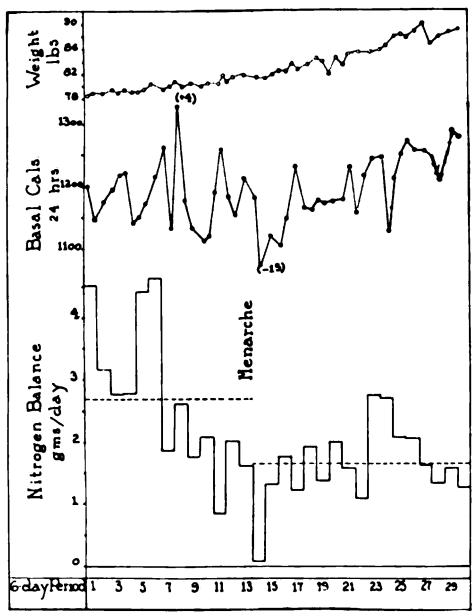

Figure 1—Metabolism at Puberty in a Normal Girl. With a constant intake of calories and protein, the amount retained declines steadily at about the time of the menarche. The expected retention will in general continue to become smaller until growth ceases.

In balance studies on the children with tuberculosis, done for the most part before the availability of chemotherapy, a number of uncomplicated recoveries with extremely high retentions were recorded in the beginning of the study, gradually declining to normal levels of retention later. We have interpreted this type of balance as characteristic of the child whose previous diet might have been calorically adequate, as judged by her weight, but was low in protein and high in carbohydrate. An example of this type of recovery is reproduced in figure 3.

Spread of the disease in association with a failure of nitrogen retention is noted in figure 4. This type of observation must always raise the question whether the disease spread as a result of the failure of storage, or whether the loss of nitrogen resulted from the spread. X-ray evidence indicates that the losses preceded the spread.

It was concluded from this study: that the increased incidence of the adult form of tuberculosis noted in adolescence could be correlated with a failure to meet the protein requirements of this age group; that this was in part a function of the diminished capacity to retain nitrogen that characterizes the decelerating phase of growth of the latter half of puberty; and that, once the disease was manifest, its course correlated positively with nitrogen storage.

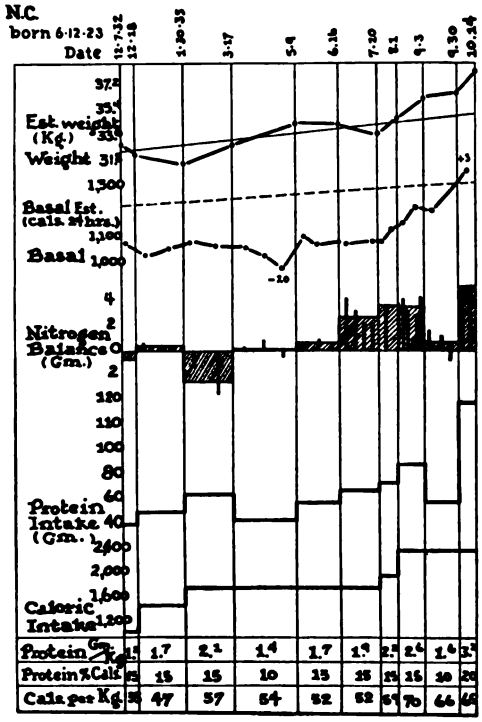

Figure 2—Example of the Study to Determine Optimal Intakes on the Normal Child. Twenty-nine subjects were studied in this way for an average of 10 months each. With calories adequate to satisfy appetite, consistently positive balances for nitrogen accompanied by normal basal metabolic rates and gain in weight were obtained when 15% of calories derived from protein.

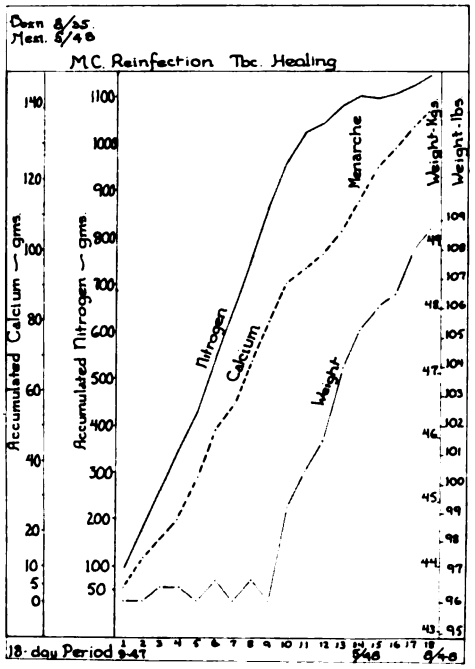

Figure 3—Failure to gain weight while storing larger than normal amounts of nitrogen and calcium characterize a change from a diet low in protein and calcium to one high in these items.

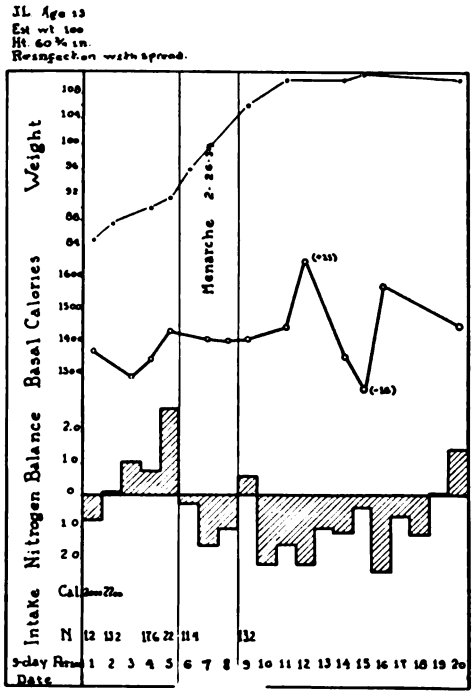

Figure 4—This girl was admitted with a minimal reinfection type lesion, and was being studied to determine nutritional requirement of this age. In the first 45 days there was a steady gain in weight but a positive nitrogen balance only with high intakes of nitrogen. The negative balance at the menarche could be attributed to a diminished intake, but immediately following the menarche, the balances were negative on amounts (13.2 gms) which before the menarche gave positive balances. At this time a frank spread of the tuberculous lesion requiring collapse therapy was detected.

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DISCUSSION

DR. PLATT: Did you have any parallel observations on the plasma proteins and plasma calcium?

DR. JOHNSTON: No, I am very sorry. I did 4 balances concurrently, with 2 helpers and that was about the extent of what we could do.

Essential Amino Acid Requirements of Adult Man

Marian E. Swendseid and Stewart G. Tuttle

THE DELINEATION OF the eight amino acids which adult man requires preformed in the diet for the maintenance of nitrogen equilibrium was accomplished by Rose and associates during the 1940s.[1] Thus, it became possible for the first time to separate the requirements for protein nitrogen into two parameters: first, the requirement for those amino acids which cannot be synthesized by the tissues, at least at a rate commensurate with need; and second, the requirement for a nitrogen source to be used in the synthesis of the nonessential amino acids.

It is possible that some of the present concepts of protein nutrition may relate specifically to one or the other of these parameters. For example, there is experimental evidence that when protein furnishes the nitrogen in the diet, the amount of nitrogen required is proportional to body weight or to body surface area.[2] However, most investigators have found no correlation between essential amino acid requirements and body weight under experimental conditions where the total nitrogen of the diet has been kept constant.[3,4] This apparent contradiction might be resolved if it were found that there is a relationship between nonessential nitrogen needs and body weight.

From a metabolic standpoint it would appear that many body processes which affect oxidation rates of amino acids might evoke changes in essential nitrogen requirements but not in the requirements for those amino acids that are readily synthesized. Therefore, it becomes important to study factors that might influence essential nitrogen requirements under conditions where the essential nitrogen is an independent parameter of protein nutrition. To accomplish this, the nonessential or total nitrogen content of the diet must either remain constant or be varied in a systematic fashion. This procedure cannot be followed when protein is the sole source of nitrogen. In such a diet a variation in essential amino acid nitrogen would automatically change the nonessential nitrogen. Hence, for most studies of essential nitrogen requirements, diets containing purified amino acid mixtures or proteins supplemented with purified amino acids must be employed. It is axiomatic that these diets must contain adequate amounts of nonessential nitrogen.

The method of keeping the total nitrogen of the diet constant while varying the amount of essential amino acids was used by Rose[1] in nitrogen balance studies, first to identify the essential amino acids and later to determine the quanti-

ties needed by young male adults. The requirements of young women have also been investigated.[3] Thus, it is now possible to begin the study of the effects of various metabolic and dietary factors such as age, sex, the amino acid pattern and the total nitrogen intake on essential amino acid requirements.

Essential Amino Acid Patterns

A great deal of experimental evidence[5] relates the efficiency with which a protein can be used as a source of amino acids to the relative proportions of the essential amino acids the protein contains. It has been suggested that the ideal amino acid proportionality pattern might be based on the essential amino acid requirements.[4] The comparison of one such pattern, the FAO amino acid reference standard,[6] with the amino acid pattern of whole egg has been conducted on healthy young men using the nitrogen balance technique.[7] In this experiment, the essential amino acids were fed either as a purified amino acid mixture or as protein supplemented with amino acids to provide the pattern under study. Egg protein from whole fresh egg was used as the protein for both patterns. The total nitrogen intake was maintained at a constant level of 10 gm per day by the addition of glycine and diammonium citrate. Each pattern was fed in quantities which furnished tryptophan, the limiting amino acid of the egg pattern and the FAO

TABLE 1
RATIOS OF ESSENTIAL AMINO ACIDS IN WHOLE EGG AND FAO PATTERN
(Tryptophan = 1)

L-amino acid	Whole Egg	FAO Pattern
Tryptophan	1.0	1.0
Isoleucine	3.7	3.0
Leucine	5.4	3.4
Lysine	5.4	3.0
Methionine and Cystine	3.7	3.0
Phenylalanine and Tyrosine	4.7	4.0
Threonine	3.1	2.0
Valine	3.9	3.0

pattern (table 1), in amounts ranging from 240 to 440 mg per day, with other amino acids in amounts proportioned to the pattern under study. In general, the procedures followed were the same as for the investigations on individual essential amino acids.[3] However, since an attempt was made to feed all amino acids in amounts approximating minimal requirements, the total amount of essential nitrogen in the diet was considerably less than in most previous studies.

In comparing purified amino acid mixtures and protein as the chief source of amino acids for a single pattern, the data showed that certain subjects seemed to retain more nitrogen when the diet contained supplemented whole egg than when it contained purified amino acids, but this finding was not consistent. These observations with supplemented whole egg, a protein with a high coefficient of digestibility, might not pertain to other food proteins.

For 5 of 6 subjects, better nitrogen balances were obtained when the dietary amino acids were in egg pattern rather than in FAO pattern proportions. This

occurred whether purified amino acid mixtures or protein supplemented with amino acids were administered. A possible explanation for the more favorable nitrogen balances can been seen from table 2, where the daily intakes of the essential amino acids and essential nitrogen are shown when each pattern furnished 360 mg tryptophan. The egg pattern furnished 60% more leucine than did the FAO pattern, as well as 50% more lysine and 20% to 30% more of the remaining essential amino acids with the exception of tryptophan. The overall effect is mirrored in a larger amount of total essential nitrogen for egg pattern, 1.28 gm compared with

TABLE 2
DAILY INTAKES OF L-ESSENTIAL AMINO ACIDS AND ESSENTIAL AMINO ACID NITROGEN

Dietary Component	FAO Pattern mg/day	Egg Pattern mg/day	Rose's Minimum Requirements mg/day
Tryptophan	360	360	250
Isoleucine	1080	1340	700
Leucine	1215	1945	1100
Lysine	1080	1640	800
Methionine + Cystine	1210	1485	1100
ϕ-aline + Tyrosine	1440	1695	1100
Threonine	720	1110	500
Valine	1080	1400	800
Total EAA Nitrogen	940	1280	730

0.94 gm for the FAO pattern. This same situation can occur also in the evaluation of food proteins of differing essential amino acid composition.[8] Here, too, a variation in the percentage composition of the essential amino acids can obscure the comparison between the amino acid patterns.

It seems therefore that, in the evaluation of essential amino acid ratios, serious consideration should be given to studies wherein the total amount of essential amino acids or the total essential nitrogen supplied by the patterns are present in equal quantities. In experiments with young men,[7] where the FAO and egg patterns have been compared in approximately isonitrogenous amounts, no appreciable differences could be observed in nitrogen retention. Hence the relative merits of the FAO and egg patterns depend upon the criteria by which the patterns are evaluated. For this reason, attention should be given to a more precise definition of an ideal amino acid proportionality pattern. It would appear that an ideal amino acid pattern could be properly defined as that pattern which requires the least amount of essential nitrogen, or of total essential amino acids, to maintain nitrogen equilibrium. On the basis of this definition egg pattern loses its evident superiority over FAO pattern. Comparison of the amounts of amino acids in egg and FAO patterns at the isonitrogenous level necessary to establish nitrogen equilibrium shows an excess of tryptophan in the FAO pattern. Reduction to the amount present in egg pattern, a reduction of 18%, results in a modified ratio that brings the FAO pattern into approximate agreement with egg pattern except for the amino acids leucine, lysine and threonine which remain relatively higher in egg pattern. It is apparent that further experimentation with varying amounts of individual amino

acids is necessary in order to evaluate both FAO and egg patterns in terms of a possible ideal essential amino acid pattern.

Essential Nitrogen Requirements of Young Men and Women

The amount of essential nitrogen required in the diet for nitrogen equilibrium is dependent, of course, upon the amino acid pattern and possibly also upon the total nitrogen intake.

Rose et al.[9] were able to maintain 2 young male subjects in nitrogen equilibrium on 1.42 gm of essential amino acid nitrogen per day with varying intakes of total nitrogen, but they did not regard this as a minimal quantity. In experiments using whole egg protein as a source of essential nitrogen[10] 3 young men showed requirements ranging up to 0.9 gm of essential nitrogen when the total nitrogen intake was 6.5 gm per day.

In more recent experiments[11] with both FAO and egg pattern in a 10 gm nitrogen diet using egg protein supplemented to the amino acid pattern under study, requirements for young men were from 1.0 to 1.2 gm of essential nitrogen per day as measured by a zero or slightly positive nitrogen balance value. As in experiments with individual amino acids, there was no correlation between essential nitrogen requirements and body weight or body surface area. It was also found that the total amount of essential nitrogen required to maintain nitrogen equilibrium was greater than the sum of the individual amino acid requirements as determined by Rose (table 2). This may be an indication that the requirement is increased when all of the essential amino acids are fed in amounts approaching their minimum. Another possibility is that the essential amino acid pattern could be improved.

Experiments with young women[11] using the same dietary regimen (10 gm nitrogen per day) have shown an essential nitrogen requirement of 0.85 gm per day. This value too is greater than the individually determined requirements for young women. This requirement for young women appears to be definitely lower than the requirement for young men. These findings, obtained with experimental conditions which were similar to those of studies with young men, lend support to the possibility that there is a sex difference in the requirements for essential nitrogen. Further experiments are necessary to ascertain whether this difference relates to all of the amino acids or whether the ideal amino acid patterns may vary for men and women.

Essential Amino Acid Requirements of Older Men

Whereas the essential amino acid requirements of young men and women have been studied in several investigations, the specific needs of older individuals have not been so defined. There is evidence, however, that their requirements for one or more of the essential amino acids are increased. When 5 healthy men over 50 years of age were given semisynthetic diets containing all of the purified essential amino acids in the ratio and amounts found in 150 gm of egg (1.2 gm essential nitrogen) they promptly showed a negative nitrogen balance.[12] Moreover, when 150 gm of egg was substituted for the synthetic mixture, they again showed a net nitrogen loss. The quantities of essential amino acids fed as purified mixtures and as protein were at least equal to and generally exceeded the amounts needed by

young adults to maintain nitrogen equilibrium (table 3). Only when the quantities of the essential amino acids in the diet were doubled was nitrogen equilibrium maintained in the older men. Thus, although these studies neither identify the specific amino acids required in larger quantities by older men nor show the magni-

TABLE 3
MINIMUM REQUIREMENTS OF ESSENTIAL AMINO ACIDS
(gm/day)

L-amino acid	Young Men	Young Women	Test Mixtures Equivalent to 150 gm egg	300 gm egg
Leucine	1.10	.62	1.72	3.44
Isoleucine	.70	.45	1.28	2.56
Lysine	.80	.50	1.39	2.78
Threonine	.50	.31	.77	1.54
Tryptophan	.25	.16	.38	.76
Valine	.80	.65	1.29	2.58
Methionine	1.10	.29	.56	1.12
Cystine	Spares Methionine	.25	.45	.90
Phenylalanine	1.10	.22	.96	1.92
Tyrosine	Spares Phenylalanine	.90	.60	1.20

(Modified from METABOLISM, Refs. 12 and 17)

tude of the increased needs except within broad limits, they do suggest that age may be an important factor in evaluating the individual amino acid requirements of adults.

Recently, attempts have been made to determine the specific essential amino acid requirements of older men. Six normal men over 60 years of age were given a mixture of essential amino acids as found in 300 gm of egg, except that cystine was omitted from the ration and methionine was added in varying amounts.[13] Nonessential nitrogen was supplied as a mixture of purified nonessential amino acids in the ratio found in egg. When these men ingested 3 gm of methionine daily along with the other essential and nonessential amino acids, they maintained nitrogen equilibrium. By gradually reducing the methionine in the test mixture, it was found that the requirements of these 6 men for this amino acid ranged from 2.4 to 3.0 gm a day. These values exceed by more than 100% the minimum requirement of young male college students as reported by Rose.

The requirement of elderly males for lysine also appears to be increased.[14] Using the same experimental plan, it has been observed that 4 men in this age category showed a net loss of nitrogen when their daily lysine ration was reduced below 2.1 gm. Preliminary observations suggest that such individuals also require larger amounts of the other essential amino acids than do young adults.

Effect of the Total Nitrogen Intake

In considering the possible effect of the total nitrogen intake on essential amino acid requirements, the source as well as the amount of nitrogen must be assessed.

Evaluation of the source of nitrogen is particularly relevant since most studies of essential amino acid requirements have been carried out using urea, diammonium citrate, glycine or combinations of these substances in place of the mixture of nonessential amino acids found in proteins. The source of supplemental nitrogen, which usually provides from 60% to 80% of the total dietary nitrogen, constitutes one of the major differences between the semisynthetic diets employed to study essential amino acid requirements and ordinary food. In a study of 4 young adult subjects,[15] glycine alone was not as effective as a mixture of nonessential amino acids (in the ratio of egg protein) in maintaining nitrogen equilibrium; however, a combination of diammonium citrate and glycine appeared to be as well utilized as the nonessential amino acid mixture. In another study[16] 8 older male subjects were unable to maintain nitrogen equilibrium on a daily diet of essential amino acids equivalent to 225 gm of egg when the chief source of nonessential nitrogen was glycine. When a nonessential amino acid mixture was substituted for glycine, equilibrium was maintained. On the basis of these limited experiments, it appears that a combination of glycine and diammonium citrate, but not glycine alone, is as effective as a nonessential amino acid mixture in nitrogen balance experiments.

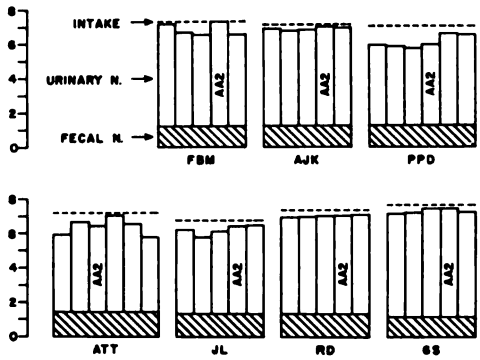

Figure 1—Mean daily nitrogen balance per period from 7 subjects fed approximately 7 gm of nitrogen. The blank bars represent the control periods; the columns labeled AA2, the semisynthetic diets containing the test essential amino acid mixture equivalent to 300 gm of whole egg. Whenever the bars extend above the intake line, the nitrogen balance is negative; when they remain below, the balance is positive. From METABOLISM 8: 61, 1959 (Ref. 17).

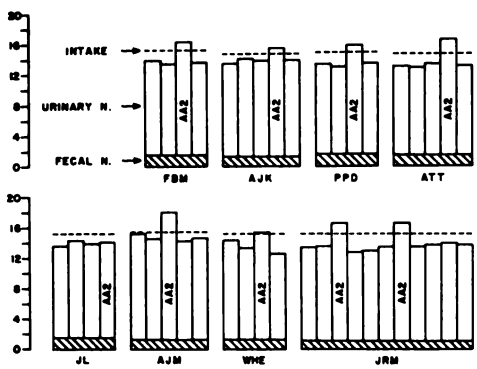

Figure 2—Mean daily nitrogen balance per period from 8 subjects fed approximately 15 gm of nitrogen. See figure 1 for key. From METABOLISM 8: 61, 1959 (Ref. 17).

The effect of the total nitrogen intake on essential nitrogen requirement was studied in 6 young adults.[10] The total nitrogen intake was increased from 6.5 gm to 13 gm by increasing nonessential nitrogen in a diet where egg protein furnished the essential amino acids and glycine and diammonium citrate were the chief sources of nonessential nitrogen. This increase in total nitrogen did not result in a consistent increase in the requirement for essential amino acids. In contrast, a study with elderly men[17] showed that they were maintained in nitrogen equilibrium on a total nitrogen intake of 7 gm on a diet containing purified essential amino acids equivalent to the amounts found in 300 gm egg

(2.4 gm essential nitrogen). When the total nitrogen was increased to 15 gm, by increasing the glycine and diammonium citrate, the subjects all were in negative nitrogen balance (figs. 1 and 2). The same men maintained equlibrium when the total nitrogen intake was reduced to 3.7 gm per day on an essential amino acid mixture equivalent to 150 gm of egg.

It would appear then that there is some evidence that the total amount of nitrogen ingested can affect the minimal requirements for the essential amino acids in elderly men. That this effect could not be demonstrated in an experiment with young men might indicate another difference in nitrogen nutrition that is related to age. For the oldster, the requirements of the essential amino acids which appear to be higher than for the young adults might also be modified to a greater degree by the total nitrogen intake.

COMMENTS AND SUMMARY

On the basis of a limited number of experiments, as reported above, some evidence has been obtained that young men require approximately 1.0 to 1.2 gm of essential nitrogen per day to maintain nitrogen equilibrium when the amino acid pattern is that of egg, a protein of high BV, or the FAO reference pattern. It remains to be determined whether an amino acid imbalance will greatly affect these requirements. The body weight or metabolic size does not appear to influence requirements for essential nitrogen. For young women, the essential nitrogen needed under similar experimental conditions was 0.85 gm per day. This lowered requirement for women indicates a sex difference in essential nitrogen but there is no evidence as to whether all or only a few of the essential amino acids are involved.

For men over 50 years of age there is an indication of an increased requirement for essential nitrogen as compared with the needs of young men. The requirements for methionine and lysine appear to be at least double those of young men. It is not known whether these increased needs apply to other essential amino acids and whether the ideal amino acid pattern will be the same for young and old adults of both sexes.

Experiments with the source and amount of total nitrogen in the diet indicate that these factors can affect essential nitrogen requirements and emphasize the necessity for studying essential nitrogen under controlled conditions of nonessential nitrogen intake. The effect of an increase in total nitrogen causing an increase in essential nitrogen requirements was demonstrated in older men and suggests that the essential nitrogen needs of these individuals can be specifically stated only in relation to the total nitrogen content of the diet.

It is difficult to translate results obtained with diets using amino acid mixtures or proteins supplemented with various nitrogen sources into meaningful criteria for practical nutrition problems. Although the findings from these limited experiments

may be modified by further study, they do raise questions that are of importance in protein nutrition. Will a change from a low to a high protein diet necessarily result in an improved nutritional state, particularly in an older individual ingesting poor quality protein? What is the proper ratio between essential and nonessential nitrogen in the diet? What is the best definition of an ideal amino acid pattern and will the ideal amino acid pattern be the same for all ages and both sexes? It should be emphasized that all experiments reported here with the nitrogen balance technique are testing minimal essential nitrogen requirements. Optimal requirements may exceed these minimal needs.

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Evaluation of Protein Foods in Premature Infants

Selma E. Snyderman, Audrey Boyer and L. Emmett Holt, Jr.

ALTHOUGH THE NUTRITIVE VALUES of protein foods for different animals show a general similarity, species differences in requirements are known to exist, such as the particular need for arginine in feathered animals[1] and an apparent greater need of hairy animals for S-amino acids.[2] In evaluating proteins it is therefore desirable to study the species for which the information is to be applied. Values for the growing infant should be determined in the growing infant rather than in the rat, the chick or the human adult. The premature infant is well suited for such evaluations. He is particularly sensitive to changes in food quality, as revealed by weight gain and nitrogen retention. We have therefore undertaken to evaluate a number of different protein foods in healthy premature infants.

The question of hazard to these presumably delicate infants presents itself. We have satisfied ourselves that such studies can be carried out without appreciable risk. There are wide variations in current feeding practices which are reflected in weight gain and nitrogen retention. The optimum is still to be determined. Submaximal weight gains and submaximal nitrogen retention for periods of a few days cannot be said to involve an appreciable hazard. Yet variations in these will quickly reflect changes in protein quality. We have not, however, felt justified in subjecting these infants to a period on a nitrogen-free diet to determine directly the endogenous nitrogen excretion according to the classical formulas for evaluating digestibility, BV and NPU. Furthermore, as is explained below, we have not felt that it was necessary to do this in order to obtain significant comparable data for different protein foods.

PROCEDURES

Subjects

Observations were made on 48 healthy premature infants whose birth weights varied from 1200 to 2070 gm, and who weighed from 1160 to 2650 gm at the time of study. Nearly all were males. An initial period of 10 days or more on a routine cow's milk formula was given to permit recovery from the initial postnatal loss of weight and establish a satisfactory weight gain. The test feeding was then instituted and its effect on weight gain and on nitrogen balance studied. In evaluat-

ing weight gain the standard grid developed by Dancis, O'Connell and Holt,[3] which readily reveals deviations from the expected growth channel for infants of different birth weights, was used.

In general there is agreement between nitrogen retention and gain in weight. Occasionally, however, discrepancies are observed which are not attributable to errors in technique. One may encounter normal weight gain with subnormal nitrogen retention and vice versa. The cause of these discrepancies, which have been observed by others, is not altogether clear. Variations in leucocyte production may be one of several factors responsible for this, for Yuile and his coworkers[4] have shown that leucocytes have an active protein metabolism. Of the two criteria, gain in weight seems to be quite as informative as nitrogen retention and can be studied with far less effort.

Balance Studies

Male infants were studied on metabolism beds permitting separate collection of urine and feces. In a few observations made on female infants urine and feces were collected together. The metabolic periods were of 4 days' duration with the exception of one instance noted. The stools were marked with charcoal to indicate the beginning and end of the study period. Nitrogen was determined in the food, urine and feces by a micro-Kjeldahl procedure.

Experimental Feedings

The chief purpose of this study was to evaluate certain vegetable protein products prepared from oil seeds, in which UNICEF was interested. These were compared with animal protein foods (milk, meat, fish), three mixed cereal products and two mixtures of pure amino acids. The protein foods studied were the following:

1. *Oil seed products*

Coconut protein. Two samples of coconut protein isolate were studied. These were prepared in the Institute of Nutrition of the Phillipines in Manila and were distributed by UNICEF under the designations "Cn-l-Io" and "Cn-l-Iw," the former being described as "fat soluble" and the latter as "water soluble."

Cottonseed flour. This product was prepared by the Traders' Oil Mill Co. of Fort Worth, Texas and was distributed by UNICEF under the designation "C 1."

Peanut flour. This was prepared by Stevens Industries of Dawson, Georgia and distributed by UNICEF under the designation "PF2."

Sunflower seed flour. This material was prepared by Cia. Productora Nac. de Aceites of Santiago, Chile, S. A. and distributed by UNICEF under the designation "Sn F2."

Sesame seed flour. This was prepared by Salada-Shirrif-Horsey, Inc. of Little Falls, N. Y. and was distributed by UNICEF under the designation "SF 4."

2. *Cereal mixtures*

"*J*" *Cereal.* This was prepared by Salada-Shirrif-Horsey, Inc. of Little Falls,

N. Y. from a formula suggested by Dr. J. B. Allison, the protein component of which was derived from soy (69%) and rice (31%).

High-protein cereal. This was a product designed by Dr. Robert Stewart and prepared by Gerber's, Inc. of Fremont, Mich. with the following composition: oat flour 27.8%, soy flour 26.7%, soy protein 14.7%, whole wheat flour 10%, corn flour 8%, cottonseed protein 4.0%, brewers' yeast 2.1%.

INCAP #8. This was a product designed by Dr. N. S. Scrimshaw and prepared at the Institute of Nutrition of Central America and Panama in Guatemala City. Its composition was as follows: dried corn masa 50%, sesame flour 35%, cottonseed flour 9%, torula yeast 3%, kikuyu leaf meal 3%.

3. *Meat.* The product studied was a preparation of homogenized beef prepared by the Armour Laboratories in Chicago.
4. *Fish.* The observations were made on a homogenized preparation of cod fish filets supplied by the Gorton-Pew Fisheries of Gloucester, Mass.
5. *Cow's milk.* Most of the studies were carried out on a proprietary milk formula for infants (Olac), supplied by Mead Johnson & Co., Evansville, Ind. Some were made on a proprietary infant formula (Similac), supplied by Ross Laboratories, Columbus, Ohio.
6. *Amino acid formulas.* Two formulas were used in which the nitrogen was supplied as a mixture of 18 L-amino acids. In one, the amino acids were supplied in the pattern of human milk.[5] In the other the essential amino acids were supplied in the pattern of the FAO reference protein,[6] the nonessentials in the proportions found in human milk.

Preparation of the Protein Feedings

The solid protein foods were incorporated into a liquid formula which could be fed by bottle. The material was first subdivided as finely as possible; it was then mixed with the appropriate amount of fat (corn oil), carbohydrate (dextrimaltose) and water and homogenized in a Waring blendor. These formulas were supplemented by a B-vitamin mixture *, a preparation of vitamins A, C and D ** and an iron preparation. *** The levels of protein intake varied from 2.0 to 9.0 gm/kg (8 to 37 calories/kg), most of the observations being made at an intake of 5.0 gm/kg (20 calories/kg). The total caloric intake was kept constant at 130 calories/kg, the differences in protein intake being compensated for by changes in carbohydrate and the fat calories kept constant at 45/kg.

Milk feedings. The two milk products were supplemented with a preparation of vitamins A, C and D as described in the preceding paragraph.

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* The B-vitamin mixture provided the following daily: thiamine, 0.38 mg, riboflavin 2.0 mg, nicotinamide 9.85 mg, calcium pantothenate 3.5 mg, pyridoxine 0.67 mg, inositol 180 mg, para-amino benzoic acid 0.5 mg, folic acid 0.05 mg, choline chloride 147 mg, biotin 0.03 mg and cyanocobalamine 0.015 mg.

** Trivisol, 0.6 cc per day.

*** Ferinsol, 0.6 cc per day.

Amino acid formulas. These were prepared from the following formula: L-amino acid mixture 10 gm, corn oil 29 gm, dextrimaltose 100 gm, mineral mixture * 4.85, water to 777 cc.

In all studies the infants were fed every 4 hours around the clock and accepted the feedings without difficulty.

Calculation of Indices of Protein Quality

The indices calculated were the *digestibility coefficient* (D), measuring the percentage of ingested nitrogen absorbed, the *biological value* (BV), measuring the percentage of absorbed nitrogen retained, and the product of these two, the *net protein utilization* (NPU), the percentage of the nitrogen intake retained. In strict usage the true absorption must be calculated by deducting from the observed fecal nitrogen the endogenous fecal nitrogen as determined on a nitrogen-free diet and, similarly, in calculating retention the observed urinary nitrogen must be corrected by deducting from it the endogenous urinary nitrogen excretion on a nitrogen-free diet.

As mentioned above, we did not feel justified in subjecting these infants to a nitrogen-free diet. We have therefore determined what may be designated as the *uncorrected D, BV, and NPU values* based on the apparent absorption and retention, using the following formulas:

$$D = \frac{\text{N intake} - \text{fecal N}}{\text{N intake}} \times 100$$

$$BV = \frac{\text{N intake} - (\text{fecal} + \text{urinary N})}{\text{N intake} - \text{fecal N}} \times 100$$

$$NPU = \frac{\text{N intake} - (\text{fecal} + \text{urinary N})}{\text{N intake}} \times 100$$

The uncorrected indices permit valid comparisons of the various protein foods. The figures, however, do not permit accurate comparisons with other data in which corrected indices were calculated. In order to permit such comparisons we have used the figures of Waterlow and Wills[7] for endogenous fecal nitrogen loss (33 mg/kg) and for endogenous urinary nitrogen loss (37 mg/kg) and have calculated "corrected" D, BV and NPU values from the classical formulas:

$$D = \frac{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N})}{\text{N intake}} \times 100$$

$$BV = \frac{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N}) - (\text{urine N} - \text{endogenous urine N})}{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N})} \times 100$$

$$NPU = \frac{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N}) - (\text{urine N} - \text{endogenous urine N})}{\text{N intake}} \times 100$$

* Composition of the mineral mixture: NaCl 18.9%, $CaHPO_4$ (anhydrous) 25.4%, $MgSO_4$ (anhydrous) 6.8%, $KHCO_3$ 44.4%, KCl 2.88%, Fe_3 Citrate 2.21%, $CuSO_4$ (anhydrous) 0.24%, $MnSO_4$ (anhydrous) 0.15%, KI 0.015%, NaF 0.03%.

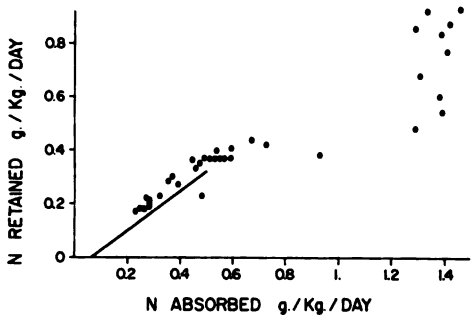

Figure 1—The line is Waterlow et al. (7)'s extrapolation of data obtained on Jamaican infants at different levels of nitrogen intake. The figure for endogenous fecal N. of 0.33 mg/kg/day was derived from this. The dots represent data obtained from our subjects at three different levels of intake of cow's milk.

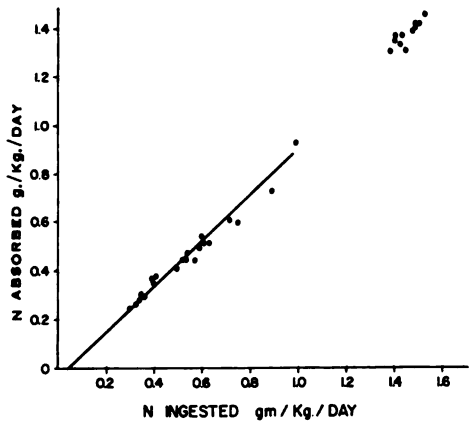

Figure 2—The line represents Waterlow's extrapolation of data from which he derived the figure of 0.37 mg/kg/day for endogenous urinary N. The dots represent data obtained in our subjects on different levels of cow's milk. The correlation is quite good at low and moderate levels of protein intake and retention. In the higher range, there is a much greater scatter of data.

The figures of Waterlow and Wills for endogenous nitrogen loss were not determined directly on a nitrogen-free diet but were extrapolations from data on Jamaican infants studied at different levels of nitrogen intake. Using this same procedure for our data on milk feedings at 2.0 and at 9.0 gm/kg, it is apparent that our data correlate closely with those of the Jamaican workers. (figs. 1. and 2.)

RESULTS

The data obtained on 153 balance studies carried out on 48 premature infants are summarized in table 1.

Effect of Varying the Protein Intake

The effect of varying the protein intake on the indices of protein quality is clearly seen in table 1. Digestibility coefficients are not affected by the intake, at least within the ranges studied, but BV and NPU may be markedly affected. Significant figures for BV and NPU are obtained only at marginal or deficient intakes, for with a surfeit of nitrogen a considerable part of this is excreted in the form of urea, lowering the proportion retained and decreasing the apparent BV and NPU figures. In the case of cow's milk, an intake of 2.0 gm/kg is apparently adequate but marginal. Increasing the intake to 3.0, 5.0 and 9.0 gm produces a progressive fall in BV and NPU; only the figures at the low intake are significant. In the case of INCAP #8, however, increasing the intake from 3.5 to 9.0 gm/kg causes no appreciable reduction in the BV or NPU figures, indicating that even at the highest level of intake a surfeit is not achieved; all the BV and NPU figures may therefore be taken as significant. A constant BV or NPU figure with increasing protein intake would suggest that minimal protein requirements had not been exceeded, whereas falling values with increasing intake point to a surfeit above requirements.

TABLE 1
SUMMARY. EVALUATION OF DIETARY PROTEIN IN PREMATURE INFANTS

	Level of Protein Fed (gm/kg)	Number of observations	Average Weight (kg)	Weight Gain Data			Measurements of Protein Quality†					
				Average gm/day	Normal range	Sub-normal range	Uncorrected			Corrected		
							D	BV	NPU	D	BV	NPU
Animal Protein Products												
Cow's Milk	2.0	32	1.97	27.3	32	0	86	78	67	95	93	88
Cow's Milk	3.0	4	1.70	20.0	3	1	85	80	69	92	80	74
Cow's Milk	5.0	4	2.23	45.0	4	0	81	72	59	85	79	67
Cow's Milk	9.0	19	2.00	32.3	19	0	93	57	53	95	60	57
Meat (beef)	3.0	4	1.72	14.5	2	2	67	83	56	70	92	64
Fish (cod)	3.0	4	1.73	20.5	2	2	62	80	50	68	91	62
Mixed Cereal Products												
High-protein	5.0	4	1.99	24.3	4	0	61	62	38	64	72	46
"J" Cereal	3.5	4	2.01	7.0	0	4	77	60	47	83	70	58
"J" Cereal	5.0	3	2.27	17.7	3	0	69	63	43	72	70	51
INCAP #8	3.5	4	1.93	6.3	0	4	68	46	31	72	59	43
INCAP #8	5.0	7	2.16	7.4	0	7	67	45	30	65	56	37
INCAP #8	7.0	2	2.19	4.5	0	2	68	47	32	72	53	38
INCAP #8	9.0	1	2.26	18.0	1	0	61	49	30	61	55	34

Single Vegetable Foods												
Coconut Protein Isolate (Cn-1-Iw)	3.5	3	1.97	5.3	0	3	70	64	45	76	71	54
Coconut Protein Isolate (Cn-1-Iw)	5.0	7	2.27	9.4	1	6	77	51	39	80	60	48
Coconut Protein Isolate (Cn-1-Iw)	7.0	1	2.31	25.0	1	0	76	60	46	79	66	52
Coconut Protein Isolate (Cn-1-Io)	5.0	8	1.88	15.4	4	4	75	61	46	81	68	55
Cottonseed Flour	5.0	3	1.88	7.3	0	3	65	48	31	71	57	41
Cottonseed Flour	7.0	1	2.63	14.0	0	1	69	39	27	72	46	33
Peanut Flour	5.0	7	2.28	12.7	2	5	73 *	27 *	27	81 *	36 *	35
Sunflower Seed Flour	5.0	6	2.19	9.1	1	5	57	28	16	71	35	25
Sesame Seed Flour	5.0	2	2.23	—28.0	0	2	75	22	17	77	33	25
Amino Acid Mixtures												
Human Milk Pattern	2.0	10	2.13	29.2	9	1	90 **	71 **	65	99 **	87 **	84
FAO Pattern	2.0	13	2.20	24.5	13	0	91 **	67 **	64	98 **	83 **	84

† The symbols D, BV and NPU represent digestibility, biological value and net protein utilization. The "corrected" values were calculated by deducting from the observed fecal nitrogen 33 mg/kg and from the observed urine nitrogen 37 mg/kg for the endogenous fecal and urinary nitrogen excretion.

* Average of 5 periods only

** Average of 7 periods only.

A phenomenon which has puzzled investigators and which appears in our data is an increase in nitrogen retention with increasing intake even when intake is adequate. This phenomenon, which is confined to the growing and maturing child, is discussed elsewhere.[8] It is not germane to the present discussion since it does not appear to affect the validity of the indices of protein quality.

Digestibility

Table 1 reveals some interesting differences of digestibility. The highest figures were observed in the case of the amino acid mixtures. None of the solid foods was the equal of milk, which showed digestibility coefficients usually in the 80's. The digestibility of the solid foods, though less than this, in no instance fell below 60. The question may be raised whether the lower digestibility of the solid foods is a peculiarity of the premature infant, whose digestive and absorptive functions may be inferior to those of the older child, or whether this lower digestibility is an inherent characteristic of the food. Data are not at hand for answering this question unequivocally. On the basis of postmortem examinations of the digestive glands, Werner[9] concluded that the digestive apparatus of the premature infant was imperfectly developed, but the studies of Madey and Dancis[10] and of Feinstone and Smith,[11] who measured proteolytic enzyme activity in the duodenal contents of living infants, failed to reveal any deficiency of these enzymes. The ability to absorb fat is known to be defective in premature infants, but a corresponding difficulty of absorbing protein split products has not been demonstrated.

Biological Values

The superiority of the animal protein foods and of the amino acid mixtures stands out clearly in the data, with little to choose between them. The meat and fish products, despite their high BV, show a lower CD and hence a somewhat lower NPU.

Among the vegetable products, the mixed cereals, the high-protein cereal and the "J" cereal showed the best performance; close to these were the coconut products, particularly the "fat soluble" preparation "Cn-l-Io." INCAP #8, cottonseed flour, peanut flour, sunflower seed flour and sesame flour showed decreasing values in the order named, the performance of the last being particularly disappointing.

Net Protein Utilization

Since there was little difference in the digestibility of the various vegetable products, it follows that the NPU values were generally parallel to the BV. The one exception was the "J" mixed cereal, which, because of its somewhat higher digestibility, showed a better net utilization than the high-protein cereal. The fish and meat products, despite their high BV, showed a poorer net utilization because of their lower digestibility.

Weight Gain

The data on weight gain are summarized in table 1. The protein foods classify themselves in almost exactly the same order as is indicated by the NPU.

In the first group are the milk proteins and the amino acid mixtures. Premature infants fed on these products followed the standard growth channel on an intake as low as 2.0 gm of protein (or its equivalent in amino acids) per kg. It is probable that this intake is very close to the minimum. In our experience all the infants we have studied with a birth weight of 1400 gm or more have performed well on such an intake, but we have encountered some with birth weights less than this who failed to do so. Apparently some of the smallest prematures require a higher protein intake.

In the case of the meat and fish products, it appears that an intake of 3.0 gm/kg is barely adequate. Several infants have gained normally on this, but not all have done so.

In no instance have infants fed exclusively on vegetable protein gained normally on an intake of less than 5.0 gm/kg. The high-protein and "J" mixed cereal products and the "fat soluble" coconut protein gave virtually a normal growth curve at this level, but there were indications that this was marginal, a change to milk causing some slight acceleration. None of the other preparations showed a satisfactory gain in weight at the 5 gm level. In one subject on INCAP #8 we achieved virtually a normal growth rate on 7.0 gm/kg and the same was true in the case of the water-soluble coconut preparation; others, however, did less well. The peanut, sunflower and sesame flours were not studied at intakes above 5.0 gm/kg.

Clinical Performance

The increase in stool volume and in stool odor on all the solid food products was noted by the nurses. Apart from the impaired weight gains, however, no abnormal clinical manifestations were observed. The one exception was the experience with the sesame flour product "SF 4." This product was given to 2 infants only. Both lost weight. The material appeared to be irritating to the gastrointestinal tract, causing mucus and blood to appear in the stools. * Because of this poor performance the feeding was discontinued before the completion of a metabolic period.

SUMMARY

A comparison of various protein products has been made in premature infants, using weight gain and nitrogen retention as criteria for evaluation.

The best performance was given by cow's milk and by a mixture of pure amino acids. No substantial difference was noted between these. Successful results were obtained with a protein intake of 2 gm/kg or its equivalent in amino acids.

* Similar observations on this product were made by Dr. E. M. DeMaeyer (personal communication) in studies upon older children.

When protein was supplied in the form of meat or fish, some impairment of digestibility was observed in the form of increased stool nitrogen. The BV of these preparations was comparable to milk, but because of the decrease in digestibility a lower NPU was observed. Satisfactory performance was obtained with an intake of 3.0 gm protein/kg.

A series of vegetable products studied also showed somewhat impaired digestibility as compared with milk protein, and BV distinctly below those of the animal proteins, both factors contributing to a decreased NPU. An intake of 5.0 or more gm protein/kg was needed to obtain satisfactory performance with some products, and with others satisfactory performance was not achieved at this level. In order of decreasing NPU value the products tested are as follows:

NET PROTEIN UTILIZATION

	Uncorrected	Corrected
Cow's milk *	67	88
Amino acids—human milk pattern	65	84
Amino acids—FAO reference pattern	64	84
Meat	56	64
Fish	50	62
Coconut protein isolate ("fat soluble")	46	55
"J" cereal	45	55
High-protein cereal	43	51
Coconut protein isolate ("water soluble")	42	51
Cottonseed flour	31	41
INCAP #8	31	39
Peanut flour	27	35
Sunflower seed flour	16	25
Sesame flour	17	25

* Based on observations with an intake of 2.0 gm/kg. Values on higher intakes were not regarded as significant, being obtained at surfeit levels.

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DISCUSSION[1]

DR. ROSE: I would like to make one comment in addition to congratulating the authors of these papers. That is that in the comparison of the FAO standard and the egg standard, these really are recommendations. To be a little fairer, I think you ought to take my recommended allowances, which are twice the minimum level, and then you would find they aren't very different.

I am very gratified, if I interpret your data correctly, that you found about the same range that we did in the young people, but these old fellows, 50 years of age, required a great deal more.

DR. SWENDSEID: I think Dr. Rose is correct in his assumption.The total nitrogen, I think, is really the only value which has merit. For young men it seems to be 1.3 gm to 1.4 gm.

DR. MERTZ: Dr. Swendseid raised the question of difference in requirements for nitrogen with regard to difference in sex. I do not know how others work out total nitrogen. In our experiment at Purdue, Dr. Helen Clark and I have always had equal numbers of female and male students on diet squads, and we have experience with about 20 males and 20 females. We have found no evidence for sex difference with respect to the lysine requirement. The requirement of the male seems to have some definite relationship with regard to lysine to parameters such as metabolic rate, body size, creatinine excretion, and so forth. There seems to be a tie-up between these and the lysine requirement for all the males which gives a nice pattern. With the exception of two females, we get the same type of relationship with the females, namely, a relationship between the lysine requirement and metabolic rate, body size, and creatinine excretion.

[1] Editor's Note—This discussion covers the two preceding papers.

DR. BENDER: I would like to ask Dr. Holt for some information, speaking with all humility as one using rats and not humans.

In the beginning you made a statement which is common in the textbooks, that the requirements for sulfur amino acids in animals like the rat are greater than in humans because the rat must grow hair, et cetera. I wish to ask if this is so, because we find that the methionine-cystine requirements as determined on the rats by ourselves and by Johnston agree quite well with the human figures for those proteins limited by methionine and cystine, which means that when the BV is determined we are measuring available methionine-cystine. Our figures and Dr. DeMaeyer's figures agree exactly. This would suggest the rat does not have a higher requirement.

DR. HOLT: The statement that the rat requires relatively more sulfur amino acid is not merely a supposition, Dr. Bender. It is based on some experimental data (for example, Cox et al. 1947, J. Nutr. *33*:437). I do not know whose data are right.

DR. SCRIMSHAW: I principally would like to ask about the sesame flour. Just as yesterday we saw the differences with different peanut samples, there were differences in the different samples that we got and used in Mixture 8. We got a 33% fat sesame flour, and then from the same place we later got what was supposed to be low fat, and it turned out to be 19%. This wasn't nearly as good. In Dr. DeMaeyer's studies he is using a 12% fat sesame, also from the same place. Whatever it is, it appears to be causing distinctly adverse effects.

DR. DEAN: Did it strike anyone else as rather peculiar or am I wrong in thinking these results show there is no disease in man due to amino acid deficiency?

DR. DARBY: You might argue pellagra is.

DR. HOLT: Under experimental conditions an amino acid deficiency may produce a definite clinical picture. We have found this to be the case in young infants who do not receive histidine. Under these circumstances a skin eruption developed, very much like eczema, except that it did not itch in any way nor was it accompanied by any eosinophilia. When we added histidine to the diet, and also to some extent when histidine was applied locally to this lesion, the lesion regressed. This phenomenon was observed only in the first few months of life. Two infants retested at the age of 6 months did not exhibit it.

DR. DEAN: There is no protein known to cause any disease in man.

DR. HOLT: I do not know of any at the moment. I can say that.

Factors Influencing Retention of Nitrogen by Normal Full-Term Infants

Samuel J. Fomon

IN THE STUDY of factors affecting retention of nitrogen by infants, there is considerable merit in controlling as many variables as possible. The data to be presented concern results of relatively long-term metabolic balance studies performed during the past few years with normal infants receiving human milk or one of several formulas providing 67 cal/100 ml and supplying approximately 7% to 20% of the calories as protein. Under these conditions, it seems probable that the major factors influencing retention of nitrogen are age and quantity and quality of protein ingested.

Subjects, Procedures and Methods

The subjects of the metabolic observations were normal, full-term infants of students of the University, resident physicians of the University Hospitals, unwed women or women with tuberculosis.

In the earlier studies[1,2] the infants lived in the metabolism ward from shortly after birth until about 6 months of age, while in later studies[3-5] some or all of the infants lived at home and were admitted to the hospital only as necessary to carry out the metabolic balances.

In each case a 3-day metabolic balance study was performed approximately every 2 weeks. Human milk or a formula were fed ad libitum and served as the sole source of calories, although additional vitamins and, in the later studies, iron were provided.

Procedures and methods have been described in detail previously.[6]

Influence of Age and Intake of Protein on Retention of Nitrogen

When normal infants receive the same feeding ad libitum from birth until 6 months of age, both intake and retention of nitrogen, expressed as functions of body weight, decrease progressively with increasing age. These relationships may be seen in figures 1 and 2, which present data concerning 6 infants fed pooled, pasteurized human milk as the sole source of calories during all of the first 6 months of life and 3 infants who received this feeding for shorter periods.[1]

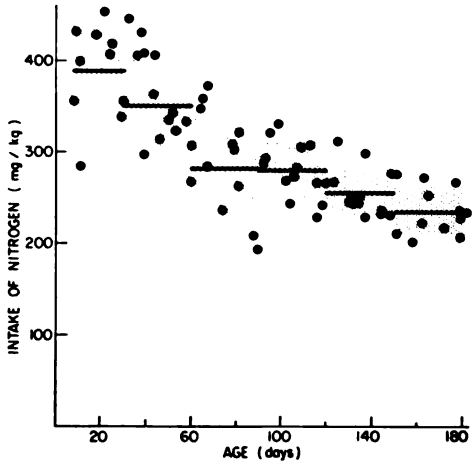

Figure 1—Intake of nitrogen vs. age of infants fed pooled, pasteurized human milk.[1] Each dot refers to the mean daily intake of nitrogen during one three-day metabolic balance study. The horizontal lines indicate the mean intakes of nitrogen during the following age periods: 8 to 30, 31 to 60, 61 to 90, 91 to 120, 121 to 150 and 151 to 182 days. The stippled areas include ± 1 standard deviation.

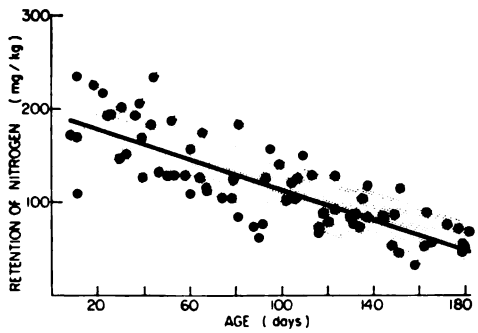

Figure 2—Retention of nitrogen vs. age of infants fed pooled, pasteurized human milk.[1] Each dot refers to the mean daily retention of nitrogen during one three-day metabolic balance study. The calculated regression line is shown. The stippled area includes one standard error of the estimate above and below the regression.

Three-dimensional diagram. In an attempt at graphic demonstration of the combined influences of age and intake of nitrogen on retention of nitrogen, the preparation of a three-dimensional diagram was undertaken[7] utilizing the data concerning infants fed pooled human milk. The effort to define such a plane was based on the computational simplicity of a plane over a more complicated surface (for example, a parabolic surface), and was justified by the lack of conclusive evidence that a surface involving terms of higher degree would result in a better fit. Because of the relatively great variability of intakes and retentions of nitrogen during the first 4 weeks of life, only data concerning metabolic balance studies performed between 29 and 182 days of age were included.

A regression plane[8] was constructed of the form $Y = a + bx + cz$, where Y = retention of nitrogen (mg/kg), x = age (days) and z = intake of nitrogen (mg/kg). The constants a, b and c were estimated by a method of least squares in such a way that the squared deviations from the regression plane were as small as possible. The values for these constants were found to be −32.64, −.235 and .587, respectively.

The regression plane, constructed as indicated above, is presented in figure 3. The formula of this plane is $Y = -32.64 - .235x + .587z$.

Retention versus intake of nitrogen for limited age ranges. In an attempt at developing a less involved approach to demonstrating the influence of age on retention of nitrogen, a comparison has been made of linear regressions[8] of retention of nitrogen on intake of nitrogen for 4 consecutive months of life: 31 to 60 days, 61 to 90 days, 91 to 120 days and 121 to 150 days (fig. 4). The respective regression equations for the four age intervals, 31 to 60 days, 61 to 90 days, 91 to 120 days and 121 to 150 days, are as follows: $Y = 192 + 0.218\,(x - 507)$, $Y =$

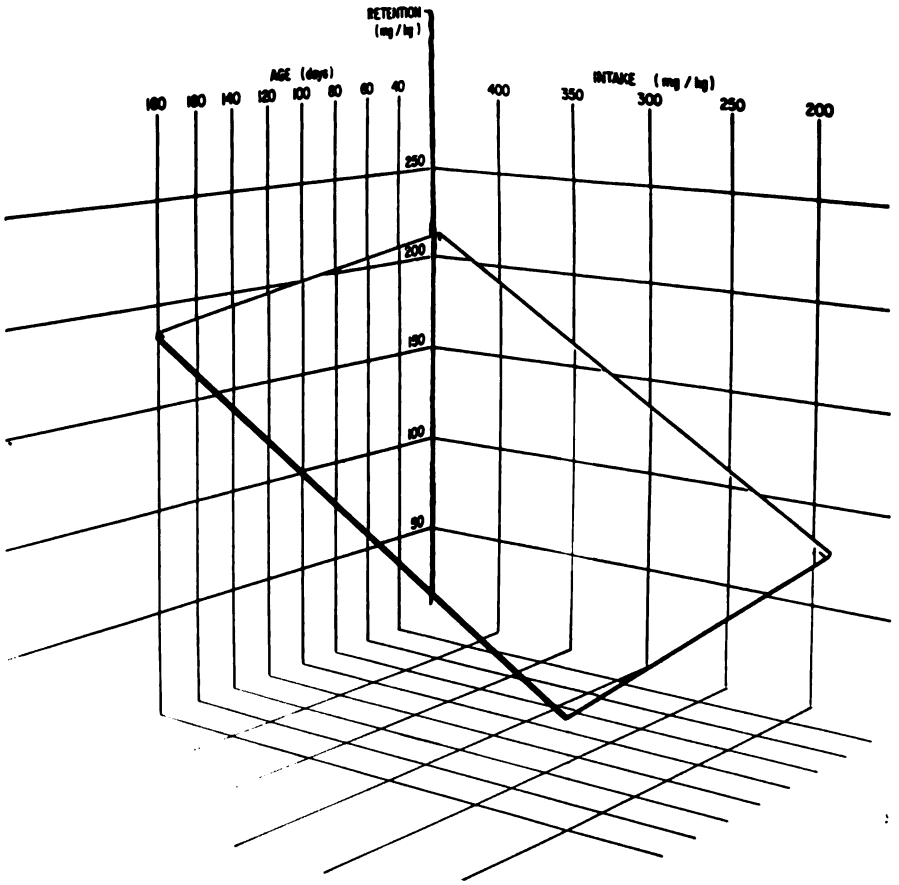

Figure 3—Regression of retention of nitrogen on age and intake of nitrogen calculated from data concerning infants 29 to 182 days of age fed pooled human milk. The vertical distance from the horizontal grid to the regression plane indicates the retention of nitrogen predicted for a given age and intake of nitrogen.

$172 + 0.258\,(x - 528)$, $Y = 148 + 0.196\,(x - 501)$, and $Y = 118 + 0.198\,(x - 436)$, where Y is a specified retention of nitrogen expressed as mg/kg/day and x is the corresponding intake of nitrogen expressed as mg/kg/day. The standard errors of the estimates of the regressions for these age intervals are 38, 41, 34 and 28 mg/kg/day, respectively. Data employed in calculating these regressions have been derived from study of infants fed pooled human milk[1] (approximately 7% of the calories from protein), or various formulas in which the protein was derived from cow milk. These formulas supplied 11% of the calories as protein,[2] 16% of the calories as protein (Nelson[9]), or 20% of the calories as protein.[3] Daily intakes of protein ranged from approximately 1.6 to 6 gm/kg between 31 and 60 days of age, and from approximately 1.4 to 5.5 gm/kg between 121 and 150 days of age.

The influence of age on the regression of retention of nitrogen on intake of nitrogen may be seen in figure 5. It will be noted that, when related to body weight, a specified intake of protein becomes progressively less effective in promoting

retention of nitrogen as age increases. The importance of the study of infants of similar age in comparisons of retentions of nitrogen with different feedings is therefore apparent.

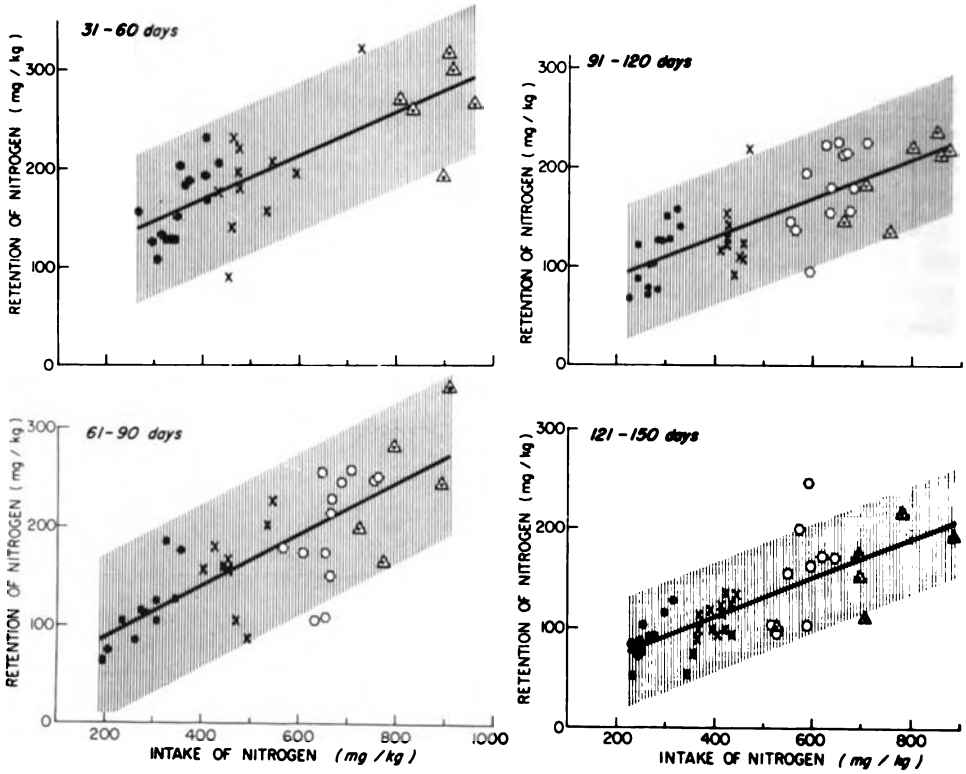

Figure 4—Regressions of retention of nitrogen on intake of nitrogen for limited age ranges: 31 to 60, 61 to 90, 91 to 120 and 121 to 150 days. Each hatched area includes two standard errors of the estimate above and below the regression. Each point pertains to one 3-day metabolic balance study. Data presented include studies with infants fed pooled human milk[1] (solid dots), a formula supplying 11% of the calories as protein[2] (x's), a formula supplying 16% of the calories as protein[9] (hexagons), and a formula supplying 20% of the calories as protein[3] (triangles with dots).

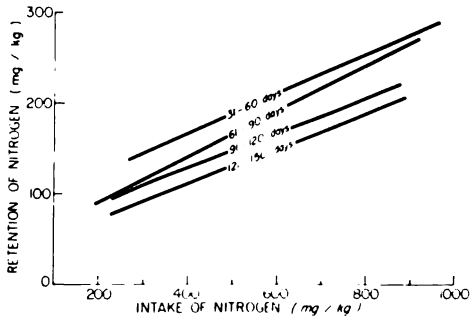

Figure 5—Composite of regression lines from figure 4.

Retention of Nitrogen by Infants Receiving Similar Intakes of Protein from Various Sources: Human Milk, Cow Milk, Soybean

Because breast feeding by a healthy, well nourished mother is generally considered to represent adequate nutrition during the early months of life, studies of the equivalence of other proteins to those of human milk may aid in establishing the requirement of infants for protein. One method of assessing

the equivalence of different proteins in infant nutrition is provided by comparison of data from nitrogen balance studies of infants receiving human milk with data from similar studies of infants receiving various formulas with the same concentration of protein.

Comparison of protein from human milk and cow milk. Eight normal full-term infants were studied[4] during ad libitum ingestion of a formula providing approximately 7% of the calories as protein from cow milk, 50% from a mixture of vegetable oils (57.5% corn oil, 37.5% coconut oil, 5.0% olive oil) and 43% from lactose. Both the mean volume of intake and the mean caloric concentration of the feeding were slightly less than those of the infants fed pooled human milk. Consequently, in 44 of the 60 metabolic balance studies with infants receiving this formula, intakes of nitrogen were less than the mean intake by infants of similar age receiving pasteurized human milk (fig. 6). Similarly, retentions of nitrogen in 37 of the 60 metabolic balance studies fell below the regression calculated from data concerning infants fed pasteurized human milk (fig. 7).

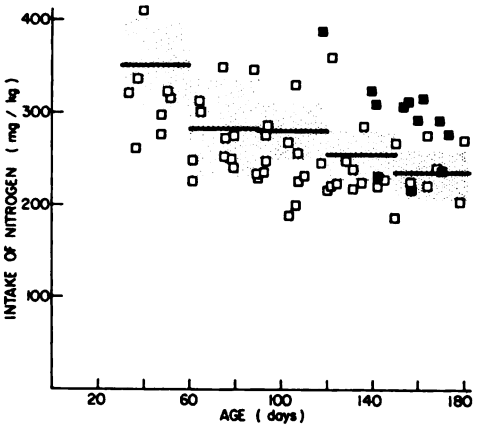

Figure 6—Intake of nitrogen vs. age of infants fed formulas supplying approximately 7% of the calories as protein from cow milk[4] (open squares) or soybean[5] (solid squares). The horizontal lines and stippled areas refer to the study of infants fed pooled human milk[1] as in figure 1.

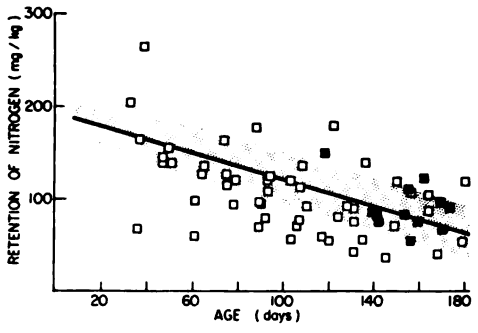

Figure 7—Retention of nitrogen vs. age of infants receiving approximately 7% of the calories as protein. Symbols are as in figure 6. The regression line and stippled area refer to the study of infants fed pooled human milk[1] as in figure 2.

Comparison of protein from human milk and soybean. Considerably less information was obtained concerning infants receiving soybean protein. Four infants, 118 to 207 days of age, were studied while receiving ad libitum a formula in which soy extract made from full-fat flour from the whole bean served as the sole source of protein and was not fortified with additional amino acids. The formula was fed at a concentration of approximately 67 cal/100 ml and provided approximately 7% of the calories as protein, 48% of the calories as fat (87% soy oil, 13% coconut oil), and 45% of the calories as carbohydrate. The mean intake of protein by infants 139 to 187 days of age receiving this feeding was 1.73 gm/kg/day, a value slightly greater than the mean intake by infants of similar age (137 to 182 days) fed pasteurized human milk (fig. 6).

As may be seen from figure 7, retentions of nitrogen by the infants fed

protein from soybean were similar to those by infants fed protein from human milk.

Comparison of proteins from human milk, cow milk and soybean: Regression of retention of nitrogen on intake of nitrogen. The regression of retention of nitrogen (mg/kg) on intake of nitrogen (mg/kg) for each of 5 consecutive months of age may be seen in figure 8. Each regression has been calculated on the basis of data applying to infants fed human milk,[1] those fed the formula with protein from cow milk[4] and, where applicable, those fed the formula with protein from soybean.[5] The respective regression equations for the five age intervals are $Y = 158 + .667 (x - 337)$, $Y = 114 + .572 (x - 276)$, $Y = 98 + .627 (x - 254)$, $Y = 88 + .576 (x - 254)$, and $Y = 76 + .553 (x - 250)$, where Y is a specified intake of nitrogen expressed as mg/kg/day and x is the corresponding intake of nitrogen expressed as mg/kg/day. The relation of retention to intake appears generally similar with the 3 feedings. *

Comparison of intakes of individual essential amino acids from human milk and formulas with protein from cow milk or soybean. Consideration of the intakes of individual essential amino acids by infants receiving the 3 feedings is pertinent because an important determinant of the ability of a protein to promote retention of nitrogen is the provision of an adequate intake of each essential amino acid. Calculated intakes of 10 amino acids by infants approximately 4½ to 6 months of age are presented in the table.

The method of calculation is as follows:

The percentage of the total protein represented by each amino acid was assumed to be that cited by Macy et al.[11] for human milk and cow milk, and was determined[5] for the soybean formula. This percentage value for the individual amino acid was multiplied by the mean intake of protein (mean intake of nitrogen x 6.25). The greater concentration of nonprotein nitrogen in human milk and the cow milk formula than in the soybean formula was not considered in the calculation. The precise age ranges of the infants were 137 to 182 days for those receiving human milk, 142 to 180 days for those receiving the formula with protein from cow milk and 139 to 187 days for those receiving the formula with protein from soybean.

In view of the greater intakes of total protein from the soybean formula (1.73 gm/kg/day vs. 1.50 gm/kg/day from human milk), intakes of amino acids that would be expected to result from an intake of 1.50 gm/kg/day of soybean protein have also been included in the table.

For comparison with these intakes, current estimations of the minimal requirements of the infant for these 10 amino acids, as determined by the "depletion

* Although the relation of retention of nitrogen to intake of nitrogen appears generally similar with the 3 feedings, a majority of the points referring to studies with infants fed the formula with protein from cow milk will be seen to fall above the regression lines, while a majority of the points referring to studies with infants fed human milk will be seen to fall below the regression lines. This observation is not interpreted as indicating that protein from cow milk is more effective than protein from human milk in promoting retention of nitrogen but is believed to be merely a reflection of the differences in mean intakes of protein from the 2 feedings in the various age intervals (fig. 6). Consideration of limited age ranges in the plot of retention of nitrogen against intake of nitrogen minimizes to some extent but does not eliminate the variable of age.

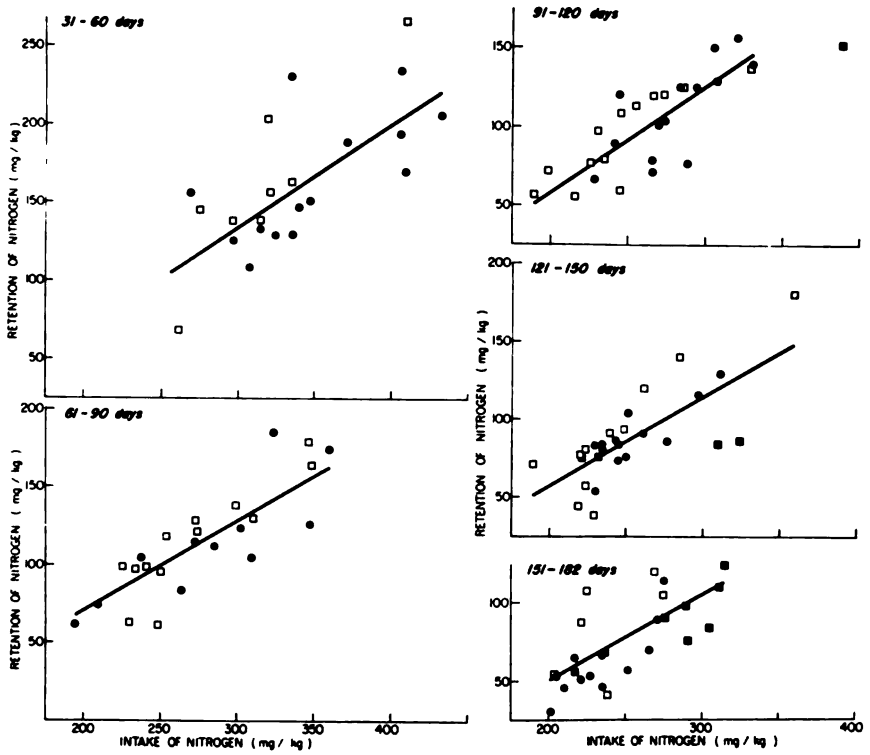

Figure 8—Regressions of retention of nitrogen on intake of nitrogen for limited age ranges: 31 to 60, 61 to 90, 91 to 120, 121 to 150 and 151 to 182 days. The regression lines are calculated from data pertaining to infants fed pooled human milk[1] (black dots) and formulas with protein from cow milk[4] (open squares) and soybeans[5] (solid squares). Data concerning the study with the soybean formula at 118 days of age have been omitted from the figure (91 to 120 days) and calculation.

technique," are given.[10] Decreasing intakes of each of the amino acids were provided until a value was reached at which gain in weight and retention of nitrogen were less than control values.

Because microbiologic methods employed in determining concentrations of individual amino acids in whole proteins are subject to considerable error, caution is necessary in interpreting differences in intakes from the feedings considered in the table. For example, concentrations of lysine in cow milk reported by 9 investigators ranged from 184 to 338 mg/100 ml and concentrations of methionine ranged from 60 to 140 mg/100 ml.[11] Such variations probably reflect a lack of precision in methods of determination as well as actual variation in content of different pools of cow milk.

Intakes of the various amino acids from human milk and from the formulas with protein from cow milk or soybean will be seen to be generally similar. It is emphasized that the true intakes undoubtedly covered a considerable range with each feeding. Probably human milk protein has somewhat lower concentrations of histidine and methionine than does cow milk or soybean protein. Cow milk protein

and soybean protein appear to have lower concentrations of leucine than does human milk, and soybean protein has a lower content of tryptophan than does protein from human milk or cow milk.

Judging from the "minimal requirements," all 3 feedings provide only borderline or actually inadequate intakes of several essential amino acids.

TABLE 1
INTAKE OF ESSENTIAL AMINO ACIDS BY INFANTS 4½ TO 6 MONTHS OF AGE RECEIVING PROTEIN FROM VARIOUS SOURCES

	FEEDING				
	Pooled Human Milk[1]	Formula with Protein from Cow Milk[4]	Formula with Protein from Soybean[5]		Minimal Requirement Depletion Technique[10] †
Mean Intake of Protein (gm/kg/day)	1.50 (observed)	1.46 (observed)	1.73 (observed)	1.50 (assumed)	
Amino Acid (mg/kg/day) *					
Arginine	63	56	100	88	0
Histidine	28	35	42	37	34
Isoleucine	108	93	112	99	119
Leucine	202	154	189	167	150 **
Lysine	99	114	137	121	103
Phenylalanine	79	76	91	80	90 ††
Methionine	28	38	41	36	45 ††
Threonine	78	67	81	71	87
Tryptophan	27	22	14	12	22
Valine	113	101	121	106	105

* See text for method of determination or calculation.
† Value listed is value shown to be adequate. Minimal requirement is probably slightly less.
** One subject required 229 mg/kg.
†† Requirement for phenylalanine was determined in presence of tyrosine and requirement of methionine in presence of cystine.

COMMENTS and SUMMARY

Although infants with malnutrition or other illness are ordinarily more readily available for study, they are likely to be less suitable than full-term normal infants for assessing the various factors influencing retention of nitrogen. Illness may interfere with retention of nitrogen in an unpredictable manner and the infant recovering from severe malnutrition retains nitrogen particularly avidly. An additional difficulty in study of the severely malnourished infant is that fluctuations in weight due to changing hydration or actual edema make it relatively unsatisfactory to express such functions as intake of calories, intake of protein or retention of nitrogen in relation to body weight.

Whether milk from the healthy, well nourished woman is adequate as a sole source of protein for the infant between 4½ and 6 months of age will probably remain unanswered until more satisfactory criteria of adequate nutrition have been established. The infants fed pooled human milk as a sole source of calories during the first 6 months of life progressed in length and weight along normal developmental channels although growth curves were generally below the 50th percentiles of the Iowa Growth Charts.[12] Retentions of nitrogen were less than those of infants of similar age receiving greater intakes of protein, a finding which does not, of course, indicate a less satisfactory nutritional state.

If human milk is not adequate as a sole source of protein between 4½ and 6 months of age, its inadequacy might result from its relatively low content of total protein or from deficiency of at least one essential amino acid. Histidine, lysine, phenylalanine or methionine might be the "limiting" amino acid. It is possible that human milk, while providing sufficient protein and essential amino acids for most normal infants during the first 6 months of life, is inadequate for a few otherwise normal infants who have somewhat greater requirements for protein.

As judged by ability to promote retention of nitrogen, a mean intake of 1.5 gm/kg/day of protein from cow milk is adequate between 4½ and 6 months of age if human milk is adequate. Similarly, a mean intake of protein of 1.7 gm/kg/day from the soybean formula resulted in retentions of nitrogen at least as great as those of infants of similar age fed human milk. The data do not indicate whether a mean intake of protein of 1.5 gm/kg/day from the soybean formula would have permitted retentions of nitrogen equal to those of infants of similar age fed human milk.

Many factors undoubtedly influence retention of nitrogen by infants fed a particular protein. Among these factors are probably the method of processing the protein and the type and amount of carbohydrate and fat associated with the protein in the diet. The demonstration that, under specified conditions, protein from human milk, cow milk and soybean promoted retention of nitrogen to the same extent does not imply that all formulas with protein from cow milk or the soybean will have similar nutritional properties. Furthermore, failure to demonstrate differences in the quality of these proteins in studies of normal full-term infants receiving approximately 7% of the calories as protein does not imply equivalence for premature infants or those recovering from gross malnutrition. Neither does it imply equivalence at lower intakes of protein (e.g., 5% or 4% of the calories as protein).

Summary

In metabolic balance studies with normal full-term infants during the first 6 months of life, it is assumed that age, intake of nitrogen and quality of ingested protein are major factors in determining retention of nitrogen. The influence of age and intake of nitrogen on retention of nitrogen is portrayed graphically by a regres-

sion plane (fig. 3). In addition, the relation of retention of nitrogen to intake of nitrogen is plotted for various age periods (fig. 4) and it is seen that the same intake of nitrogen results in a lesser retention of nitrogen as age increases (fig. 5).

Ability to promote retention of nitrogen was studied with 3 feedings having similar concentrations of protein: pooled human milk, a formula with protein from cow milk and a formula with protein from the soybean. Infants 4½ to 6 months of age had mean intakes of protein of 1.50 gm/kg/day from human milk, 1.46 gm/kg/day from the formula with protein from cow milk and 1.73 gm/kg/day from the formula with protein from soybean. Retentions of nitrogen were similar with the 3 feedings. The data do not demonstrate whether retentions of nitrogen would have been significantly less with the soybean formula if the mean intake of protein had been 1.5 gm/kg/day.

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DISCUSSION

DR. GARN: The nitrogen retention per kilogram of body weight would not be parallel if you used the fat-free weight, because in that same time period, zero to 180 days, the children are markedly increasing their proportion of fat. It occurred to me that using the fat-free weight as a reference standard might give you more nearly a straight line.

Knowing your interest in the area, I wonder if you have already done it.

DR. FOMON: We are not yet able to measure fat-free weight.

Experimental Protein Malnutrition in Animals

Lipemia Associated With Protein Depletion

J. B. Allison and R. W. Wannemacher, Jr.

A LIPEMIA HAS BEEN OBSERVED in dogs and rats depleted in body nitrogen.[1] The suggestion was made that this alteration in lipid metabolism was the result, in part, of an overloading of enzyme systems which were reduced in activity by depletion in body proteins.[1,2,3] The following study was done to determine in more detail the characteristics of the lipemia in dogs depleted in body proteins, and the effects of varying the amount and kind of dietary fat upon the serum lipids. The study also included the effects of repletion while feeding casein or wheat gluten.

Methods

Adult dogs were divided into 4 groups of 6 dogs each and were fed the protein-free diets recorded in table 1. Diet A had 30% of the calories and diet B had 5% of the calories as either lard or corn oil. The dogs were fed these diets at

TABLE 1
PROTEIN-FREE DIETS FED TO DOGS

Ingredients	Diet A (gm)	Diet B (gm)
Sucrose	22.9	32.7
Dextrose	38.7	45.2
Dextrin	18.7	16.1
Lard or corn oil	15.3	2.2
Salt mixture	1.7	1.5
Agar	2.7	2.3
Water	132.4	132.4
Vitamins	7.6	7.6
Total	240.0	240.0

80 cal/day/kg. Eight dogs were repleted with either casein or wheat gluten protein at 0.6 gm N/day/kg until the nitrogen lost during depletion was regained. The total caloric intake on the repleting diets was 140 cal/day/kg. This higher caloric intake promoted the most rapid rates of repletion.[4]

Urine and fecal collections were made throughout the depletion and repletion periods. Micro-Kjeldahl nitrogen determinations were made on these samples in

order to calculate the nitrogen lost from the bodies of the animals by assuming an overall protein content of the animal to be 16%.

At various times, during depletion and repletion periods, blood was drawn and total protein, protein and lipoprotein electrophoretic patterns, free and esterified cholesterol and phospholipid phosphorus were determined on serum samples. The protein content of the serum was measured by the Biuret method,[5] the protein electrophoresis on paper was estimated by the alcoholic bromphenol blue method and the lipoproteins were determined by the oil red 0 procedure of Jencks et al.[6] The serum lipids were extracted in a 2:1 chloroform methanol mixture from which the cholesterol was separated by method of Wycoff and Parsons.[7]

Results

The percentage losses of body weight and of body nitrogen by dogs fed the protein-free diet were linear with time as illustrated by the data in figure 1. These data demonstrate the greater rate of loss in nitrogen than in body weight. Similarly Standard, Wills and Waterlow[8] found that a body weight deficit underestimates loss in body nitrogen in malnourished infants.

The effects of depletion in body nitrogen upon serum proteins and lipids are illustrated in figure 2. The open circles in the lower left-hand portion of the figure record the reduction in the albumin: globulin ratio with loss in body nitrogen in dogs fed the diet containing 30% of the calories as lard. The triangles record similar data obtained while feeding the diet containing 30% of the calories as corn oil.

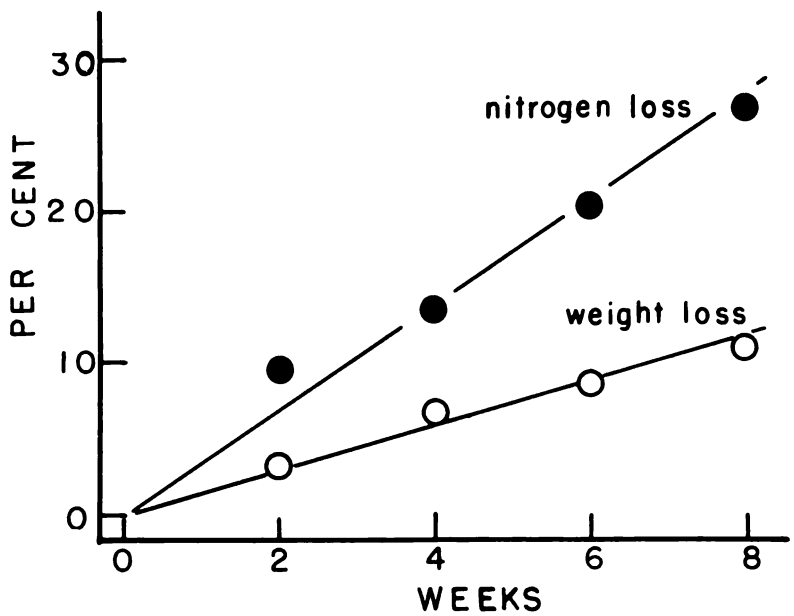

Figure 1—The percent loss in body nitrogen (black circles) and loss in body weight (white circles) correlated with time dogs were fed a protein-free diet.

Substituting corn oil for lard did not alter the rate of reduction in the ratio with loss in body nitrogen.

Corn oil in the diet, however, did have a different effect than lard upon the amount of lipid migrating electrophoretically with the alpha and beta globulins, as illustrated by the data plotted in the lower right-hand portion of figure 2. There was a marked increase in lipid associated with the globulins in the dogs fed the lard following a loss of approximately 10% of body nitrogen. This increase in lipid was slight, possibly insignificant in the animals fed the corn oil. There was an increase also in the phospholipid phosphorus and in serum cholesterol ester with loss in body nitrogen in animals fed lard, less so in those fed the corn oil.

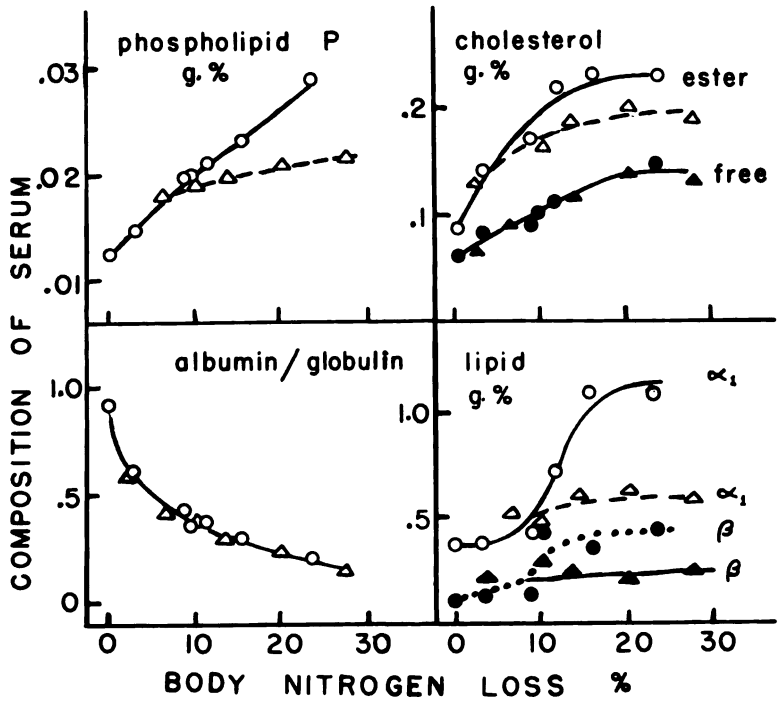

Figure 2—Correlation between loss in body nitrogen and the serum albumin/globulin ratio and various serum lipid fractions. The Circles—protein-free diet with thirty percent of the calories as lard; Triangles—corn oil.

The rate of increase in phospholipid phosphorus or of cholesterol, however, was reduced when the amount of fat in the diet was lowered to 5% of the calories. The open circles in figure 3 illustrate the serum phospholipid phosphorus and cholesterol ester in dogs fed a protein-free diet with 5% of the calories as lard. The open triangles record similar data obtained when dogs were fed the diet containing 5% of the calories as corn oil. The closed circles and triangles represent the serum free cholesterol in dogs depleted in body nitrogen by feeding the protein-free diet containing 5% of the calories as lard or as corn oil respectively. Thus with the

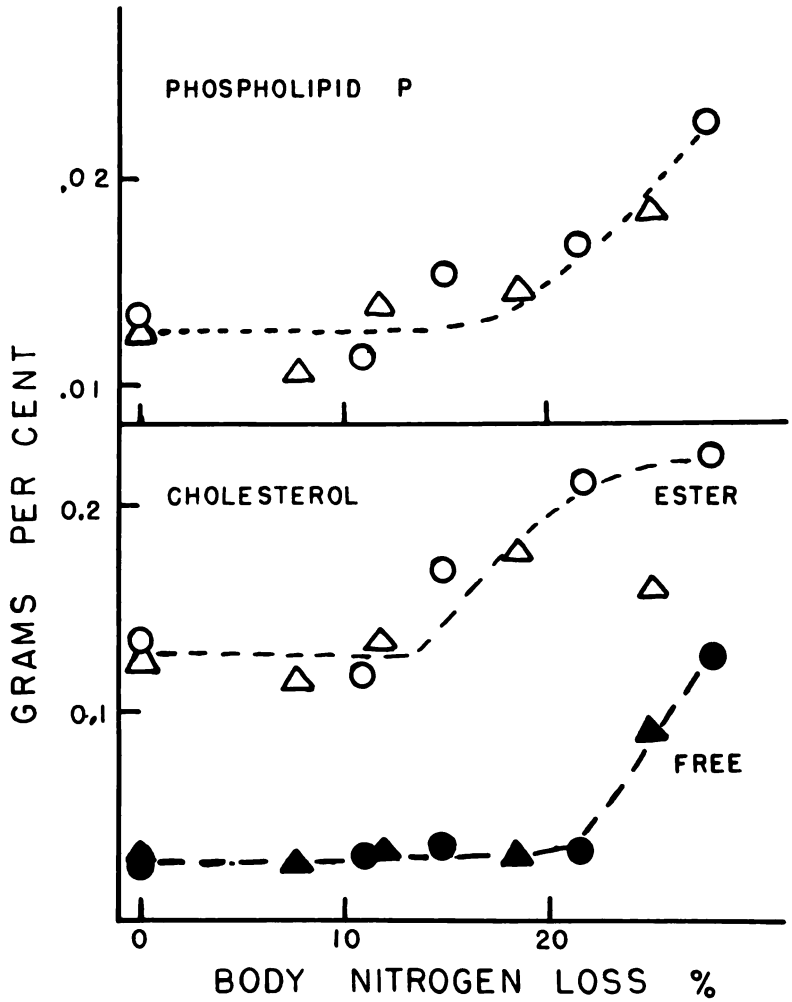

Figure 3—Correlation between loss in body nitrogen and serum phospholipid phosphorus and cholesterol. Five percent of the calories were fed in the form of lard (Circles) or corn oil (Triangles).

reduced fat intake there was no difference in response to the different types of fat. These results are consistent with the suggestion that this protein-depletion lipemia may be the result, in part, of overloading reduced oxidation and transportation mechanisms in the depleted animal. With a low fat intake, approximately 20% of the body nitrogen must be lost before a rise will take place in serum cholesterol or phospholipid phosphorus.

This lipemia observed in depleted dogs and rats has not been reported in children suffering from kwashiorkor. Indeed, serum lipids tend to be low in children with protein malnutrition. However, Schwartz and Dean[9] and Arroyave et al.[10] found an initial rise in serum lipids during repletion of depleted children,

followed by a return to normal value as repletion became complete. Possibly the initial rise in lipids during repletion could be the result of overloading of the depleted enzyme systems.

The effects of repletion with casein or with wheat gluten on serum albumin and serum lipids are summarized in figure 4. The white bars (C) record data obtained while feeding dogs the control diet containing casein and the fat as lard equivalent to 30% of the calories. These animals were fed 80 cal/day/kg. The bars with vertical lines (C') record data obtained while feeding another group of animals this same diet but at a higher caloric intake of approximately 140 cal/day/kg. This

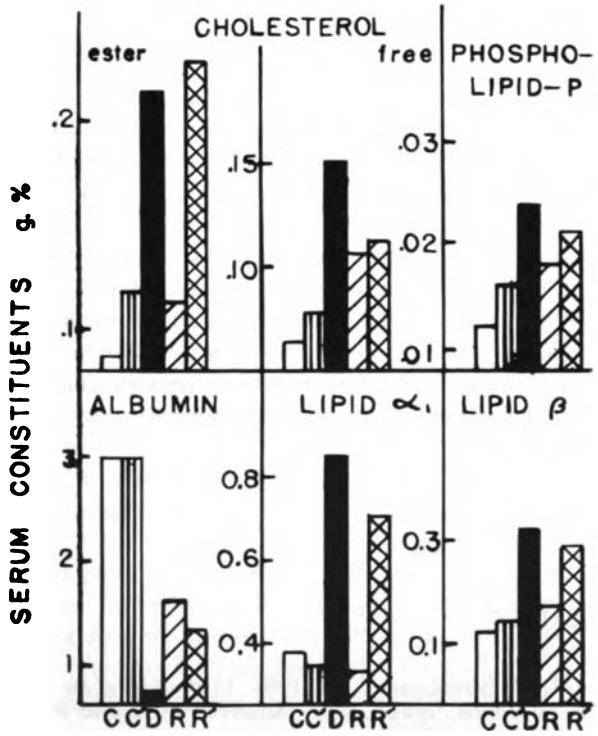

Figure 4—Effects of repletion with casein or with wheat gluten on serum albumin and serum lipids. C = normal dogs, C^1 = obese dogs fed excess calories, D = protein-depleted dogs, R = dogs repleted with casein, R^1 = dogs repleted with wheat gluten.

higher caloric intake represented the amount consumed during repletion when the depleted animals were fed ad lib. Such a high intake produced obesity in normal animals and tended to increase serum cholesterol ester and phospholipid phosphorus slightly but did not alter the magnitude of serum albumin or the lipid migrating electrophoretically with the alpha or the beta globulins. The normal animals gave the same response whether they were fed lard or corn coil. The black bars (D) in figure 4 illustrate the reduction in serum albumin and the rise in lipid migrating

electrophoretically with the serum globulins and the increase in cholesterol ester and in phospholipid phosphorus associated with a loss in approximately 25% of body nitrogen. The bars with slanted lines (R) record the rise in serum albumin and the reduction in the serum lipids when approximately 90% of the body nitrogen had been repleted by feeding casein. The bars with crossed lines (R') illustrate data obtained after 90% of the body nitrogen had been repleted while feeding wheat gluten. The serum lipids were not returned to normal during repletion with wheat gluten as with casein, the cholesterol ester remaining particularly high. Approximately 40% of the body nitrogen was repleted by feeding casein before the serum cholesterol ester decreased significantly. Following this 40% repletion with casein the cholesterol ester dropped rapidly to control values.

Summary

Depletion in body nitrogen in dogs fed a protein-free diet resulted in an increased serum cholesterol, phospholipid phosphorus, and lipid migrating electrophoretically with globulin fractions. The increase was marked when the diet contained 30% of the calories as lard, less so when the fat intake was reduced to 5% of the calories. A high fat intake, whether from lard or corn oil, had little or no effect upon serum lipids in dogs with normal protein reserves. The lipemia was less marked, however, at the high fat intake when corn oil was substituted for lard in the diet of the depleted dog. Repletion with casein brought serum lipids back toward normal more adequately than repletion with wheat gluten. Thus the balance between dietary amino acids and between dietary protein and lipid are factors in maintaining the biochemical environment of the serum.

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DISCUSSION

DR. RAMALINGASWAMI: I was very much interested in the comment that was made on the cholesterol values in protein-depleted dogs. As my paper to follow will show, the total serum cholesterol decreases in monkeys. We have not fractionated the cholesterol. In view of the total fall in cholesterol, there is probably a fall in serum cholesterol, too.

Do you think this could be a species difference or is it a matter of fat intake? In the animals I shall discuss, we fed no fat at all, but we had a group in which a certain percentage of the calories were supplied in the form of coconut fat. The group that received no protein and 30% of calories in the form of coconut fat also showed a fall in its cholesterol, but it was not as marked as in the group which did not have any fat in the diet. So, really, a fall in total cholesterol is related to the protein deficiency. The rapidity with which it began to rise when we fed protein led us to believe that there is some kind of relationship. I would very much like you to comment on that situation.

The second point is the rise in γ-globulin which seems to occur in the absence of obvious infection in protein-depleted monkeys. Of course, one cannot be sure that the internal microflora or the intestinal microflora have been changed. We do not have any data on that at all. That increase persists when the animal is apparently back again to normal and has been a puzzling feature in our studies. I wonder if Dr. Allison would comment on that, too.

DR. ALLISON: There may be a species difference which would explain the data in part. However, I think this difference is probably more in degree than in kind. The rise in serum cholesterol ester in the dog depends upon the amount and kind of fat in the diet. When the amount of fat is very low, there is even a tendency for the cholesterol ester to drop a bit. The depleted dog, however, does seem to respond to an increase in the amount of fat in the diet by a rise in cholesterol ester much more than the rhesus monkey. I think the amount of unsaturated fat, of course, plays an important role here, too.

As far as the γ-globulin is concerned, we have observed this rise in γ-globulin in the majority of the animals but not in all of them. We considered this rise to be associated with a challenge of some kind.

DR. KAYE: Do you think that these results have anything to do with the situation in the experimentally nephrotic animal. When protein loss in the kidney is induced, the β-globulins apparently are produced more rapidly than the albumin. Dr. Swan and Dr. Stokes have hyperimmunized human volunteers to produce tetanus antisera, pertussis and other things. When they bleed these men 8 or 9 months after they have been hyperimmunized and withdraw a good portion of the γ-globulin, there is a rise in the next 2 weeks in the γ-globulin and an induced rate of synthesis of γ-globulin. Pulling it out of the body again stimulates it toward the production side.

I wondered whether your results of protein depletion in the dog receiving fat are analogous to the situation of protein loss which decreases the input side and still provides a reasonable fat intake through the diet.

DR. ALLISON: Yes, I think there could be an analogy. Possibly there is correlation between rate of synthesis and rate of utilization of some of the fractions. We have observed such an imbalance during the growth of certain types of tumors where kidney damage does not occur but where there is loss in plasma albumin.

DR. HARPER: I just wanted to ask Dr. Allison if he knew the amount of lipids in the wheat gluten.

DR. ALLISON: We do not know but I assume it is about the same as in the wheat gluten that you are using.

DR. HARPER: Our experience with the rat is a little different. We are primarily using diets that are high in cholesterol. We observed that the wheat gluten as compared with casein tended to bring the serum cholesterol levels down. When we extracted all of the lipids and there was something between 10% and 12% of fairly unsaturated lipids still in the wheat gluten, we found that this effect was lost. Then when we did this with animals that received no additional cholesterol in the diet, we found something a little more comparable to what you found, that the wheat gluten which still contained the lipid tended to increase our cholesterol fat a little bit, as if the unsaturated fat was stimulating or increasing the transport, or at least increasing the quantity of cholesterol in the serum. I wonder if you have any explanation for that.

DR. ALLISON: I have no explanation, but we have been studying the effects of unsaturated fatty acids on this type of increase in cholesterol and we seem to be getting data similar to yours.

Protein Deficiency in the Rhesus Monkey

V. Ramalingaswami, M. G. Deo and S. K. Sood

IN HUMAN DISEASE, one often deals with "a constellation of events in time and place and with multiple causes" (Fremont-Smith 1955). Nevertheless, it is essential to know the effects attributable to individual causative factors; these effects, when summated, provide a basis for understanding the total picture. This is no less true of kwashiorkor than of any other disease.

The fundamental importance of protein deficiency in the aetiology of kwashiorkor is now widely recognised. The effects of pure protein deficiency in the animal organism and their relation to the pathological picture of kwashiorkor are engaging the attention of several investigators (Shils et al., 1954 a and b; Best et al., 1955; Follis, 1957; Wilgram et al., 1958). We have been interested in studying the response of the rhesus monkey to various types of liver injury. By employing the technique of tube feeding the animals measured quantities of food daily, it has been possible to study the short and long term effects of uncomplicated protein deficiency in the rhesus monkey while at the same time keeping the intake of other nutrients optimum and constant throughout the experimental period. Thus a periportal fatty change, similar to that in kwashiorkor, has been produced readily in monkeys by feeding low-protein diets, a change which can be influenced remarkably by the level of protein intake (Deo and Ramalingaswami, 1960).

We present here further observations with the aid of this experimental model and discuss them in the light of the known pathology of kwashiorkor.

ANIMALS AND DIETS

The composition of diets used, techniques of feeding and liver biopsies and care of animals have been described elsewhere (Deo and Ramalingaswami, 1960). Here, only the principles will be outlined. Observations have been made to date on 25 rhesus monkeys maintained on low-protein diets for varying periods of time. The protein intakes varied from negligible to an amount contributing up to 8% of total calories in the form of casein. The control group consisted of 14 monkeys which were maintained concurrently on a diet in which 15% of the calories were derived from casein. Both groups received 100 calories/kg/day and optimum quantities of all other known nutrients. The body weights of the animals ranged from 2 to 4 kg at the start of the experiment and the animals were considered to be adolescent or in early adult life. Basal studies were made on all animals before feeding the test diets. Reversibility of lesions was studied by increasing the protein

intake of some of the deficient animals without altering the intake of other essential nutrients. Most of the animals studied were males, whose gonads showed full maturation. Liver biopsies at intervals and complete autopsies at the elected time were performed. Serial determinations were made of some chemical constituents of the serum plasma.

General Features

The animals on low-protein diets maintained their body weights for the first 2 to 4 weeks and declined gradually in weight later on. The plasma albumin concentration showed a fall early in the course of deficiency and reached significantly low levels. The plasma γ-globulin concentration showed a concomitant rise to significantly higher levels (table 1). The α- and β-globulins showed fluctuations which were not consistent. On refeeding protein to the deficient group, there was rapid restoration of plasma albumin level, but the γ-globulin showed a delayed fall and did not reach basal values even after 2 months of feeding the 15% casein diet following an equivalent period of deficiency (fig. 1). The elevation of γ-globulin in deficient animals, which was not explicable on the basis of obvious infection, and

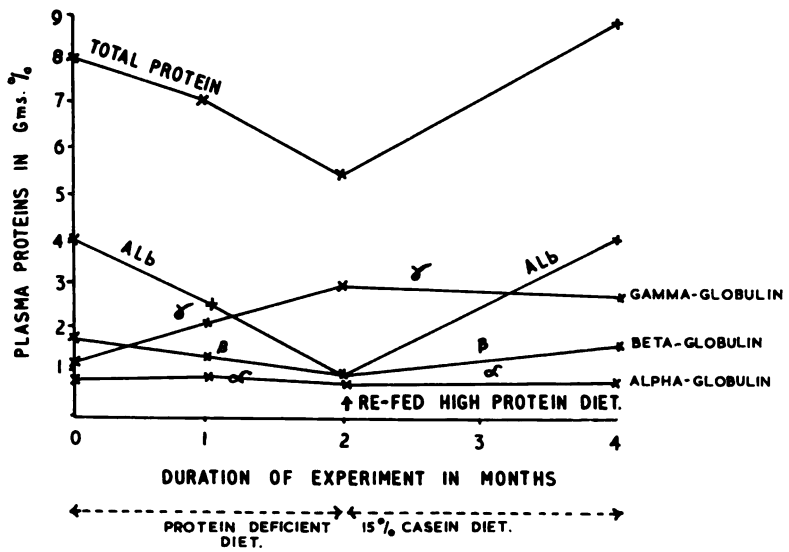

Figure 1—Typical response of serum proteins during protein depletion and repletion.

its persistence even after plasma albumin and liver morphology had returned to basal state are of particular interest and may indicate tell-tale evidence of previous protein deficiency. Its mechanism awaits further study. Total plasma protein was estimated by Biuret method (King and Wootton, 1956) and the protein fractions were estimated electropheretically, using "Spinco" paper electrophoresis apparatus. Five animals developed generalised oedema, most conspicuous over the eyelids after 2 to 3 months of deficiency (figs. 2 and 3). It is not clear why these animals came down with oedema and the others in the deficient groups did not.

Figure 2—Oedema of eyelids in a monkey after 6 weeks of protein depletion.

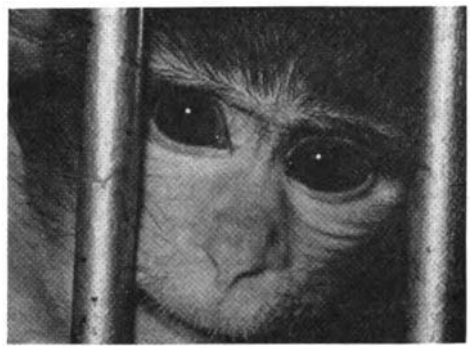

Figure 3—Control monkey being fed on 15% casein from the beginning of the experiment.

Total serum cholesterol was reduced significantly in the protein-deficient animals (table 2). The reduction occurred early in the deficiency. The cholesterol level appeared to be a sensitive indicator of protein nutrition and its level declined whenever the animal was fed low-protein diets irrespective of the fat content of the diet. On the other hand, in the control group the cholesterol levels either remained at the basal level or rose (table 2). Total cholesterol was determined according to the method of Zak et al. (1954) after extracting cholesterol with hot alcohol: acetone mixture.

Iron-binding capacity of plasma was markedly and consistently reduced in protein-deficient monkeys, while the fasting plasma iron was reduced only in some animals (table 3). They were restored to normal basal levels on correcting the protein deficiency (fig. 4). These changes occurred irrespective of the fact that the animals received 10 to 20 mg of elemental iron in the form of ferric ammonium citrate daily throughout the experiment. The method of Williams and Zak (1957) was modified for the estimation of total plasma iron, and plasma iron-binding capacity was estimated by the method of Ressler and Zak (1958).

TABLE 1
PLASMA PROTEINS IN PROTEIN-DEFICIENT MONKEYS
(gm/100 ml)

	Total Protein			Albumin			Gamma-Globulin			A:G. Ratio		
Monkey No.	Initial	1 mon. deficient	2 mon. deficient	Initial	1 mon. deficient	2 mon. deficient	Initial	1 mon. deficient	2 mon. deficient	Initial	1 mon. deficient	2 mon. deficient
116	7.25	6.70	5.75	4.18	3.67	1.81	1.40	1.80	1.85	1.42	1.22	0.47
119	7.90	6.90	5.20	3.84	2.38	0.80	1.11	2.03	2.85	1.06	0.58	0.18
127	8.90	5.60	5.30		3.15	1.79		1.18	1.79		1.35	0.51
154	8.25	7.60	5.60	4.13	1.4	1.01	1.78	2.20	2.45	1.00	0.23	0.22
162	7.20	5.80	4.60	3.81	2.50	0.70	1.76	2.04	2.48	1.15	0.76	0.18
172	6.80	6.60	5.10	3.50	2.14	1.75	1.78	2.34	1.97	1.12	0.48	0.521
130	6.45	4.90		3.51	2.45		1.27	1.15		1.27	1.00	
134	7.35	5.20		4.03	2.11		1.37	1.90		1.23	0.68	
Mean	7.51	6.16	5.25	3.85	2.47	1.31	1.49	1.83	2.23	1.11	0.67	0.33

TABLE 2
SERUM CHOLESTEROL IN MONKEYS

Diet	Monkey No.	Duration of experiment	Serum cholesterol Initial (mg/100 ml)	Serum cholesterol After exptl. period (mg/100 ml)
		PROTEIN-DEFICIENT GROUP		
No protein, and no fat	13	3-4 weeks	165.0	130.0
	15	"	202.0	160.0
	51	"	215.0	120.0
	55	"	180.0	156.0
	57	"	192.0	136.0
	61	"	200.0	128.0
	71	4-5 weeks	236.0	212.0
	73	"	232.0	204.0
Mean			202.7	155.7
		CONTROL GROUP		
15% casein, no fat	52	3-4 weeks	215.0	244.0
	56	"	128.0	200.0
	58	"	168.0	192.0
	62	"	164.0	215.0
	72	4-5 weeks	286.0	280.0
	74	"	248.0	258.0
Mean			201.5	231.5

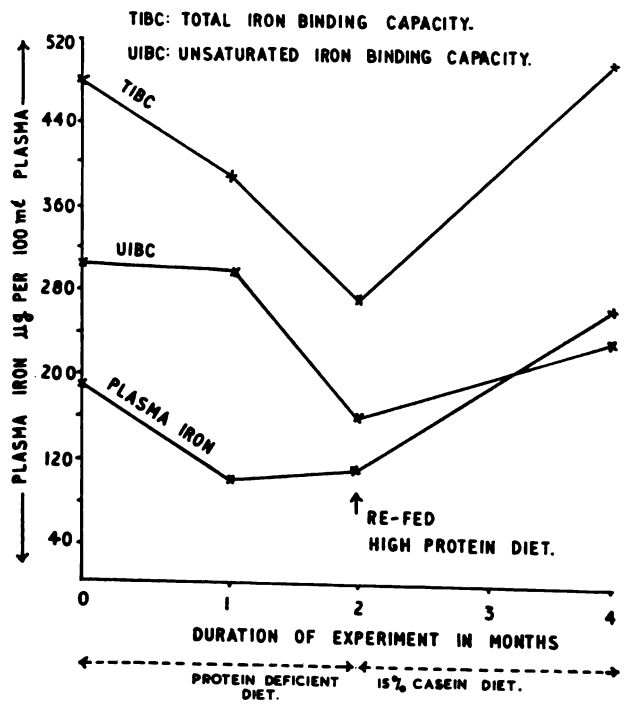

Figure 4—Typical serum iron responses during protein depletion and repletion.

TABLE 3
PLASMA IRON AND IRON-BINDING CAPACITY IN PROTEIN DEFICIENCY
(μg of iron/100 ml)

Monkey No.	Fasting Initial	Plasma Iron in μg% One month defic.	Two months defic.	Unsaturated iron-binding capacity (U.B.I.C.) Initial	One month defic.	Two months defic.
116	83	70	68	339	259	125
119	193	98	110	303	296	156
127	137	177	125	313	164	185
154	98.5	—	75	317	—	272
162	163	110	100	300	266	106
172	127	145	120	343	178	153
130	115	210	—	305	—	—
134	221	79	—	300	235	—
Mean	142.2	112.0	99.6	315.0	233.0	166.1

These changes in serum constituents are not explicable on the basis of plasma volume changes. Although plasma volumes have not been estimated, the haematocrit showed variable and insignificant fluctuations.

LIVER

In the manner vividly described by Davies in discussing kwashiorkor (Davies 1948), the animals on low-protein diets developed periportal fatty change which, as the deficiency progressed, spread to involve the rest of the lobule and, when the deficiency was corrected, regressed first in the centri-lobular areas and lastly in the immediate periportal areas. The rapidity with which the fatty change developed depended upon the severity of protein deficiency. When an animal weighing 2 kg is placed on a diet containing negligible protein, it develops well marked periportal fatty change in 15 to 20 days (fig. 5) and the lesion becomes diffuse in 3 to 4 weeks time (fig. 6). Animals have been maintained on such a regimen up to five and a half months. Their livers remained intensely fatty, containing 40% to 45% of fat by wet weight. Occasional foci of necrosis of liver cells were observed but, in general, necrosis, inflammatory cellular infiltration and desmoplastic reaction were

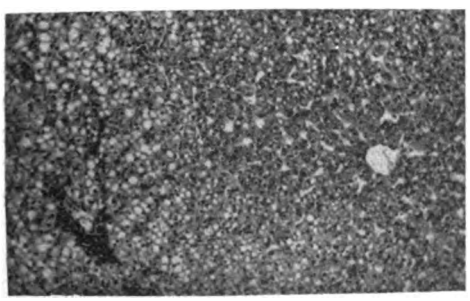

Figure 5—Section of liver showing periportal fatty change within 3 weeks of placing the animal on a diet containing negligible protein. H & E x 90.

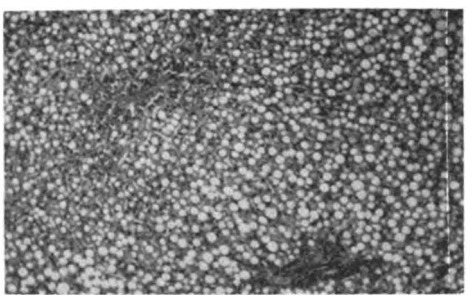

Figure 6—Section of liver of animal shown in figure 5, after 4 weeks of protein deficiency showing diffuse fatty change. H & E x 90.

conspicuous by their absence. The connective tissue of Glisson's capsule in the portal canals was less conspicuous than at the beginning of the experiment, and the sinusoidal reticulin was compressed and stretched by the fat-laden liver cells. This is a picture completely different from the familiar one of prolonged choline deficiency in the rat. It is also a picture closely similar to kwashiorkor wherein no progression of fatty liver to cirrhosis has ever been observed (Waterlow and Bras, 1957). It may be mentioned that all our monkeys, whether in the deficient or control group, received a daily ration of 100 mg of choline chloride. The protein-deficient livers showed neither ceroid nor haemosiderin at any stage. Glycogen could be demonstrated in fair quantities in tissue sections histochemically and seemed to be progressively pushed towards the cell boundaries by the accumulating lipid. Loss of cytoplasmic basophilia, at first in periportal areas and later generalised, was conspicuous. The cytological alterations were similar to those found in the rat in protein deficiency (Ramalingaswami et al., 1954).

Pancreas

Acinar atrophy, crowding of acinar nuclei and relative prominence and increase in size of the islets of Langerhans were observed in all deficient animals (figs. 7 and 8). The zymogen granules were absent; the lumina of the acini were

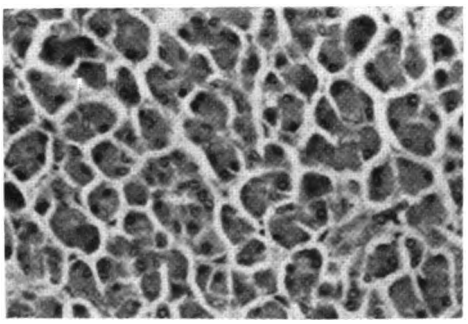

Figure 7—Pancreas of a control monkey. H & E x 400.

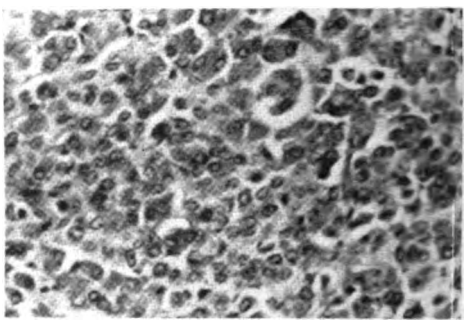

Figure 8—Pancreas of a protein-deficient monkey at the end of 2 months of feeding a diet containing negligible protein. H & E x 400.

obliterated and the interrelated ducts approximated and apparently increased in number. These changes were reversed completely by refeeding protein to the deficient animals, and the acini regenerated to normal appearance (fig. 9). The reticulin and collagen framework did not appear to be altered and no fibrosis or inflammatory reaction was seen in the deficient animals.

Salivary Glands

The changes in the parotid and submaxillary glands were inconspicuous and equivocal. Some animals showed an apparent shrinkage of acini in the parotid, but inflammatory reaction and fibrosis were again absent.

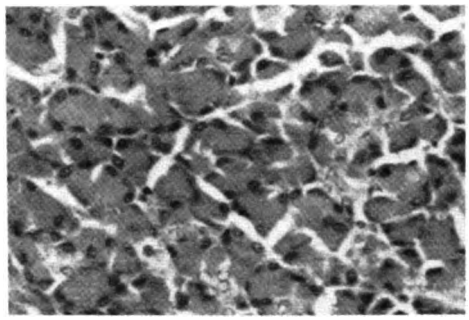

Figure 9—Pancreas of a monkey fed a protein-deficient diet for 2 months followed by an equal period of feeding with 15% casein. H & E x 400.

Spleen and Lymphnodes

There was an apparent reduction in the number of lymphoid follicles in the spleen and in particular an atrophy of the germinal centres (figs. 10 and 11).

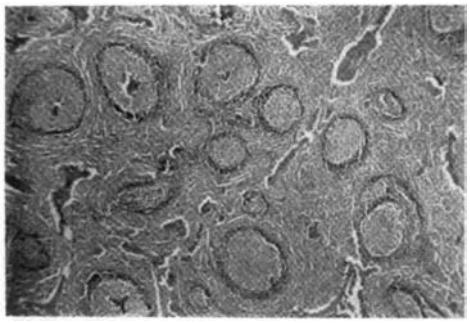

Figure 10—Spleen of a control monkey. H & E x 27.

Figure 11—Spleen of monkey at the end of 2 months of protein deficiency. H & E x 27.

COMMENTS and CONCLUSIONS

This study of uncomplicated protein deficiency in the rhesus monkey has revealed several features in common with kwashiorkor. Failure of growth, hypoalbuminaemia, hypocholesterolaemia and oedema are clinical characteristics of kwashiorkor whenever it occurs (Trowell, Davies and Dean, 1955). The protein-deficient monkeys in this experiment showed the first two of the three features consistently and the last infrequently. Fatty change in the liver, predominantly periportal in distribution, and acinar atrophy of the pancreas and salivary glands are the hall marks of kwashiorkor. The protein-deficient monkeys showed the first

two lesions consistently and the third equivocally. All these alterations in the monkeys were reversible on correcting the protein deficiency. Some essential features of the pathology of kwashiorkor have thus been reproduced in rhesus monkeys under conditions which may be considered to represent uncomplicated protein deficiency.

The absence of desmoplastic reaction in the liver and pancreas in spite of severe parenchymal degeneration and long-continued deficiency is of particular interest. This observation is in line with the contentions of Brock (1954) and Higginson (1957). It indicates that the fibrotic reactions observed in these organs in some cases of kwashiorkor are epiphenomena, unrelated to protein deficiency and attributable perhaps to an associated factor. Sriramachari, a former colleague of ours, has in fact adduced evidence that the severely protein-deficient rat is unable to form as much collagen as one receiving optimal protein when both are exposed to identical hepatotoxic influences (Sriramachari 1959).

It has been suggested that the pancreatic lesion was primary in kwashiorkor and that the fatty liver developed secondarily to this (Davies 1948). We were unable to study the pancreas in the early stages of protein deficiency as we did the liver; comparing the intensity of changes in the liver and pancreas at various intervals, however, we are not convinced that pancreatic lesions are primary.

The reduction in the iron-binding capacity of plasma protein in the protein-deficient animals was indeed quite striking. Anaemia was inconsistent in these animals and, when it occurred, was mild (Sood and Ramalingaswami, 1960 to be published). It appears that the iron transport mechanism is altered, which may be an adaptation to the altered metabolic state in severe protein deficiency. It would be of interest to know the state of the transport mechanisms in the case of other minerals and trace elements in protein deficiency.

Conclusions

1. The changes resulting from uncomplicated protein deficiency in rhesus monkeys were studied. Observations were made on animals before and during the development of deficiency. The recovery following the correction of the deficiency was also studied.

2. A lowering of the concentration of plasma albumin, total serum cholesterol and plasma iron-binding capacity and an elevation of plasma γ-globulin were constant features of the deficiency state. All these changes except the changes in γ-globulin were reversed to normal by correcting the protein deficiency alone without altering the intake of any other nutrient.

3. Severe fatty change developed in the liver, periportal in the early stages of deficiency and diffuse later on. Pancreatic acinar atrophy was marked and consistently observed. These changes were reversible on correction of protein deficiency.

4. Some essential features of the pathology of kwashiorkor have thus been produced in the rhesus monkey exposed to a quantitative deficiency of protein alone.

5. Proliferation of fibrous tissue was not observed in the liver and pancreas of the deficient animal. Such a lesion seen only in some cases of kwashiorkor appears to be incidental and not a consequence of protein deficiency.

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DISCUSSION

DR. SCRIMSHAW: During the last ten years, every meeting of this sort has lamented the lack of ability to produce kwashiorkor in an experimental animal. Now all of a sudden, at this meeting, not only in the presentation you have just heard but in the presentations of Dr. Platt and Dr. Follis, this apparently has been achieved.

DR. BROCK: I would like to echo what you have just said about the immense value of this new development of experimental work in protein deficiency.

May I ask Dr. Ramalingaswami how long he kept his monkeys on the protein-deficient diet? Secondly, when he stopped giving them the protein-deficient diet, did he put them back onto a full or normal diet? Thirdly, in those cases where the full diet was restored, was he able to follow, either by biopsy or autopsy, the late results in the liver where there had been early protein malnutrition and restoration through a normal diet?

I ask this question because of the work which I have heard of several times, of Suckling and Campbell, showing that, in children recovered from kwashiorkor in our nontropical region, there is no evidence whatsoever of persisting liver damage up to five years after recovery from kwashiorkor.

DR. PICKERING: I would like to commend the author of this study for once again bringing out the value of these animal studies. We have conducted a series of studies over several years pertaining to other aspects of the nutrition and biochemistry of animals.

To start with, I think it is of importance to recognize the relative age group with which you are dealing in this experiment. You are dealing with an animal comparable to the young adult. What you have observed here is equally well observed in investigations particularly into carbohydrate intermediary metabolism as well as fat metabolism and protein metabolism during the period equal to the first four or five years of life. However, you throw upon the scene the problem of growth development. I think this is obviously of importance in understanding kwashiorkor.

We have standardized our diet at a level of protein and varied the fat source. I think a few very significant observations which you may be ascribing to your protein are equally important in this regard; and in going into the use of C14 labeled acetate as the road into the synthesis of intermediaries in cholesterol and fat metabolism, one can associate this very distinctly. I cannot help but feel that this is important in all that we have heard at this meeting. I would like at least to say it with regard to the experimental animal.

Getting to the alterations in lipoproteins and as another facet of this, the studies with regard to the fractionation of proteins relate almost specifically to very carefully documented data we have in which we know the life of the animal from within one hour of the time of conception.

DR. RAMALINGASWAMI: I must say at the outset, limitations of time did not permit me to do justice to the enormous amount of work that is going on and has been done exploring this type of approach. So I would apologize if I have not mentioned any of those very significant studies from which we ourselves have received inspiration.

Professor Brock's comment is very important. As I said, we had animals which were sacrificed at the end of two months, animals sacrificed at the end of five and a half months, and there are some that are still going on the low-protein diet. Some have been biopsied at intervals in order to study tissue chemistry and tissue histology and correlate the two. In the animals that have been refed with protein, we have not observed residual liver damage, nor did we observe significant stromal response so far. Obviously, this is important, particularly in view of some recent studies which Dr. Hartroft reported this year. When he put back protein, he observed a very curious disturbance in the liver, "meta-nutritional cirrhosis," I believe he called it.

I am aware of Professor Brock's original reminder, surveying the African scene, telling us we should be rather careful in transplanting animal work to humans.

Dr. Pickering's observations I value most because of the very excellent work he is doing with monkeys. I would agree, that these are probably young adults. I have not been able to breed monkeys in our laboratory. It would be an expensive and very difficult thing for us to do. I have been consulting with veterinarians as to how they could help me get the ages of these monkeys carefully. Apparently bone studies, teeth studies and things of that sort are necessary.

C14 acetate incorporation into cholesterol is obviously an important technique for the study of cholesterol synthesis in protein deficiency.

Your comment on lipoproteins is significant because we believe that this first appearance of stainable lipid in periportal zones might be due to a breakdown of lipoprotein which exposes the lipid there and converts it into a globular fat.

Studies on a Kwashiorkor-like Syndrome in Monkeys

Richard H. Follis, Jr.

IT IS WITH some apprehension that I speak before this audience, for I have no tables and charts dealing with food efficiency and protein efficiency ratios or with nitrogen balances and data on nitrogen retention—absorption ratios, such as have been presented during the past few days. Moreover, the studies which I should like to describe are not even concerned with uncomplicated protein deficiency. Their purpose is somewhat different. Several years ago I came to the conclusion that since certain human deficiency disease syndromes, such as beriberi and pellagra, were not available for study in this country, it might be worth while to attempt to reproduce them in the laboratory in primates. Need I tell this audience that much of our understanding concerning the pathogenesis of these disease syndromes is obscure.

This presentation will deal with the effects of feeding diets composed predominantly of maize to growing monkeys (Cercopithecus Aethiops). As you well know, ground whole maize is deficient in certain inorganic elements such as calcium, sodium and iodine. The quality of its protein is poor, since the amino acids tryptophan and lysine are much too low, and the leucine content may be too high. In addition, maize is low in certain vitamins and at least one, nicotinic acid, may be largely unavailable. Hence it was to be expected that this cereal diet would lead to disease in the experimental animal. Such, of course, has been the experience during the past half century since the initial studies of McCollum and of Hopkins. When these experiments with maize were begun, I was hoping to see the development of a disease which might resemble pellagra, about which there remain many unsolved problems. (Incidentally, is it worthy of note that on the fourth day of this conference this is only the second time the word "pellagra" has been uttered by a speaker from this rostrum?) Instead of pellagra there has developed a syndrome more resembling kwashiorkor, which I should now like to describe.

When growing monkeys are placed on a diet of ground unbolted maize, cooked or raw, which is allowed ad libitum, they immediately cease to gain in weight and soon begin to lose varying amounts. The first sign of disease is revealed by needle biopsy of the liver. After approximately 4 weeks on the maize regimen, fat appears in the periportal areas and as time goes on this accumulation of lipid

spreads to involve the entire liver lobule. If the animal loses weight excessively, the fat in the liver may actually decrease in amount. In association with this hepatic change a reduction of serum-protein concentration occurs, at the expense of the albumin fraction. Puffiness of the eyes may then be noticed. Animals on corn diets for prolonged periods become apathetic and lose interest in their surroundings. Loss of strength is apparent when they are handled preparatory to vena puncture. No change in the color of the coat and no abnormalities of the skin or mucous membranes are found. At autopsy the most conspicuous change is extensive fatty infiltration of the liver, so that the organ is yellow in color. On chemical analysis the lipid content may be over 60% of the weight of the dry tissue. No necrosis or increase in connective tissue fibers have been observed. No alterations have been found in the pancreas. The skeleton exhibits retardation of maturation with respect to remodeling sequences. This syndrome of growth retardation, loss of weight, weakness, apathy, edema with hypoalbuminemia and periportal fat accumulation in the liver appears to have a number of the characteristics of kwashiorkor in children.

Certain modifications have been made in the maize diet so as to make the deficiency state more severe or to improve the ration. When the maize is diluted with sugar, weight loss is greater and true marasmus appears. No fat accumulates in the liver; in fact, on chemical analysis the lipid content of this organ may be even less than normal. Moreover, little or no reduction in plasma protein concentration occurs. Such a state as this more closely resembles the picture of true marasmus or semistarvation as it is seen in children and thus has graphically exemplified in the experimental subject the transition from kwashiorkor-like syndrome through the spectrum of marasmic-kwashiorkor to complete marasmus, as has been so well depicted in children by the now famous INCAP pyramid.

Since maize is deficient in quality protein, salts and vitamins, it appeared desirable to explore the effects of adding these materials singly or together to the maize diet. These studies are not yet complete. Certain observations may, however, be of interest. When 6% casein and 5% standard salt mixture are added to the corn regimen, growth of the animals is somewhat improved. No change in plasma protein concentration is found. Fat is found in the liver, but with a different distribution than that found with maize alone. Instead of being aggregated in the periportal areas, the lipid tends to be found about the central portions of the liver lobules. This is of course reminiscent of the central deposition which one encounters in the rat as a result of choline deficiency. However, lack of other vitamins may well be playing a role, since, as is well known, lipid may accumulate in hepatic cells as a result of deficiency of riboflavin, pyridoxine, pantothenic acid, vitamin B_{12} or folic acid.

The mazie diet of 2 animals was supplemented with a standard salt mixture and all of the vitamins in appropriate amounts. The primary deficiency in these animals was therefore in the quality and quantity of ingested protein. These animals did not gain in weight but each lost 23.5% and 20.0% of their original weights in 51 and 70 weeks respectively. At autopsy no excess fat was found in the liver; no other pertinent microscopic changes were found in other tissues. In this

instance, therefore, vitamin supplementation appeared to have prevented the accumulation of fat which might be expected to have occurred on the maize diet alone.

These experiments with a naturally occurring foodstuff, maize, illustrate that a specific syndrome may be produced in monkeys. The results obtained in tests on supplementation with carbohydrates, protein, vitamins and salts, which are still in progress, serve to illustrate the complexity of problems faced when one essays to go from the field of single deficiency states to that of multiple deficiencies.

Studies such as these on animals fed an unsupplemented or supplemented natural foodstuff emphasize another facet of the complexity of multiple deficiency disease states: the appearance of other syndromes which may even modify the response of the host to the primary nutritional deficiency. You will recall that some years ago the classical Steenbock-Black rachitogenic diet was found to be goitrogenic. It was this observation that led Remington and his coworkers to utilize ground maize as the principal ingredient of the iodine-deficient ration which has achieved such widespread use. It was therefore not too unexpected to find evidences of thyroid hyperplasia of an extreme degree in the monkeys which had been fed nothing but corn meal. When the glands of these animals are compared with those from normals under the microscope they show loss of colloid, extreme epithelial hyperplasia and increase in vascularity, just as do the tissues from naturally occurring iodine-deficient goiters in humans and animals, and these are similar to those which may be produced in the laboratory by iodine deficiency or various goitrogens. Only recently I have begun to study the goiter problem specifically in monkeys; however, when cod liver oil was added to the diet of an animal which had been subsisting on the maize regimen for some weeks, colloid goiter appears to have been inadvertently produced. Cod liver oil is, of course, a potent source of iodine, which could effect an involutionary change in a gland already hyperplastic as a result of the iodine-deficient maize ration.

One other important point which was briefly alluded to above might be further discussed at this time, for it involves a principle which many nutritionists lose sight of in studies of naturally occurring or experimental deficiency disease states. This principle is that, in order to have biochemical or anatomical lesions develop in cells, such cells must attain a certain level of metabolic activity so that they can react. If cells are totally starved, they become atrophic and metabolically reach sort of a state of suspended animation, a kind of hibernation if you will. Life continues, and may do so for prolonged periods: witness the life span of rats which Dr. Holt placed on a diet of nothing but thiamine and dextrose. Now if the metabolic activity of certain cells or tissues is elevated by the inclusion of certain essential nutrients in the diet, imbalance is created and the basal state is changed into a detrimental one for the entire organism; then biochemical lesions, anatomical changes or death may ensue.

Certain of the classical deficiency disease syndromes provide excellent examples of this principle. The relation of growth of the skeleton to the development of rickets or scurvy probably provides the most dramatic example. Rickets occurs most flagrantly at an age when growth of the skeleton is most rapid and in those

bones which are increasing in length the fastest. The dictum: no growth, no rickets, is familiar to all nutritionists. Moreover, as you well know, the fact that rickets may heal if growth ceases exemplifies this principle. Again, the classical anatomical lesion of scurvy occurs in those bones which are growing most exuberantly. When skeletal growth slows up after the first year, scurvy no longer manifests itself so prominently at the cartilage-shaft junctions of the ribs or long bones. The effects of the state of nutrition on the development of skin lesions, on the presence of alterations in the gastrointestinal tract and on many other areas provide further examples of this principle. The experiments of Dr. Ramalingaswami, who, as you recall, force-fed his monkeys rather than allowing them to eat ad libitum as mine, also exemplify this principle. The increased intake of an unbalanced dietary causes more change than would have probably ensued had the animals been allowed to effect a certain degree of homeostasis, on their own, albeit at a lower level.

I hope that these studies with naturally occurring foodstuffs, though complex, may help in unraveling some of the complexities of nutritional disease in the human. Moreover, it may be possible at a later date to evaluate the influence of certain other factors, such as diarrhea, infection and anemia, which concern everyone who is studying the pathogenesis of human deficiency disease syndromes.

DISCUSSION

DR. ARROYAVE: We have seen how by dietary means one can produce in animals some specific clinical or histopathological pictures of severe protein deficiency, but we also must recognize some inconsistency in the results obtained. At the risk of confusing the picture a little more, I would like to introduce a new variable.

Dietary factors have been considered somewhat independently of the organism itself. One must remember that the malnutrition state which results from consuming a poor diet is an interaction between the diet and the organism. As a general concept, agent and host both participate in the production of disease. We have made some pertinent observations in children, both with kwashiorkor and with marasmus, of clinical conditions which differ in their histopathological picture in several respects.

The marasmic child has lost a large amount of muscle but has a relatively normal-looking liver with a relatively high protein content. His serum proteins, particularly the albumin fraction, may be decreased, but not nearly as much as in the child with kwashiorkor. In kwashiorkor one sees a very altered histopathology of the liver, and a markedly decreased protein content of the liver and serum. Furthermore, in Jamaica at least, kwashiorkor is found in children with relatively good adipose tissue reserves and sometimes with relatively good quantities of muscle which could serve as protein

reserves, but they appear to have been unable to use them for some reason. What could this reason be?

We have noticed, in studying the excretion of steroids in the urine, that there is a marked difference between marasmic children and those with kwashiorkor. The marasmic children have a high 17-hydroxycorticosteroid excretion and the kwashiorkor children a very low one. Another difference is that the marasmic children have a very low eosinophil count while the kwashiorkor children have a relatively high count. These findings indicate higher adrenoglucocorticoid activity in marasmus.

This difference in glucocorticoid activity in these two conditions suggested some experiments in rats which we have done by taking rats immediately after weaning and putting them on a low-protein diet (5%) with all of the protein coming from corn. We produced, not kwashiorkor, but a severe protein deficiency as indicated by lower plasma protein and albumin levels, increased liver fat, decreased liver protein content of about 13 to 14 gm %, completely altered electrophoretic pattern, and so forth. The animals increased in weight only slightly. At the end of 21 days we divided them into 2 groups, one of which was given cortisone and the other a placebo.

The animals given cortisone showed some marked changes which are very suggestive. In the first place, at the end of one week the serum proteins had increased about 1½ gm per 100 ml, and this increase could be accounted for exclusively by the albumin fraction. There was some slight decrease in γ-globulin, which is probably a consequence of the effect of cortisone on the reticuloendothelial system.

At the end of even one week, the proteins of the liver were up to about normal levels in the cortisone group and no abnormal fat was found either histopathologically or chemically. However, the muscles of these animals given cortisone had wasted at a rapid rate. Both histopathologically and by the weight of the isolated soleus muscle from the back of the rat, we could demonstrate a rapid wasting of muscle. Something had happened in these animals; evidently the protein from the muscle had gone somewhere else, and the changes observed could be accounted for by an efficient utilization in the cortisone-treated rats of amino acids from the muscle protein for the synthesis of albumin and liver proteins.

The concept derived from these observations is that, for some reason, probably adrenocortical failure, the kwashiorkor patient is unable to utilize his own body proteins from muscle for the maintenance of other more important proteins, exemplified by the albumin and enzymes in the body. Once the organism is able to put to work this endogenous mechanism for the utilization of protein, he is protected.

This is done very beautifully by the marasmic child, who is slowly becoming more undernourished but who is defending himself in a relatively efficient manner. However, if for some reason the child is unable to put this mechanism to work, an acute clinical emergency situation results which puts the child in the hospital or to death, that is, kwashiorkor in children.

Kwashiorkor is a very unfortunate episode because it indicates a complete failure of the organism to defend itself against the nutritional insult. In a normal animal, cortisone would remove protein from the muscle and this would result particularly in gluconeogenesis with waste of nitrogen; there is some synthesis of protein at the level of the viscera, but this is a secondary process to gluconeogenesis. We feel that in malnourished, severely protein-depleted animals, on the other hand, the same process of protein resynthesis from the amino acids of muscle takes priority over the waste of nitrogen in the form of urea. In our experimental rats we found that the blood urea nitrogen, after cortisone, decreased instead of increasing as we would expect, suggesting that the nitrogen was being reutilized efficiently.

Experimental Protein Malnutrition

B. S. Platt

TYPICAL CONDITIONS CORRESPONDING to marasmus and kwashiorkor have been produced in pigs by feeding a low protein (LP) diet and the same diet to which carbohydrate (LP+CH) has been added (figure 1). The low protein diet is representative of the type of diet which is eaten in Gambia. The changes in weight, radius length and skin composition of pigs on various diets are shown in figure 2. The CLP diet is one with 5% of the casein replaced with starch. There is no evidence to support the common statement that the marasmus subject is deficient in calories and the kwashiorkor subject is deficient in protein. When carbohydrate is added to the LP diet calories are still deficient. The skin of the LP+CH animal is much fatter and edematous than that of the LP animal (figure 3).

In regard to urinary N excretion, the LP animals excreted 55% of the intake as compared with 41% for the LP+CH animals. Urea N as percent of total urinary N was 81% in the LP and 63% in the LP+CH animals.

The hemoglobin and plasma protein response to LP and LP+CH diets are shown in figure 4. The hemoglobin and plasma protein decrease more rapidly with LP+CH than on LP alone. Added carbohydrate clearly aggravates the pathology of protein malnutrition.

One example of interrelationships of nutrients in deficiency states is that of the increase of serum vitamin A and serum albumin (figure 5). There appears to be no deficiency of vitamin A in the livers of the animals with low serum albumin but it appears to be primarily a problem of transport or mobilization. Since vitamin A is stored in the liver its reduction in serum is not due to poor absorption.

Some of the observations made in the course of the experiments are presented in figures 6-20 (Platt 1958a).

Skin changes in figure 6 show edema and break-up of the collagen.

The bone changes in figures 7, 8 and 9 illustrate an "arrest line" phenomenon, usually attributed to some intercurrent infection or endocrine disturbance, which can be produced regularly by protein deficiency.

Liver fat (figure 10) is not prominent on the LP diet but increases notably with LP+CH.

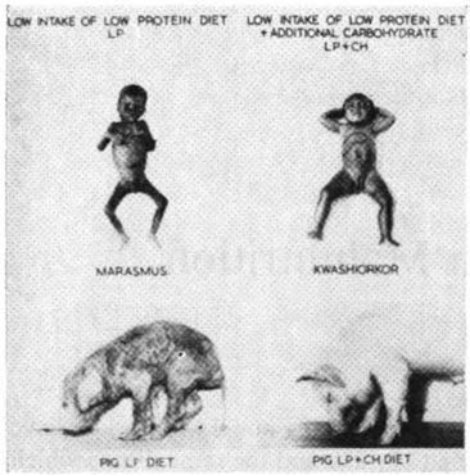

Figure 1—Appearance of Young Children and Pigs with Protein Malnutrition.

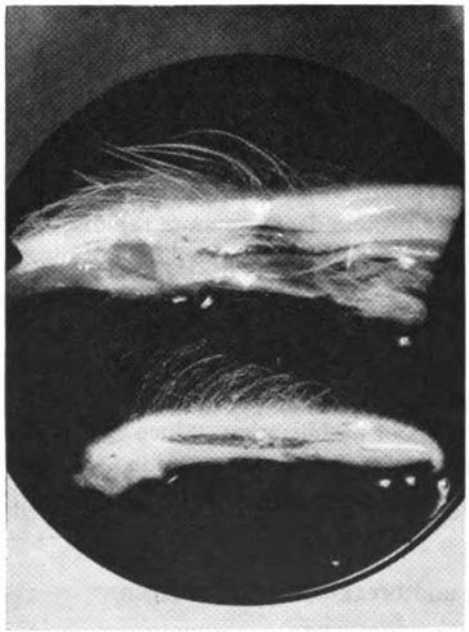

Figure 3—Skin taken from corresponding sites in two pigs. Both animals received the same daily diet; animal from which skin in upper section was taken received, in addition, 100 gm of carbohydrate daily.

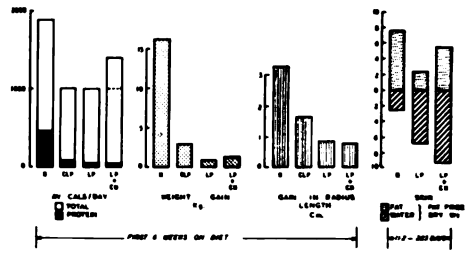

Figure 2—Histogram showing weight, bone length and skin composition in pigs on various diets (N:CLP:LP:LP+CH) in experimental malnutrition.

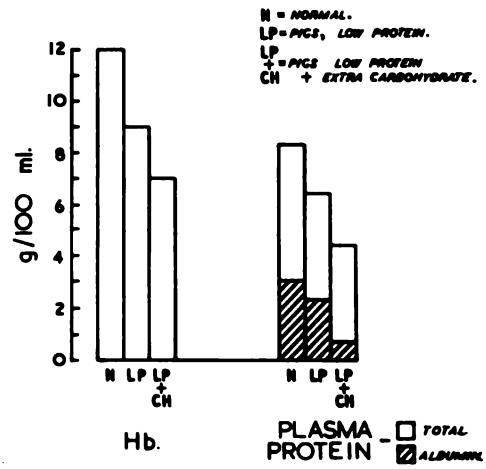

Figure 4—Differences in hemoglobin and plasma proteins in experimental protein malnutrition.

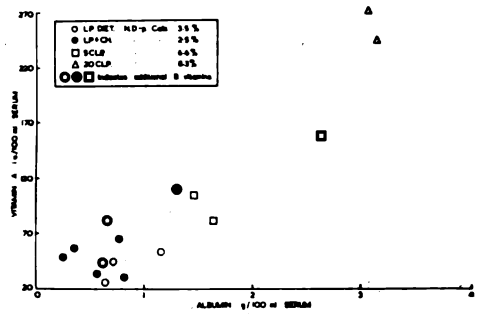

Figure 5—Correlation between serum "albumin" and serum vitamin A.

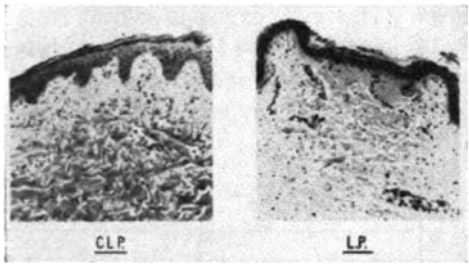

Figure 6—Photomicrographs of skin of pigs, showing appearance during protein malnutrition.

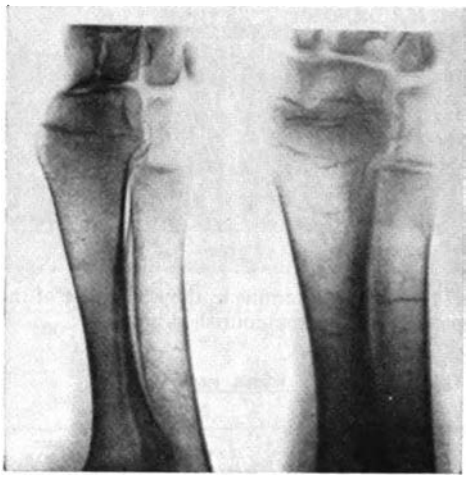

Figure 7—"Arrest line" in the radius of a pig following renewal of growth when given a stock diet after a period of protein malnutrition.

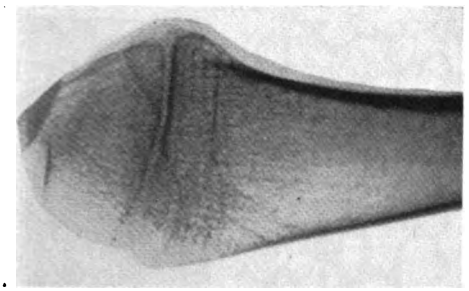

Figure 8—Radiograph of distal metaphysis of the radius of a malnourished pig, showing transverse trabeculae which resemble "arrest lines."

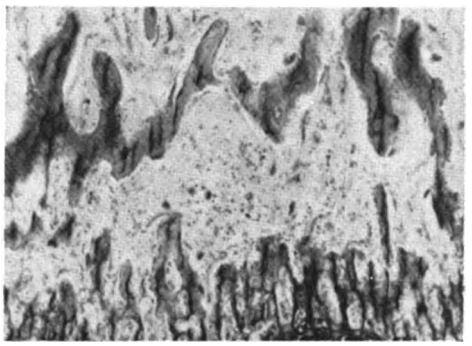

Figure 9—Photomicrograph of distal end of the radius of a malnourished pig, showing transverse trabeculae.

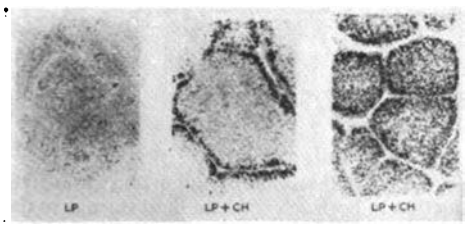

Figure 10—Fat in liver (pig).

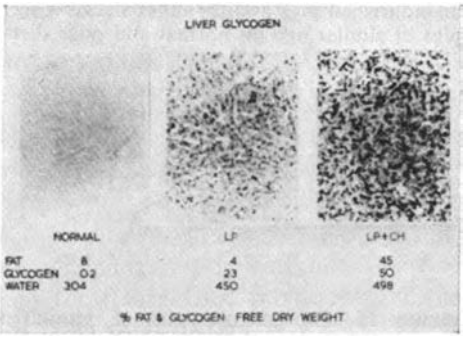

Figure 11—Glycogen in liver (pig).

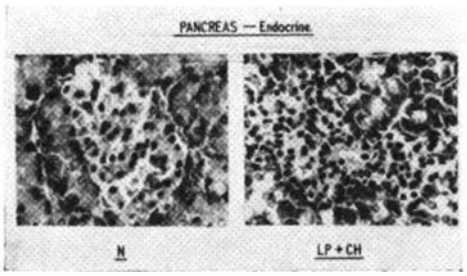

Figure 12—Alterations in the islet tissue of the pancreas of the malnourished pig.

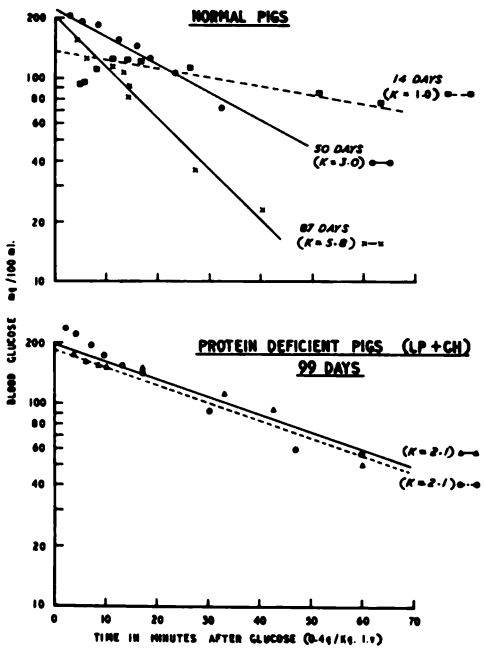

Figure 14—Glucose tolerance curves illustrating (a) similarity between young normal and older malnourished pigs and (b) differences between pigs of similar age on normal and poor diets.

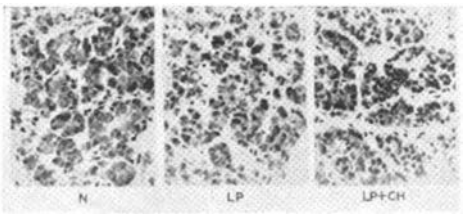

Figure 16—Photomicrographs of pituitaries showing alterations in malnourished pigs as compared with the normal.

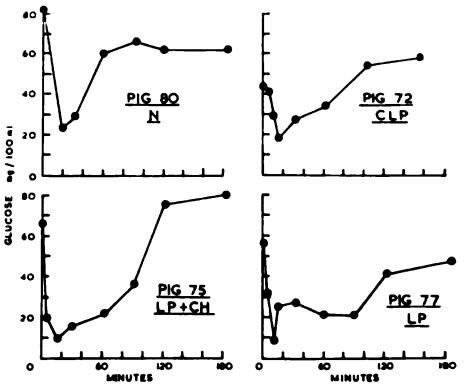

Figure 13—Insulin tolerance (0.1 i.u./Kg.) of pigs fed on low protein and normal diets.

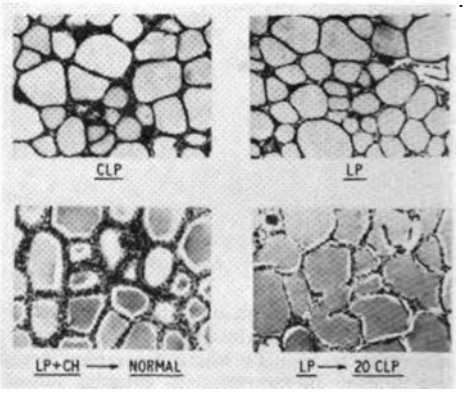

Figure 15—Photomicrographs of pigs' thyroids showing appearance during protein malnutrition and recovery on improved protein intakes.

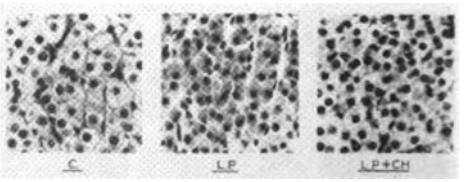

Figure 17—Photomicrographs illustrating reduction in both cell size and lipid storage in the zona fasiculata of the adrenals of malnourished pigs.

Liver glycogen (figure 11), normally 0.2%, goes up to 23% with the LP diet and to 50% with LP+CH. This observation is not well known but calls for further attention to changes in carbohydrate metabolism. Alterations in the endocrine portion of the pancreas are summarized in table 1. (See also figure 12.)

TABLE 1
SUMMARY OF ALTERATIONS IN THE ENDOCRINE PORTION OF THE PANCREAS OF THE PIG DURING PROTEIN MALNUTRITION

	DIET			
	N	CLP	LP	LP + CH
Islet cell size as % normal cell size	100	88	65	59
α cells (phloxin stained) as % total cells	25	26	38	32
β cells (aldehyde fuchsin) as % total cells	71	68	58	59
β cells/α cells	2.8	2.6	1.5	1.8

The insulin tolerance responses are shown in figure 13. The glucose tolerance changes with age. Figure 14 illustrates that the maturation of ability to handle sugar is delayed in the LP+CH animals. The liver glycogen lingers in the liver and the glucose-6-phosphatase activity becomes undetectable. (Table 2.)

TABLE 2
DATA RELATING TO AVAILABILITY OF LIVER GLYCOGEN OF PIGS

	DIET				
	ad. lib.		iso-caloric		
	N	CLP	CLP	LP	LP + CH
Glycogen (gm/100gm liver)	0.8	2.6	6.3	8.8	10.0
Glucose-6-phosphatase (units/gm liver)	10	15	10	0	0
Recovery time after insulin (minutes)	30	45	120	>120	>120

The thyroid is grossly affected (figure 15) probably directly rather than through the pituitary although notable changes from normal are evident in the pituitary (figure 16). The adrenals are not so much affected (figure 17) although there is a reduction of the lipid in the cytoplasm of the cortex.

The central nervous system is affected as illustrated in figures 18, 19 and 20. Deaths in some acute cases of protein malnutrition resemble those seen in beriberi and may result from central nervous system involvement (Platt 1958b).

PRACTICAL EVALUATION OF DIETARY PROTEIN VALUES

The practical use of protein foods for maintenance of health or prevention of disease requires consideration of protein quality and quantity and of total calories. A method has been devised to unite these factors in one value which has been designated Net Dietary-protein Value. It can be expressed as NDpVgm per 100 gm of food or NDpCals% i.e. protein calories per cent of total calories (Platt, Miller & Payne, 1961). The quality of the protein can be measured by determining the Net Protein Utilisation (operative) = NPU(op) for freeze-dried mixtures or dishes, meals or diets. The quantity is determined from total dietary N x 6.25. Protein value then = quality x quantity = NPU (op) x (N x 6.25).

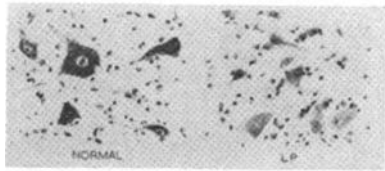

Figure 18—Nerve cells of the reticular formation of the medulla in a normal and a malnourished pig. *Note:* Diffuse granules and satellitosis in LP.

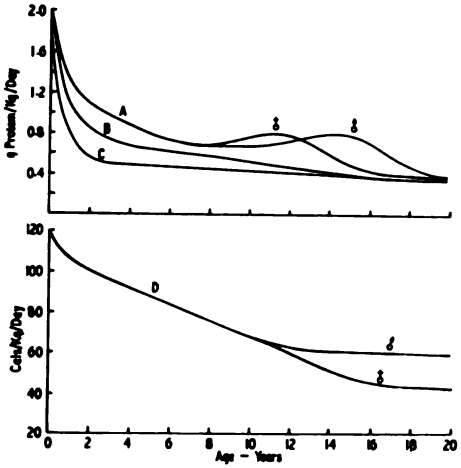

Figure 21—Protein and Calorie Requirements for Human Subjects at Various Ages.
Curve A: From "Average Minimum Requirements for Protein" (FAO, 1957b).
Curve B: From "Minimum Protein Requirements" (Hegsted, 1959).
Curve C: From N.R.C. (1959).
Curve D: From FAO (1957a).

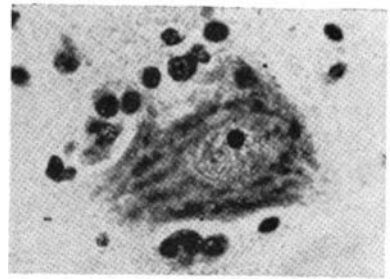

Figure 19—Anterior horn cell (cervical cord) of a pig recovering from protein malnutrition. *Note:* Cell recovery, Nisal granules good, some concentration of oligodendroglia.

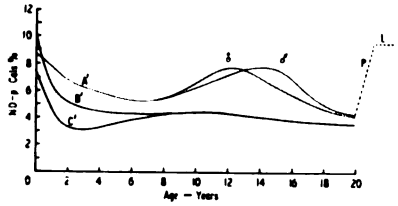

Figure 22—Protein Allowances for Human Subjects at Various Ages expressed as NDpCals%, using data set out in Figure 21.
Curve A' from A and D.
Curve B' from B and D.
Curve C' from C and D.
p = pregnancy.
L = lactation.

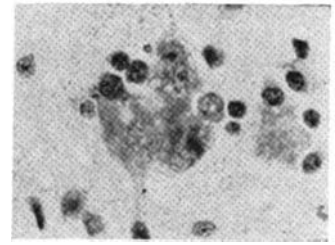

Figure 20—Anterior horn cell from same section and region as Figure 19. *Note:* Cell irreversibly damaged and being removed by glial cells.

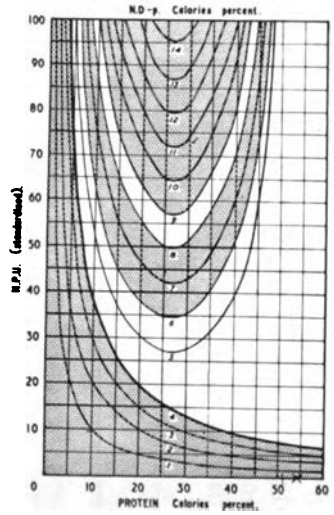

Figure 23—Nomograph for the prediction of Protein Values.

Protein and calorie requirements for human subjects at various ages from various sources are presented in figure 21 in terms of daily needs per kilogram of body weight. The corresponding requirements for protein expressed as NDpCals% are presented in figure 22. For the infant the requirement is around 8 which approximates that of human milk. If the NPU (standardised) is less than 50 a protein value of NDpCals% of 8 is not attainable. A nomograph for prediction of protein values is presented in figure 23. The solid black line indicates maintenance requirements and the curves show lines of equal protein value. If the energy value of the diet is deficient in relation to the consumer's needs the NPU value of the diet *to the consumer* decreases.

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Basic Principles of Protein and Amino Acid Evaluation and Potential Protein Resources

Utilization of Vegetable and Animal Protein in Human Subjects

Some Obscure Aspects

V. N. Patwardhan

Nitrogen Balances

PATWARDHAN, MUKUNDAN, RAMA SASTRI AND TULPULE[1] reported in 1949 that, at comparable nitrogen intake of about 10 to 13 gm per day, young healthy adult subjects excreted more nitrogen in urine on diets in which animal protein was 50% or more of the total dietary protein than on predominantly vegetarian diets. These differences were of the order of 2 to 4 gm per day. They could not be explained on the basis of differences in the digestibility of protein, for the maximum observed difference in faecal nitrogen was 0.80 gm for one subject and lower still for three others (table 1). Vegetable proteins in the basal experimental diet were mainly derived from cereals and pulses, with minor contributions from vegetables and milk and milk products. Meat, eggs or large quantities of milk provided the protein in the high animal protein diet. In the basal diet animal protein was at the level of 4.7% to 16% of the total dietary protein, whereas on the high animal protein diets its contribution was 55% to 60%. The animal protein effect resulting in an increase in urine nitrogen was seen irrespective of the source of the animal protein. It was also observed that return to a diet rich in vegetable protein resulted in the lowering of urinary nitrogen, although the total nitrogen intake had not changed. These observations led to the tentative conclusion that the human body seemed to retain more nitrogen on predominantly vegetable protein diets than it did on the high animal protein diets. Karambelkar, Patwardhan and Sreenivasan[2] made similar observations in 7 other subjects and also examined the relationship between body weight changes and nitrogen retention. They observed that on the high animal protein diet 6 of the 7 subjects showed increases in body weight expected from the small amount of nitrogen retained, according to the Rubner concept, whereas on the predominantly vegetarian diet most of the subjects showed little change or even some loss of weight in spite of the fact that positive nitrogen balance in this period was higher.

TABLE 1
INTAKE AND EXCRETION OF NITROGEN: MAXIMUM AND MINIMUM VALUES ON ALL DIETS

Subject	Intake N, gm			Faecal N, gm			Urine N, gm		
	Maximum	Minimum	Difference	Maximum	Minimum	Difference	Maximum	Minimum	Difference
K.M.	12.67	12.23	0.44	2.92	2.12	0.80	8.34	4.40	3.94
S.	13.08	12.20	0.88	3.09	2.35	0.74	8.43	4.45	3.98
P.G.T.	9.83	9.62	0.21	1.56	0.90	0.60	8.46	4.97	3.49
B.V.R.S.	10.09	9.87	0.22	1.96	1.40	0.56	6.87	4.86	2.01

N.B. The maximum and minimum values quoted in table 1 were those observed when "steady state" was reached on each diet. Earlier fluctuations have not been taken into account.

The above findings suggested that the source of dietary protein influenced the nitrogen metabolism. Obviously the question needed closer examination. Karambelkar, Patwardhan and Sreenivasan[3] have observed an 8% to 21% increase in total creatinine in urine on diets rich in animal proteins. The animal protein component in these diets was made up chiefly from milk with a minor contribution from eggs. The low animal protein diet had a creatine content of 10 to 15 mg and the high animal protein diet had a higher creatine content, viz. 20 to 40 mg. It is doubtful whether this small difference in dietary intake of creatine could have had any influence on creatinine excretion, in view of the observations of Folin (quoted by Karambelkar et al.) and of Beard and Jacob[4] that ingestion of large quantities of creatine had little influence on creatinine excretion. A detailed analysis of urine on the vegetable- and animal-protein-rich diets was therefore considered desirable, in order to elucidate the effect of animal proteins on urinary nitrogen excretion.

Partition of Urinary Nitrogen

Phansalkar and Patwardhan[5] again confirmed in 3 subjects maintained on constant nitrogen intake the observation that an increase in the animal protein component to 50% of total dietary protein caused an increase in total urinary nitrogen. Raising the proportion of animal protein still higher had little further effect on the total urinary nitrogen excretion. The results are summarised in table 2.

TABLE 2
PARTITION OF URINE NITROGEN ON LOW AND HIGH ANIMAL PROTEIN DIETS

	Phansalkar and Patwardhan 1954							
	Subject S				Subject K			
	Diet I		Diet III		Diet I		Diet III	
	gm	%	gm	%	gm	%	gm	%
Dietary N	13.3		13.5		13.3		13.5	
Animal protein N	0.6		10.4		0.6		10.4	
Total urine N	5.464		8.794		5.347		7.971	
Urea N	3.368	61.6	6.623	75.3	3.544	66.3	6.123	76.8
Ammonia N	0.477	8.7	0.443	5.0	0.340	6.4	0.486	6.1
Uric acid N	0.159	2.9	0.216	2.5	0.150	2.8	0.201	2.5
Creatinine N	0.477	8.7	0.519	5.9	0.417	7.8	0.488	6.1
Total amino acid N	0.171	3.1	0.250	2.8	0.184	3.4	0.250	3.1
Undetermined N	0.813	14.9	0.742	8.4	0.712	13.3	0.423	5.3

It will be seen that practically the entire rise in total urine nitrogen could be accounted for by the increase in urea nitrogen. There were minor alterations in ammonia nitrogen, uric acid nitrogen, creatinine nitrogen and amino acid nitrogen, all of them showing a slight tendency to increase. Since the major product of protein metabolism in man is urea, a rise in the latter was to be expected with the rise in total urinary nitrogen. However, it was felt that a closer examination of the relationship between urea nitrogen and total urinary nitrogen was necessary in order to determine how far the distribution of nitrogen between urea and other nitrogenous constituents in urine was likely to be influenced by the fact that the dietary protein was of vegetable or animal origin.

Folin[6] had clearly pointed out that "the distribution of nitrogen in urine among urea and other nitrogenous constituents depended upon the absolute amount of total nitrogen present." We found a high correlation between the total urinary nitrogen and urea nitrogen in the values reported by Folin. The regression equation was $Y = -1.1832 + 0.9444\ \chi$ where Y is urea nitrogen and χ the total urinary nitrogen. The results obtained by Phansalkar and Patwardhan[5] in Indians on vegetable and animal protein diets when similarly treated gave the relationship by the expression $Y = -1.5980 + 0.971\ \chi$. The correlation coefficients for the two equations were 0.998 and 0.978 respectively. The two regression equations did not differ significantly. Thus one could conclude, as Folin had done 55 years before, that it is the level of total urinary nitrogen and not the source of dietary protein which determines the urea nitrogen excreted. Expressed as percentage of the total urinary nitrogen, the urea nitrogen reaches a high percentage (87%) of total urine nitrogen when the latter is high; the proportion of urea nitrogen falls linearly as the total urine nitrogen decreases. This is best illustrated by the values for total urine nitrogen and urea nitrogen reported for healthy Indian adults (figs. 1 and 2).

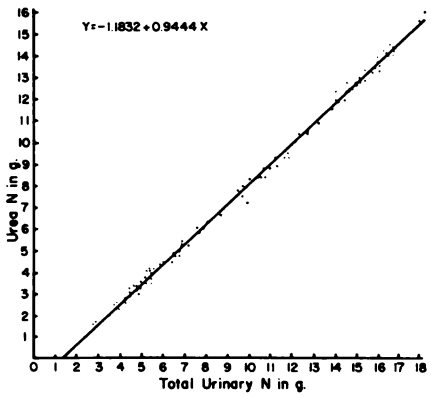

Figure 1—Correlation of urea nitrogen and total urinary nitrogen in healthy Indian adults.

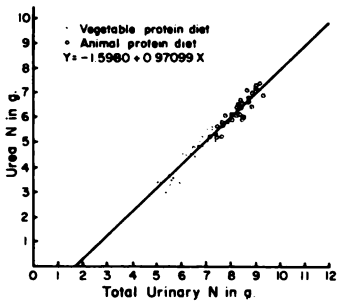

Figure 2—Correlation of urea nitrogen and total urinary nitrogen from vegetable and animal protein diets.

Gokhale[7] had found an average urinary nitrogen excretion amounting to 6.09 gm per day in 47 healthy Indian men in Bombay. Urea nitrogen was 4.65 gm, being 76.4% of the total nitrogen. This figure and that found for 15 Indian subjects

by Phansalkar and Patwardhan indicate that urea nitrogen found in Indians forms a lower proportion of the total urinary nitrogen than the values reported by Folin,[6] Smith[8] and Rheinberger[9] principally because the total urinary nitrogen in Indian subjects is low. The total urine nitrogen may be low due to the following reasons: a) protein intake itself may be low; b) although protein intake is sufficient, its digestibility may be low (Subrahmanyan et al.[10]) leading to a lower absorption of ingested protein; or c) the dietary protein may be almost entirely made up of vegetable protein. The effect of the last-mentioned factor, however, is variable depending upon the composition of the diet and is most often seen to advantage when the intake is about 10 gm nitrogen per day.

A feature of the results of Phansalkar and Patwardhan[5] to which attention must be drawn is the unusually high value and proportion of what we call "undetermined" nitrogen. It was 15% and 13% of the total urinary nitrogen in subjects S and K respectively when they were on predominantly vegetarian diets. An average of 17% undetermined nitrogen was found in 93 observations made on 15 subjects on uncontrolled diets which were predominantly vegetarian in composition. The values for undetermined nitrogen found in the observations of Folin[6] and Smith[8] are 3.75% and 6.8% respectively. They would be still lower if amino acid nitrogen had been determined by these investigators.

It is sometimes argued that Indian subjects (including those studied by us) could not have been normal since they were excreting in urine a large proportion of nitrogen not accounted for by the common nitrogenous constituents. It is interesting to see, therefore, the change in the undetermined nitrogen when subjects S and K were placed on a diet containing 50% or more of total protein in the form of animal protein without changing the total nitrogen intake. The absolute amount of undetermined nitrogen was considerably reduced in one subject (K) and when expressed as per cent of total urinary nitrogen the undetermined nitrogen was found reduced in both (8% and 5%). The values, although not yet comparable with those reported by Folin and Smith, indicate the possibility that at the still higher levels of total urinary nitrogen, the undetermined nitrogen would form a negligible proportion. In view of this, one has to conclude that a larger value for undetermined nitrogen in Indian subjects was not an indication of an abnormality in protein metabolism. We had another proof to indicate that we were not dealing with subjects with abnormal protein metabolism. Phansalkar and Patwardhan[11] fed to 4 healthy adults diets in which dietary protein provided 5% of the total calories. This is the marginal level of protein intake used by Murlin and associates[12,13,14] to determine the milk and egg replacement value and BV of various dietary proteins. The results obtained by Phansalkar and Patwardhan[11] with egg and refined wheat flour were similar to those reported by Murlin et al.[14]

The constituents of undetermined nitrogen are likely to be several compounds which are excreted daily in small quantities. Among the known compounds could be included purine and pyrimidine bases, imidazole, indole, amines and their derivatives and vitamins of the B complex and their metabolites. Of these, the last mentioned class of compounds is not likely to make a major contribution to undetermined nitrogen and hence slight or even marked variations in their excretion on

animal and vegetable protein diets will not account for more than a negligible fraction of the total undetermined nitrogen. Since one has to account for 500 to 800 mg of undetermined nitrogen per day in the urine of Indian adults, one will have to identify the major components to find the answer.

Some published work had indicated that vitamin B_{12} possibly influenced the utilization of vegetable protein. Hartman, Dryden and Cary[15] suggested that vitamin B_{12} improved the utilization of dietary protein in rats. Marfatia and Sreenivasan[16] observed a significant increase in the BV of a mixture of wheat and peanut protein in rats when fed with vitamin B_{12}. On the other hand Chow and Barrows[17] could find no effect on nitrogen retention in rats fed a soybean diet and Chow[18] had similar experience in infants fed diets in which soybean was the sole source of protein. Tulpule, Rama Sastri and Patwardhan[19] also could not demonstrate any effect on nitrogen excretion in urine in 2 Indian adults on vegetable protein diets. They had used a comparatively small dose of 30 μgm vitamin B_{12} in divided doses. Phansalkar[20] later used in 2 subjects 300 μgm vitamin B_{12} in 3 daily doses of 100 μgm each but could not detect any appreciable effect on total urine nitrogen or in the common nitrogenous constituents determined by him during the basal, the injection and the postinjection periods. It would appear, therefore, that vitamin B_{12} had little to do with the shift in urine nitrogen described by Patwardhan and coworkers.

Utilization of Animal and Vegetable Proteins in the Absorptive and Early Postabsorptive Stages

It was therefore decided to study the utilization of animal and vegetable proteins in the absorptive and early postabsorptive phases after a test meal.[21] The subjects were adult males given a light meal early in the evening, the test meal being given the next morning at 8.00 A.M. The total nitrogen content of the test meal was 4.5 to 4.8 gm and the total calories were between 1440 and 1540. Eggs and milk supplied the animal protein, whereas vegetable proteins were derived from cereals and pulses. A sample of capillary blood was drawn and the bladder was emptied before the test meal. Urine and capillary blood were collected half an hour after the test meal and every hour thereafter for 8 hours. The total fluid intake during this period was adjusted so as to be approximately equal on the 2 test meals. The average results obtained in 4 adult male subjects are illustrated in figure 3.

Larger amounts of nitrogen were excreted in urine within 8 hours after the animal protein test meal than after the vegetable protein meal; the difference varied with each subject, the variation being 11% to 35%. Most of the excess urinary nitrogen was excreted in the first 3 to 4 hours, which suggested that possibly the rate of digestion and absorption of animal protein was faster than that of vegetable protein.

It is interesting to find that differences in the rate of protein utilization were reflected in the reduction of blood sugar levels of the subjects; a more rapid rate of decrease in blood sugar was found in the subjects after the animal protein than after the vegetable protein test meal. Munro and Thompson[23] have reported a decrease in free amino acids in the blood of fasting human subjects after the feeding of

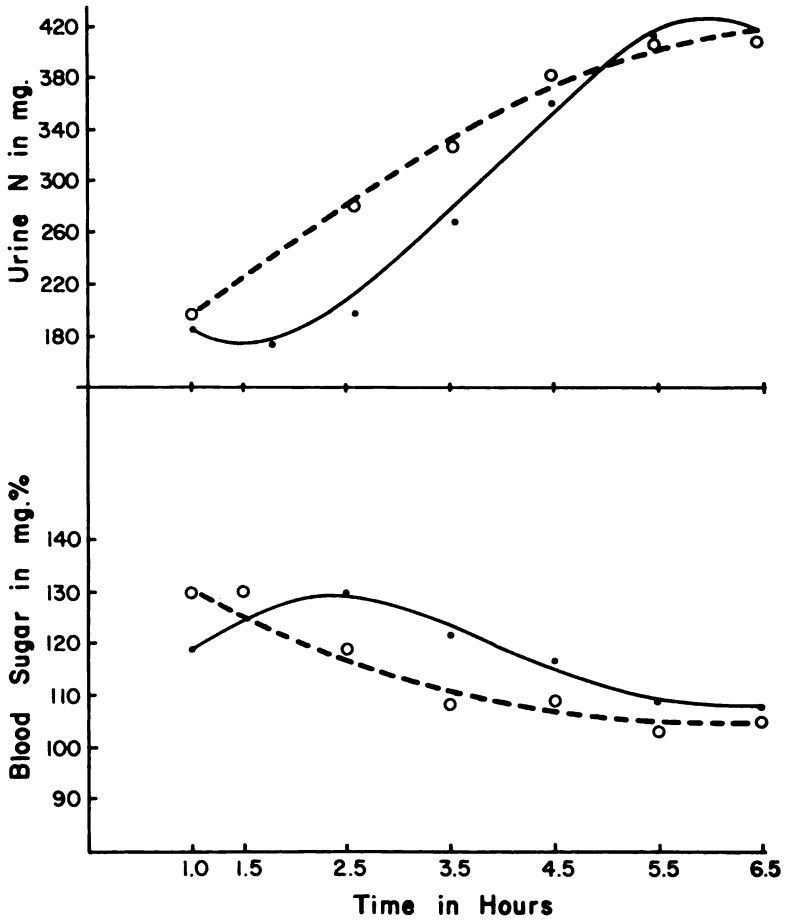

Figure 3—Blood sugar and urinary N concentration following test meal with animal (dotted line—open circle) vs. vegetable (solid line) protein.

50 gm of glucose. The observation made by Phansalkar and Patwardhan[21] is the reverse; both these, however, show the interdependence of carbohydrate and protein metabolism.

It must be mentioned that Coleman, Tuttle and Daum[24] found no differences in the blood sugar curves after animal and vegetable protein breakfasts. The peak was reached at 1 hour, and thereafter the blood sugar decreased at the same rate after either of the 2 breakfasts. It is difficult to explain the discrepancy between their findings and ours. Their diets provided just as much protein as our test meals. On the other hand, their diets supplied 700 to 800 calories only as against the 1400 to 1500 calorie value of ours. Whether this large difference in calorie intake was responsible for differences in the behaviour of blood sugar after the test meals is a point worth investigating.

It was possible that vegetable proteins, being enclosed within the cellulosic cell wall, were less easily digested and absorbed than animal proteins like milk,

eggs and meat; although cooking results in swelling of the cell and probably also bursting of the cell wall, the protein seemed to be still difficult to get at by the digestive enzymes. This possibility was tested on whole wheat and wheat gluten separated from wheat by a physical procedure.[22] When the subjects were given test meals containing gluten, its absorption was quicker, the peak in blood amino nitrogen being reached in 3 hours as compared with 5 hours on whole wheat test meal of identical protein content. Further, 16% more nitrogen was excreted in the urine in 7 hours after the former test meal than after the latter (fig. 4). It appeared,

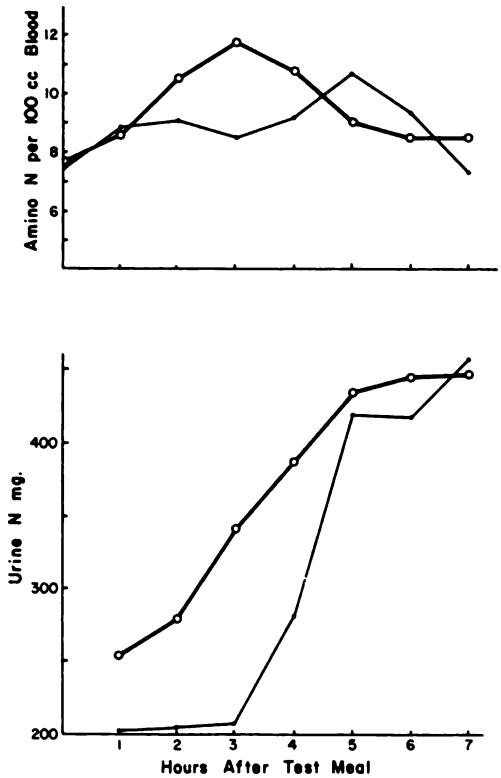

Figure 4—Urinary N excretion and blood amino acid concentration following test meal of whole wheat (solid dots) vs. wheat gluten (open circles).

therefore, that a vegetable protein freed from its natural surroundings would behave, so far as digestion and absorption were concerned, like an animal protein. Differences in the rate of utilization of animal and vegetable proteins would seem to originate in the alimentary tract and to be due to physical causes, since they did not seem to depend upon the amino acid composition of the protein or upon some other substances accompanying it within the foodstuff.

COMMENTS

These experiments yielded information on the different rates of digestion and absorption and also on utilization of animal and vegetable proteins, but they failed to throw light on the underlying reasons for the higher retention of nitrogen on vegetable protein than on animal protein at comparable protein intake. That the type of the dietary protein may cause changes in urinary nitrogen and hence in nitrogen retention has been reported by other workers also. Leverton and Gram[25] found in 14 girl students a 10% higher retention of nitrogen when breakfast alone of the 3 meals was free of any animal protein. On a later occasion, Leverton, Gram and Chaloupka[26] could not observe a similar effect when protein intake was 63 gm but did observe it on an intake of 43 gm per day. In this latter experiment milk was withheld or added at lunch and not at breakfast as in the earlier experiments. The authors suggest that vegetable and animal proteins should be taken together to permit better protein utilization. I cannot see how this conclusion arises from their recorded observations on nitrogen retention, which was higher when animal protein was omitted from the meal. Daum et al.[27] also reported that on a cereal-milk breakfast nitrogen retention in 7 aged subjects was significantly higher than on a bacon-egg-milk breakfast.

There is no mention of body weight changes in the observations of Leverton et al. and Daum et al. However, in our experiments mentioned at the outset the body weight changes were found to be small and could not account in most instances for the nitrogen retained on vegetable protein diets. Here I might refer to the observations of Holmes, Jones and Stanier,[28] who found extraordinarily high retention of nitrogen with no corresponding weight increase in adult Africans. Although these subjects were malnourished to begin with, positive nitrogen balances were found even after there was reason to believe that rehabilitation had been fully accomplished. Holmes et al. have not been able to offer any satisfactory explanation. I have discussed this question in greater detail elsewhere, hence I confine myself to a mere mention of it and of the problem it poses.

Considering all the observations referred to in this paper, one finds oneself confronted with some questions for which correct solutions have to be found. These are:

1. How does the proportion of vegetable protein in the diet or the intake of animal and vegetable proteins separately or together influence the total urinary nitrogen excretion at constant nitrogen intake?

2. What constitutes the undetermined urine nitrogen which has been observed to be unusually high in Indian subjects presumably on diets which contain a high proportion of vegetable protein, and how does dietary protein influence its constituents?

3. What does positive nitrogen balance unaccounted for by expected weight changes in a healthy adult signify?

It has to be admitted that we do not have today satisfactory answers to any of these questions. They seem to show up an obscure corner in the picture of

protein metabolism. It is hoped that this paper will have stimulated interest at this conference so that future investigations may help in filling the lacunae in our knowledge.

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DISCUSSION

DR. FOMON: I think Dr. Patwardhan might be interested to know that his findings are not limited to adults. If you study full-term normal babies from birth to 6 months of life, you find that a baby fed 7% of the calories as protein retains relatively little nitrogen throughout the first 6 months. A baby fed 20% of the calories as protein retains considerably more nitrogen. Yet there is very little difference in weight of these babies at 6 months. This has been a problem that has bothered pediatricians doing balance studies for years. We cannot explain where this extra nitrogen goes in the baby fed the high-protein diet. We have attempted to pursue this in a number of ways and have not been able to demonstrate greater concentrations of nitrogen through the methods of indirect measurements of body composition of living subjects.

DR. ARROYAVE: Dr. Patwardhan in his writings has suggested that the skin losses of nitrogen might be an important variable here. For some reason, different diets might be determining different losses of nitrogen through the skin, accounting for this undetermined nitrogen.

Another comment is that in regard to this ratio of urea to total nitrogen, as some of us have been conceiving the concept to estimate nutritional status,

we have followed it as a determination run on fasting urine without the effect of the previous dietary intake of nitrogen. From your curves, I remember that you have shown that, in 8 hours or so, you get rid of the effect of the previous protein intake. So, by determining the ratio of urea to total nitrogen in a fasting urine, you could be measuring the metabolic level in the subject determined by his nutritional status but independent of the immediate previous intake of protein.

DR. GYÖRGY: As a pediatrician I should like to follow up the remarks of Dr. Fomon. Many of us feel that there is a systematic error in our technique when we do balance studies. Dr. Wallace figured from the balance studies of Dr. Levin and his group that, should the retention have gone on for many, many months, not only should the whole body have been protein but somewhere protein should have been oozed out.

In addition to that, there are no data on special changes in the body composition. I think in some parts of the world now we could have some fairly good cadaver studies due to accident or some similar happenings. I would like to ask Dr. Patwardhan whether he has some cadaver composition studies on people on animal and vegetable diets. I have the feeling that we should definitely consider a systematic error either because we cannot measure the nitrogen in the feces or for many other possibilities which have been discussed. I just cannot see how nitrogen should go on and go on with such a high retention and the body composition should not change.

DR. WILLIAMS: I would like to comment on an experiment back in the late thirties in Dr. Macy-Hoobler's laboratory. One group of children were on pasteurized milk for 9 months and another group were on evaporated milk for the same period. The children on evaporated milk had a slightly higher retention of nitrogen over this period than those on the pasteurized milk.

We were curious about this. It has never been published because we did not have an explanation. We did measure the amino nitrogen in the blood, which was the approved method at the time. The evidence indicated it was slightly higher with the pasteurized milk, indicating a more rapid absorption and therefore a more rapid deamination in the liver and excretion of the nitrogen. This might be explained on the basis of digestibility. On the other hand, it does not explain what Dr. György has just pointed out, what happened or why it should be retained, except a more rapid deamination would cause a greater excretion.

DR. HARPER: Dr. György's comment on nitrogen balance studies does not explain the difference between animal and vegetable protein. Surely the same experimenter using the same subjects should get the same results with both.

But in ruminants a fair amount of the nitrogen from the rumen apparently is converted into gaseous materials, various oxides of nitrogen, and a reasonable percentage can be accounted for in this way as being given off in gases.

I wondered, particularly when one looks at the vegetable proteins which have a fairly high content of amide nitrogen which is not amino acid nitrogen, whether the microorganisms of the intestinal tract convert some of this to a gaseous nitrogenous substance, which is not accounted for in the nitrogen balance experiments.

DR. PATWARDHAN: I was aware of the observations in children where the nitrogen retention could not be explained in terms of increase in body weight, but of course up to the age of 3 years, one can explain some of this by changes in body composition which take place. After chemical maturation of the body occurs, no further change in body composition is expected. It is under these conditions that one finds it difficult to explain the nitrogen retention in terms of increase in body weight when the latter is not observed.

I have read the interesting paper of Dr. Wallace which was presented at the Federation meetings with Dr. György in the Chair. Dr. Wallace does point out what is called systematic error, but his point is that it is far more important when you are changing the intake of nitrogen from low to high or high to low. Here, as you see, we kept the nitrogen levels constant as far as possible. I would admit to a certain extent the existence of what you call systematic error, but the changes observed in the changeover from vegetable protein to animal protein and back again are far more than could be accounted for merely on the basis of systematic error. That is the point which has to be explained.

Dr. Harper has suggested that some of the nitrogen on vegetable protein diets may be lost as gaseous nitrogen due to the activity of bacteria in the gastrointestinal tract, which is a consideration to which some thought should be given and probably some experimental observations made. There is no doubt that on a diet which contains a large proportion of pulses and beans the tendency to flatulence is there. It is quite possible that microorganisms in the intestinal tract are doing something to the nitrogen which we cannot account for in our balance studies.

Dr. Arroyave's reference to skin losses does not really apply here, because these studies were done in subjects continuously in a situation where there was no visible perspiration at all. At that time we were in Coonoor. It has a temperate climate where, unless you really exert yourself, you do not perspire. There could not be a difference in the nitrogen loss in one period unaccounted for because of this particular factor.

With regard to the proportion of urinary nitrogen to total nitrogen and its significance in terms of the determination of nutritional status in terms of protein intake, I think that again is probably a controversial point and may require further observation. All I can give is the result of the protein intake on the immediately preceding date rather than any information on the nutritional status with regard to protein intake over a longer period. To my mind, the ratio of urea nitrogen: total urinary nitrogen does not appear to be a very reliable index of protein nutrition.

We have had no cadaver studies. As you know, in the whole world there are only a few cadaver studies on which we base so much of our concept of body composition and the changes that take place here and there. More such studies are needed, not only in this field but in every field where the body composition is presumed on certain physical measurements in live people. What applies there would also apply to the storage of nitrogen in the body.

Determination of the Nutritive Value of Proteins by Chemical Analysis

A. E. Bender

WHEN BLOCK AND MITCHELL introduced the concept of chemical score (CS) in 1946 it became feasible to deduce the nutritive value of proteins from chemical analysis instead of carrying out long, laborious and reputedly inaccurate biological assays.

The relation between CS and nutritive value compiled by Block and Mitchell is shown in figure 1 together with the postulated theoretical relation suggested by Bender in 1954. The relation depends on three factors: an accurate estimation of the limiting amino acid, the ratio of this figure to the target figure, and the biological value (BV) of the protein.

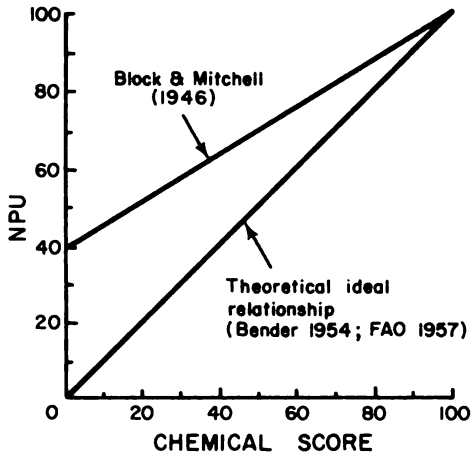

Figure 1

The straight line relationship was based in the first instance on the theoretical reasoning that the BV of a protein should be numerically equal to the relative content of its limiting amino acid, and that if one essential amino acid is absent, then that protein should have a BV of zero. It was supported by the finding that the BV of gelatin was zero and the literature value of 29 was an error (Bender et al. 1953). The only other reference to the CS/BV relation is that of Fisher (1954) who

also postulated a straight line running from zero to 100. The Report of the FAO Committee (No. 16, 1957) accepted the theoretical 0-100 line to represent the ideal relationship. This paper offers evidence for a more detailed relationship between CS and BV, and some discrepancies.

The Ideal Protein (for the rat)

Of the three factors needed to determine the CS/BV relation, the target figures are open to doubt. All the literature calculations take the amino acids of defatted egg as the target, and CS's are the ratio of the limiting amino acid in the test protein to the same amino acid in egg. Egg clearly has not less than 100% of the requirements of every amino acid (although Mitchell (1959) suggested a possible slight deficiency in lysine) but it could contain more than 100% of the requirements of one or more of the amino acids. If this were so, then CS's would be underestimated.

Experiment showed that egg has, in fact, a surplus of all the essential amino acids. Defatted egg protein has Net Protein Utilisation (NPU) 97 (mean of 5 determinations); "dilution" by adding 15% by weight of a mixture of nonessential amino acids did not reduce this value, NPU 99. Further dilution with 30% did reduce NPU to 82, and 45% dilution reduced it to 59. (NPU's were determined by the carcass analysis method of Bender and Miller (1953) and Miller and Bender (1955). Where BV's are quoted they are derived from NPU divided by digestibility.)

The next step was to determine the extent of the excess of each amino acid. This was done by first making a mixture of synthetic amino acids to simulate egg protein. We used the analysis found in the Rutgers Cooperative Determinations (1950) ("best" average values) as this could be expected to be more accurate than any other analysis. The figures show some differences from those of Block and Mitchell (1946) that are usually used in calculating CS. For example, both lysine and cystine + methionine are 15% lower in the Rutgers analysis. In making this mixture we were forced to use five amino acids in the DL form; for the four other than methionine we used double quantities. This mixture gave duplicate NPU's of 91 and 87; and when diluted 15% with nonessential amino acids it gave values of 92 and 99. This appears to be the first reported experiment in which a mixture of amino acids has given a BV of approximately 100. There appears to be no trouble from the unnatural isomers, nor from the absence of "strepogenin." It has previously been claimed that maximum growth cannot be achieved on amino acid mixtures; we were measuring NPU, not growth, but we have repeatedly found that rats eat less of a diet composed of amino acids, and this may be one of the reasons for the suboptimal growth previously reported on these diets.

The second stage was to feed "half-mixtures." Each amino acid in turn of the mixture simulating egg protein was halved in quantity in a series of diets, the other amino acids being maintained at the full level. If the original mixture had been "perfect" we should expect with every diet that the NPU would fall to 50, as there is now a limiting amino acid present at 50% of the old target. If any diets showed results greater than 50, then the amino acid in question must have been present in the original mixture at more than 100% of the requirements. The results

are shown in table 1: 6 amino acids were in surplus, although if the amino acid mixture simulating egg could be diluted by 15% all should be in surplus.

We now had two sets of apparent target figures; one was the composition of "diluted" egg, and the other was calculated from the "½ amino acid" diets. Of these two, the lowest value for each amino acid was taken as the test target figure (table 2). Thus for histidine, 1.8% (100/120 x 2.1), for threonine 4.1% (100/120 x 4.9), for valine 5.0% (100/140 x 7.0). Lysine and methionine + cystine figures, although their "half values" gave NPU's of 50, were lower in "diluted" egg, and the latter values were therefore used. Leucine and isoleucine were taken as in "diluted" egg, in view of the difficulty of interpreting the apparent imbalance effect obtained when the isoleucine was halved. The value of 10.8% for DL-isoleucine (corresponding to 5.4% L-isoleucine in diluted egg) was used in a mixture that gave NPU of near 100, but as this was subsequently found to be only 72% pure, the target figure was taken to be 72% of 10.8, i.e. 8.6 gm of the DL-form per 16 gm nitrogen.

TABLE 1
NET PROTEIN UTILISATION OF "HALF-AMINO ACID" MIXTURES

A mixture of amino acids simulating egg protein according to the analysis of the Rutgers Bureau, with each amino acid in turn halved in quantity

	Replicates	% requirement in original
½ lysine	49, 54	100
½ histidine	45, 65, 67	120
½ tryptophan	92, 82, 96, 77	160
½ phenylalanine	75, 65, 82, 75	150
½ (cystine + methionine)	54, 58	100
½ threonine	63, 61	120
½ leucine	63, 58	120
½ isoleucine	37, 29	
½ (leucine + isoleucine)	62, 49	100
½ valine	71, 71	140

Tryptophan should have the target level of 0.7%, but mixture V suggested that this was inadequate and the level of tryptophan in diluted egg was used. The high NPU's of the "½ tryptophan" mixture have not been explained. The phenylalanine content of "diluted" egg was taken as the target value for similar reasons. All these values are tentative, but as most proteins are limited by lysine or methionine + cystine, only these are of consequence and experimental evidence suggests that these two target figures are correct.

When a mixture of amino acids was made according to this recipe, NPU's of 92 and 99 were obtained. The tentative target figures for amino acid requirements are these values, halved in the appropriate cases where DL-mixtures had been used. The accurately known CS's of the amino acid mixtures in table 2 show good agreement with the measured NPU's.

Table 3 shows the target amino acid figures, the FAO Provisional Pattern (1957), the mixtures used by Rose (1948) and by Rao et al. (1959) for maximum growth, and the analysis of egg protein, both according to the Rutgers Bureau and

TABLE 2
COMPOSITION AND NET PROTEIN UTILISATION OF VARIOUS AMINO ACID MIXTURES

	TARGET AMINO ACID LEVELS	Mixture V	Mixture VI	Mixture I	Mixture X	L forms		
						Egg analysis according to Block & Mitchell	Egg analysis according to Rutgers	Egg "diluted" with 15% nonessential amino acids
Histidine L	1.8	2.2	2.2	2.0	2.4	2.1	2.1	1.8
Lysine L	5.2	6.7	6.5	7.3	7.3	7.2	6.1	5.2
Tryptophan DL	1.9	*1.4* *	*0.7* *	1.5	2.1	1.5	1.1	1.0
Phenylalanine DL	9.8	7.5	3.8 *	6.3 *	10.5	6.3	5.6	4.9
Methionine	2.7	2.0	2.0	4.1	3.3	4.1	3.2	2.8
Cystine L	2.0	2.8	2.7	2.4	2.2	2.4	2.3	2.0
Threonine DL	8.2	8.3	4.1	9.8	*4.6* *	4.9	4.9	4.3
Leucine L	7.8	7.5	7.5	9.2	8.5	9.2	9.0	7.8
Isoleucine DL	8.6	9.3	4.3	16.0	11.8	8.0	6.2	5.4
Valine DL	10.0	10.0	5.0	14.6	13.3	7.3	7.0	6.1
Chemical Score	100	74	38	64	56			
NPU	92 } 99 }	77 (5 assays)	45	68 (3 assays)	53			

* Limiting amino acid.

to the figures used by FAO (1957). FAO Provisional Pattern for leucine is lower than in egg and also lower than our target figure, but in view of the leucine-isoleucine imbalance, the leucine target figure is not necessarily the minimum. Lysine in the Provisional Pattern is only 80% of the target but may be more accurate for man, as so many cereals, limited by lysine, give higher NPU's for man than for the rat. Threonine is also lower in the Provisional Pattern, but the presence of isomers in the synthetic threonine used to compile the target values may account for this. The provisional value for valine is much less than is present in egg, but we know that this is in excess in egg.

TABLE 3
COMPARISON OF AMINO ACID TARGET FIGURES WITH FAO PROVISIONAL PATTERN OF AMINO ACIDS
(gm/100 gm protein)

	Target	FAO Pro-visional	Rao et al. (1959)	Rose (1948)	Egg FAO	Egg Rutgers
Isoleucine	4.3	4.2	5.5	5.0	6.8	6.2
Leucine	*7.8*	*4.8*	6.9	8.0	9.0	9.0
Lysine	*5.2*	*4.2*	9.0	10.1	6.3	6.1
Phenylalanine	4.9	2.8	7.2	4.6	6.0	5.6
Tyrosine		2.8			4.4	
Methionine + cystine	4.7	4.2	4.9	7.5	5.4	5.5
Threonine	*4.1*	*2.8*	5.1	5.0	5.0	4.9
Tryptophan	*1.0*	*1.4*	1.1	1.0	1.7	1.1
Valine	*5.0*	*4.2*	5.6	7.0	7.4	7.0
Histidine	1.8		2.1	4.0		2.1

The two major differences between these target values (for maximum BV), and the requirements for maximum growth of Rao, Metta and Johnson (1959) are that the target values are 60% of the growth figures for lysine and 65% for the phenylalanine. The methionine + cystine values agree remarkably well. Johnson's experiments, however, showed only a small difference in PER (from 3.9 to 4.1), but a more marked difference in growth rate (from 3.5 to 4.4 gm) on increasing lysine from 0.8% to 0.9% of the diet, and no levels of lysine below 0.8% were tested. Hence it is possible that a level near to our target values might still have given near maximum PER's. Moreover, Johnson's lysine requirement of 9.0% is 50% greater than the lysine content of egg, which is usually regarded as the perfect protein, and Howe, Gilfillan and Allison (1960) found no improvement on adding lysine to the FAO provisional pattern. The discrepancy between the two phenylalanine figures cannot be so explained, unless it is because the target mixture contained double quantities of the DL-isomer. We used five amino acids in the DL-form; Johnson's team used only the L-form, thus it does not appear that the D-isomers caused any (other) difficulty.

Zero End of CS/BV Correlation

The critical part of the CS/BV correlation, where the theoretical line differs from that of Block and Mitchell, is the lower range. To determine the zero end.

a series of amino acids was made from which each amino acid in turn was omitted. The NPU's were determined. These results were replicated many times because of the unusual variations.

The results are in table 4 and put the amino acids into three groups. In group I, containing valine and methionine + cystine, the NPU approximates to the expected value of zero. In group II, containing tryptophan, threonine, histidine, phenylalanine and leucine plus isoleucine, the NPU in the absence of the amino acid lies between 10 and 30. Lysine is the sole amino acid in group III and has an NPU varying in different experiments between 20 and 50.

TABLE 4
NET PROTEIN UTILISATION OF MIXTURE COMPLETELY LACKING IN ONE AMINO ACID

Amino acid omitted	Individual NPU's	Mean
Valine	0, 8,	*4*
Cystine + methionine	17, 10, 0, 0	*7*
Tryptophan	2, 26, 20, 21, 17	*17*
Threonine	10, 7, 17	*11*
Histidine	21, 15	*18*
Phenylalanine	9, 14, 32, 20, 32	*21*
Leucine + isoleucine	7, 21, 23, 27	*20*
Lysine	17, 33, 18, 39, 39 46, 51, 56	*37*

When CS is now correlated with NPU the line is not the same for all proteins but depends on the limiting amino acid. When group I amino acids are limiting, the line follows its theoretical course from 0 to 100. When amino acids of group II are limiting, the line runs from 100 to about 50 and then curves off so that CS zero corresponds to NPU about 20. When lysine is limiting, the line runs from 100 to 50 and ends at NPU 30-50, corresponding to CS zero (fig. 2).

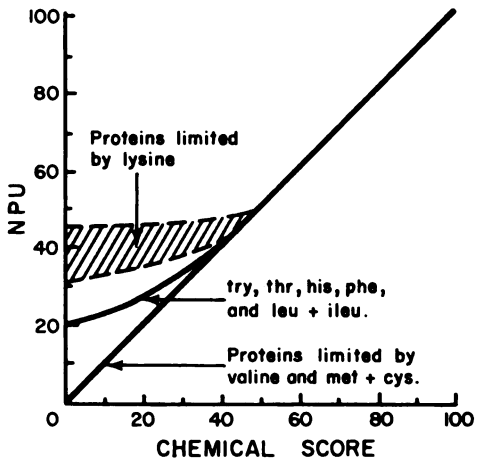

Figure 2

Lysine

The replicated NPU's of a mixture of amino acids lacking lysine show great variability, and for this reason the CS/BV curve for proteins low in lysine cannot be defined precisely and is given in figure 2 as a range.

It is accepted that lysine differs from other amino acids in its slow turnover rate. It seems likely that when the animal breaks down tissue protein in the normal turnover processes, part, at least, of the lysine can be reused for new protein synthesis. We have been able to keep rats alive for periods longer than six months on an amino acid diet from which lysine was omitted. There was an initial weight loss during the first two weeks, then the rats maintained constant body weight for the rest of the time. As essential protein structures, including the synthesis of enzymes, must have continued, the animal presumably obtained lysine from some source. One possible reason, therefore, for the variable NPU's obtained on lysine-free diets might be individual variation in the animal's ability to reuse lysine. (A similar reason might explain the poor duplication of the NPU's of the other mixtures of this series.)

Another source of lysine is the faeces. In one day's collection from rats on a lysine-free diet we found 4 mg to 8 mg of lysine. This quantity must be considered in relation to a daily intake of 7 gm to 8 gm of food, containing 0.7 gm to 0.8 gm of an amino acid mixture (lacking lysine). The ideal protein, according to table 3, contains 5.2% lysine, thus 0.7 gm to 0.8 gm of protein would contain 36 mg to 42 mg of lysine. If all the faeces were consumed the rat could obtain a maximum of 4 mg to 8 mg of lysine per day from its faeces; in relation to a daily need of 36 mg to 42 mg, this is 10% to 25% of its requirements. This alone, assuming lysine CS/BV line to run in accordance with theory from 0-100, would permit BV of 10-25 when CS is zero. Experiments are in progress to examine this point.

An unusual feature of the postulated CS/BV relation for lysine-limited proteins is the almost horizontal part of the curve, i.e. proteins or amino acid mixtures with lysine at any level below about 40% to 50% of the target values, all have the same NPU's of about 30-50. We have confirmed this generalisation by an experiment in which increments of lysine were added to a lysine-free diet and body weight determined (fig. 3). Weight was maintained at a constant level on the lysine-free diet; increments of lysine up to 1% of the "protein" of the diet had no effect whatever on weight. When the supplement of lysine was increased to 2% there was an immediate but very small increase in weight, of only a few grams, then this new weight was maintained. When, however, the lysine was increased to 3% of the protein diet, rapid and continued growth followed (fig. 3). As the ideal protein contains 5.2% lysine, these results agree with figure 2 in that levels of lysine from 0 to about 40% to 50% of the target figures do not increase the NPU to any appreciable extent.

It is difficult to suggest any explanation for this effect. If the rat can reuse lysine from degraded proteins, why cannot it add to its protein synthesis from the lysine added to its diet? It would almost appear that when the rat is provided with small doses of lysine it automatically ceases to reuse lysine from degraded tissue proteins to an equivalent extent.

TABLE 5
AMINO ACID COMPOSITION, CHEMICAL SCORE AND BIOLOGICAL VALUE OF A VARIETY OF PROTEINS

	Nigerian dried fish FD-1	Isolated coconut protein C_n-I-(I_w)	Coconut 2:1 mix C_n-I-(I_w+I_o)	Cottonseed Flour C 1	Cottonseed Flour CF 2	Reference Skim Milk A. E. B. (chromatograph)	Reference Skim Milk UNICEF (microbiol.)
Histidine	2.4	2.1	1.8	3.2	3.3	2.7	1.9
Lysine	9.7	4.1	3.7	4.3	4.3	7.9	8.3
Tryptophan				1.1	1.1	0.9	1.4
Phenylalanine	4.2	4.4	4.5	5.9	5.5	4.5	4.4
Methionine	2.7 *	1.5 *	1.4 *	1.5 *	1.2 *	1.9	2.5
Cystine	0.6	1.1	1.1	1.7	1.7	0.9	0.9
Threonine		2.5	2.8	3.5	3.4	4.0	4.1
Leucine	8.1	7.3	7.3	6.5	6.1	7.7	7.7
Isoleucine	5.2	3.8	3.4	3.3	3.2	5.2	5.5
Valine	4.9	5.7	5.5	5.3	4.7	6.4	6.6
CS	69	56	54	68	62	60	72
BV	67	66	54	65	63	80	

* Limiting amino acid (Methionine + Cystine).

Evidence Supporting the CS/BV Relation

1) A number of proteins have been analysed for their amino acid composition by the Moore and Stein resin chromatographic technique and their NPU's forecast from the postulated CS/BV curve. The NPU's were then determined by the carcass analysis method and the results in table 5 show very good agreement (within the limits of amino acid determination).

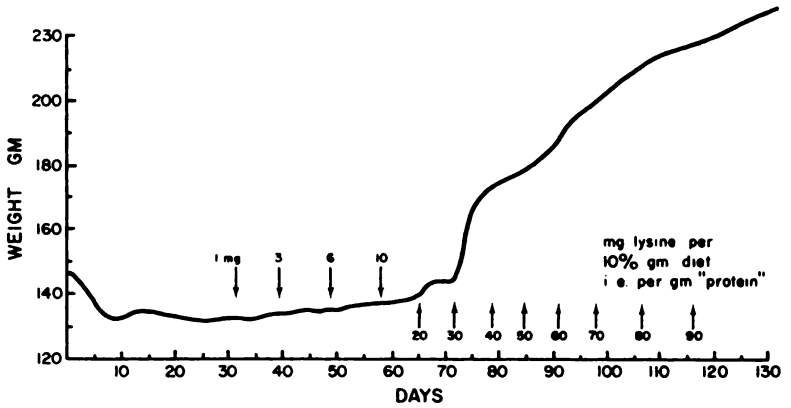

Figure 3—Body weights of rats started on lysine-free diet and supplemented with various increments of lysine.

FAO reference skim milk. The limiting amino acid is methionine + cystine and the results of two independent analyses are shown. One gives a CS of 62 and the other of 72: NPU determination was 74, digestibility 93%, BV 80, not too far removed from the CS of 72, but in poor agreement with CS 62. It seems that we can often obtain better reproducibility (and accuracy ?) by biological than by chemical assay (table 5).

2) *Complementation of proteins.* The pea flour and maize meal used by Dr. Brock were analysed for amino acid composition and their BV's forecast. The composition of the mixture of two parts maize meal to one part of pea flour was calculated and its BV forecast at 72, as in table 6. The BV was determined and found to be 70.

TABLE 6
FORECAST OF BIOLOGICAL VALUE OF PROTEIN MIXTURE FROM CHEMICAL SCORE

	Yellow Pea Flour 27.7% protein	Maize Meal 11% protein	Mixture 2 parts maize: 1 part pea flour (calculated)
Lysine	5.6	2.6	4.3
Methionine	0.9 } 2.0	2.8 } 5.3	1.7 } 3.4
Cystine	1.1 }	2.5 }	1.7 }
Limiting amino acid	methionine + cystine	lysine	methionine + cystine
CS	43	50	72
BV (determined)	43	35	70

3) *Serial supplementation of bread.* Table 7 shows our analysis of bread protein (Palgrave, 1958) and other recent figures for comparison. It is, of course, limited by lysine, and the BV, derived from figure 2, is about 50: determined value 50 (NPU 46, D. 93%). When bread is supplemented with lysine, the limiting amino acid, according to Palgrave's analysis, becomes threonine, BV calculated 66, determined 61. After this three amino acids are close together, valine at 82, methionine + cystine at 87 and isoleucine (+leucine) at 88. Biological assay shows that methionine + cystine is the third limit at 80, valine the fourth at 85. Supplementation of bread protein with all four amino acids, lysine, threonine, methionine and valine, gave the highest value we have obtained, namely 93, which we accept as fully supplemented bread.

Discrepancies

We have encountered two outstanding discrepancies, namely meat meal and peanut meal, that do not fit in with the expected pattern set by bread as described above.

Meat meal. A series of 20 commercial samples of meat meals showed a range of NPU's from 9 to 40, with digestibilities ranging from 70% to 90%. Those of lowest NPU were not of low digestibility, and BV's covered the same range as NPU's.

The amino acid analysis (chromatographic) of one sample (MM 10) is given in table 8 and shows tryptophan limiting at 32%, followed by methionine + cystine at 45%, then isoleucine at 56%, and threonine, phenylalanine, histidine and lysine at 70%. The NPU's on table 8a show MM 10 much lower than analysis expects (18 instead of 32-45) and no effect of supplementation with methionine and tryptophan.

A series of analyses of meat products (animal muscle, two meat meals, meat and bone scraps and processed meat) is given in table 8 and the CS is calculated from the lowest figure for each amino acid in an attempt to forecast NPU. NPU's are shown in table 8a for three samples of commercial meat meal, not individually analysed. MM 2 shows an improvement only when lysine, tryptophan, methionine and leucine are added together, and this is only from 33 to 42. MM 3, very poor at NPU 11, is improved to 22 with methionine, and MM 18, mediocre at 28, is increased to 50 with methione. All four meat meals are much lower in NPU than warranted by amino acid analysis, and all fail to show the expected increases on amino acid supplementation.

Peanut meal. The NPU's of a variety of peanut samples have been measured. Whole peanuts showed NPU's of 45-55, prepared flours ranged from 30 to 55, a selection of commercial peanut meals ranged from 40 to 47; digestibility in all samples was 92% to 95%.

Table 9 shows six amino acid analyses of peanut meal. The methionine + cystine and the available lysine values on samples were also assayed biologically. The BV's agree fairly well with the CS's for methionine + cystine.

TABLE 7
ESSENTIAL AMINO ACID CONTENT OF BREAD
(gm per 16 gm N)

	1 Palgrave (1958)	2 McDermott & Pace (1957)	3 Block & Weiss (1956)	4 Wertz et al (1956)	5 McDermott & Pace (1960)	Target	CS (from column 1)	BV	No. of estimations
Histidine	2.1	2.12	2.7	—	2.1	1.8	—	—	—
Lysine	2.0	1.95	2.5	2.03	1.9	5.2	29	50	(4)
Tryptophan	1.0	1.15	0.9	0.71	1.1 (grist)	1.0	—	—	—
Phenylalanine	5.3	5.29	4.0	4.22	4.9	4.9	—	—	—
Cystine	2.7	2.52	2.1	—	2.3	4.7	87	80	(4)
Methionine	1.4	1.79	2.2	1.30	1.9				
Threonine	2.7	2.79	3.1	2.39	2.8	4.1	66	61	(3)
Leucine	7.0	7.22	6.3	5.71	7.2	7.8	90	93	(2)
Isoleucine	3.8	3.82	4.1	3.98	3.7	4.3	88		
Valine	4.1	4.39	3.8	3.94	4.2	5.0	82	85	(9)

TABLE 8
ANALYSES OF MEAT MEALS

	Commercial Sample MM 10	CS	Animal Muscle Block & Weiss [1]	Meat meals Pritchard & Smith [2]		Processed meat Eastoe [3]	Meat & bone scraps, Lyman, Kuiken & Hale [4]	Minimum CS
Lysine	5.0		8.5	8.3	9.9	4.8	5.5	
Available lysine	3.7	71						71
Histidine	1.3	72	2.8	2.9	2.9	1.5	1.7	72
Threonine	2.7	66	4.6	3.6	3.7	3.3	3.3	66
Valine	5.5	—	5.5	3.3	3.8	3.9	4.8	66
Phenylalanine	3.3	70	4.5	4.9	4.2	3.1	3.4	65
Methionine	1.3	*45*	2.5	2.1	1.6	1.0	1.3	?
Cystine	0.8		1.4					
Isoleucine	2.4	56	4.7			2.5	3.4	56
Leucine	6.5	87	8.0	5.3	6.3	5.4	6.2	71
Tryptophan	0.32	32	1.1	0.4	0.6		0.7	32

[1] Amino acid handbook.
[2] J. Sci. Fd Agric., (1957) *8*, 669.
[3] J. Sci. Fd Agric., (1960) *11*, 87.
[4] J. Agric. Fd Sci., (1956) *4*, 1008.

TABLE 8a
NET PROTEIN UTILISATION OF FOUR COMMERCIAL MEAT MEALS, WITH AMINO ACID SUPPLEMENTS

MM 10	17, 19 *	MM 2	33
+ methionine	26, 22 *	+ methionine	36
+ lysine	19, 20 *	+ lysine	30
+ methionine, lysine	20 *	+ tryptophan	30
+ isoleucine	20	+ leucine	30
+ methionine, isoleucine	19 *	+ lysine, tryptophan	33
+ methionine, lysine, isoleucine	22 *	+ lysine, tryptophan, methionine, leucine	42
+ methionine, tryptophan	18 *		
MM 3	11	MM 8	28 Ø
+ methionine	22	+ methionine	50 Ø
+ lysine	12	+ lysine	29 Ø
+ methionine, lysine	24	+ methionine, lysine	44 Ø
		+ methionine, tryptophan	48
		+ methionine, threonine	48

* Protein Retention Efficiency (i.e. Net Protein Ratio x 16).
Ø These determinations were carried out by Mr. J. Bunyan of Vitamins Ltd, London.

According to the analyses in table 9 (which were not carried out on any of the samples assayed biologically) lysine and threonine are equally limiting after methionine, and when these three amino acids are added to peanuts, the BV should be about 75. Table 9a shows the failure to achieve this. There was only a small improvement when methionine was added to commercial meal samples GN 2, GN 3 and GN 10, which was not increased when lysine and threonine were also added to GN 2 and GN 3. The only increase, and that was not marked, was when methionine, threonine, lysine, leucine, isoleucine, phenylalanine and tryptophan were added together. Valine is not limiting according to five of the six analyses in table 9; it has not yet been tested biologically.

TABLE 9
ANALYSIS OF PEANUT PROTEIN
(gm per 16 gm N)

	1a	1b	2	3 B & B	5	4 FAO	C. S.
Lysine	3.3	3.3	3.6	3.0	3.5	3.5	58— 69
Histidine	2.9	2.4	2.5	2.1	2.2		100
Threonine	2.6	2.8	2.5	(1.6)	2.8	2.7	61— 68
Valine	3.7	4.9	4.5	4.4	4.6	4.9	74— 98
Phenylalanine	5.1	5.2	5.4	5.1	4.9	5.1	100
Methionine + cystine	2.0	2.3	2.8	2.6	2.2	2.4	43— 60
Isoleucine	3.3	4.2	3.5	4.6	4.1	4.1	77—100
Leucine	6.4	6.5	6.5	6.7	7.1	6.0	80— 90
Tryptophan	0.9	1.1			0.8	1.1	100

1. Rosen, G. D. in "Processed plant protein foodstuffs" by A. M. Altschul. a) chromatographic, b) microbiological.
2. Private communication—chromatographic.
3. Block & Bolling.
4. "Protein requirements," FAO, 1957.
5. Rutgers Cooperative Determinations ("best" average values).

TABLE 9a
BIOLOGICAL VALUE OF THREE DIFFERENT SAMPLES OF PEANUTS, ALONE AND SUPPLEMENTED WITH VARIOUS AMINO ACIDS

	BV
GN 2	49
+ methionine	53
+ methionine + lysine	54
+ methionine, lysine, threonine	52
+ methionine, lysine, threonine, isoleucine	47
+ methionine, lysine, threonine, isoleucine, leucine	48
GN 3	39
+ methionine	48
+ methionine, lysine	41
+ methionine, threonine	44
+ methionine, lysine, threonine	50
GN 10	50
+ methionine, threonine, lysine, leucine, isoleucine, phenylalanine	59
+ methionine, threonine, lysine, leucine, isoleucine, phenylalanine, tryptophan	66

Whether this discrepancy is a criticism of the method of chemical score, or of peanuts as a source of protein, is not clear.

It has been reported (Balasundaram et al. 1958) that peanut protein is improved with lysine, isoleucine and threonine separately, as well as with methionine, but as conclusions were based on 3 gm to 4 gm differences in body weight of rats of over 100 gm weight after 7 weeks feeding, the results are doubtful.

CONCLUSIONS

It can be concluded from this work that there are circumstances in which the nutritive value of a protein can be forecast from chemical composition. It is necessary to know the amino acid composition with some degree of accuracy, which is all too often questionable in many literature analyses. It is obvious that methionine and cystine must always be considered together, yet many analyses of whole diets omit cystine and this renders all the data almost useless. The greatest obstacle to the use of chemical values is the question of availability. Chemical analysis shows the amino acids present after hydrolysis, and this is often not the same as that quantity biologically available to the animal. The problem appears to be solved in the case of lysine, where chemical analysis of "available lysine" agrees well with that biologically available. Little is known, however, of the availability of methionine and cystine, nothing is known of any chemical method of measuring this, and further, the analyses for methionine and cystine appear to be, in many laboratories, unreliable. We are thus still forced to rely on biological methods. An examination of some of the amino acid analyses available in the literature suggests that biological

methods, in addition to giving more information, may even be more accurate than chemical analysis.

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DISCUSSION

DR. LONGENECKER: I just wanted to offer my congratulations to Dr. Bender for the fine work they have been doing and to point out a little bit of the work we have been doing.

We have been doing some amino acid studies where we show a direct relationship between the plasma amino acid and the amino acid composition of the protein. We have started recently, still very preliminarily, and I was struck by the correlation we have here with your work.

When we were testing whole albumin with human subjects we found that actually whole albumin did not seem to be as ideal as we thought it should be, because we postulated our plasma amino acid changes should be essentially all equal if all the amino acids are in the proper proportions. We found that methionine and cystine appeared to be lower for the human subject (you were using the rat), that lysine and valine were in excess to a greater extent, and that tryptophan and threonine were also in excess. I would be interested in being able to complete our work and compare it with yours further.

DR. ROSENBERG: I would like to raise two points. No. 1, in your report on BV, I suppose you are using NPU's rather than total BV. No. 2, when you gave us figures for peanut meal to which you have added various amino acids, you have not mentioned the levels of the amino acids you have added. That is, of course, a very important item. I cannot take the time to go into details, but I can well visualize that the failure of your experiments is merely due to the fact that you have not used the proper levels.

DR. BENDER: With regard to BV and NPU, we use the carcass analysis method which gives us NPU, that is to say, the percentage of the eaten protein. At the same time, we determine digestibility and, by dividing one into the other, we achieve BV, which is the percentage of the absorbed protein that is retained. The figures used are all BV's, not NPU's, because if the material hasn't been digested, then we wouldn't expect it to correlate.

On the second point, the level of supplementations of amino acids, we usually use half the gap between the protein being tested and the target figure. There is no point in adding the whole, because of the second bottleneck. We are always dealing with relatively small amounts, never exceeding the quantity that would be present in the whole protein.

DR. PATWARDHAN: I wish to ask Dr. Bender whether he has had the experience of determining NPU's of protein in the natural state in which the matter is complicated by tryptic inhibitor. Have you tried soybeans or one of the beans when your CS and BV probably would be affected by the presence of the tryptic inhibitor in the sample?

DR. BENDER: It is generally accepted that raw soybean has a lower NPU because of the tryptic inhibitor. As soybean is heated the NPU rises to a maximum,

and at that point all the inhibitor is apparently destroyed. With further heating the NPU begins to fall as the material is damaged. That is said to be true also of peanut, but most of the peanut samples that we have examined have been heated, and if there were a tryptic inhibitor present, it is destroyed.

We get lower answers in general with tryptic inhibitors present. There is also the possibility that we get lower answers, or certainly badly reproducible answers, if there are toxic factors present. About that there is very little known. We are beginning to believe that when a material is toxic for any reason, we get not so much a lower answer as very great variability between different animals.

DR. HARPER: I just want to haggle over one or two points. One is, Dr. Bender sometimes tends to overlook the problem of food intake. We don't dispute at all, I think, the work with crystalline amino acid diets, that you can get very high BV's with these, whether you determine BV or NPU. At the same time, it is very difficult to get the food intake of animals on these diets up to that of animals receiving whole protein. I think this is a point that is overlooked in some of the studies of NPU and BV.

Another one is with regard to the requirement of young, growing rats for lysine, which Dr. Rose determined as very close to Rao's figure and which we have also shown to be very close to this figure. It happens to be one of the amino acids which shows a very great drop as the animal matures. So I suspect probably everybody is right in this case.

The other point, I should like to emphasize that one of the things that comes out of this study is that one of the big gaps in our nutrition work at the present time is information about the availability of amino acids from proteins, the effect of treatment, the effect of processing on the availability, the problem that Dr. Patwardhan mentioned of tryptic inhibitors, and the actual lack of availability because of poor solubility of some of the natural plant proteins.

DR. BENDER: I agree with those two points. We are measuring the quantity of lysine, let us say, for nitrogen balance when "protein" is fed at 10%. Other workers have measured the particular amino acid makeup for maximum growth. Therefore, we might not expect to agree. Surprisingly we do agree, although we are measuring two different things.

We were very gratified to hear Dr. DeMaeyer's paper 2 days ago in which the same proteins had been tested on children. Our measurement, which can be called an analytical laboratory procedure, does agree with his experiments on children.

I would offer one word of self-warning on that. I think every protein that we have both examined has been limited by methionine and none by lysine. I would like to know whether we shall get agreement between children and rats when lysine is the limiting amino acid.

The third point was that of the quantity of intake. For BV and NPU estimations, the answer is quite independent of food consumption, but for

growth experiments quite clearly the food consumption is most important, and also for PER's. I have in the past criticized PER's because, if for any reason the animal's appetite is reduced, then the answer is artificially low. We always find, incidentally, that the consumption by the rat is approximately proportional to the nutritive value of the protein. The rats eat more of a better quality protein diet and less of a poor quality protein diet. But this does not hold for amino acid mixtures. When fed these, they eat only half what we would expect. Therefore, when people are measuring growth on an amino acid mixture and do not achieve the same growth as they would on protein of the same composition I suggest that it might sometimes be due to poorer food intake.

The Concept of Amino Acid Availability and its Bearing on Protein Evaluation

Jean Mauron

THERE IS LITTLE doubt today that protein quality is primarily fixed by its essential amino acid content. Nevertheless, evaluation of the nutritive value of a protein from knowledge of its amino acid composition still leaves much to be desired. Two main reasons may be put forward for this. First, although we know the approximate amino acid needs of several species, these needs are variable according to the metabolic state of the subject (Henry and Kon, 1958; Tremolières 1959) and second, amino acid content as revealed by classical amino acid analysis does not necessarily reflect amino acid availability to the organism. We are concerned here with this second point only. The basic assumption implied in all methods of chemical scoring for evaluation of protein value is that the total amount of amino acid as determined by classical methods is available to the organism. There is, however, good experimental evidence that in certain foods a proportion of the analytically determined amino acids may not be available for assimilation. Thus Gupta et al. (1958) found that lysine availability to the weanling rat was only about 50% for corn, 70% for wheat, 85% for rice, 90% to 95% for spray-dried milk powder and 68% for a roller-dried milk sample.

Using *in vitro* digestion procedures, several workers (Eldred and Rodney 1946; Pader et al. 1948; Hankes et al. 1948; Evans and Butts 1949) found that lysine and, to a lesser extent, methionine and tryptophan are made unavailable when proteins are submitted to severe heat treatments. Even under less severe conditions, as encountered in milk processing (Mauron et al. 1955) or milk storage (Lea and Hannan 1950), in extraction of oil seeds (Riesen et al. 1947; Mauron and Bujard 1960) and in industrial manufacture of fish flour (Carpenter et al. 1957), lysine, and to a lesser extent methione, may be made less available.

In several of these studies (Lea and Hannan 1950; Riesen et al. 1947; Mauron et al. 1955) it was shown that the usual chemical amino acid analysis after acid hydrolysis is unable to detect correctly the decreased availability. In these processed foodstuffs the assumption that amino acid content equals amino acid availability therefore does not hold. This is one of the reasons why the concept of amino acid availability must be introduced and means have to be found for its determination.

The availability of a given amino acid may be defined as the amount or percentage of that amino acid in the food which is utilized for protein synthesis in

the organism (growth or maintenance), when this amino acid is the only limiting factor of the diet.

This definition excludes all situations where the amino acid is not used efficiently for protein synthesis because other amino acids or factors are lacking or because there is an amino acid imbalance.

It includes, however, all cases where an amino acid, although absorbed through the intestinal wall, is not used for protein synthesis because of a retarded absorption (Cannon et al. 1947; Geiger 1947) due to a delayed liberation of the amino acid from certain proteins or because it is absorbed in an inactivated form. It follows that the non-reappearance of an amino acid in the feces does not necessarily mean that the amino acid is available.

To be sure, the introduction of the concept of amino acid availability does not dispense with the usual chemical amino acid analysis, but the latter should be considered as a first approximation only as long as nutritional problems are involved. Most people will agree that the introduction of the concept of amino acid availability is theoretically sound. However, opinions diverge on the question of how far this concept may be used in practice to improve the chemical scoring of proteins.

The difficulties encountered in trying to measure amino acid availability are formidable. Devices to estimate *in vivo* availability of individual amino acids have been developed by several authors. Schweigert and Guthneck (1953, 1954) determined the availability of lysine and methionine using the growth of protein-depleted rats as a basic criterion for this value. Deshpande et al. (1957) evaluated the availability of isoleucine using growth response in young rats. Gupta and collaborators measured availability of amino acids on the basis of both growth response and amino acid determination in food and feces of rats (lysine, Gupta et al. 1958; tryptophan, Gupta and Elvehjem 1957). Kuiken and Lyman (1948) and Kuiken (1952) estimated the availability of the 10 essential amino acids in different foods by measuring the amount of the ingested amino acids excreted in the feces. Watts et al. (1959) attempted to evaluate amino acid availability using human subjects. They computed the percentage of the availability of amino acids by determining the amount of each amino acid excreted in the feces.

In regard to these *in vivo* availability determinations, the interpretation of any study involving the amino acid content of feces is limited by the ability to measure the extent of degradation and synthesis of amino acids by intestinal bacteria. Also, we have just seen that an amino acid may be absorbed through the intestinal wall and yet not be available. Even the gross implications of data based on fecal amino acid excretion must, therefore, be viewed as tentative. The methods based on growth response are, from the theoretical point of view, the most valid ones, since they satisfy the definition for availabity. However, to test the availability of a given amino acid, it must be the sole limiting factor of the basic diet. Since in practice it is almost impossible to find a protein devoid of one essential amino acid, all the others being present in adequate amounts, the basic diet always contains a relatively unbalanced protein which has to be corrected for its deficiencies with synthetic amino acids, except for the amino acid to be tested, which is kept

at a low level. Another possibility is to utilize only synthetic amino acids for the basic diet, with protein being supplied by the food under investigation. In each case pure amino acids as well as protein are the dietary source of essential amino acids, so that in case of a slow digestibility of the protein the failure of all essential amino acids to be present simultaneously (Cannon et al. 1947; Geiger 1947) may reduce the utilization of the latter by the organism and thus impair the test for their availability.

Other difficulties arise because the amino acid requirement may be influenced by the type of dietary carbohydrate (Gupta et al. 1958). Therefore, not only must the conditions of the *in vivo* availability be carefully checked, but for each essential amino acid the method has to be specially adjusted. The determination of the availability of the 8 essential amino acids in this way would represent a huge task. Even if one confines oneself to the 2 or 3 most important amino acids, the work involved remains extensive and unpractical for serial analyses.

Several authors tried, therefore, to measure amino acid availability *in vitro*. This may be performed by enzymatic or chemical methods. Among the former, a series of *in vitro* digestion procedures has been developed, some of which have already been cited (Eldred and Rodney 1946; Pader et al. 1948; Hankes et al. 1948; Evans and Butts 1949; Riesen et al. 1947). These methods were not intended to establish analytical procedures, but to demonstrate that amino acid availability and amino acid content differ sometimes quite strikingly.

Enzymic *in vitro* analytical procedures were developed by Sheffner et al. (1956) and Mauron et al. (1955). The former determined the pattern of essential amino acids released by pepsin, the latter the release of the key amino acids, tryptophan, methione and lysine by pepsin and pancreatin. The results obtained with the method of Sheffner et al. showed remarkable agreement with biological values determined by Mitchell and Beadles (1950) in adult rats. There was, however, poor agreement for many proteins with the chemical score calculated by Oser (1951). The volume of work involved in the procedure of Sheffner et al. is considerable indeed, since 10 amino acids have to be determined in the acid hydrolysate as well as in the pepsin digest. It is, therefore, questionable whether this method may ever be used for large series of determinations. By confining itself to the 3 amino acids most likely to be limiting factors in foodstuffs, the method of Mauron et al. reduces substantially the amount of work involved. This procedure has been used extensively in the quality control of heat-processed milk, and the agreement with the protein efficiency ratio measured on growing rats has been excellent (Mauron and Mottu 1958).

To our knowledge the only useful *chemical* method developed to measure amino acid availability is that employing Sanger's reagent (Carpenter and Ellinger 1955; Bruno and Carpenter 1957) * for determining available lysine in foods. It makes use of the reaction of fluorodinitrobenzene (F-DNB) with free amino groups in proteins. ϵ-dinitrophenyl-lysine (ϵ-DNP-lysine) released after subsequent hydrolysis is measured colorimetrically. This method showed good agreement with

* Bensabat et al. 1958.

biological value in a wide range of processed foods like milk powder, fish flour, cottonseed flour and peanut flour.

Closing this necessarily incomplete survey, we find that reproducible analytical methods to measure amino acid availability are very few, although quite an amount of work has already been done on this subject. We retained two methods which lend themselves to use on a large scale with relatively good reproducibility, the chemical procedure of Carpenter and our enzymic *in vitro* digestion, and applied them to processed foods.

So far, the problem of amino acid availability as discussed in this report might be found to be a rather academic one and to have but remote connection with the subject of this conference, namely protein malnutrition. However, if one considers the list of additional high-protein foods selected for study by UNICEF to be used in the prevention of protein malnutrition one reads, in the same order, fish flour, soybean products, peanut flour, sesame flour, cottonseed flour and coconut. All these foodstuffs, as well as skim milk powder which is extensively used in curing kwashiorkor, are heat-processed foods. The sole determination of amino acid content is, therefore, not enough for judging the value of these high-protein foods, as processing may impair amino acid availability more than it does amino acid content.

The aim of this report is to demonstrate the usefulness of the concept of amino acid availability on three examples, namely the quality control of processed milk, peanut flour and high-protein biscuits.

EXPERIMENTAL PROCEDURE

For all experimental details as well as standard errors, the original publications should be consulted (Mauron et al. 1955; Mauron and Mottu 1958; Mauron et al. 1960; Mauron and Bujard 1960; Mauron and Mottu 1960). We shall briefly set forth here the principle of the *in vitro* digestion procedure and Carpenter's method for available lysine.

Overall digestibility and availability of tryptophan, methionine and lysine were determined by *in vitro* digestion with simultaneous dialysis. An amount of sample corresponding to 8.4 gm protein is first dialyzed against tap water to eliminate low-molecular substances and then digested in the dialysis bag with 50 mg pepsin at a pH of about 2 and at 37° C. After 15 hours pepsin digestion the pH in the dialysis bag is adjusted to 8 and 200 mg pancreatin are added. The digestion is now continued for 24 hours at 50° C under stirring. The pH is kept between 7.5 and 7. The dialyzed fractions containing the amino acids are siphoned every hour and analyzed by appropriate means. Tryptophan and methione are determined colorimetrically, lysine with a specific decarboxylase and amino nitrogen gazometrically according to van Slyke. The methods for tryptophan and lysine are specific for the free, completely unsubstituted amino acid.

The availability of lysine was also determined with fluorodinitrobenzene (F-DNB) according to Carpenter, 1958. The principle of this method is: since the reduced availability of lysine seems to be largely due to its ϵ-NH_2 group combining with other active groups under conditions of moist heat to form a linkage

that resists hydrolysis by enzymes but not by acids, only lysine molecules with reactive ϵ-NH_2 groups are nutritionally available. Lysine with a reactive ϵ-NH_2 group yields a coloured ϵ-DNP-compound. N-terminal amino acids form α-DNP-derivatives which are previously eliminated by ether extraction, the ϵ-DNP-lysine remaining in the aqueous phase.

In processed milk we utilized the original method of Carpenter and Ellinger (1955) designed for animal proteins. In peanut products the modification for vegetable proteins using the extra separation stage with methoxy-carbonyl chloride (Bruno and Carpenter 1957) was adopted. Histidine interferes slightly with this method.

For those interested in the determination of available lysine in foods, we may mention that recently Carpenter modified his method so that it may be used for animal and vegetable material as well without interference from histidine (Carpenter 1960). In this recommended modification, the methoxy-carbonyl chloride stage is done only to obtain a blank value for non-lysine interfering colour. This method is called "corrected straight-acid" procedure.

RESULTS AND COMMENTS

Processed Milk

Amino acid content. The study of amino acid availability in milk requires special attention for two main reasons: first, milk used to cure or prevent protein malnutrition in underdeveloped countries is for the most part processed milk which has often been stored for a long time; second, the results with milk are especially clear cut and best illustrate the concept of amino acid availability.

Amino acid content of fresh milk and several types of processed milk is given in table 1.

TABLE 1
AMINO ACIDS IN PROCESSED MILK
(gm amino acid per 100 gm protein)

	Amino N	Trypto-phan	Tyrosine	Methio-nine	Lysine
1. Fresh and boiled milk	11.6 ± 0.04 [a]	1.6	5.0	3.2	8.3 ± 0.05
2. Spray-dried milk *A* and *B*	11.6	1.6	5.0	3.2	8.0 ± 0.07 [b]
3. Roller-dried milk *A*	11.5	1.6	5.0	3.3	7.2 ± 0.10 [c]
4. Roller-dried milk *B*	10.9 ± 0.15 [c]	1.6	4.8	3.3	6.1 ± 0.05 [c]
5. Evaporated milk *B*	11.6	1.6	4.9	3.1	7.6 ± 0.07 [c]
6. Sweetened cond. milk	11.6	1.5	4.9	3.2	7.9 ± 0.08 [c]

[a] Standard error of the mean = $s/\sqrt{n}$.
[b] Difference between sample and fresh and boiled milk significant at the 5% level.
[c] Difference between sample and fresh and boiled milk significant at the 1% level.

Processing of the milk does not alter the content of tryptophan, tyrosine or methione. Amino nitrogen is affected in the slightly scorched roller-dried milk B. Lysine is always reduced by processing operations. In evaporated milk lysine destruction amounts to 8%, in usual commercial roller-dried milk to 13% and in slightly scorched sample to 27%. As a whole, even for lysine, the modifications by processing are relatively small.

In vitro digestion. When milk is submitted to the *in vitro* digestion procedure and the percentage of dialyzed amino acids is plotted against time, a graph is obtained as shown for lysine in figure 1.

The liberation of lysine sets in as soon as pancreatin has been added. The plateau observed after 23 hours of digestion is an artifact due to the reduced dialysis rate during the night.

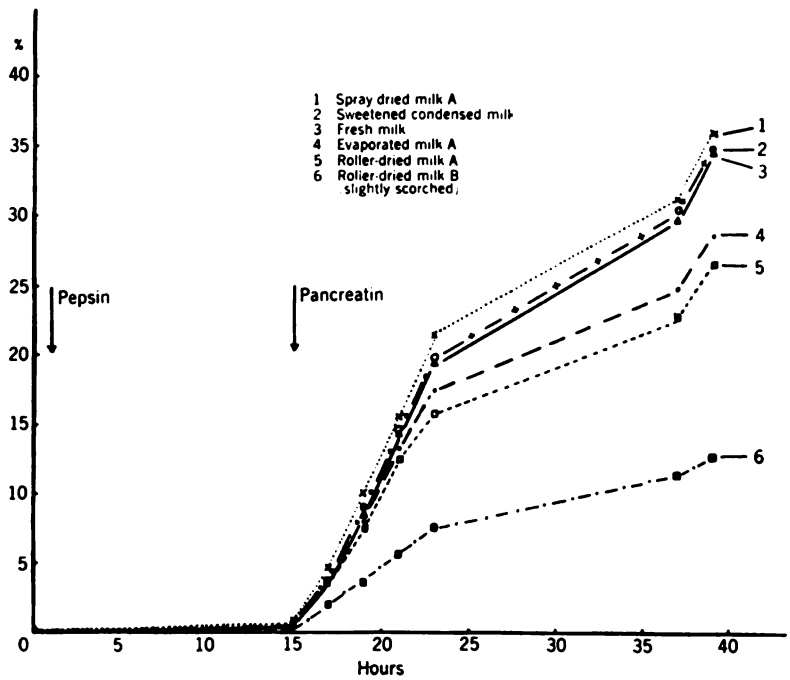

Figure 1—Liberation of lysine by *in vitro* digestion as percentage of dialyzed amino acids.

Although the percentage liberation of lysine is represented here as the percentage of the lysine actually present in the sample, so that lysine destruction is already accounted for, several processed milk samples showed a lower lysine liberation than fresh milk throughout the digestion-dialysis. The rate of digestion was, however, the same in all samples. It follows that in those samples showing lower liberation lysine is present in a blocked or inactivated form which is not attacked by the digestive enzymes. We can, therefore, express the results of the *in vitro* digestion in terms of inactivation of lysine. There is no lysine inactivation

in fresh milk, sweetened condensed milk and spray-dried milk powder. Some inactivation occurs in evaporated milk and in commercial roller-dried powder, and in the slightly scorched powder the inactivation is quite important.

For the other amino acids the *in vitro* digestion yields the following results: There is no inactivation whatsoever of tryptophan and tyrosine in any milk sample tested. Methionine is inactivated in roller-dried powder only, 11% in the commercial sample and 20% in the scorched sample.

The results obtained for lysine availability in processed milk are shown in table 2. The exact definitions of the words employed are given below the table in a form which explains how the values were computed.

TABLE 2
IN VITRO LYSINE AVAILABILITY IN MILK

	De-struction [a] %	In-activation [b] %	Deterior-ation [c] %	Avail-ability [d] %
Fresh milk	0	0	0	100.0
Boiled milk	0	5	5	95.0 [e]
Spray-dried milk A	3.6	—3.4	0.2	99.8 [e]
Spray-dried milk B	3.6	0	3.6	96.4 [e]
Roller-dried milk A	13.2	20.0	33.2	66.8 [f]
Roller-dried milk B slightly scorched	26.6	45.8	72.4	27.6 [f]
Evaporated milk B	8.4	11.2	19.6	80.4 [f]
Sweetened condensed milk	4.8	—1.6	3.2	96.8 [e]

[a] Difference between lysine content of fresh milk and that of the sample, determined after acid hydrolysis, expressed in per cent of lysine content of fresh milk.
[b] Difference between lysine deterioration and destruction.
[c] Difference between amount of lysine liberated enzymically from fresh milk and that freed from the sample, expressed in per cent of lysine liberated from fresh milk.
[d] Lysine freed enzymically from the sample, expressed as per cent of lysine liberated from fresh milk.
[e] Availability not significantly different from that of fresh milk.
[f] Availability in sample significantly different from that of fresh milk at the 1% level.

From table 2 we derive the practical conclusion that the processing of spray-dried milk powder and sweetened condensed milk does not alter lysine availability, while the manufacture of evaporated milk and roller-dried milk powder does. The diminution of lysine availability is due one third to destruction and two-thirds to inactivation, a relationship which has been found to be constant in many more samples of processed milk than are mentioned here. Hence, the *in vitro* digestion procedure yields very clear-cut results for lysine in milk. We propose to extend the nomenclature introduced here for lysine to the other amino acids. The word "destruction" should be reserved for the disappearance of an amino acid as determined after acid or alkaline hydrolysis of the protein. The expression "inactivation" should denote the fact that an amino acid which is still measurable after chemical hydrolysis is not set free any more by the digestive enzymes. Finally, "deterioration" should designate the fact that an amino acid is rendered unavailable by processing, whatever the mechanism of this deterioration may be. Strictly speaking, these definitions apply to *in vitro* work (chemical and enzymic), whereas availability may be related to both *in vitro* and *in vivo* experimentation.

Comparison of results obtained by the enzyme, chemical and *in vivo* procedure of Gupta et al. (1958) is shown in table 3.

Enzymic *in vitro* digestion best allows prediction of *in vivo* lysine availability, while the Carpenter F-DNB method gives good correspondence as long as lysine availability is not too low.

TABLE 3
DIFFERENTIAL ANALYSIS OF LYSINE IN MILK
(in % of protein)

	Lysine content	Lysine availability enzymic	Lysine availability with F-DNB	in vivo (rats)
Fresh milk	8.3	8.3	8.0	—
Milk powder (spray)	8.0	8.3	7.9	8.0
Evaporated milk	7.6	6.2	6.7	6.4
Milk powders:				
roller-dried, commercial	7.1	5.4	6.0	5.8
roller-dried, commercial lower quality	6.8	4.5	5.3	4.6
roller-dried, slightly scorched	6.2	2.3	4.0	1.9

These findings have a practical implication for the quality control of skim milk powder so widely used in underdeveloped countries. Part of this skim milk powder is still of roller-dried quality and part is used after long storage. Storage under humid and hot conditions is injurious to lysine, as was shown by Lea and Hannan (1950).

Peanut Flour

Peanut flour prepared from peanut press cakes is an important potential protein source for underdeveloped countries. World production of peanuts or groundnuts reached 13.4 million tons in 1957 (FAO Yearbook 1958). The primary objective of peanut culture is the oil yield, which leaves the protein-rich press cake as a relatively cheap byproduct.

In order to be utilizable for human consumption, peanut flour has to meet certain specifications of purity and composition that have been elaborated by UNICEF jointly with WHO and FAO. An investigation of protein quality of peanut flours related to the manufacturing conditions is rendered difficult by the fact that these conditions are rarely known. UNICEF organized, therefore, the analysis of a series of peanuts and peanut flours manufactured under well defined conditions. Having collaborated in this study, we may briefly summarize our results as far as they are of general interest.

Description of samples analyzed. Two samples of peanuts, one of the Spanish type from Georgia (USA) with the code no. P1-C3 and the other from South Africa and designated P-4, were analyzed. Of the five peanut flours under investigation, three, namely PF-1, PF-2 and PF-3, had been prepared from the same peanuts P1-3C under varying heating conditions.

Peanut flour PF-4 was prepared from peanuts P-4 under mild conditions, since the temperature of the cake emerging from the expeller never exceeded 108° C.

Peanut flour PF-5 was produced from peanuts P-5 of Senegal (French West Africa). The temperature of the oil emerging from the expeller was about 105° C, whereas in the expeller the temperature reached 140° C. We had no sample of peanuts P-5 for analysis, so that we were not able to compare the flour PF-5 directly with the original peanuts. For more details of the manufacturing conditions of these flours, see Mauron and Bujard 1960.

Amino acid content. A complete amino acid analysis was performed in peanuts P1-3C and P-4, as well as in flours PF-1 and PF-2 using the Moore and Stein chromatographic technique (1951). Tryptophan and the basic amino acids were determined in samples PF-3, PF-4 and PF-5. The results are summarized in table 4.

TABLE 4
AMINO ACID CONTENT OF PEANUTS AND FLOURS
in % of protein (Nx6.25)

	P1-3C	PF-1	PF-2	PF-3	P-4	PF-4	PF-5
Aspartic acid	11.0	10.9	11.2		11.1		
Threonine	2.9	2.8	3.0		2.9		
Serin	5.1	4.8	4.9		4.8		
Glutamic acid	18.5	18.9	18.5		18.8		
Glycine	5.5	5.5	5.6		5.4		
Alanine	3.8	3.7	3.7		3.6		
Valine	4.2	4.1	4.4		4.5		
Methionine	1.2	1.0	1.1		1.0		
Isoleucine	3.3	3.4	3.7		3.6		
Leucine	6.2	6.2	6.7		6.3		
Tyrosin	4.3	4.2	4.2		4.2		
Phenylalanine	5.0	5.0	5.5		5.7		
Tryptophan *	1.2	1.0	1.1	1.0	1.1	0.9	1.0
Lysine	*3.19*	*2.89*	*3.18*	*3.01*	*3.72*	*3.41*	*3.19*
Histidine	2.31	2.40	2.28	2.32	2.57	2.28	2.38
Arginine	10.3	10.6	10.2	11.1	11.1	10.2	10.8

* Determined colorimetrically according to Portner and Högl (1953).

No significant differences in amino acid content are apparent except for lysine, the content of which is higher in peanuts P-4 from South Africa. Lysine content is somewhat reduced in peanut flours according to the heat applied during oil extraction. However, even in the most heated flour PF-1, the lysine content is but 9% lower than in the original peanuts. Usual amino acid analysis is, therefore, not very efficient for detecting possible differences due to processing.

In vitro digestion. In submitting the peanut products to the *in vitro* digestion procedure, difficulties were encountered, since the digestibility of the raw peanuts was less than that of the peanut flours in the products derived from P1-3C but not from P-4. This may be explained by a different content in antitryptic factor of peanuts P1-3C and P-4. The presence of an antitryptic factor in raw peanuts was demonstrated by Borchers and Ackerson (1950).

The enzymic liberation of amino nitrogen and lysine in the peanuts P1-3C and the products produced from them proceeds in a parallel manner. It is first augmented with increasing heat treatment and reaches its highest value in flour PF-2. With further increase of heat processing it diminishes again. The explanation of this phenomenon is probably the following: The first effect of heat treatment is to denaturate the protein and to destroy the antitryptic factor, thus increasing the digestibility. The second effect is to inactivate the ϵ-amino group of lysine that results in decrease of lysine liberation as well as of protein digestibility, because the number of active centers for trypsin action is reduced in the protein molecule. We do not mention here the detailed results of the *in vitro* digestion of all samples, as this procedure is not the method of choice for determining lysine availability in peanut products, although it gives interesting information on digestibility.

Comparison of lysine availability by the F-DNB method (Bruno and Carpenter, 1957) and NP-4 values (Miller and Bender, 1955) are shown in table 5.

TABLE 5
LYSINE CONTENT AND AVAILABILITY AS COMPARED WITH NET PROTEIN UTILIZATION IN PEANUT PRODUCTS

	P1-3C	PF-1	PF-2	PF-3	P-4	PF-4	PF-5
Lysine content % of protein	3.19	2.89	3.18	3.01	3.72	3.41	3.19
Lysine availability (F-DNB)	*2.71*	1.97	2.61	2.03	*3.0*	2.57	2.00
NPU	*47*	32	41	35	*47*	45	37

The higher value for "available lysine" in peanuts P-4 reflects their higher lysine content. The difference found between lysine content and "available lysine" in the raw peanuts does not necessarily mean that in the latter some lysine is present in an unavailable form, but might be due to some ϵ-amino groups being sterically hindered from reacting with F-DNB in raw peanuts. Be it as it may, it is obvious from table 6 that Carpenter's procedure allows us to detect differences due to manufacturing more efficiently than does the usual analytical method; thus, the difference in lysine availability between the low-heat flour PF-2 and the overheated PF-1 is 25%. The values (x) for "available lysine" correlate well with those (y) for NPU. The regression equation is $Y = 7.43 + 13.7x$. It permits estimation of the nutritive value of peanut protein from lysine availability with good accuracy. The correlation coefficient (0.93) is significant at the 1 o/oo level.

Although lysine availability is lower in peanuts P1-3C than in P-4, NPU has the same value of 47. This may be explained by the fact that methionine (+ cystine) and not lysine is the limiting amino acid in raw peanuts.

According to our data, lysine becomes limiting when its availability falls below a value of about 2.7% to 2.5%. Certainly, the three peanut flours with an "available" lysine value of about 2.0% have a significantly reduced nutritive value. Note also that peanuts P1-3C have the same lysine content as flour PF-5, yet lysine availability and NPU are much lower in PF-5.

We propose to introduce Carpenter's method for "available lysine" as a routine method for the quality control of peanut flours to be used for human consumption in underdeveloped countries. A minimum "available lysine" value of

2.5% of the protein should be recommended for these flours. This would correspond to a NPU value of 42 according to our regression equation.

High-Protein Biscuits

The biscuit is a suitable form for utilization of a high-protein food complement, except for small infants. It allows efficient control of supplementary food intake and is easy to distribute in schools, homes etc. However, in the preparation of ordinary biscuits, intense heat treatments are involved which might well deteriorate certain amino acids so as to make the value of this form of high-protein food complement illusory. We studied, therefore, the effect of biscuit preparation on the key amino acids (tryptophan, methionine and lysine) and on nutritive value in a series of biscuits prepared from the same initial mixture. The effect on amino acids was measured by analysing amino acid content and availability, the effect on nutritive value by determining NPU. Amino acid availability was evaluated by *in vitro* digestion only, as there were no more samples of these biscuits left when Carpenter's method was introduced in our laboratory. For experimental details reference is made to the original publication (Mauron et al. 1960).

Preparation. The composition of the biscuits is given in table 6. In this formula, about 1/3 of the proteins comes from fish flour, 1/3 from peanut flour and 1/3 jointly from yeast, skim milk and corn. The formula as such is not to be discussed here, the only point under investigation being the heat effect.

TABLE 6
COMPOSITION OF BISCUITS

Ingredients	%	Protein %
Fish flour	9.31	7.6
Corn flour	22.75	1.8
Peanut flour	14.25	6.7
Skim milk powder	5.70	2.0
Dried yeast	5.70	2.8
Sugar	28.50	—
Peanut oil	8.40	—
Coconut	4.48	0.4
Salt and flavour substances	0.91	—
	100.00	21.3

From this mixture biscuits were made according to two procedures:

I) Preparation of a dough with water, addition of bicarbonate and cooking in an oven in the usual manner.

II) Preparation of a diluted paste without the sugar in a mixer, cooking for an hour and subsequent drying in the mixer, until the humidity drops to 18%. (This takes about two hours and the temperature falls slowly from 98° to 60° C.) Addition of sugar, pressing of the mass in biscuit form and final drying in hot air stream (air temperature max. 70° C).

TABLE 7
PREPARATION OF BISCUITS

Sample	III	IV	V	VI	VII
Cooking in oven					
Temperature	140°C	140°C	170°C	170°C	170°C
Duration	8′	8′	5′	8′	16′
Thickness mm	4.9	3.7	4.0	3.8	7.6
Taste	well done	crisp	well done	slightly roasted	well done

The raw mixture (Sample I) was used to prepare biscuits III, IV, V, VI, and VII under conditions summarized in table 7, and the amino acid content is indicated in table 8.

In the pressed biscuit (sample II) no destruction of any of the 3 amino acids occurs. In biscuits cooked in the oven, methionine and lysine are destroyed according to the intensity of the heat treatment. Tryptophan content is not altered at all. The term destruction has been put in quotation marks in the case of tryptophan and methionine, because it does not strictly conform to the definition proposed earlier in this paper, where destruction was related to amino acid content as determined after acid or alkaline hydrolysis of the protein. Neither tryptophan nor methionine was measured here after chemical hydrolysis of the protein, but they were determined colorimetrically within the peptide chain after peptonization of the protein with papain. Since these were accepted methods of determining the 2 amino acids which suffer losses during chemical hydrolysis, we maintained the term destruction.

TABLE 8
AMINO ACID CONTENT AND DESTRUCTION AS DETERMINED IN FIVE SAMPLES OF BISCUITS

Sample	Tryptophan [1]		Methionine [2]		Lysine [3]	
	% of protein	"Destruction" %	% of protein	"Destruction" %	% of protein	"Destruction" %
I	1.05	—	1.64	—	6.88	—
II	1.02	0	1.64	0	6.98	0
IV	1.03	0	1.16	29	4.64	33
VI	1.07	0	0.97	41	4.04	41
VII	1.11	0	1.30	21	5.60	18

[1] Portner and Högl (1953).
[2] Horn et al. (1946).
[3] Gale (1945).

All samples were submitted to the *in vitro* digestion procedure and the results are given in table 9. The values obtained for enzymic amino acid liberation in the unprocessed mixture I were taken as standard of reference for calculation of amino acid deterioration in the biscuits II to VII.

Amino nitrogen and tryptophan deterioration are about the same in all samples, that of methionine is somewhat higher, lysine deterioration is strongest. Although here again lysine is the amino acid most affected by heat treatment, amino acid deterioration is much more uniform here than it was in processed milk, and impairment of digestibility, as measured by deterioration of amino nitrogen, is more important.

TABLE 9
IN VITRO DIGESTION OF BISCUITS
(Enzymic liberation of amino acids in mg per 8.4 gm protein and deterioration in %)

Sample	Amino nitrogen		Tryptophan		Methionine		Lysine	
	Liber. mg	Deterior. %	Liber. mg	Deterior. %	Liber. mg	Deterior. %	Liber. mg	Deterior. %
I	239	—	39	—	104	—	170	—
II	238	0	39	0	100	4	165	3
III	214	10	36	8	88	15	125	27
IV	172	28	28	28	69	34	89	48
V	204	15	35	10	85	18	131	23
VI	131	45	22	44	54	48	66	61
VII	203	15	34	13	86	17	133	22

The only biscuit in which the amino acids were practically unaltered during processing is the pressed biscuit (II).

All biscuits which were cooked in the oven showed some amino acid deterioration proportionate to the heat action. In other words, cooking of the biscuits in an oven reduces amino acid availability in any case.

The *in vitro* digestion procedure is best suited to arrive at this result in an unequivocal way. Determination of amino acid content gives an incomplete answer, although it permits detection of heat damage in case of methionine and, to a certain extent, lysine, but not tryptophan, the content of which is not affected by heat treatment, while its availability is.

For lysine, which is determined after acid hydrolysis of the protein, the term "destruction" holds strictly, and lysine deterioration may, therefore, be attributed separately to destruction and inactivation (table 10). We find then that 2/3 of lysine deterioration is due to destruction and 1/3 to inactivation which is just the reversed ratio of what is found in processed milk.

TABLE 10
LYSINE AVAILABILITY IN BISCUITS

Sample	Destruction	Inactivation	Deterioration	Availability
I	0	0	0	100
II	0	3	3	97
III	20	7	27	73
IV	33	15	48	52
V	—	—	23	77
VI	41	20	61	39
VII	18	4	22	78

In order to correlate *in vitro* amino acid availabilities with the nutritive value of the proteins *in vivo*, NPU was determined according to Miller and Bender (1955) in 4 representative samples, nos. I, II, VI and VII. The results are found in table 11.

The raw mixture and the pressed biscuit manufactured in our pilot plant display identical values for NPU, a result which could be foreseen from the *in vitro* digestion. In samples VI and VII the loss in protein value is quite proportional to the amino acid deterioration as shown in table 9. It could be almost accounted for

TABLE 11
NET PROTEIN UTILIZATION IN BISCUITS

Sample	NPU	Loss in protein value
I	69	—
II	70	0%
VI	33	52%
VII	51	26%

by the diminution of digestibility (deterioration of amino nitrogen) or by the deterioration of any of the 3 key amino acids. Since methionine is almost certainly the limiting amino acid in these biscuits, its deterioration should actually be the immediate cause of impairment of nutritive value.

We infer from this study that in manufacturing high-protein supplements in form of oven-cooked biscuits, very severe controls of amino acid availability are to be made, especially when the mixture contains skim milk powder, as is the case here. In our opinion, the distribution of high-protein supplements in underdeveloped countries as ordinary oven-cooked biscuit cannot be recommended. Whenever feasible, some kind of low-heat biscuit should be prepared, avoiding usual cooking in an oven.

CONCLUSION

If we follow the evolution of protein evaluation by chemical means since Magendie established the indispensability of organic nitrogen in 1816, we notice that the determination of total nitrogen was the sole practical tool for this purpose for about a hundred years. The discovery of the amino acids as essential factors in nutrition (Abderhalden, Osborne, Mendel, Hopkins 1906—1912) opened the way to protein rating by amino acid analysis. It was, however, not until the classic work of Rose (1936) establishing the definitive list of amino acids essential for the rat that amino acid analysis could be used as a solid base for protein evaluation. Since then, several systems for empirical rating of proteins based on essential amino acid content were devised (Block and Mitchell 1946; Oser 1951; Mitchell 1954; *) which agree to a certain extent with biological values determined *in vivo*. Chemical scores should be further improved by taking amino acid availability rather than amino acid content as basic value.

From a practical point of view, however, we notice that, in spite of this remarkable development, nitrogen analysis remains the sole method for measuring the protein value of foods in too many laboratories. The fact that total amino acid analysis is still very expensive and relatively time consuming is certainly a reason for this. Because many laboratories have no facilities for keeping animals and,

* Bender 1960.

even when they do, quick decisions cannot wait for the outcome of feeding trials, the need for rapid *in vitro* methods of protein evaluation is felt. Such a method consists in determining the content of S-amino acids and lysine. This is, however, not entirely satisfactory, as no allowance is made for differences in availability.

We think that a first step in meeting this lack has been made with enzymic *in vitro* digestion and especially with Carpenter's method for available lysine. Time and apparatus required for these procedures are less than for determining total amino acids. In processed foods, they offer the supplementary advantage of being much more efficient in detecting heat damage to proteins than classic amino acid analysis. It is to be hoped that in the near future more and improved methods for measuring *in vitro* amino acid availability may be developed, as this kind of procedure should be of greatest help in countries with reduced laboratory facilities. The results in the quality control of high-protein food supplements obtained with such methods seem to demonstrate the practical usefulness of the concept of amino acid availability.

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DISCUSSION

DR. ALTSCHUL: I would like to congratulate Dr. Mauron on the beautiful demonstration of the differences in nutritive value of things that are called the same, like peanut meals or milk powder. I would like to ask him whether he has had any experience with soybean products in using the Carpenter method for measuring availability.

DR. MAURON: We have not used the Carpenter method extensively in soybean products. We have just made a few trials. So far, even with processed soybeans, we could not find with Carpenter's method the same value as with the Moore and Stein testing. We find, for instance, in soybeans 6.5% lysine according to Moore and Stein, or Gale, and only about 4.5% according to Carpenter in uncooked soybeans and in slightly autoclaved soybeans where the lysine should not be destroyed. We have not done extensive studies, so we cannot give a definitive answer. In soybeans the amino groups of lysine may be partially masked.

DR. HARPER: There is one point I want to make. You dealt very kindly with the biological method, the rat assay method of determining biological availability. We have gradually lost our enthusiasm for this method because we

have found cases in which not only does it measure availability but also apparently there is a measurement included for the poor balance in some of the proteins. We thought we have made corrections for this, but in other cases apparently we are getting a summation of both availability and something that might be termed amino acid imbalance just in the technique itself.

The other point: Do you do your analyses for the free amino acids after the enzymatic digestion by microbiological assay? If so, is there some possibility of there being stimulation by some of the peptides that would still be present in the enzymatic digestion?

DR. MAURON: We did not use the microbiological assay for the amino acid determination after enzymatic digestion. For lysine we use the decarboxylase method, which is specific for free lysine; and for tryptophan and methionine we use colorimetric methods. For methionine we are not so satisfied for the moment. For lysine, the decarboxylase method certainly operates quite well in enzymatic digests. We had a lot of trouble in the amino acid determinations before we performed the digestion with simultaneous dialysis. In the dyalysate, amino acid determinations are much easier.

Amino Acid Imbalance, Chemical Score and Efficiency of Protein Utilization*

A. E. Harper

THE CONCEPT OF amino acid imbalance originated from observations made by Krehl, Elvehjem and their associates[3] when they were attempting to develop a diet low in tryptophan which would be suitable for the study of niacin deficiency in the rat. In one series of experiments they used a diet containing 9% of casein as the basal source of protein and, to raise the overall protein content, they supplemented it with the tryptophan-deficient protein, gelatin. As shown in table 1, the growth rate of the group fed the supplemented diet *ad libitum* was inferior to that of the group fed the unsupplemented diet. This was considered to be a result of an amino acid imbalance due to the addition of gelatin. It was corrected by the addition of either niacin or tryptophan with the gelatin.

TABLE 1
EFFECT OF A SUPPLEMENT OF GELATIN ON THE GROWTH OF RATS FED A DIET CONTAINING 8% OF CASEIN AND 0.3% OF DL-METHIONINE

Supplements	Weight gain gm/2 wks.
None	15
6% gelatin	5
6% gelatin + 2.5 mg. niacin	27
6% gelatin + 0.1% L-tryptophan	32

The effect was thought for some time to be intimately linked with niacin deficiency; but, when other similar examples were observed in experiments that were not complicated by niacin deficiency, it appeared that this might be a general phenomenon. Such effects have since been observed in experiments on a variety of dietary proteins and with mice, rats, dogs, pigs and chicks as experimental animals.[2] Also, two examples from studies on human subjects appear to fall into this category.[6,7]

Such effects can be produced regularly by adding a quantity of a protein (or an amino acid mixture) lacking one indispensable amino acid to a diet that is

* Unpublished results presented in this paper are from studies carried out by L. G. Elias and U. S. Kumta.

low in protein. Similar effects have also been produced by adding a smaller quantity of one of two almost equally limiting amino acids to such diets.

Both of these conditions are illustrated in table 2 for rats fed *ad libitum* on diets containing 6% of beef blood fibrin as the basal source of protein.[8,9] In this protein methionine, phenylalanine, leucine, isoleucine, valine and histidine are almost equally limiting. Leucine, isoleucine, valine and histidine appear to be in greatest deficit; these, grouped as A, may be thought of as the most limiting amino acid. Phenylalanine and methionine appear to be slightly less limiting and, grouped as B, may be considered the second most limiting amino acid.[10] The addition of B (group 2) causes retardation of growth which can be prevented by the further addition of A (group 3). Similarly, if an amino acid mixture lacking histidine is added to the basal diet, growth is retarded and this is prevented by the further addition of histidine (groups 4 and 5). Thus, the overall effect in both of these cases in which the addition of an amino acid mixture to the diet causes an amino acid imbalance is that the severity of an existing amino acid deficiency is increased.

TABLE 2
EFFECT OF A SUPPLEMENT OF METHIONINE AND PHENYLALANINE OR AN AMINO ACID MIXTURE LACKING HISTIDINE ON THE GROWTH OF RATS FED A DIET CONTAINING 6% OF FIBRIN

Supplements	Weight gain gm/2 wks.
1. None	32
2. B [methionine (0.2%), phenylalanine (0.3%)]	20
3. B + A[leucine (0.1%), isoleucine (0.1%), valine (0.15%), histidine (0.05%)]	45
4. Amino acid mixture lacking histidine	13
5. As for group 4 + histidine (0.05%)	33

The two examples differ in one respect. The first type can be demonstrated only if the diet contains close to 6% of fibrin; the second can be demonstrated over a fairly wide range of dietary levels of protein.[9]

Two questions may be raised at this point: 1) Should any growth depression caused by adding amino acids to a diet be attributed to an imbalance? 2) Can amino acid imbalances be demonstrated when the protein content of the diet is adequate for maximum growth?

In answer to the first question, it is evident that any amino acid addition alters the amino acid balance of the diet and that any adverse effect from such an addition could be attributed to an amino acid imbalance. Nevertheless, and this I would like to emphasize, if the term imbalance is restricted to those conditions which can be corrected by a supplement of the amino acid that is most limiting for growth a good deal of confusion can be avoided. This removes from consideration as imbalances two conditions that we have described as antagonisms and toxicities.

The first of these, antagonism, is illustrated in table 3. An excess of L-leucine causes a severe retardation of the growth of rats fed *ad libitum* a diet containing 9% of casein as the source of protein. Supplementation with the most limiting amino acids in the diet does not prevent the depression (group 3, table 3). However,

supplementation with isoleucine reduces the effect of an excess of L-leucine and, if valine is added as well, excess leucine is quite well tolerated. It may be worth noting that growth retardations have been observed when as little as 0.6% of L-leucine has been added to low-protein (5-6%) diets containing rice[11] or fibrin.[12]

TABLE 3
EFFECT OF EXCESS LEUCINE ON THE GROWTH OF RATS FED A DIET CONTAINING 9% OF CASEIN

Addition	Weight gain gm/2 wks.
None	52
5% L-leucine	8
5% L-leucine + (Thr. + Try. + Val. + Lys. + Hist.)	7
5% L-leucine + 1.3% DL-isoleucine	20
5% L-leucine + 0.16% L-iso. + 0.15% L-val.	44

The second, toxicity, is well illustrated by the results shown in table 4 from a paper by DeBey, Snell and Baumann.[13] In this case a high intake of methionine causes a severe depression in growth rate. It is particularly severe when the pyridoxine content of the diet is low but can be demonstrated when both the protein content and the pyridoxine content of diet are more than adequate. Tyrosine in excess may also be toxic and can cause severe eye and skin lesions.

TABLE 4
GROWTH OF RATS FED VARIOUS LEVELS OF DL-METHIONINE AND PYRIDOXINE (Basal diet contained 18% of casein)

DL-methionine added	Weight gain (gm/4 wks.) Pyridoxine (mg/kg diet) 0.6	6.0	30
%	gm	gm	gm
0	78	121	—
0.4	85	131	—
1.0	25	114	—
2.5	3	59	54

We exclude from consideration as amino acid imbalances examples of adverse effects from dietary additions of amino acids that resemble either of the examples cited above. We also exclude from consideration a number of examples in which excesses of one or more amino acids have been shown to cause growth depressions, because these defy classification.

The second question, Can amino acid imbalances be demonstrated when the protein content of the diet is adequate for maximum growth, can be answered by referring to table 5. The basal diet in this experiment contained 11% of casein supplemented with methionine. This is a well balanced protein and provides most of the indispensable amino acids in about the quantities needed for maximum growth of the rat. Addition of an equal amount of nitrogen from gelatin gives a diet with the same quantity of tryptophan but a large excess of other amino acids. Despite its poor balance, such a diet will still support maximum growth. However, when the gelatin level is increased much above this, growth is depressed and an

TABLE 5
EFFECT OF INCREASING INCREMENTS OF GELATIN ON GROWTH OF RATS FED A DIET CONTAINING 11% OF CASEIN AND 0.3% DL-METHIONE

Addition	Weight gain gm/2 wks.
None	87
10% gelatin	88
18% gelatin	73
22% gelatin	50

amino acid imbalance occurs even though the diet still contains the same amount of tryptophan.[14]

These observations parallel very closely observations on the effect of protein level of the diet on individual amino acid requirements, expressed as a percentage of the diet. Figure 1, from a paper by Grau[15] describing a study on chicks,

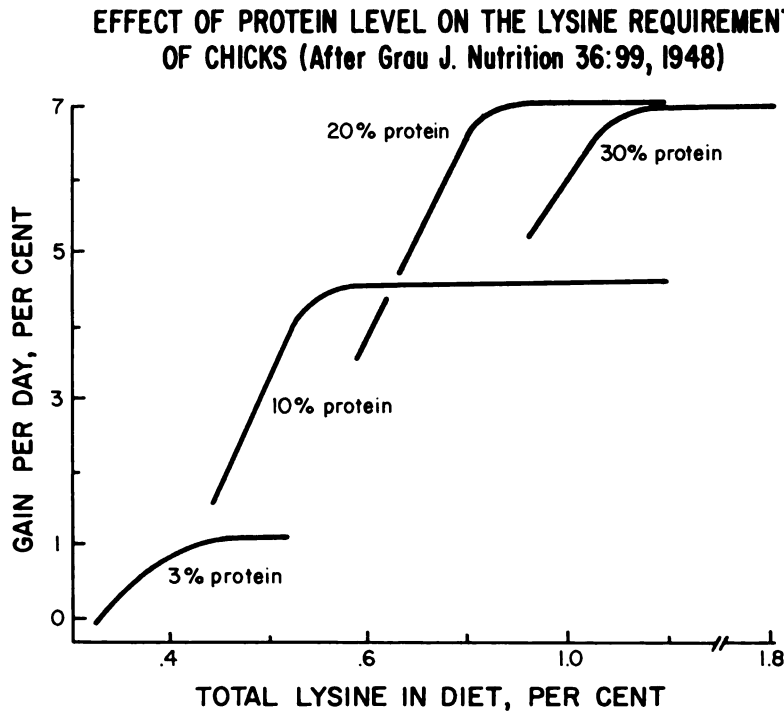

Figure 1—Effect of protein level on the lysine requirement of chicks (after Grau, J. Nutrition *36*:99, 1948).

illustrates this point. The lower curves, of course, do not represent an effect of protein level on amino acid requirement; they merely represent cases in which growth is limited first by a deficiency of one amino acid, then by protein generally. However, the upper curves do show that, if the protein level of the diet is increased sufficiently, a point is reached at which growth is depressed unless the level of the

limiting amino acid is also increased. This situation is very similar to that shown in the previous table and might be considered as the point at which an amino acid imbalance occurs.

The experimental procedure in both of these studies consisted of diluting the balanced portion of the dietary protein with unbalanced protein. This results in the nutritional value of the dietary protein being gradually lowered. Efficiency of protein utilization falls as the dietary protein becomes more unbalanced so the PER, the BV and the CS should all fall in proportion to the degree of dilution. However, a point is reached at which the fall is steeper than would be predicted from the degree of dilution of balanced with unbalanced protein. This is illustrated in figure 2 which shows the relationship between PER determined experimentally

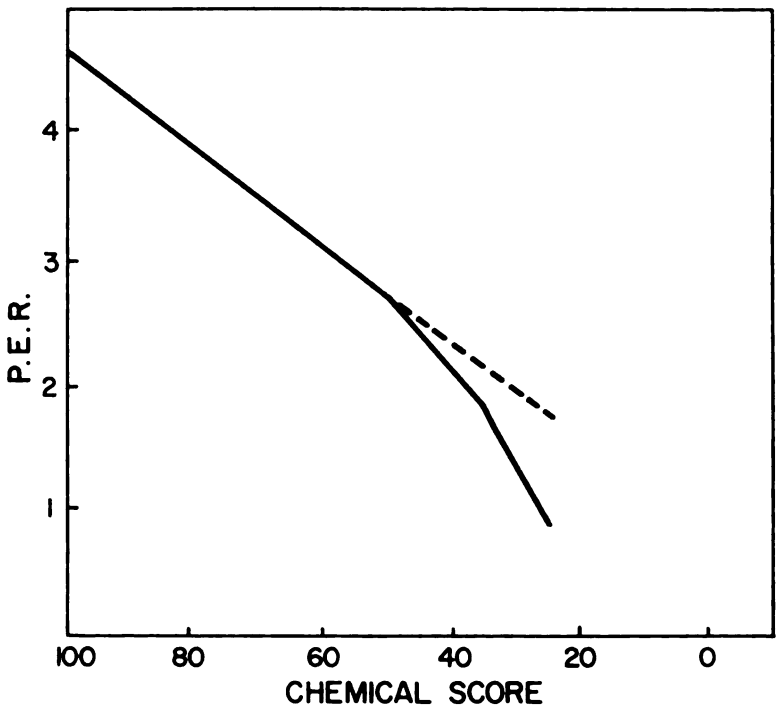

Figure 2—Relationship between PER and CS. Casein supplemented with methionine was arbitrarily given a CS of 100. The methionine-supplemented casein was diluted with gelatin to give protein mixtures with lower CS but the mixtures were included in the diets at levels that would provide 11% of casein. All of the diets, therefore, contained the same percentage of tryptophan, the amino acid in lowest concentration in relation to the requirement of the rat.

and CS calculated from the composition of the dietary protein for a series of diets containing 11% of casein and gradually increasing increments of gelatin. The dotted line represents the theoretical relationship. In appraising these results it should be recalled that all of the diets contained the same amount of the most limiting amino acid, tryptophan, an amount sufficient to satisfy the accepted requirement of the rat.

This brings us to the relationship between amino acid imbalance and efficiency of protein utilization. In general, the amino acid requirements of a subject can be met using a protein of low CS or BV if the quantity of that protein in the diet is raised sufficiently. However, the observations mentioned above lead to the conclusion that there should be a CS or a BV below which optimum performance cannot be obtained even when the dietary level of protein is high enough to satisfy what is considered to be the requirement for the most limiting amino acid in the diet.

An attempt to demonstrate this experimentally is shown in figure 3. The diets for this experiment all contained 11% of casein supplemented with methionine,

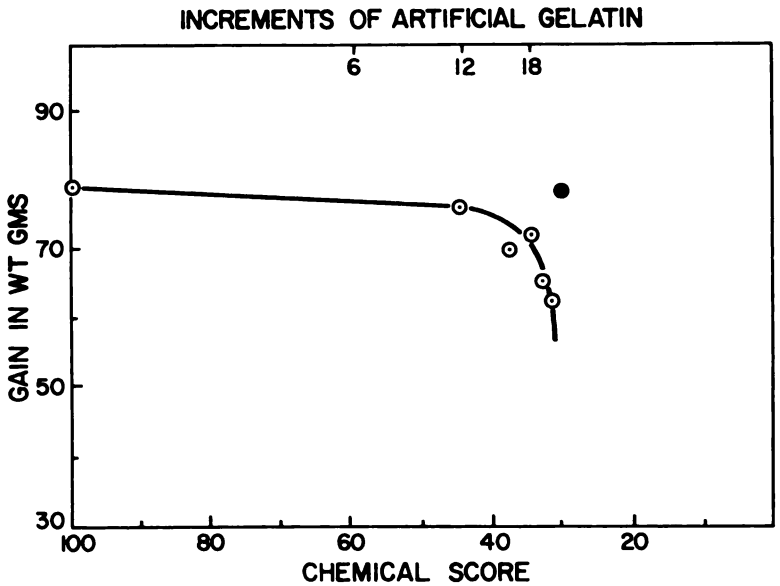

Figure 3—Effect of CS of dietary protein on the growth of rats fed on diets containing the same amount of the most limiting amino acid, tryptophan (see legend of figure 2 for details). The black dot indicates the growth response observed when the diet containing a protein mixture of CS 30 was supplemented with tryptophan. The numbers at the top of the figure indicate the percentage of the gelatin-amino acid mixture included in the diet.

i.e., about 10% of protein. This is sufficient protein to enable the young rat to grow at close to the maximum rate. As it is a well balanced protein and provides just the requirement for most of the indispensable amino acids, we have arbitrarily assumed that it has a CS of 100. Substitution of 10% of a gelatin-indispensable amino acid mixture for part of the carbohydrate gives a diet containing about 20% of protein with a CS of roughly 50, based on the relative concentrations of tryptophan in the casein and in the casein-gelatin mixture. Indispensable amino acids, excepting tryptophan, were added with the gelatin to give an unbalanced protein with about the same ratio of dispensable to indispensable amino acids as is found in most food proteins. By increasing the amount of the gelatin-indispensable amino acid mixture in the diet still further, diets containing protein mixtures with different CS yet all having exactly the same tryptophan content could be prepared. In essence, what

was done was to prepare, artificially, proteins having CS of from 30 to 100 in which tryptophan was the amino acid most limiting for growth. These were then included in a series of diets in such quantities that all of the diets had exactly the same tryptophan content.

As can be seen from figure 3, the growth rate of rats fed *ad libitum* and receiving 20% of a protein mixture of CS 50 was almost the same as that of rats receiving 10% of protein of CS 100. The rat can therefore tolerate a diet of this type that is unbalanced to the extent of 50%. However, as the CS of the protein fell below 50, growth was retarded even though the protein mixtures were included in the diets in quantities sufficient to meet the requirement for the most limiting amino acid, tryptophan. Additional tryptophan, which would increase the CS of the protein, prevented the growth retardation as indicated by the black dot in figure 3. The same pattern of results was obtained when growth was plotted against CS if the casein content of the diets was dropped to 7% or increased to 15%.

It is probable that the CS below which performance is suboptimal will vary with the amino acid composition of the protein mixture and with the amino acid that is most limiting in the diet. Wheat gluten, which is deficient in lysine, appears to be tolerated better than the casein-gelatin mixtures described here even though its CS is well below 50.[16] This may be explained by the observation of Bender[17] that for proteins and amino acid mixtures deficient in lysine, NPU values are considerably higher than those predicted from CS values when the lysine content is very low.

In conclusion, it appears that if the CS of a protein or protein mixture falls below a certain point (about 50 for a casein-gelatin mixture, using casein supplemented with 0.3% DL-methionine as the standard) an amino acid imbalance will occur and the PER will fall below that calculated from a knowledge of the amino acid pattern of the protein. Both the food intake and the growth of rats fed on diets containing such proteins are depressed below the values expected from the concentration of the limiting amino acid in the diet. In order to restore food intake and growth to equal the values obtained for animals fed a diet containing the same concentration of the limiting amino acid from a well balanced protein, it is necessary to increase the concentration in the diet of the amino acid in shortest supply. This can be done either by adding a small quantity of the crystalline amino acid or by increasing the proportion of well balanced protein in the diet.

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Multiple Amino Acid Supplementation of Proteins

Hans R. Rosenberg and R. E. Eckert

AMINO ACID SUPPLEMENTATION of a protein is carried out in order to increase the amount of protein that is effective for tissue growth and maintenance and to decrease the ineffective portion.[9] In some proteins, such as wheat protein, single amino acid supplementation can greatly increase the amount of effective protein,[14] but in others, such as corn and rice protein, multiple amino acid supplementation is needed for good results. If the ideal protein is defined as that in which all essential amino acids are properly balanced against each other and against the needed amount of the nonessential amino acids, then it follows that multiple amino acid supplementation can bring a protein considerably closer to the ideal than single amino acid supplementation. In any protein, only that amount which corresponds to the definition of ideal protein is considered effective. The rest of the protein is not effective, although it is conceivable that under special conditions a small amount of ineffective protein may by mass action help in the utilization of effective protein. Multiple amino acid supplementation of a protein is, then, only an extension of supplementation with a single amino acid. The latter subject has been studied extensively.[11]

There are only a few simple rules which have to be followed in order to carry out a successful supplementation. The first limiting essential amino acid should be used unless the protein is primarily deficient in nonessential amino acids. Furthermore, the supplementation should be carried out in such a manner that the total amount of the first limiting amino acid in the diet is balanced against the second limiting amino acid in the protein according to the needs of the organism. This is achieved when the amount of supplementary amino acid chosen will give maximum response according to the measurement applied, e.g., growth, efficiency of food utilization, nitrogen balance, etc. A protein thus supplemented is improved but is not necessarily a "balanced" protein. It is in most cases far from being an ideal protein, which is probably the only one that deserves the term "balanced."

The results of single amino acid supplementation are illustrated in the first two figures. Figure 1 shows the 5-week growth data of weanling male rats maintained on a diet of 90% dried commercial white bread supplemented with graded levels of lysine and having a protein content of 12.5%. With increasing amounts of the essential amino acid, increasing gains were obtained until the optimum was

reached. Supplementation with larger amounts did not bring about any particular change; growth had reached a plateau. This type of response is the most common one encountered when foods are supplemented with the first limiting amino acid. When the level of protein is lowered, the response curve to amino acid supplementation shows a different shape. Supplementation with amounts larger than needed for optimum response causes an imbalance[3] and results in growth de-

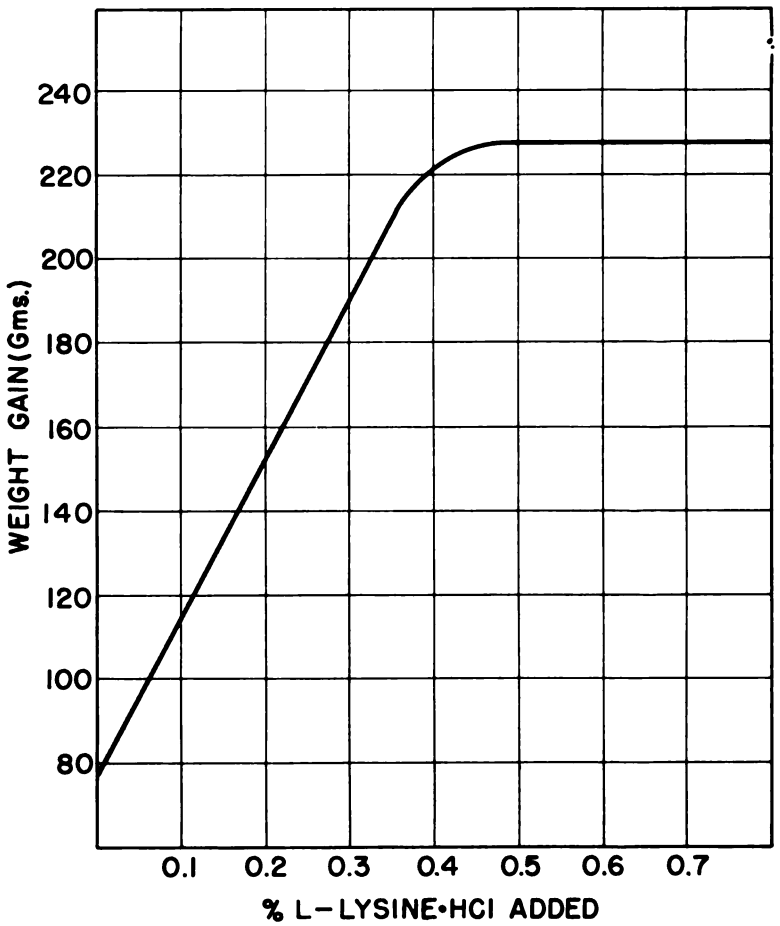

Figure 1—5-week growth of weanling male rats on 90% bread diet supplemented with graded levels of lysine.

pression. This is illustrated in figure 2, which demonstrates the gains experienced when a 7.75%-protein precooked rice diet is supplemented with graded levels of lysine.[12]

In an effort to extend the findings on single amino acid supplementation to multiple systems, supplementation with two amino acids was investigated. The hypothesis on which the design of the first series of experiments was based is illustrated in figure 3. Using the same rice diet discussed in the last illustration, the

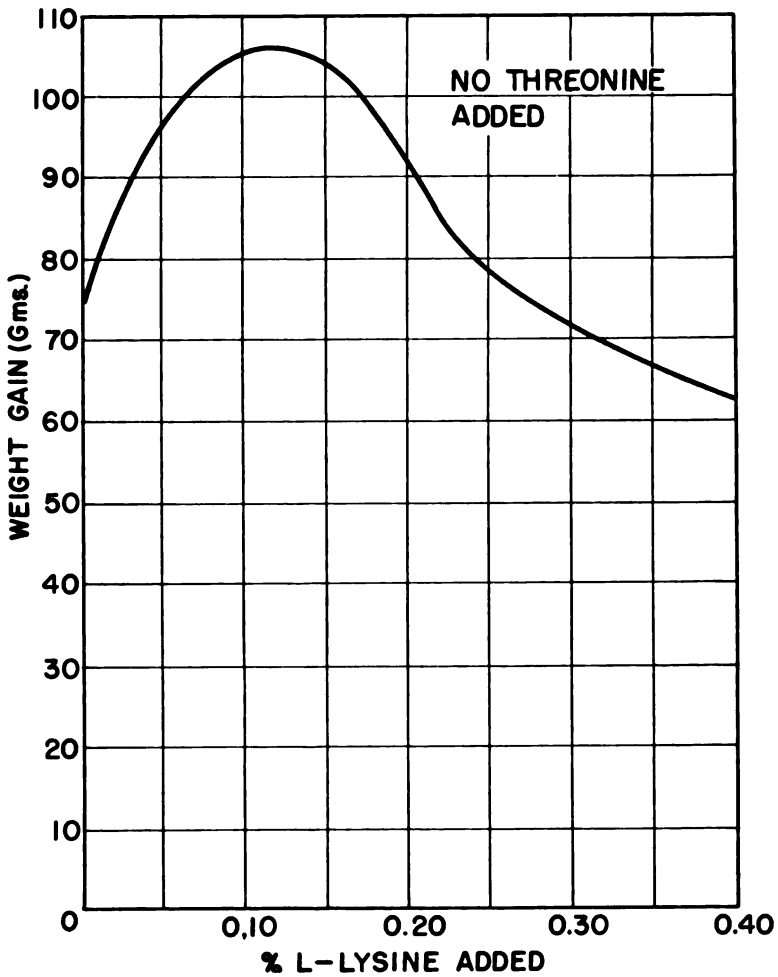

Figure 2—5-week growth of weanling male rats on 90% precooked rice diet supplemented with graded levels of lysine.

first two limiting amino acids are lysine and threonine.[6] These form the ordinates in figure 3. Then the analytically determined amounts of the two amino acids in the rice diet, 0.24% lysine and 0.23% threonine, are plotted on the ordinates. No significant improvement results when threonine is added to the rice diet. When, on the other hand, lysine is added in gradually increasing amounts, considerable improvement results. Optimum response is obtained when the lysine concentration indicated at point "A" has been reached. According to our original working hypothesis, in order to obtain further improvement in growth the next limiting amino acid, threonine, should now be added simultaneously with lysine and in the proportions of the animal's requirements, until balance is reached with the next limiting amino acid. The diagonal line from the origin of the system to the upper right hand corner represents the hypothetical ratio line based on the requirement

data for the growing rat as found by Rose.[7] At any point along this line lysine and threonine are in the same ratio of 1.66:1.

Supplementation of the rice diet with lysine and threonine was studied first by adding fixed amounts of L-lysine • HCl, e.g., 0.05%, 0.1% and 0.2%, and varying the level of threonine at each amount of lysine. Optimum responses were obtained at combinations quite different from those on the hypothetical ratio line. Moreover, when lysine was varied at fixed threonine levels, optimum responses

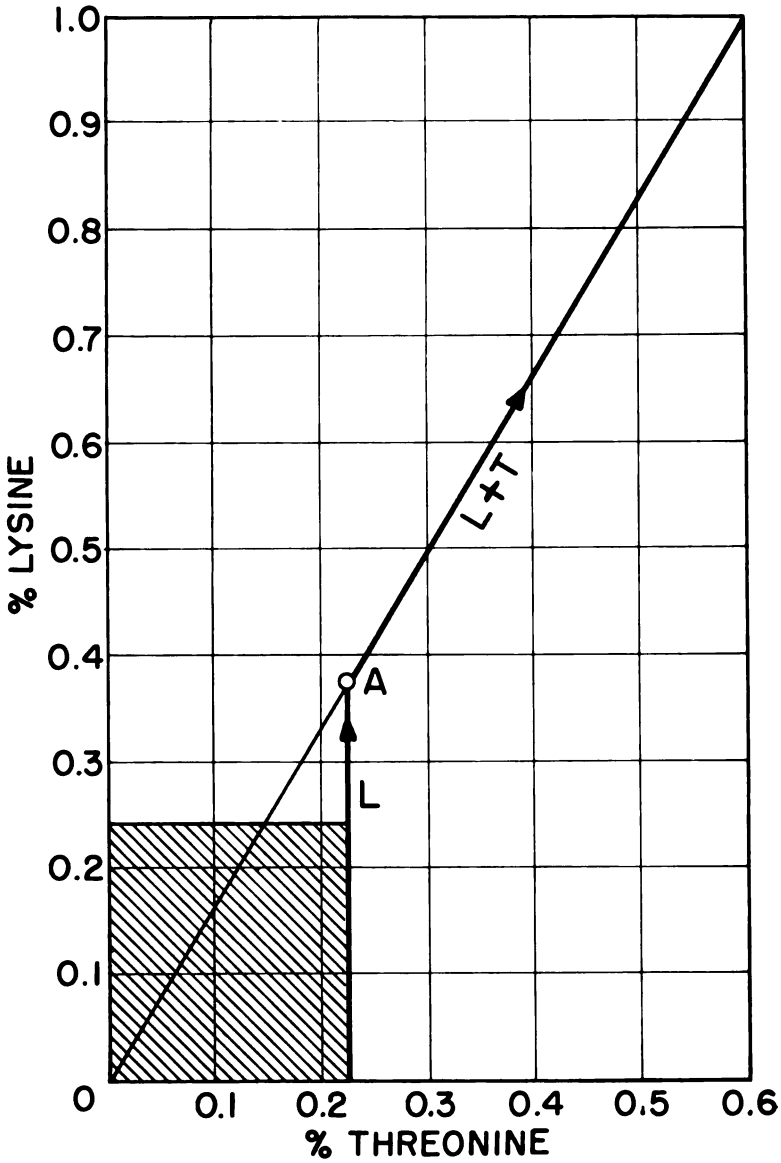

Figure 3—Plan for supplementation of rice diet with lysine and threonine.

were obtained at combinations remarkably different from those observed when threonine was varied. All data fitted, however, into a pattern[13] when the gain data were plotted on the earlier graph as a third dimension. At each lysine-threonine combination the experimentally determined gain was noted and points of equal gain were connected. After smoothing out the lines of equal gain, the graph obtained resembled a mountain ridge. Once the existence of a mountain ridge was recognized, it was desirable to find a mathematical model that agreed with the data. Gain (G) in such a model should be a function of the concentration of lysine (L) and threonine (T). Thus the simple formula

$$G = k\, L^{l}\, T^{t} \tag{1}$$

is obtained in which l and t are constants expressing the concentration of the respective amino acids. The logarithmic form of this model is more convenient for calculation of the constants, thus

$$\ln G = \ln k + l \ln L + t \ln T \tag{2}$$

This equation describes the general pattern of the experimental results and is an expression of mass action. This equation does not, however, account for the observed detrimental effect of excesses of lysine. Therefore, quadratic terms, which describe the gain reaching a maximum and then decreasing, were added

$$\ln G = \ln k + l \ln L + t \ln T + g (\ln L)^2 + d (\ln T)^2 + f \ln L \ln T \tag{3}$$

Because of the importance attached to the ratio of the essential amino acids to each other, equation 3 is rewritten in terms of ratio and product of the two amino acids.

$$\ln G = \ln k + r \ln \frac{L}{T} + p \ln LT + m\left(\ln \frac{L}{T}\right)^2 + n(\ln LT)^2 + q \ln \frac{L}{T} \ln LT \tag{4}$$

This model was found to fit the experimental data. The model can be used to find the amounts of the two limiting amino acids that give "peak" performance and to test if the ratio of the two amino acids at the peak also represents proper balance when smaller amounts of amino acids are added. The constants k, r, p, m, n and q were obtained from the experimental data by regression analysis (and with the help of a computer). All results from experiments with a ratio of L-lysine to L-threonine between 1.0 and 1.8 were used. Optimum weight gain of the animals as calculated from this equation occurs when 0.57% lysine and 0.41% threonine are present in the rice diet. Similar equations have been developed for efficiency of food utilization. Best efficiency was calculated to occur at concentrations only slightly higher than for optimum gain. This deviation is in accord with many observations on single amino acid supplementation of proteins. In fact, practical use is made of this phenomenon by the feed industry in order to achieve the highest degree of efficiency.

Figure 4 shows the predicted average weight gains. This figure is a contour map of the expected responses due to supplementation of the basal rice diet with

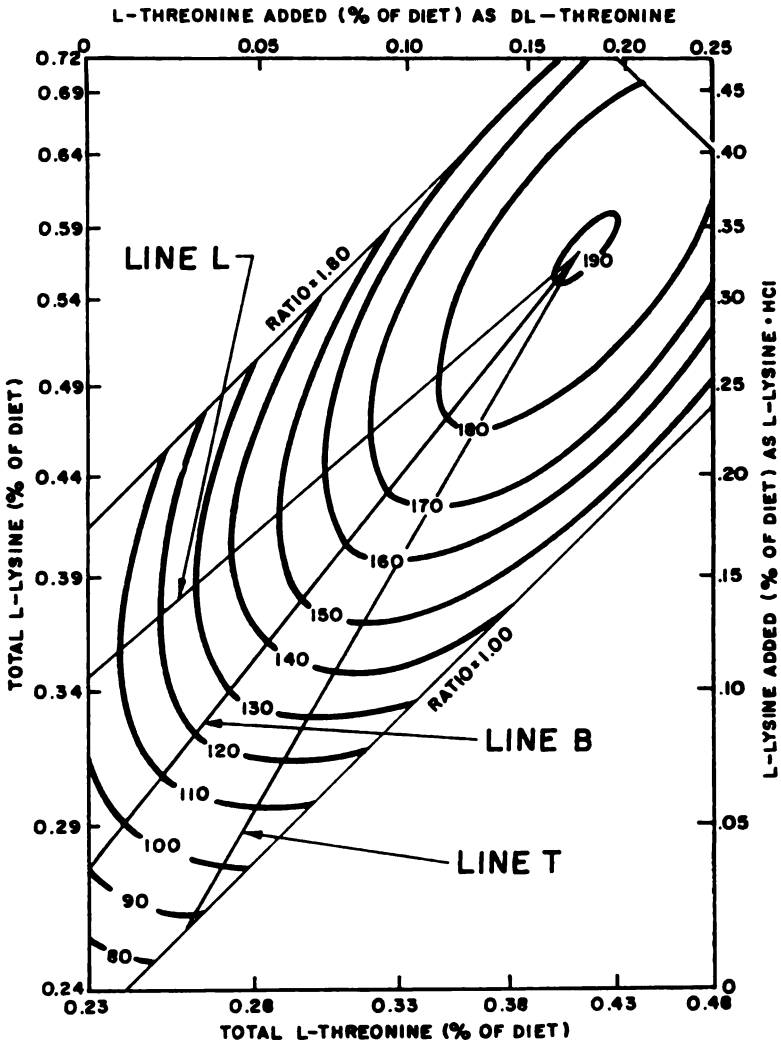

Figure 4—Predicted 5-week weight gains of weanling male rats on rice diet supplemented with lysine and threonine (contour map).

different amounts of the first two limiting amino acids. Changes in weights gained correspond to changes in altitude on a geographical contour map. The total percentages of L-lysine and L-threonine are plotted on logarithmic scales because of the type of the equation. In this system, the ratio lines are parallels with a 45° slope to the amino acid axes. It is to be noted that the mountain ridge, line "B" in figure 4, does not coincide with a ratio line, but deviates slightly. This means that "proper balance" between the two amino acids changes as the level of supplementation changes. At optimum performance the ratio of lysine to threonine is 1.4:1. When no threonine is added the ratio is 1.2:1. Such a shift in ratio would not occur if the analytical values used for the lysine and threonine content of the

diet were identical with the biologically available amounts. Since a shift was observed it must be assumed that the availability of the two amino acids differs from the amounts determined analytically.

At suboptimal levels of lysine and threonine, as seen in figure 4, the same rate of gain can be obtained by an infinite number of different combinations of the two limiting amino acids. Of all these combinations, the one which requires the smallest product of the two amino acids represents their *proper balance. The line of proper balance* connects the point of optimum performance with the points which give best response for a given product. The line of proper balance is, of course, the mountain ridge on the contour map and should coincide with a ratio line unless, as pointed out above, the amino acids of the protein are not fully available.

When only lysine is added to the basal rice diet, optimum response is predicted with about 0.10% supplementation. As seen in figure 4, optimum response does not occur at the line of proper balance. In fact, at any suboptimal level of threonine the amount of lysine predicted to give optimum response is greater than needed to reach the balance line. *The line of optimum response* from lysine supplementation at constant threonine levels is shown as line L in figure 4. There is also an *optimum response line* T from threonine supplementation at constant lysine levels. These two lines of optimum response meet at the point of maximum performance.

The ratio of lysine to threonine at the point of maximum performance was found to be 1.4:1. This ratio is different from the 1.6:1 and 2:1 ratios suggested by Rose in 1937 and 1949, respectively[7,8] but agrees with a similar figure found by Steffee et al.[19] This difference in ratio cannot be accounted for by biological variation and must be considered real. The question arose then as to whether other proteins would require the same low ratio.

To study this question, we decided to carry out an experiment with bread protein. From past experience of ourselves[15] and others,[5] it was known that air-dried bread when fed at a 12.5% protein level was not further improved by threonine supplementation after lysine had been added. However, if the level of bread protein is reduced to about 9%, it is possible to demonstrate that threonine is the second limiting nutrient in this protein, even though supplementation with lysine alone will still provide a substantial increase in the content of effective protein. An experiment was carried out, therefore, to determine the ratio of lysine: threonine needed to optimally supplement bread protein when fed at a 9.3% level (N x 6.25) in the diet.[17]

The principle of the design of this experiment is shown in figure 5. The basis of the plan follows from equation 4 used for the interpretation of the data. Arranging the amounts of the two amino acids in the test diets on an equally spaced grid is the best cover for the region of interest. The region is selected to be sure that the "peak" growth is included.

In order to find the maximum, the angle of the grid in relation to the amino acid axes is not important. However, in order to test if the ratio of the two amino acids at the "peak" also represents proper balance when smaller amounts are added, the grid is rotated 45° from the amino acid axes. In this position, the grid lines become constant ratio and constant product lines. In equation 4, the last term

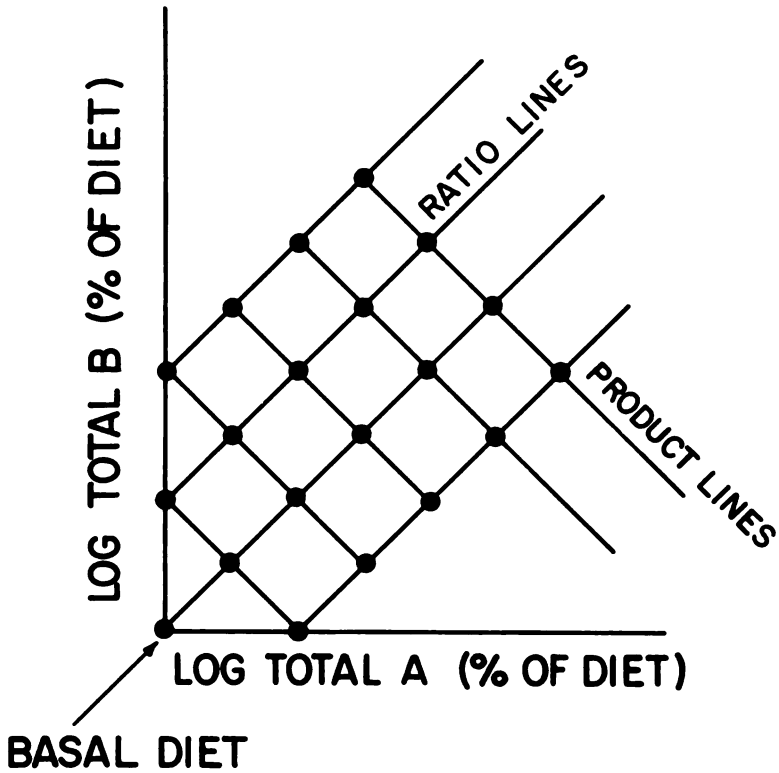

Figure 5—Design of a feeding experiment with two amino acids added in varying amounts.

is due to the deviation of proper balance from a constant ratio. The statistical test is whether the last term contributes significantly to predicting the weights of the test animals on the various diets. If it does not, the ratio of the amino acid at the "peak" is also proper balance for smaller amounts.

The amounts of the two essential amino acids in the test diets have been selected to form a factorial design. The actual diets fed form an incomplete factorial design since it is not possible to include diets with an amino acid content below that of the basal diet. The two factors studied are the log ratio and log product of the total lysine and threonine contents of the diet. The log form of the dietary amino acid is used because the relative amounts of the amino acids are more important than the absolute amounts. With this design all the essential information can be gained from a single experiment, provided there is sufficient prior knowledge available to place the experimental groups so that they include the point of optimum performance, the peak of the mountain ridge. Eighteen different combinations of lysine and threonine were used in the experiment. In order to obtain confirmation and greater precision of the results, the study was repeated with minor changes.

Figure 6 represents the results of these experiments. The point of optimum performance was located in the expected region. The ratio of lysine to threonine

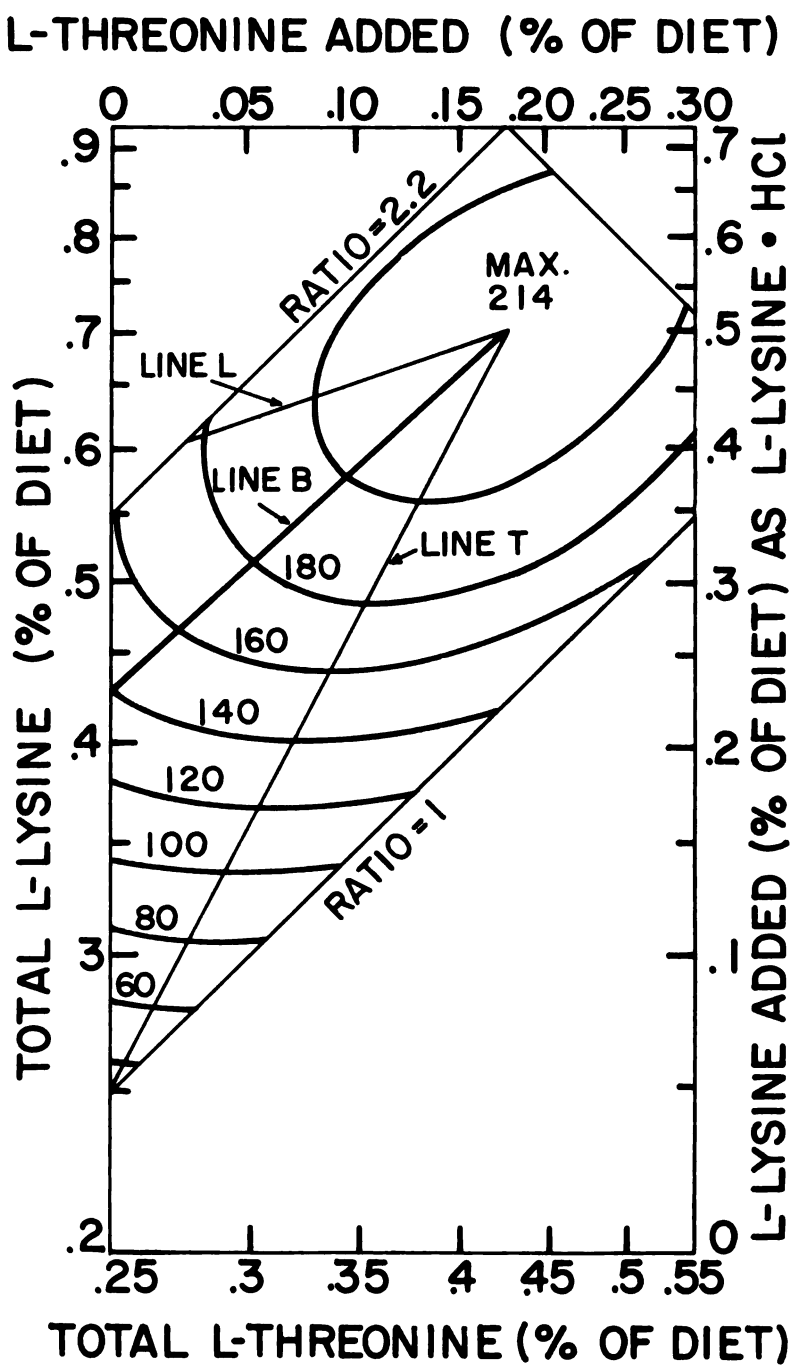

Figure 6—Predicted 5-week weight gains of weanling male rats on 63.5% dried bread diets supplemented with lysine and threonine.

at this point is 1.67:1. This result, then, is different from that found in the study on rice and agrees with the ratio of Rose's (1937) amino acid requirements for the growing rat. The difference between these two ratios is believed to be real and each value is believed to be well founded on experimental evidence. The reason for the difference in results obtained with rice and with bread protein is most probably the limited availability of the amino acids in the rice protein. It is conceivable that there is a specific balance characteristic for each protein, as the absolute amount of any one essential amino acid required for best balance may be affected by the amounts of all the other amino acids in the test protein and by the extent to which they become available for growth and maintenance. This may apply particularly to diets of low protein content. Further research is needed to clarify this matter.

The general shape of the mountain ridge representing the responses to lysine and threonine supplementation of the bread diet is similar to that obtained on the rice diet. There are, besides the line of proper balance, the two lines of optimum response for the two amino acids. The line of proper balance in figure 6 coincides with the 1.67 ratio line. Apparently, then, the two amino acids in the bread protein as measured analytically are fully available to the organism.

These studies on the supplementation of rice and bread protein with lysine and threonine support the principle that *multiple amino acid supplementation of a protein should be carried out in such a manner that, according to the needs of the organism, the supplemented amino acids are present in the proper ratio to each other and in balance with the next limiting amino acid or nutrient in the diet.*

This conclusion has been affirmed further by investigation of multiple amino acid supplementation of Southern white corn meal.[16] This material was chosen because it is a low-protein food of unusual interest in the diet of many people. It was fed untreated and uncooked, and was supplemented with lysine and tryptophan. It had been shown earlier[10] that appropriate lysine supplementation improved the gain of the male rat by about 40%. Overall growth was, however, still relatively poor. The design of the experiment on the supplementation with the first two limiting amino acids followed the principle discussed earlier for the bread protein investigation. The 18 combinations used covered ratios of lysine to tryptophan of 4:1 to 9:1. Because of the low protein level (0.97% nitrogen), only moderate gains were obtained in this study, and a few of the animals on the unsupplemented diet suffered a small loss in weight. A change of the mathematical model was indicated, therefore, since the log of a negative gain or weight loss cannot be taken. Also, the variability of the gain is not likely to be the same for all values if gain is used as the response. Equation 4 was, therefore, modified. Instead of gain, the total weight of the animals was used. The model in which the response is log weight is more general and gives good results for both gains and losses of weight. Since the weanling rat is likely to vary in size, a term, W_0, was added for the initial weight. Equation 4 was thus transformed into equation 5.

$$\text{Ln weight} = b_0 + b_3 \ln W_0 + b_1 \ln LT + b_2 \ln \frac{L}{T} + b_{11} (\ln LT)^2 + b_{22} \left(\ln \frac{L}{T}\right)^2 + b_{12} (\ln LT)\left(\ln \frac{L}{T}\right) \quad (5)$$

The combined results of 2 experiments are seen in figure 7. Growth on the basal corn meal diet was exceedingly poor. At optimum levels of lysine and tryptophan supplementation gains were improved over 400%. The ratio of lysine to tryptophan at the greatest gain was found to be 5.5:1. This value is in excellent agreement with Rose's ratio of 5:1. The proper balance line shifts noticeably, showing a ratio of about 4:1 with low lysine addition. There are also the two lines of optimum response, line L showing the amount of lysine predicted for maximum gain at fixed tryptophan levels, and line T giving the quantity of tryptophan at fixed lysine levels.

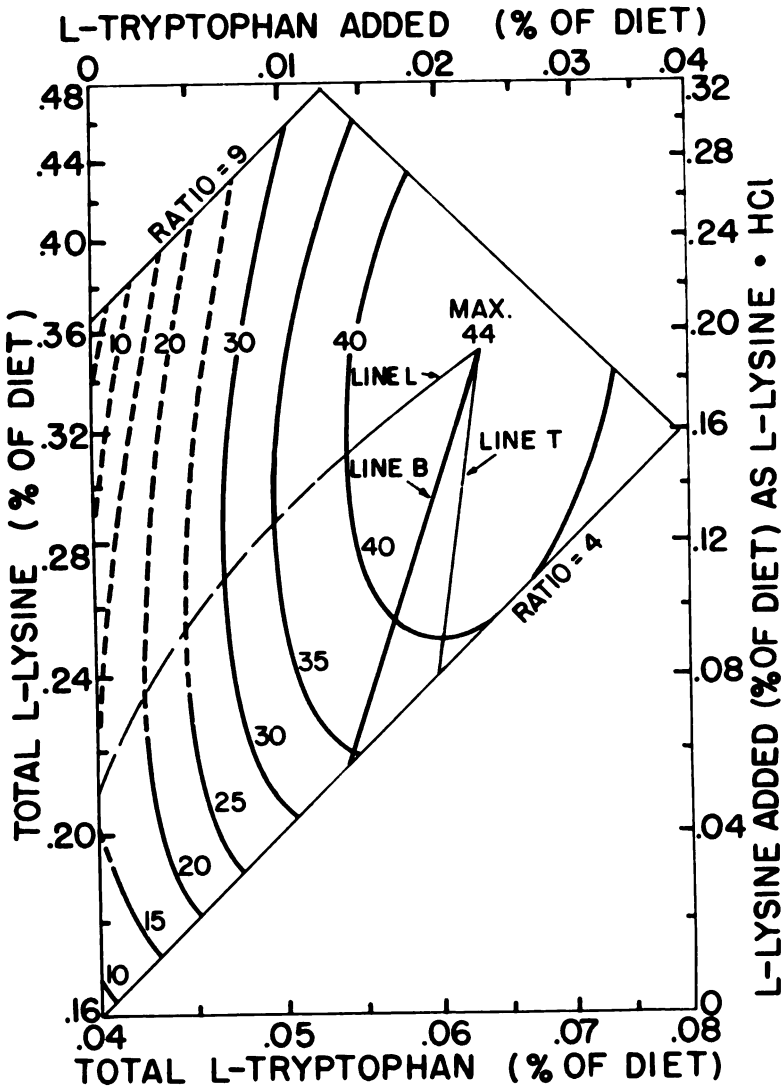

Figure 7—Predicted 5-week weight gains of weanling male rats fed white corn meal diets supplemented with lysine and tryptophan.

Line L is shown partly in dotted form since not enough data were available to fix the line accurately.

Thus the usefulness of the general principle of multiple amino acid supplementation has been demonstrated with three proteins and their first two limiting amino acids. It may be safe to generalize and to predict that this principle will apply to all similar systems. To prove this for a particular protein it is necessary to know the identity of the first two limiting amino acids and to have an analysis of the essential amino acid pattern of the protein. The total protein content of the diet must be adjusted so that it is possible to measure accurately the effect of supplementation.

An unexpected finding was the occurrence of two lines of optimum response, one for each of the supplementing amino acids. This was observed when one amino acid was held constant and the other varied. Two *lines of optimum response* were encountered because the maximum response depends upon the direction of the supplementation experiments. In terms of the mountain, position of a maximum depends upon the angle at which the mountain ridge is approached. If the series of diets had been selected to fall on a straight line at a 90° angle to the axis of the ridge, the *line of proper balance* would have been found to coincide with the *line of optimum response* (figure 8). However, when the mountain ridge is approached at a different angle, e.g. at about 45°, as is the case when either lysine (figure 9) or threonine (figure 10) is varied at a constant level of the other amino acid, maximum response does not occur at the mountain ridge (line of proper balance) but at some point beyond the ridge.

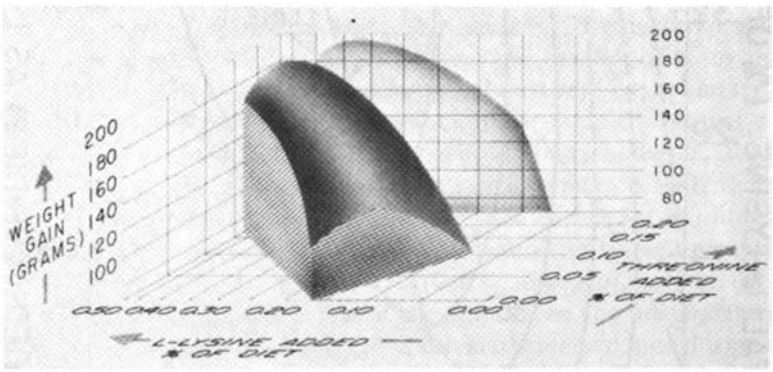

Figure 8—Mountain ridge representing growth responses to rice diet supplemented with lysine and threonine (compare contour map, Figure 4). Note the cut perpendicular to the mountain ridge. The maximum at the cut coincides with the mountain ridge.

Now that we accept the lines of optimum response, the prediction can be made that at the point of optimum response from supplementation with the first and the second limiting amino acid the ratios of these two to the third limiting essential amino acid are likely to be somewhat beyond those of "proper" balance.

At one particular stage of our investigations we entertained the thought of investigating this prediction. It seemed to us that such a study might be made profitably with corn meal, for any new knowledge on this protein could be of some

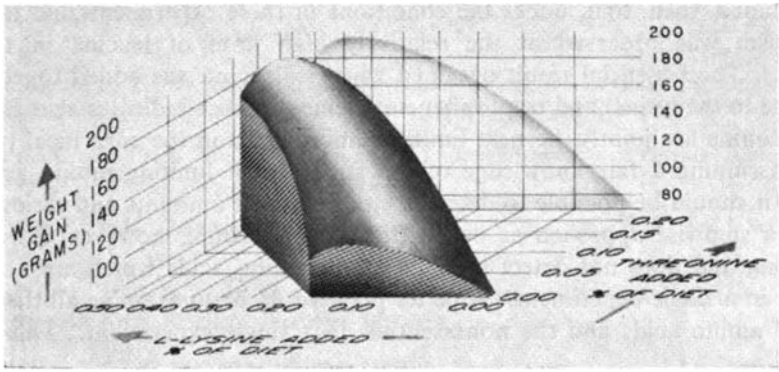

Figure 9—Mountain ridge as in Figure 8, but cut made parallel to lysine axis. The maximum at the cut does not coincide with the maximum of the ridge but occurs with greater amounts of lysine.

direct use in those areas where corn is the main food item of many protein-starved people. The first step in such a study is the identification of the third limiting amino acid. An experiment was carried out, therefore, in which the basal diet contained lysine and tryptophan in amounts slightly in excess of the optimum combination. Four other essential amino acids,[18] threonine, isoleucine, valine and methionine, were added at a low level of 0.05%, singly and in all possible combinations of two, three and four. The results, seen in table 1, show clearly that under these conditions no one amino acid singly improved the basal diet. Among the combinations of two there is one, threonine and isoleucine, which improved weight gain 70%. It is quite likely, therefore, that these two amino acids as a group constitute the third limiting nutrient in the corn meal protein. A quantitative study should be made, however, before this result is finally accepted.

These and other experiments revealed that isoleucine, although supplied in graded amounts, did not improve the nutritional value of corn meal, nor of corn meal supplemented properly with lysine or with lysine and tryptophan. It must

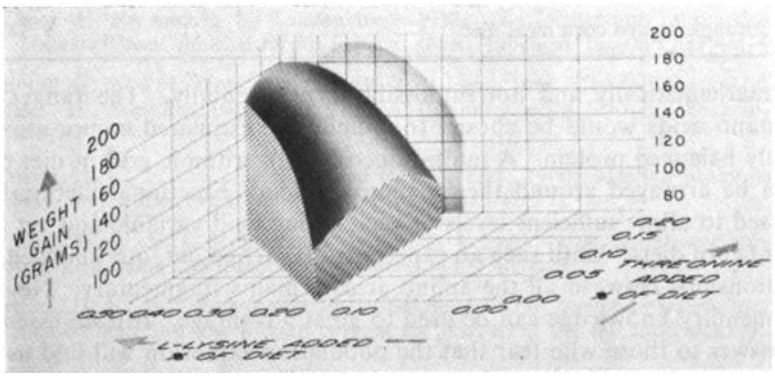

Figure 10—Mountain ridge as in Figure 8, but cut made parallel to threonine axis. The maximum at the cut does not coincide with the maximum of the ridge but occurs with greater amounts of threonine.

be concluded, then, that, under the conditions of these experiments, no isoleucine antagonism was observed at the relatively high level of leucine in the corn meal.[1,2,4] The beneficial result obtained when isoleucine was added together with threonine to the lysine- and tryptophan-supplemented diet indicates that isoleucine and threonine are jointly the next limiting amino acids in the corn meal protein.[16]

Assuming a fair knowledge of the sequence of limiting amino acids in a protein, it should be possible to determine the absolute amounts and ratios of each one by a stepwise approach as outlined. This procedure, however, is very time consuming and does not detect interactions of amino acids which are not varied together in a single experiment. A better plan would be to examine all the limiting essential amino acids and the nonessentials in a single experiment. This is quite

TABLE 1
EFFECT OF 0.05% AMINO ACID ADDITIONS TO WHITE CORN MEAL DIETS SUPPLEMENTED WITH 0.40% L-LYSINE • HCL AND 0.06% L-TRYPTOPHAN

Group	Supplementation	Avg. Weight Gain (gm)	Feed Gain
1	None	61	6.17
2	Threonine	43	7.38
3	Isoleucine	45	6.86
4	Valine	46	6.95
5	Methionine	46	6.91
6	Threonine + isoleucine	105	4.36
7	Threonine + valine	35	8.03
8	Threonine + methionine	45	6.58
9	Isoleucine + valine	54	6.14
10	Isoleucine + methionine	53	6.05
11	Valine + methionine	54	6.16
12	Threonine, isoleucine + valine	111	4.47
13	Threonine, isoleucine + methionine	120	4.13
14	Isoleucine, valine + methione	57	6.15
15	Threonine, valine + methionine	70	5.20
16	Threonine, isoleucine, valine + methionine	130	4.13
17	Unsupplemented corn meal diet	21	14.59

feasible mathematically and not impossible experimentally. The ranges of each of the amino acids would be chosen to include the estimated composition of the completely balanced protein. A multi-dimension logarithmic grid of diet compositions can be arranged around the balanced protein. Fractional factorial designs can be used to allow sufficient levels of each amino acid variable and yet limit the total number of diets. Until such an experiment is carried out, our knowledge of the interrelationships between all the amino acids remains fragmentary. Nevertheless this fragmentary knowledge can be used to great advantage. It is an essential part of our answer to those who fear that the population explosion will find us without an adequate food supply. There should never be a fear of want as far as protein is concerned if we make proper use of our knowledge of the principles of single and multiple amino acid supplementation of proteins.

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DISCUSSION

DR. BROCK: I should like to make a correction on something which I said last Sunday which has a bearing on Dr. Rosenberg's results.

In showing you two slides on an experiment on children aged 4 to 8 years on a diet in which 90% of protein was derived from maize, I was talking mainly to the importance of quantity, and I was not talking to the importance of quality at that time. I left the impression that we had not got a lysine effect in this experiment. Comparing the lysine-glycine groups, in respect to weight and serum albumin regeneration there was a statistically significant difference in favor of the lysine group. I wanted to make that correction of a misimpression which apparently I left last Sunday.

Then I would like to ask Dr. Rosenberg two questions, both of which arise from unanswered questions from the du Pont Conference a year ago.

First, is it legitimate to use glycine as a control substance, or is there at present evidence of any adverse effect of glycine?

The second question is, he is presumably talking about uncooked diets, and is it correct that, when working on humans with cooked diets, we have to make variable allowance for the effect of cooking? I should like to know particularly in respect to threonine what the present position is.

DR. ROSENBERG: As far as the first question is concerned, whether or not glycine is used depends entirely upon the objective of your experiment. If you are of the opinion that you should have isonitrogenous diets, then you may use glycine in order to compare it with other added amino acids. On the other hand, I know of at least one experiment in which we found glycine not to be very beneficial. This happened to be in a study on rice. The data from the first experiment with and without glycine showed that statistically there was no difference. After additional experiments had been carried out it became obvious that 1% of glycine in the diet was not beneficial. It decreased the growth of rats by between 5% and 10%. Low-protein diets are probably more sensitive to this effect than high-protein diets.

As far as the question of cooked versus raw materials is concerned, we have tried in all our work to use the food in the form most similar to that consumed by human beings. In our rice work we used precooked rice. In the work with corn which I have described, we did not use corn meal in cooked form. First of all, we don't know how to get cooked corn meal in a form which would be suitable for our experiment. We have, however, experimented with baked corn in the form in which it is sometimes eaten in the southern part of the United States, namely, corn pone. We have compared corn pone with unbaked corn as such and with lysine supplementation. Unfortunately, in these experiments the baked pone was not as nutritionally beneficial as the uncooked corn meal. Something was destroyed by the heat, resulting in a lower biological value. It is for that reason that we continued our work with untreated corn.

DR. BROCK: Is threonine particularly susceptible to heat?

DR. ROSENBERG: I would think threonine would be, but I do not have any real data on it. I would expect lysine to be the first amino acid attacked by heat, and then threonine, but I do not know.

DR. BENDER: Might I ask whether the growth curves were at all affected by food intake? All these figures were growth only.

DR. ROSENBERG: All these experiments were run by ad libitum feeding. Pictures of malnutrition, very similar to those shown for growth, have been drawn for feed efficiency. For most efficient food conversion, a little higher supplementation may be required than for optimal growth. In experiments with equalized food intake, we have observed, as you would expect, that food intake governs the growth rate. Multiple amino acid supplementation apparently affects food intake in the same manner as supplementation with a single amino acid.

DR. DARBY: Dr. Rosenberg, there is some evidence that cooking corn, steaming corn, makes either niacin or tryptophan more available, which I think has been published by at least two laboratories. So you were speaking, I assume, of lysine.

DR. ROSENBERG: I am speaking only of lysine. We always add enough nicotinic acid to the experimental diets to avoid the question of a possible deficiency.

DR. DARBY: One other point in terms of the form of corn which is used. While bolted corn meal is used in the South, many parts of the world use whole corn. Perhaps there is much more whole ground maize consumed than Southern-type corn meal.

DR. ROSENBERG: Whole corn, of course, differs from the Southern-type corn meal essentially in the total amount of protein. The Southern meal contains only about 6% to 7% of protein, whereas whole corn is anywhere from 10% to 12%, depending somewhat on the water content. As you well know, the nutritional value of many types of corn has often been investigated. There are many differences between species but, generally speaking, the higher the protein content the higher the nutritional value. As far as I know, the first two limiting amino acids in corn of any origin are lysine and tryptophan. The amounts of the two needed for optimal supplementation may vary somewhat, but the same principles will apply.

DR. HOWE: I carried out some experiments with steamed corn with adequate niacin present, and I find that I do not get a much better growth rate with added lysine and tryptophan than I do with the unsteamed corn. So I do not think the effect is entirely due to the niacin.

Relationship Between Plasma Amino Acids and Composition of Ingested Protein

III. EFFECT OF DIETARY PROTEIN ON PLASMA AMINO ACIDS AND CLINICAL CHEMISTRY OF DOGS

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IT IS BECOMING more and more apparent each year that protein malnutrition is one of the foremost problems facing the nutrition and medical fields today. The experimental approach to this problem is difficult, since protein deficiency can be created by an inadequate dietary intake of protein or an inadequate intake of any one of the essential amino acids. Complicating this situation further is the fact that protein deficiency can also result from disease, even though protein intake by the organism appears to be quite sufficient. In the latter case, the situation is probably created by an increased protein or essential amino acid requirement and/or a disturbance in utilization of the protein caused by the disease.

It is known that plasma protein, in particular the albumin fraction, and blood urea concentrations are reduced during dietary protein deficiency. Waterlow[1] has recently reviewed the effect of protein nutrition upon the enzymes of the blood, digestive secretions and body tissues. A translation has been published of an excellent Russian article by Kaplanskiy,[2] in which disorders of amino acid metabolism in protein deficiencies are discussed. Subnormal levels for some of the plasma amino acids have been reported for malnourished babies,[3] undernourished postsurgical patients[4] and pregnant women.[5]

It is quite possible that changes in the total plasma protein levels and the various blood and liver enzymes due to protein depletion may result in significant concentration changes of the fasting level plasma amino acids. These changes may be a more sensitive and an earlier index of protein malnutrition than the currently available methods.

A Plasma Amino Acid (PAA) ratio technique has been developed in this laboratory.[6,7] Utilizing this procedure, it has been shown that the post absorptive concentration changes of the plasma amino acids for the normal adult dog and the normal adult human are directly dependent upon the amino acid composition of the protein ingested if it is postulated that the individual essential amino acids are removed from the blood by the body tissues at rates proportional to the amino acid requirements of the test subject. During the course of these studies, we have

observed that, if adequate time is not allowed to recover from the blood loss, the changes in the concentrations of the plasma amino acids after a protein meal are usually altered from the normal when replicate experiments are conducted with the same subjects. It also has been observed that the postprandial plasma amino acid changes are different from the normal when the dog is infested with intestinal parasites.

With this background, a long-term feeding study with dogs was set up to determine whether the fasting level concentrations of the plasma amino acids and/or the postabsorptive plasma amino acid changes as determined by our published PAA ratio procedure[7] could serve as an index of protein nutritional status. The results of this study are reported here.

Experimental Procedure

The three dogs used for this study were healthy, adult mongrel dogs. They were maintained in our laboratory on Purina Laboratory Chow plus vitamins for at least 6 months prior to the start of the experiment. Control values for blood and urine chemistry, and the fasting level concentrations for the amino acids, are the average of four determinatons made during the 3-month period immediately preceding the feeding of the special diets.

The dogs were transferred from Purina Laboratory Chow to the special diets (table 1) during the first 15 days. The Purina Laboratory Chow was gradually reduced as the special diet was increased, as shown in footnote d, table 2. The diets were mixed with water (45% by weight) and offered each day to provide 83 cal/kg/day. Protein was provided in diets A and B at a level of 2.0 gm/kg/day. These levels are just slightly higher than the minimum caloric and protein requirements for maintenance of the adult dog.[8] Dogs 110 and 109, with a few exceptions, ate the entire meal each day. The food consumption for dog 69 on the nonprotein diet decreased gradually during the first 100 days. At this point, in an attempt to

TABLE 1
COMPOSITION OF TEST DIETS

Ingredients	Diet A	Diet B	Diet C
		Percent	
Casein [a]	11.482	0.000	0.000
Wheat gluten [b]	0.000	13.582	0.000
Sucrose	18.000	17.500	18.000
Dextrose	33.300	33.300	33.300
Dextrin	18.000	17.500	29.482
Corn oil	14.000	14.000	14.000
Salt mixture W [c]	5.000	5.000	5.000
Vitamins [d]	0.218	0.218	0.218

[a] A.N.R.C. Reference Protein, Sheffield Chemical Company, 87.5% protein (N x 6.29).
[b] Pure Gluten Company, Columbus, Ohio, 75.4% protein (N x 5.70).
[c] Nutritional Biochemicals Corporation.
[d] Provides in 100 gm diet: 0.20 mg thiamine • HCl; 0.32 mg riboflavin; 1.6 mg nicotinic acid; 1.3 mg calcium pantothenate; 0.20 mg pyridoxine • HCl; 200 mg choline; 5,500 units Vitamin A; 1.120 USP units Vitamin D; 6.0 mg α-tocopherol; 0.06 mg biotin; 0.06 mg folic acid. In addition every day each dog was given a vitamin tablet containing: 3.0 mg thiamine • HCl; 1.2 mg riboflavin; 10 mg niacinamide; 5.0 mg calcium pantothenate; 2.0 mg pyridoxine • HCl; 75.0 mg Vitamin C; 0.5 mg Vitamin K; 5000 USP units Vitamin A and 500 USP units Vitamin D.

improve his food consumption, the dry diet was mixed with applesauce (1:1) and offered in this form for the remaining 30 days of the nonprotein period.

Blood was drawn into heparinized syringes from the cephalic or jugular vein. The plasma amino acids were determined by the chromatographic methods of Hamilton[9] or Spackman et al.[10] Tryptophan was determined microbiologically using *Lactobacillus plantarum*. The PAA ratios were determined by the "Short" procedure recently developed in this laboratory.[7] The cyanmethemoglobin standard method was used to determine hemoglobin, and Reinhold's[11] adaptation of Kingsleys' method was employed for serum protein, albumin and globulin. Cholesterol and alkaline phosphatase were determined by the methods of Saifer and Kammerer[12] and Bodansky,[13] respectively. Methods described in the Manual of Clinical Biochemistry[14] were employed for blood urea nitrogen and urine protein.

General Clinical Picture

The data showing the weight changes, food consumption and clinical chemistry for dogs 69, 109 and 110 are given in table 2. During the protein depletion period for dog 69 (0-130 days), there was a marked reduction in food intake, weight, plasma protein (caused entirely by the decrease in the albumin fraction), blood urea nitrogen and urine protein. At the same time the alkaline phosphatase increased slightly along with the blood cholesterol, which rose to a maximum for this period by the 60th day.

After being on the nonprotein diet for 130 days dog 69 was placed on the wheat gluten diet (130 to 199 days). This dietary change caused little change in the dog's weight, serum protein, or urine protein, but produced an increase in food intake and blood urea nitrogen and a sharp increase in the alkaline phosphatase. The blood cholesterol increased further above the maximum level reached during the preceding nonprotein period, making a total elevation of approximately 250% above the dog's normal value. L-lysine was added to the diet (200 to 319 days) to present a better balanced dietary amino acid pattern. After this dietary change, the dog regained his normal weight in approximately 60 to 70 days and returned to a near normal clinical state by the time the experiment was terminated.

The clinical condition for dog 109, on the wheat gluten diet, declined similarly to that for dog 69 (while on the nonprotein diet) but to a lesser degree. The hypercholesterolemia for dog 109 was of the same magnitude but developed more slowly than that for dog 69. A hypoproteinemia began to appear for dog 109 after 185 days on the wheat gluten diet, similar to that which occurred for dog 69 after being on the nonprotein diet for 50 days.

The overall picture for dog 110 was fairly constant throughout the experiment, except for a decrease in the blood urea nitrogen and urine protein and an elevation of the blood cholesterol. In this case, the hypercholesterolemia was less pronounced and developed more slowly than for dogs 69 or 109.

Fasting Level Plasma Amino Acid Concentrations

The fasting level concentrations for the plasma amino acids for dogs 69, 109 and 110 are given in table 3. During the 130-day nonprotein period for dog 69,

TABLE 2
WEIGHT CHANGE, FOOD CONSUMPTION AND CLINICAL CHEMISTRY FOR DOGS No. 69, 109, AND 110

Dog No. 69		Ml		Clinical Chemistry [b]									
Days	Wt Kg	Blood Taken	Caloric Intake [a]	Hgb	Hct	Prot	Alb	Glob	A/G Ratio	Chol	Alk Phos	BUN	Urine Prot
Control [c]	31 ± 1	—	2050 ± 250	16.0 ± 0.5	51 ± 1	6.45 ± 0.10	2.98 ± 0.10	3.49 ± 0.20	0.85 ± 0.10	129 ± 10	2.83 ± 0.20	11.4 ± 2.0	6.8 ± 3.3
Mixture of Purina Laboratory Chow and Nonprotein Diet C [d]													
1	29.9	25	2515	17.0	50	6.62	2.93	3.69	0.79	148	4.37	8.8	—
7	—	25	2515	15.6	51	6.37	2.17	4.20	0.52	168	2.85	6.5	10.0
16	30.1	25	2515	16.7	51	6.09	3.24	2.85	1.14	175	4.79	1.7	9.5
Nonprotein Diet C Starting the 16th Day													
37	28.0	40	1918	18.2	49	5.36	2.03	3.33	1.61	243	7.13	2.2	5.0
49	27.7	25	1380	17.1	51	5.57	1.96	3.61	0.54	317	6.09	2.0	3.0
60	26.8	40	1257	18.6	54	5.20	1.77	3.43	0.52	408	4.86	1.6	0.0
70	26.2	10	1058	18.4	50	5.38	2.14	3.24	0.66	366	6.44	1.5	2.0
91	24.4	45	988	18.1	40	5.04	2.05	2.99	0.69	366	6.44	1.8	1.5
101	23.6	10	1005	17.7	48	5.10	1.51	3.59	0.42	388	6.80	0.0	0.5
112	23.2	40	1274	18.4	51	5.13	1.36	3.77	0.36	390	6.80	3.5	3.0
129	22.1	40	1455	17.7	52	5.15	1.54	3.61	0.43	363	7.25	4.5	1.5
Conversion from Nonprotein Diet C to Wheat Gluten Diet B Starting the 130th Day [e]													
144	22.4	40	1363	16.8	50	5.06	1.27	3.79	0.34	336	9.60	5.5	1.5
157	22.5	10	1676	15.8	46	5.31	1.47	3.84	0.38	432	19.12	7.5	0.0
171	22.7	40	1720	14.5	48	5.21	1.63	3.58	0.46	455	23.80	4.0	1.0
185	23.3	40	1782	14.7	47	—	—	—	—	510	19.85	8.7	0.0
199	22.9	40	1702	13.7	44	5.44	1.70	3.74	0.46	480	17.67	7.4	0.0
Wheat Gluten Diet B Plus L-lysine Starting the 200th Day [f]													
210	25.0	10	2059	15.0	45	5.54	1.59	3.95	0.40	442	17.60	5.7	—
220	26.2	40	2068	14.0	43	5.63	1.57	4.06	0.39	411	22.43	6.8	1.5
227	27.2	10	2214	15.3	49	5.69	1.35	4.34	0.31	394	17.80	6.4	4.5
235	27.8	40	2143	14.6	45	—	—	—	—	360	17.20	1.0	0.5
241	28.6	10	2515	13.9	45	5.70	1.97	3.73	0.53	363	13.03	7.5	0.0
262	30.0	40	2196	16.1	48	6.12	2.40	3.72	0.65	298	11.08	6.6	4.0
276	30.9	40	2306	16.5	50	6.42	2.79	3.63	0.77	258	11.31	6.0	2.5
290	31.9	10	2381	16.5	50	6.72	2.83	3.89	0.73	230	9.60	6.2	—
311	32.3	40	2400	16.6	51	6.40	2.75	3.65	0.75	231	7.32	7.0	2.0

TABLE 2 (Continued)

Dog No. 109 Days	Wt Kg	Ml Blood Taken	Caloric Intake[a]	Clinical Chemistry[b] Hgb	Hct	Prot	Alb	Glob	A/G Ratio	Chol	Alk Phos	BUN	Urine Prot
Control[c]	15.5 ± 0.5	—	1239 ± 180	15.6 ± 0.9	48 ± 2	6.07 ± 0.10	2.51 ± 0.10	3.56 ± 0.10	0.7 ± 0.1	115 ± 10	1.20 ± 0.40	8.2 ± 2.0	2.5 ± 3.0
					Mixture of Purina Laboratory Chow and Wheat Gluten Diet B[d]								
1	15.6	10	1256	15.0	47	5.04	2.56	3.08	0.83	126	2.13	7.7	—
7	15.7	25	1256	15.2	46	5.74	2.48	3.26	0.76	146	3.61	6.0	4.0
16	15.6	25	1256	16.0	49	5.81	2.94	2.87	1.02	172	4.74	6.3	4.5
					Wheat Gluten Diet B Starting the 16th Day								
37	15.8	25	1256	16.6	50	5.61	2.64	2.97	0.89	175	5.74	6.5	2.0
49	16.3	25	1256	16.1	47	5.91	3.06	2.85	1.07	257	3.49	4.8	0.0
70	16.8	25	1256	17.6	51	5.68	2.66	3.05	0.88	283	—	4.0	1.0
91	17.1	25	1256	17.9	52	5.83	3.17	2.66	1.19	335	2.82	7.5	4.5
112	17.8	25	1256	17.3	48	5.56	2.39	3.17	0.75	309	2.64	4.5	3.5
129	18.5	40	1256	17.4	49	5.78	2.95	2.83	1.04	320	—	5.5	—
157	18.6	40	1160	15.6	47	5.37	2.30	3.07	0.75	309	4.00	6.9	2.5
185	18.2	10	935	15.9	48	5.28	1.73	3.55	0.49	345	7.33	3.5	0.0
213	17.7	40	922	14.7	47	5.57	2.02	3.55	0.57	339	8.20	5.0	5.5
241	17.7	40	1032	14.2	46	5.64	2.15	3.49	0.62	347	7.25	10.6	2.0
262	18.2	10	1256	14.2	42	5.45	2.34	3.11	0.75	310	5.56	6.6	—
290	18.6	40	1256	14.0	43	5.60	1.91	3.69	0.52	326	6.08	7.0	—
311	18.5	10	1256	14.9	45	4.99	1.74	3.25	0.54	332	6.08	8.2	4.0
Dog No. 110 Control[c]	11.0 ± 0.5	—	955 ± 40	16.8 ± 0.2	49 ± 1	6.68 ± 0.3	2.68 ± 0.3	4.18 ± 0.3	0.65 ± 0.1	74 ± 9	1.24 ± 0.2	12.2 ± 3.0	0.8 ± 1.0
					Mixture of Purina Laboratory Chow and Casein Diet A[d]								
1	10.8	10	926	17.5	53	6.80	2.56	4.24	0.60	84	1.64	11.8	—
7	10.7	25	926	16.7	49	6.64	2.99	3.65	0.82	92	2.16	8.5	1.5
16	10.8	25	926	16.6	49	6.89	2.75	4.14	0.66	100	1.71	9.3	7.5
					Casein Diet A Starting the 16th Day								
37	10.5	25	926	16.7	48	6.73	2.58	4.15	0.62	142	—	5.6	0.0
49	11.0	25	926	16.4	49	6.92	2.94	3.90	0.74	143	1.83	5.8	0.0
70	10.9	25	926	17.9	48	6.70	2.54	4.16	0.61	144	—	5.0	3.0
91	11.0	25	926	17.6	49	6.22	2.69	3.53	0.80	156	1.96	5.4	0.0

TABLE 2 (Continued)

Dog No. 110		Ml		Clinical Chemistry[b]									
Days	Wt Kg	Blood Taken	Caloric Intake[a]	Hgb	Hct	Prot	Alb	Glob	A/G Ratio	Chol	Alk Phos	BUN	Urine Prot
112	11.0	25	926	17.7	49	6.43	2.27	4.16	0.55	173	1.40	6.3	2.0
129	10.6	40	926	17.9	49	6.86	2.89	3.97	0.73	204	—	7.5	—
157	10.8	40	926	15.4	45	6.60	2.21	4.39	0.50	203	2.20	9.0	0.0
185	11.1	10	926	15.9	45	6.36	2.36	4.00	0.59	199	1.95	8.3	0.5
213	10.6	40	926	15.0	45	6.33	2.30	4.03	0.57	200	3.40	8.2	5.0
241	10.6	40	926	14.2	45	6.09	2.03	4.06	0.50	190	1.33	7.5	0.0
262	10.8	10	926	16.6	48	6.35	2.40	3.95	0.61	210	1.84	6.0	2.5
290	10.5	40	926	16.6	47	6.28	2.34	3.94	0.59	267	2.08	6.8	—
311	10.8	40	926	15.6	45	6.74	2.05	4.69	0.44	236	2.03	7.2	4.0
Normal[g]	—	—	—	16.1 ± 1.7	49 ± 5	6.41 ± 0.37	2.84 ± 0.30	3.58 ± 0.44	0.84 ± 0.17	121 ± 28	2.12 ± 1.08	17.1 ± 4.8	—

[a] Average caloric intake per day for period from last blood sampling. 80 cal/kg/day has been reported (8) as the minimum caloric requirement for the adult dog.

[b] Key for abbreviations: Hgb—hemoglobin, gm/100 ml; Hct—hematocrit, per cent; Prot—serum protein, gm/100 ml; Alb—serum albumin, gm/100 ml; Glob—serum globulin, gm/100 ml; Chol—plasma cholesterol, mg/100 ml; Alk Phos—alkaline phosphatase, Bodansky Units; BUN—Blood Urea Nitrogen, mg/100 ml; Urine Prot—urine protein, mg/100 ml.

[c] Three-months' period preceding the feeding of the special diets. Dogs were fed Purina Laboratory Chow plus vitamins with all values being the average of 4 determinations.

[d] Mixture of Purina Laboratory Chow and Special Diets A, B, or C, respectively, in the following proportions: 3:1 starting the first day; 2:2 starting the 6th day; and 1:3 starting the 11th day.

[e] Mixture of diet C and diet B, respectively, in the proportion of: 2:1 starting the 130th day; 1:2 starting the 134th day; and 0:3 starting the 138th day.

[f] 0.25gm L-lysine monohydrochloride per 100 gm of diet B.

[g] Values determined over a 4-year period for 50 dogs on Purina Laboratory Chow by the Biochemical Section, du Pont Haskell Laboratory, under the direction of Dr. J. R. Barnes. In each case, except for the serum proteins, approximately 600 to 800 determinations were performed. For the serum protein values triplicate analyses on 15 dogs were performed over a 2-month period.

TABLE 3
FASTING LEVEL CONCENTRATIONS OF PLASMA AMINO ACIDS FOR DOGS NO. 69, 109, AND 110

Dog No. 69

Days	Thr	Val	Cys	Met	Iso	Leu	Tyr	Phe	Lys	His	Arg	Try
	Amino Acid Concentrations, mg/100 ml											
Control [a]	2.30 ± 0.20	2.00 ± 0.30	0.90 ± 0.18	0.82 ± 0.22	1.02 ± 0.23	1.80 ± 0.16	0.70 ± 0.10	0.76 ± 0.14	3.96 ± 0.54	1.26 ± 0.24	2.66 ± 0.28	1.50 ± 0.05
	Mixture of Purina Laboratory Chow and Nonprotein Diet C [b]											
7	3.35	2.77	—	0.86	0.91	1.80	1.09	1.24	4.34	1.54	3.59	1.31
16	1.99	2.22	—	0.51	0.81	1.74	0.89	0.99	3.75	1.37	2.09	1.84
	Nonprotein Diet C Starting 16th Day											
27	1.58	1.76	—	0.45	0.86	1.72	0.70	1.07	4.46	1.64	2.08	0.76
39	1.65	1.82	—	0.28	0.58	1.47	0.47	0.78	3.52	1.81	1.52	0.86
53	1.30	1.33	0.48	0.54	0.70	1.27	0.37	0.64	3.33	1.88	1.52	0.61
74	1.40	1.25	0.32	0.38	0.58	1.25	0.32	0.76	3.10	2.01	1.19	0.70
88	1.56	1.12	0.28	0.37	0.48	1.07	0.23	0.65	2.84	2.07	1.04	0.68
102	1.57	1.48	0.37	0.46	0.66	1.27	0.33	0.64	4.09	2.26	1.23	0.60
116	1.40	1.67	0.25	0.25	0.37	0.92	0.17	0.49	3.46	2.36	0.90	0.64
130	1.68	1.16	0.25	0.33	0.52	1.13	0.27	0.66	4.32	2.54	1.31	0.59
	Conversion From Nonprotein Diet C to Wheat Gluten Diet B Starting the 130th Day [c]											
158	1.04	0.25	0.86	0.35	0.61	0.98	0.21	0.56	1.92	2.27	1.60	0.73
171	1.90	1.50	0.75	0.43	0.61	1.00	0.44	0.69	1.72	2.52	1.44	0.92
193	2.15	1.57	0.75	0.63	0.75	1.22	0.49	0.73	2.24	—	—	—
	Wheat Gluten Diet B Plus L-lysine Starting the 200th Day [d]											
214	1.67	1.20	1.05	0.47	0.69	0.84	0.28	0.63	1.65	1.72	1.38	1.04
235	0.97	1.21	0.90	0.48	0.71	0.99	0.34	0.62	2.17	1.79	1.84	1.14
256	0.96	1.53	0.89	0.52	0.73	1.23	0.63	0.88	2.88	2.06	2.47	1.38
277	1.23	1.89	1.01	0.74	0.90	1.61	0.91	1.20	3.73	2.13	3.14	1.58
298	1.18	1.47	1.38	0.54	0.70	1.39	0.69	1.06	2.54	1.96	1.99	1.84
319	0.98	1.74	1.16	0.57	0.81	1.47	1.26	0.59	2.10	1.30	1.58	1.97

TABLE 3 (Continued)

Days	Thr	Val	Cys	Met	Iso	Leu	Tyr	Phe	Lys	His	Arg	Try
Dog No. 109												
					Amino Acid Concentrations, mg/100 ml							
Control [a]	2.75 ± 0.47	2.00 ± 0.22	0.85 ± 0.20	0.64 ± 0.20	0.78 ± 0.16	1.28 ± 0.13	0.78 ± 0.08	0.78 ± 0.08	2.33 ± 0.52	1.18 ± 0.17	2.09 ± 0.38	1.40 ± 0.24
					Mixture of Purina Laboratory Chow and Wheat Gluten Diet B [b]							
7	4.53	1.92	—	0.68	0.74	1.42	0.99	1.12	2.63	1.32	2.54	0.99
16	2.09	1.72	—	0.60	0.94	1.19	1.07	0.92	3.88	1.24	3.08	0.66
					Wheat Gluten Diet B Starting 16th Day							
27	3.92	1.99	—	0.31	0.71	1.38	1.40	1.05	3.22	1.37	2.88	0.96
39	2.98	1.93	—	0.64	0.63	1.27	0.97	0.99	3.94	1.50	2.56	0.84
49	2.40	1.65	—	0.57	0.54	1.14	0.90	0.91	2.58	1.34	2.19	0.85
70	2.29	1.39	—	0.45	0.49	0.97	0.83	0.89	2.32	1.34	2.04	0.99
91	1.62	0.96	—	0.51	0.54	0.93	0.74	0.76	1.53	1.33	1.68	1.11
112	2.04	1.55	—	0.50	0.49	0.91	0.84	0.85	2.10	1.41	1.75	0.84
133	1.75	1.28	—	0.41	0.55	1.09	0.75	0.69	1.39	1.34	1.70	1.35
154	1.59	1.12	1.40	0.35	0.51	0.93	0.77	0.93	1.56	1.57	1.69	1.38
182	1.11	1.26	0.66	0.28	0.48	0.95	0.49	0.73	2.48	2.44	1.70	1.58
210	1.62	1.02	1.07	0.40	0.48	0.67	0.56	0.71	0.79	1.79	0.83	1.10
238	1.81	0.98	—	0.41	0.46	0.69	0.50	0.91	2.17	2.02	1.82	0.95
266	1.84	1.01	—	0.44	0.50	0.83	0.66	1.00	3.06	1.80	2.46	0.97
294	2.07	1.41	1.00	0.51	0.47	0.84	0.40	0.83	0.93	2.43	1.04	1.18
322	2.62	1.19	1.10	0.55	0.48	0.86	0.67	0.99	1.97	2.03	1.76	0.98
Dog No. 110												
Control [a]	2.45 ± 0.42	1.85 ± 0.25	0.90 ± 0.15	0.93 ± 0.31	0.68 ± 0.05	1.38 ± 0.10	0.85 ± 0.21	0.78 ± 0.05	2.33 ± 0.35	1.43 ± 0.21	2.17 ± 0.54	1.17 ± 0.15
					Mixture of Purina Laboratory Chow and Casein Diet A [b]							
7	1.71	1.70	—	0.60	0.62	1.38	0.73	1.02	2.52	0.71	2.82	0.85
16	2.54	1.97	—	0.60	0.64	1.27	1.03	1.07	2.21	1.18	2.39	0.71

TABLE 3 (Continued)

Days	Thr	Val	Cys	Met	Iso	Leu	Tyr	Phe	Lys	His	Arg	Try
	Amino Acid Concentrations, mg/100 ml											
	Casein Diet A Starting Day 16											
27	1.59	1.71	—	0.57	0.64	1.27	0.91	0.86	2.85	1.08	2.47	0.51
39	1.59	1.99	—	0.52	0.70	1.12	0.80	0.73	3.08	0.95	2.21	0.66
49	1.54	1.79	—	0.63	0.56	1.22	1.03	0.67	3.54	1.09	2.78	0.68
70	1.60	1.56	—	0.48	0.55	0.92	—	—	2.70	0.99	1.95	0.52
91	1.48	1.33	0.79	0.58	0.54	0.93	0.71	0.62	2.63	0.87	1.69	0.54
112	2.52	1.38	1.12	0.75	0.64	0.93	0.93	0.82	3.69	1.16	2.29	0.54
133	1.76	0.93	0.97	0.52	0.40	0.75	0.57	0.54	2.42	0.65	1.01	0.86
154	2.36	1.42	—	0.70	0.70	0.95	1.05	0.80	4.18	1.03	0.90	0.86
182	1.70	1.44	—	0.55	0.45	0.81	0.85	0.67	3.07	1.79	1.79	0.92
210	1.91	1.27	0.68	0.61	0.53	0.90	0.76	0.69	2.72	1.32	1.79	0.88
238	2.76	1.49	1.19	0.88	0.63	1.07	0.93	0.78	3.79	1.41	2.39	0.88
266	—	—	—	—	—	—	—	—	3.11	—	1.76	0.95
294	2.36	0.98	0.93	0.75	0.56	0.91	0.84	0.57	2.60	1.18	1.52	0.74
322	2.65	1.54	0.91	1.58	0.65	1.17	0.85	0.71	3.21	1.33	1.95	0.71

[a] Three-months' period preceding the feeding of the special diets. Dogs were fed Purina Laboratory Chow plus vitamins with all the values being the average of 4 determinations.

[b] Details given in table 2, footnote d.

[c] Details given in table 2, footnote e.

[d] 0.25gm L-lysine monohydrochloride per 100 gm of diet B.

a 20% to 70% reduction in the fasting level concentrations occurred for 10 of the 12 amino acids determined, with little overall change occurring for lysine and an increase of approximately 80% above the normal level for histidine. The changes in the fasting level concentrations for the amino acids for dog 109 during the 322 days he was on the wheat gluten diet were very similar to those for dog 69 during the 130-day nonprotein period, but in most cases less pronounced. As was found for dog 69, the plasma concentration for histidine increased approximately 80% above the normal level for dog 109.

For dog 110, the fasting level plasma amino acid concentration changes during the 322-day period the dog was on a casein diet were of a lesser magnitude than for dogs 69 or 109. While this overall change was small, a greater reduction did occur for most of the amino acids during the first 150 days, with a gradual increase back toward the normal concentrations taking place during the last half of the test period. Apparently this result shows the dog's ability to adapt to a minimum dietary protein intake. This adaptation did not occur for dog 109 on the wheat gluten diet, due probably to the poor amino acid pattern of the wheat gluten caused by the lysine deficiency.

Plasma Amino Acid (PAA) Ratios for Wheat Gluten

During the course of this study the postabsorptive plasma amino acid changes after the ingestion of a wheat gluten meal were determined by our recently published PAA ratio procedure. For dog 109 and dog 110 some fluctuation in the postabsorptive plasma amino acid changes did occur during the 322 days the dogs were on the special diets, but the overall changes were not significantly different from those found when the dogs were on Purina Laboratory Chow except for methionine and methionine-plus-cystine. In the latter two cases the PAA ratios increased two- to three-fold during the 322 days these dogs were on the wheat gluten and casein diets.

The results of the postabsorptive plasma amino acid changes and thus the PAA ratios for wheat gluten with dog 69 as determined at various intervals during this study are presented in table 4. As was found with dogs 109 and 110, the PAA ratios for methionine and methionine-plus-cystine increased two- to three-fold during the protein depletion period and remained at this high level until the dog had recovered.

The general changes in the PAA ratios for the other amino acids appear to be a 20% to 30% lowering during the first half of the nonprotein period, with a gradual increase back toward the normal range toward the latter part of this period. During the wheat gluten and the early stage of the wheat gluten-plus-lysine dietary periods, the ratios show a tendency to remain at the normal or slightly above normal levels. Progressing into the lysine-supplemented wheat gluten dietary period, there seems to be a tendency for the ratios to decrease below the normal levels, followed by a gradual increase back toward the normal values toward the end of the protein repletion period.

TABLE 4
PAA RATIOS[a] OF WHEAT GLUTEN FOR DOG NO. 69

	Lys	Met	Cys[b]	Arg	Val	Thr	Iso	Try	Leu	His	Phe[c]
Expt. No. 1[d], Day 0											
1. X[e]	2.44	1.23	2.29	3.14	4.26	3.20	2.21	1.86	3.85	2.81	2.90
2. A[f]	3.70	1.09	1.99	2.40	3.01	1.89	1.11	1.61	2.11	1.19	1.45
3. (X-A)	—1.26	0.14	0.30	0.74	1.25	1.31	1.10	0.25	1.74	1.62	1.45
4. PAA Ratio[a]	—17.8	2.8	4.2	13.0	19.5	32.8	17.7	22.7	20.5	81.0	15.8
Expt. No. 2[d], Day 53											
1. X[e]	2.35	0.83	1.63	2.09	2.47	1.95	1.59	0.82	2.77	3.12	2.33
2. A[f]	3.30	0.54	1.02	1.52	1.33	1.30	0.70	0.61	1.27	1.88	1.01
3. (X-A)	—0.95	0.29	0.61	0.57	1.14	0.65	0.89	0.21	1.50	1.24	1.32
4. PAA Ratio[a]	—13.4	5.7	8.5	11.2	17.8	16.3	14.4	17.7	17.7	62.0	14.4
Expt. No. 3[d], Day 74											
1. X[e]	2.24	0.70	1.33	1.89	2.14	2.62	1.37	0.86	2.52	3.32	2.49
2. A[f]	3.10	0.38	0.70	1.19	1.25	1.40	0.58	0.70	1.25	2.01	1.08
3. (X-A)	—0.86	0.32	0.63	0.70	0.89	1.22	0.79	0.16	1.27	1.31	1.41
4. PAA Ratio[a]	—12.1	6.3	8.7	12.3	13.9	30.5	12.7	14.5	14.9	65.5	15.3
Expt. No. 4[d], Day 88											
1. X[e]	2.42	0.16	1.23	1.63	1.83	2.19	1.26	0.77	2.27	3.25	2.07
2. A[f]	2.84	0.37	2.65	1.04	1.12	1.56	0.48	0.68	1.07	2.07	0.88
3. (X-A)	—0.42	0.24	0.58	0.59	0.71	0.63	0.78	0.09	1.20	1.16	1.19
4. PAA Ratio[a]	—5.9	4.7	8.1	10.3	11.1	15.8	12.6	8.2	14.1	58.0	13.0
Expt. No. 5[d], Day 102											
1. X[e]	2.92	0.73	1.33	2.04	2.30	2.74	1.41	0.98	2.52	3.42	2.23
2. A[f]	4.09	0.46	0.83	1.23	1.48	1.57	0.66	0.60	1.27	2.26	0.97
3. (X-A)	—1.17	0.27	0.50	0.81	0.82	1.17	0.75	0.38	1.25	1.16	1.26
4. PAA Ratio[a]	—16.5	5.3	6.9	14.2	12.8	29.3	12.1	34.5	14.7	58.0	13.7
Expt. No. 6[d], Day 116											
1. X[e]	2.70	0.74	1.47	1.91	2.62	2.55	1.68	0.94	3.19	3.74	2.65
2. A[f]	3.46	0.25	0.50	0.90	1.67	1.40	0.37	0.64	0.92	2.26	0.66
3. (X-A)	—0.76	0.49	0.97	1.01	0.95	1.15	1.31	0.30	2.27	1.48	0.99
4. PAA Ratio[a]	—10.7	9.6	13.5	17.7	14.8	28.8	21.1	27.0	26.7	74.0	21.6

TABLE 4 (Continued)

	Lys	Met	Cys [b]	Arg	Val	Thr	Iso	Try	Leu	His	Phe [c]
Expt. No. 7 [d], Day 130											
1. X [e]	3.67	0.68	1.29	2.04	2.56	2.75	1.45	0.91	2.69	3.82	1.42
2. A [f]	4.32	0.33	0.58	1.31	1.61	1.68	0.52	0.59	1.13	2.54	0.93
3. (X-A)	—0.65	0.35	0.71	0.73	0.95	1.07	0.93	0.32	1.56	1.28	1.49
4. PAA Ratio [a]	—9.2	6.9	9.9	12.8	14.8	26.8	15.0	29.0	18.3	64.0	16.2
Expt. No. 8 [d], Day 158											
1. X [e]	0.90	0.77	2.39	1.70	3.32	—	1.84	—	3.23	4.16	2.61
2. A [f]	1.92	0.35	1.21	1.60	1.31	—	0.61	—	0.98	2.27	0.77
3. (X-A)	—1.02	0.42	1.18	0.10	2.01	—	1.23	—	2.25	1.89	1.84
4. PAA Ratio [a]	—14.4	8.2	16.6	1.8	31.4	—	19.8	—	26.5	99.5	20.0
Expt. 9 [d], Day 171											
1. X [e]	0.81	0.73	1.81	1.65	2.74	2.81	1.49	1.48	2.64	3.75	2.74
2. A [f]	1.72	0.43	1.18	1.44	1.50	1.90	0.61	0.92	1.00	2.52	1.13
3. (X-A)	—0.91	0.30	0.63	0.21	1.24	0.90	0.88	0.56	1.64	1.23	1.61
4. PAA Ratio [a]	—12.8	5.9	8.9	3.7	19.4	22.5	14.2	50.8	19.3	61.5	17.5
Expt. No. 10 [d], Day 193											
1. X [e]	1.24	1.03	1.53	—	3.67	3.44	1.98	1.97	3.30	—	3.81
2. A [f]	2.24	0.63	1.38	—	1.57	2.15	0.75	0.94	1.22	—	1.22
3. (X-A)	—1.00	0.40	0.15	—	2.10	1.29	1.23	1.03	2.08	—	2.59
4. PAA Ratio [a]	—14.1	7.8	2.1	—	32.8	32.3	19.8	93.7	24.5	—	28.2
Expt. No. 11 [d], Day 214											
1. X [e]	0.80	1.01	2.59	2.47	3.36	2.04	2.18	1.93	3.73	3.34	4.37
2. A [f]	1.65	0.47	1.52	1.38	1.20	0.78	0.69	1.04	0.84	1.72	0.91
3. (X-A)	—0.85	0.54	1.07	1.09	2.16	1.26	1.49	0.89	2.89	1.62	3.46
4. PAA Ratio [a]	—11.2	10.6	15.1	19.1	33.8	31.5	24.0	81.0	34.0	81.0	37.6
Expt. No. 12 [d], Day 235											
1. X [e]	1.11	1.05	2.39	2.53	3.20	2.35	1.96	1.90	3.39	3.53	3.78
2. A [f]	2.17	0.48	1.38	1.84	1.21	0.97	0.71	1.14	0.99	1.79	0.96
3. (X-A)	—1.06	0.57	1.01	0.69	1.99	1.38	1.25	0.76	2.40	1.74	2.82
4. PAA Ratio [a]	—14.9	11.2	14.2	12.1	31.1	34.5	20.2	69.0	28.2	87.0	30.7

TABLE 4 (Continued)

	Lys	Met	Cys [b]	Arg	Val	Thr	Iso	Try	Leu	His	Phe [c]
Expt. No. 13 [d], Day 256											
1. X [e]	1.45	1.16	2.20	2.15	2.96	1.28	1.60	1.84	2.72	3.15	3.06
2. A [f]	2.88	0.52	1.41	2.47	1.53	0.96	0.73	1.38	1.23	2.06	1.51
3. (X-A)	—1.43	0.64	0.79	0.04	1.43	0.32	0.87	0.40	1.49	1.09	1.55
4. PAA Ratio [a]	—20.1	12.5	11.1	0.7	22.3	8.0	14.0	41.8	17.5	54.5	16.8
Expt. No. 14 [d], Day 277											
1. X [e]	1.98	1.11	2.16	2.73	2.69	1.47	1.25	1.85	2.65	2.98	3.36
2. A [f]	3.73	0.74	1.75	3.14	1.89	1.23	0.90	1.58	1.61	2.13	2.11
3. (X-A)	—1.75	0.37	0.41	—0.41	0.80	0.24	0.35	0.27	1.04	0.85	1.25
4. PAA Ratio [a]	—24.6	7.3	5.8	—7.2	12.5	6.0	5.6	24.5	12.2	42.5	13.6
Expt. No. 15 [d], Day 298											
1. X [e]	1.59	1.03	1.85	2.99	2.84	2.07	1.66	1.91	3.03	3.32	4.90
2. A [f]	2.54	0.54	1.92	1.99	1.47	1.18	0.70	1.84	1.39	1.96	1.75
3. (X-A)	—0.95	0.49	—0.07	1.00	1.37	0.89	0.96	0.07	1.64	1.36	3.15
4. PAA Ratio [a]	—13.4	9.6	—1.0	17.5	21.4	22.3	15.5	6.4	19.3	68.0	34.2
Expt. No. 16 [d], Day 319											
1. X [e]	1.57	0.97	2.01	2.55	3.29	1.98	1.73	2.41	3.15	3.00	1.82
2. A [f]	2.10	0.57	1.73	1.58	1.74	0.98	0.81	1.97	1.47	1.30	0.59
3. (X-A)	—0.53	0.40	0.28	0.97	1.55	1.00	0.92	0.44	1.68	1.70	1.23
4. PAA Ratio [a]	—7.5	7.8	4.0	17.0	24.2	25.0	14.8	40.0	19.8	85.0	25.2

[a] Determined by the "Short" procedure (7).
[b] Includes methionine.
[c] Includes tyrosine.
[d] For each feeding test 84.9 gm wheat gluten, 95.1 gm sucrose, 20.0 gm corn oil mixed with 150 ml water was ingested.
[e] Analysis of pooled samples containing equal volume of 5 hourly samples after meal. Concentrations as mg/100 ml plasma.
[f] Fasting level concentration, mg/100 ml plasma.

COMMENTS

The clinical condition of dog 69 after being on a nonprotein diet for 130 days was similar to that of dog 109 after being on a wheat gluten diet for 322 days. Both dogs developed a hypoproteinemia (caused primarily by the decrease in the albumin fraction) and a hypercholesterolemia. Liver impairment is indicated for each dog by an increase in the alkaline phosphatase enzyme. While the hemoglobin and hematocrit for dog 109 decreased significantly, no such change was apparent for dog 69 during the protein depletion period. This difference may be due to a hemoconcentration occurring for dog 69 due to weight loss. The reduction in blood urea nitrogen and urine protein shows that protein metabolism had been curtailed markedly for each dog.

The clinical chemistry for dog 110 on the casein diet remained quite constant throughout the entire experiment. A hypercholesterolemia appeared for dog 110 which developed more slowly for this dog than was the case for dog 109 on the wheat gluten diet. This finding indicates that the blood cholesterol level is dependent upon the dietary protein quality. It is also interesting to note that the hypercholesterolemia developed more slowly for dogs 109 and 110 than for dog 69 on the nonprotein diet. This finding shows that the blood cholesterol level is dependent upon the dietary protein concentration and confirms similar results reported for the rat,[15] swine[16] and chick.[17] These findings reveal the importance of dietary protein quantity and quality as controlling factors for the development of hypercholesterolemia. The general cholesterol increase experienced by dog 110 on a supposedly good protein diet is probably due to the lower protein content and the higher fat content of the special diet, compared with the Purina Laboratory Chow which the dogs were fed during the control period. A small percentage of the cholesterol increase could have been caused by the blood loss during the course of the experiment. This effect has been noted for rats.[18]

After 130 days of protein depletion for dog 69, it is interesting to note the worsening of the dog's clinical condition when the wheat gluten diet was substituted for the nonprotein diet. The blood cholesterol increased another 30%, and additional liver impairment is strongly suggested by the sharp increase in the alkaline phosphatase. After the animal had been on the wheat gluten diet for 70 days, lysine monohydrochloride was added to the diet. A remarkable recovery in weight was attained in approximately 60 days, and in the dog's general clinical condition in 80 to 110 days.

It should be noted that during the protein depletion period (0-130 days) the dog became somewhat nervous and excitable. Within the first several weeks of the wheat gluten dietary period several of the animal caretakers, who had no knowledge of the dietary change, reported that this dog was becoming extremely nervous and much more excited than he had been during the previous month. And in the same manner, these men reported a general improvement in the dog's behavior during the first week that lysine was added to the diet.

Fasting Level Plasma Amino Acid Concentrations

Several investigations have shown that poor nutrition in general, and poor protein nutrition in particular, generally results in a reduced fasting level concentration for most of the essential amino acids.[3,4] Our results with the dog verify this generalization and indicate that the greater the protein depletion the greater the reduction in the fasting level concentrations for most of the essential amino acids. It should be pointed out that, while the fasting level concentrations did tend to decline during the first 150 days dog 110 was on a minimal casein diet, a return to normal concentrations took place during the latter part of the experiment. This type of change may indicate the animal's ability to adapt to a minimal protein intake.

The amino acid concentration changes which occurred for dog 109 on a wheat gluten diet for 322 days were similar, but of a lesser magnitude, than those which took place for dog 69 on a nonprotein diet for 130 days. While most of the amino acid concentrations were reduced, lysine and phenylalanine showed little change and histidine increased approximately one fold. The reason for or significance of this histidine elevation is not known.

It is interesting to note that the fasting level of lysine for dog 109 on wheat gluten (most limiting in lysine) was not affected as greatly as some of the other amino acids. This may indicate that metabolic activity is more important than dietary adequacy in controlling plasma amino acid fasting levels.

Along with these changes that took place during protein depletion, it can be seen that during the protein repletion period for dog 69 the fasting level concentrations for the amino acids gradually returned to the normal range. Since the dietary protein intake has been controlled and the clinical chemistry followed throughout this experiment, it appears that the fasting level concentrations for the amino acids do reflect to some degree protein nutritional status.

Plasma Amino Acid (PAA) Ratios

It is interesting to speculate as to the meaning of the PAA ratio changes during protein depletion and repletion. The elevation of the PAA ratios for methionine and methionine-plus-cystine for all 3 dogs suggests that the metabolism of these amino acids is quite sensitive to dietary protein status. The general decreases in the postabsorptive concentration changes for the other amino acids during the first 50 to 80 days of the protein depletion period for dog 69 may reflect a disturbance with the digestive enzyme system. It has been reported in the literature that this enzyme system is affected in the early stages of protein depletion.[19,20] The general tendency for the PAA ratios to come back to normal during the latter part of the protein depletion period may reflect an impairment of the enzymes involved with the removal of the amino acids from the plasma. Thus, with disturbances in protein digestion and also with amino acid utilization, the net effect is near normal postabsorptive amino acid changes.

The general trend in the postabsorptive changes for the majority of the amino acids during the wheat gluten and the wheat gluten-plus-lysine dietary period appeared to be just the opposite of that which occurred during the protein depletion

period. The elevation of the PAA ratios above the normal values during the early stages of recovery may reveal that the digestive enzyme system is restored to normal first, and during the latter part of the repletion period the enzymes involved with the utilization of the amino acids from the plasma are repaired.

On the basis of these preliminary observations, it is interesting to speculate as to possible indices that may be useful in detecting protein nutritional status. These indices are outlined below.

Possible Indices for Predicting Protein Nutrional Status for the Dog.

I. *Minimal Dietary Protein*
   a. High PAA ratios for methionine and methionine-plus-cystine.
   b. Normal A/G ratio.
   c. Normal fasting level plasma concentrations for the essential amino acids.

II. *Moderate Protein Depletion*
   a. High PAA ratios for methionine and methionine-plus-cystine.
   b. Slightly low A/G ratio.
   c. Slightly low (0-40% below normal) fasting level plasma concentrations for all the essential amino acids except histidine.
   d. High (80% above normal) fasting level concentration for histidine.

III. *Moderately Severe Protein Depletion*
   a. High PAA ratios for methionine and methionine-plus-cystine.
   b. Low A/G ratio.
   c. Low (0-70% below normal) fasting level plasma concentrations for all the essential amino acids except histidine.
   d. High (80% above normal) fasting level concentration for histidine.

The results of this preliminary study indicate that a combination of the fasting-level plasma amino acid concentrations, the postabsorptive plasma amino acid changes after the ingestion of a protein meal and the serum albumin concentrations may serve as a more sensitive index of protein nutritional status than the use of the serum albumin concentrations alone. Of course, additional studies will be necessary to determine how useful these indices might be in determining protein depletion. A study with dogs duplicating the experiment reported here is now in progress to confirm or reject these preliminary findings.

SUMMARY

Long-term feeding studies with 3 dogs are presented. These indicate that the changes in the fasting level concentrations of the plasma amino acids and the postprandial plasma amino acid concentration changes after a wheat gluten test meal are dependent upon the subject's protein nutritional status. The use of these two indices along with blood albumin concentrations appears to be a more sensitive index of protein nutritional status than the use of blood albumin levels alone.

In this study with dogs it was observed that severe protein depletion accompanied by a caloric deficiency caused a marked weight loss and a poor clinical condition. Recovery was not obtained by the addition of a poor protein (wheat gluten) to the diet. However, with the addition of L-lysine to the wheat gluten, the dog regained his original weight and his clinical picture returned to normal. During the course of these studies, it was found that serum cholesterol levels for the dog are dependent upon the dietary protein content and quality. The significance of these findings is discussed.

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DISCUSSION

DR. HOLT: I would like to raise one question in connection with the basic assumption Dr. Longenecker has made, and that is that removal of amino acids from the blood is in proportion to the requirements. As I see it the renal thresholds also enter into the picture. Those have been studied for individual amino acids. I am not aware that they are in any way parallel to requirements.

The second point I wanted to bring out was that he quoted us in regard to the plasma amino acid level as an index of protein efficiency. That is perfectly true. We did find in comparing kwashiorkor bloods in several countries with normal values that the plasma amino acid nitrogen was lower, but the urea was much more strikingly lower and, therefore, we think it is a more valuable index.

DR. LONGENECKER: As to your first comment, assuming that the amino acids are removed from the blood in proportion to the requirements, I do not know how much effect the renal threshold has on this. I do know there are several reports in the literature, one in particular by Munro, who fed several

nonprotein diets and found the plasma amino acids actually were lowered, and they seemed to be lowered in proportion to the requirements of the animal. I would not say that this assumption is exact. I am sure it is not. However, I think the overall assumption is logical and employing this postulation enables one to interpret postabsorptive plasma amino acid changes more meaningfully than by considering only absolute concentration changes.

Your second comment on urea. I think I said that your data indicated that the plasma amino acids may serve as an index of protein efficiency. I did not want to infer that you said they were or should be used as an index, because I do not believe we have enough data yet to say that they are. I think we can say there is an indication they might possibly be an index. As for urea, I hesitate to use urea because I think there is so much fluctuation in urea values. Possibly this fluctuation is due to analytical difficulties.

Practical Synthetic Routes to the Essential Amino Acids

H. C. White

IT IS MY AIM to summarize our conclusions as to the most practicable commercial routes of synthesis for the individual amino acids, based both on our own experience and on the patent and periodical literature. Availability of raw materials will be mentioned only in those cases where an adequate supply for commercial-scale production might be a problem.

Before amino acid supplementation of animal or human diets can be realized, not only must the nutritional value be demonstrated, but the amino acids must be available in adequate supply and at reasonable cost. Development of commercially feasible synthetic routes to the essential amino acids has been a goal of our group for almost 20 years.

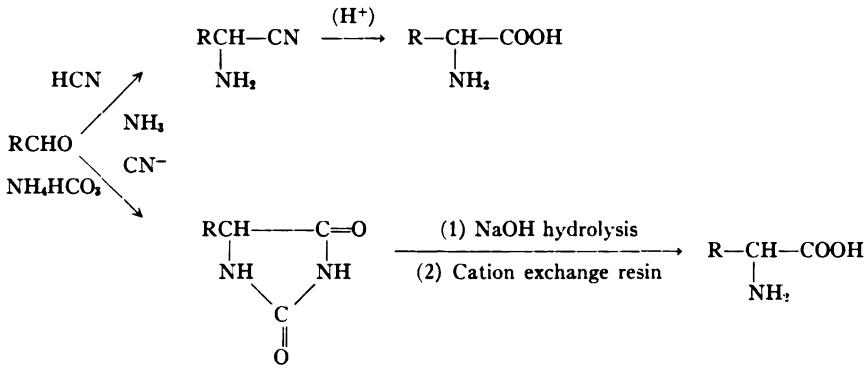
Except for L-lysine, our efforts have been directed to the DL-amino acids. As both isomers of methionine are utilized, its resolution would be uneconomical. Resolution of the remaining essential amino acids, while nutritionally desirable, must wait for further advances in technique before becoming commercially feasible.

For convenience, I have grouped the eight amino acids essential for man in three categories: Valine-leucine-isoleucine, threonine-lysine-methionine, phenylalanine-tryptophan.

Group I

In the first group are the simple aliphatic amino acids. An obvious synthetic route consists of α-halogenation of the corresponding acid followed by ammonolysis. While this is a practical route for glycine and alanine, the aliphatic acids for valine, leucine and isoleucine are not adequately available.

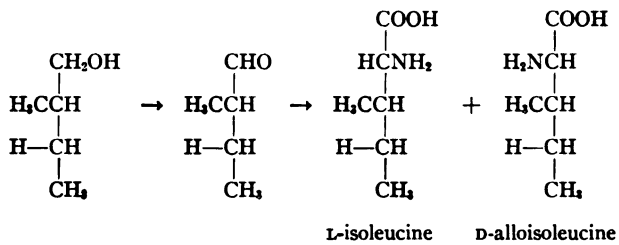
The preferred synthesis starts with an aldehyde containing one less carbon and proceeds by way of either the Strecker synthesis or the corresponding hydantoin as follows:



On the basis of ease of operation and lower corrosion of metal equipment, we prefer the hydantoin process.

As isobutyraldehyde is commercially available at a reasonable cost, valine is potentially the cheapest of the essential amino acids.

Leucine and isoleucine will be considered together, as the same raw material, fusel oil, is used for both. Fusel oil, a byproduct of the fermentation industry, is primarily a mixture of isoamyl alcohol and D-2-methyl-l-butanol. These alcohols result from bacterial action on small amounts of L-leucine and L-isoleucine present during the fermentation process. The configuration of the β-carbon atom of the L-isoleucine gives the optically active D-2-methyl-l-butanol, and this activity is preserved during the oxidation of the alcohol to D-2-methyl-butyraldehyde and subsequent conversion to the amino acid[2]:



The resulting product is thus a 50-50 mixture of the diastereo isomers L-isoleucine and D-alloisoleucine. As there is no convenient name to apply to this mixture, it is unfortunate that significant amounts have been marketed as its nutritional equivalent, DL-isoleucine. Probably the easiest way to check the composition is by optical rotation.

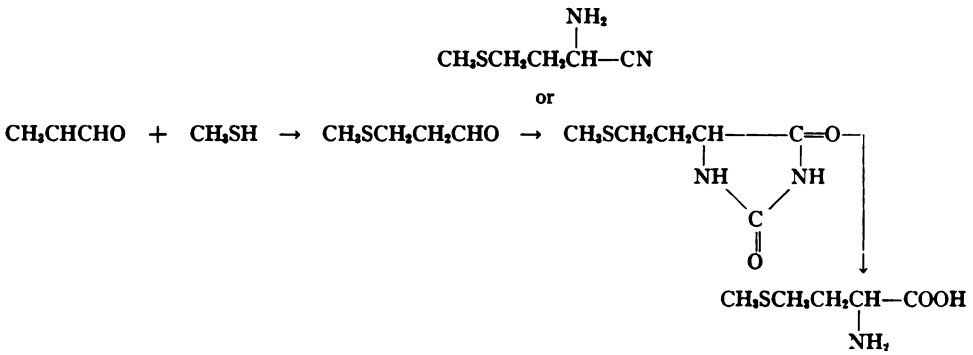
As fusel oil is a byproduct, its supply is dependent upon the fermentation industry and this supply varies considerably from year to year. The quantity of fusel oil available has been declining over the last decade until the outlook for large commercial quantities of isoleucine (and to a lesser extent leucine) by this process is not particularly promising. In addition, the separation of the two alcohols, boiling at only 2.7° apart, is a difficult one requiring at least 100 theoretical plates to achieve a purity of about 98%. Increasing the tolerance of leucine in the isoleucine mixture or vice versa would lower the cost considerably. Synthetic isoamyl

alcohol probably could be substituted for the fermentation product for DL-leucine, but synthetic DL-2-methyl-l-butanol would give all four isomers of isoleucine.

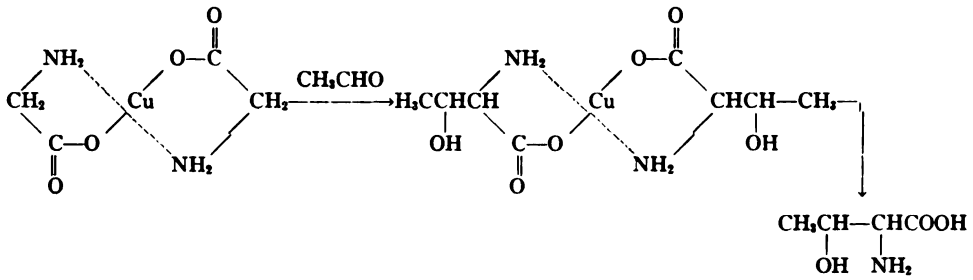
Group II

There is no general synthesis for the second group of amino acids consisting of methionine, threonine and lysine.

DL-Methionine is the first essential amino acid to reach commercial-scale production. It is also the only essential amino acid of which both isomers are normally utilized. Commercial syntheses begin with the addition of methyl mercaptan to acrolein to form β-methylmercaptopropionaldehyde, which is then converted to methionine via either the aminonitrile or the hydantoin.[3] Raw materials for the synthesis are abundantly available.



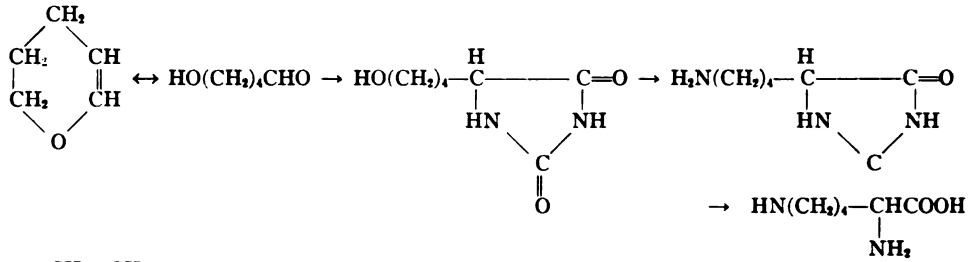
Threonine is complicated, as was the case with isoleucine, by two asymmetric carbon atoms and therefore the possibility of four isomers. Unfortunately, the erythro pair, DL-allothreonine, is considerably easier to prepare than the desired threo pair, DL-threonine. Although methods of interconverting DL-allothreonine to DL-threonine are known, they are too complex for commercial use. Stereospecific addition to crotonic acid derivatives has been studied by many, including ourselves, but the best method appears to be the partially stereospecific addition of acetaldehyde to cupric glycinate of Akabori and coworkers[4]:

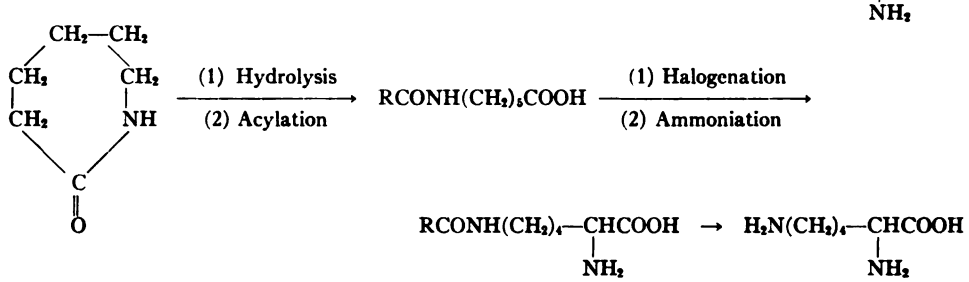


This method gives 70% to 75% yield of crude product assaying about 75% DL-threonine with the remainder DL-allothreonine. Separation of pure DL-threonine can be accomplished through the differential solubilities of the sodium

salts in alcohol (Shabica of Merck)[5] or, according to recent Belgian patents,[6] by crystallization of the cupric bisacetaldehyde bisthreonine complex.

Several practical commercial syntheses of L-lysine have been developed starting with abundant raw materials such as dihydropyran[7] and caprolactam[8]:



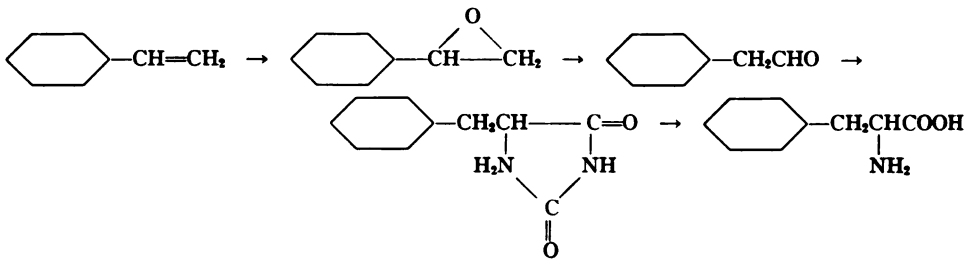


DL-Lysine can be resolved using L-glutamic acid[9] or D-camphoric acid,[10] with the result that L-lysine can be produced more economically than the L-lysine content in DL-lysine. This requires the recovery of the D-lysine followed by racemization to DL-lysine and recycle to the resolution procedure.

Recently microbiological procedures have been developed for production of L-lysine[11] which appear to be as cheap as or cheaper than chemical synthesis for moderate scales of production. This might not be true, however, if lysine reaches the scale of production that has been forecast if lysine supplementation of bread becomes accepted. Other potential large markets could be supplementation of cereals in those countries more dependent upon cereals for their protein supplies.

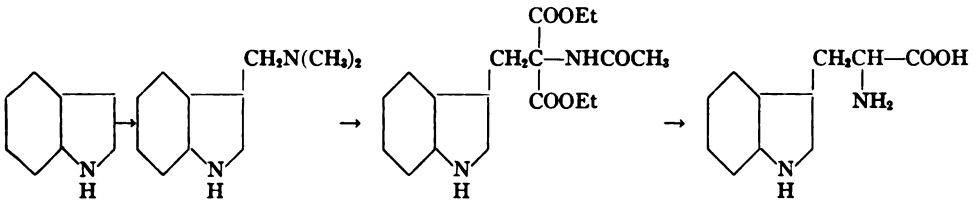
Group III

The third group consists of the aromatic amino acids phenylalanine and tryptophan. Although phenylalanine can be prepared using the diethyl acetamidomalonate procedure, we believe that the most suitable procedure for large-scale production involves the conversion of styrene to styrene oxide and then to phenylacetaldehyde followed by the hydantoin procedure to give the phenylalanine[12]:

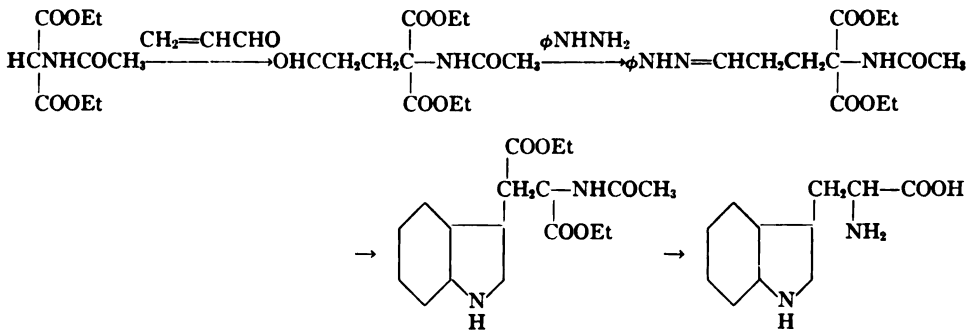


Tryptophan is the essential amino acid required in the smallest amount by humans and by animals and also appears to be potentially the most expensive to produce on a commercial scale. Practical syntheses start with indole by the procedures (a) developed by Snyder and Smith,[13] or (b) from phenyl hydrazine, acrolein and diethylacetamidomalonate as developed by Moe and Warner[14]:

a.



b.



With the present cost of indole, the General Mills process is preferable for large-scale production but, if there were a large demand, it is possible that the cost of indole could be lowered sufficiently to make it the preferred starting material.

In conclusion, there are tabulated below our long-range estimates for the selling prices of the individual amino acids at an arbitrary level of 5,000,000 pounds per year.

	Price Range $/lb.
DL-Valine	0.85—1.45
DL-Leucine	0.90—1.60
L-Isoleucine/D-alloisoleucine	1.05—1.80
L-Lysine • HCl (synthetic)	2.00—3.50
DL-Threonine (75% assay)	1.75—3.10
DL-Phenylalanine	1.05—1.80
DL-Tryptophan	4.70—8.00

In addition to the table, DL-methionine is currently available, feed grade, at $1.43 per pound.

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DISCUSSION

DR. HOWE: Were your prices based on the content of DL amino acid?

DR. WHITE: They were based on the amino acid as shown; the DL except in the case of lysine.

DR. HOWE: In the synthesis of isoleucine, you were starting with an optically active material and went through synthesis which might not give necessarily equal quantities of the two distereo isomers. Do you actually get 50-50?

DR. WHITE: The actual composition, as far as we can determine, is about 48% L-isoleucine and 52% DL. So it is very close to 50-50.

Summary of Progress on the Use of Purified Amino Acids in Foods

E. E. Howe

SINCE THE DISCOVERY that certain of the constituent amino acids of proteins are essential to mammalian life, man has been intrigued with the potential of these compounds as food supplements. Clearly, if dietary proteins are deficient in one of these essential nutrients, its addition must increase the nutritional value of the diet greatly out of proportion to the quantity of the amino acid added. Mendel[1] advanced this important concept as early as 1923 and, in addition, noted that an animal ate primarily to satisfy his energy requirements and that use of a diet of high caloric density might under certain conditions precipitate an amino acid deficiency. Because of the validity of this latter concept, for the last decade and a half methionine has been used to supplement broiler rations. It is the purpose of my discussion, in the light of the papers presented earlier in the conference, to attempt to evaluate our progress in realizing the vast potential of amino acid supplementation.

Of course, the prime requisite for supplementation is to establish that there is a need for it, i.e., to demonstrate that segments of the world's population would benefit from the addition of one or more amino acids to their staple diets. Before discussing this matter, however, it seems desirable to consider a number of other factors which play an important role in the realization of our primary objective. These are economic availability, safety of use, and methods of determining supplementary requirements.

Economic Availability

Until relatively recently, pure amino acids have been in short supply for extensive animal and human study. Classically they have been isolated from protein hydrolysate or have been built up by chemical procedures from readily available raw materials. Obviously, preparation by the former method for large-scale supplementation is not a likely prospect. If a protein which has a high content of a desirable amino acid can be produced in quantity, why not feed it alone or with others which are mutually supplementary? Indeed, this is a widely used and highly effective practice.

Chemical synthesis is a logical method of large-scale production, since the ingenuity of organic chemists is almost unlimited in finding simple means of converting cheap raw material into complex molecules. During the past 20 years

progress in this area has been spectacular, with the result that many of the amino acids which were previously laboratory curiosities are now relatively common research material.

Yesterday Dr. White gave us an excellent summary of the progress made in recent years in developing methods of synthesis of the amino acids. He estimated that the cost of the racemic essential amino acids in large-scale production might range from $0.85 to $8.00 per pound. I am somewhat more optimistic. I believe that, given a sufficiently large market incentive, any of the L-amino acids might eventually be synthesized to sell for $1.00 to $4.00 per pound. This method, however, also has a serious disadvantage in that each of the essential amino acids has at least one center of asymmetry which in most cases necessitates development of costly resolution and racemization procedures. DL-Methionine, both isomers of which are utilized by man, is now being produced synthetically and sells for $1.45 per pound.

Recently a third source of amino acids has become available. This source, production by fermentation, will undoubtedly become more important than either of the others. It has the great advantage that none but the L-isomers are produced and these in a free and easily isolatable form. Already glutamic acid and lysine are being produced commercially by this method, and the recent issuance of a U. S. patent[2] indicates that it is also applicable to L-threonine. With an adequate mutation program and a sufficiently high production level, any of the naturally occurring amino acids can probably be made microbiologically for less than $1.00 per pound. It may therefore be concluded that the problem of availability of pure amino acids for supplementation is no longer of any great importance. If the demand for large quantities of these substances arises, it will be met by the chemical industry.

Safety of Use

Harper[3] has suggested use of the term "unbalanced" to describe proteins that are "low in one or more essential amino acids or that are generally poorly balanced from a nutritional point of view." For example, the BV of wheat gluten is much improved by the addition of lysine and because of this fact wheat gluten is said to be "unbalanced" or "deficient" in lysine. On the other hand, it has been shown that excesses of certain amino acids may increase the requirement for the limiting amino acid in a protein. This phenomenon Harper calls "imbalance." In experimental animals he has demonstrated many delicate imbalances involving nearly all of the essential amino acids.[4] It has been shown that these imbalances are manifest, not only by a decreased growth rate, but in some instances by other changes such as increased deposition of liver fat or a decrease in liver enzymes. Amino acid imbalances have made themselves apparent only on low-protein diets.

As Dr. Harper remarked yesterday, amino acid antagonisms have also been demonstrated, as illustrated by the relationship of leucine and isoleucine in zein. Dr. Bender observed this effect in his experiments with amino acid mixtures patterned after egg protein. You will recall that it was necessary to halve, not only the isoleucine of the mixture but the leucine as well, in order to obtain optimum growth at the 50% isoleucine level. Finally, toxicities without involvement of other amino

acids have been observed with excessively high levels of two amino acids, methionine and tyrosine.

Experiments in which the individual amino acids were administered intraperitoneally have shown that they have a low order of toxicity.[5] Much more extensive experiments have demonstrated the harmless nature of large quantities of lysine,[6-8] and the Food and Drug Administration has placed it on the "generally recognized as safe" list of food components.[9] It is now generally recognized that judicious supplementation of foods with amino acids, rather than creating imbalances as was feared earlier, will correct them and in so doing produce a nutritionally superior protein.

Determination of Supplementary Requirement

Block and Mitchell[10] some time ago introduced the concept of determining the nutritional value of a protein on the basis of its amino acid composition. The composition of the protein under test was compared with that of egg protein and the limiting amino acid expressed as a percentage of the same amino acid in egg. This value was called the "chemical score" of the protein. This concept suffers from two shortcomings.

In the first place, egg protein is not an ideal protein in the sense that it contains all of the amino acids in just the proportions needed, but it is excellent nutritionally because it contains an exceptionally high percentage of essential amino acids many of which are utilized to a considerable extent as sources of energy and nonspecific nitrogen. Bender[11] has shown that whole-egg protein may be diluted with 15% of a mixture of nonessential amino acids without affecting its NPU value. The FAO Committee on Protein Requirement[12a] has attempted to eliminate this disadvantage by substituting for egg protein a provisional reference amino acid pattern formulated from the amino acid requirement of man as determined by Rose, Leverton, Holt and others.

In the second place, the CS of a protein does not take into consideration its digestibility. Evidence is accumulating that vegetable proteins may in general be more difficult to hydrolyze than those of animal origin. Failure of CS to correlate with biological availability is shown by a simple experiment carried out in the Merck Institute. By chemical analysis lysine is the most limiting amino acid in corn protein, and supplementation of a corn-based diet with this amino acid might be expected to produce a growth response in weanling rats. Such, however, is not the case. On the other hand, a diet containing a mixture of amino acids with a corn protein pattern, while supporting a less rapid rate of growth than corn itself when supplemented with lysine, was found to be superior to corn. These data are shown in table 1.

The paper presented by Dr. Mauron at this conference indicates that a chemical method of determining biological availability based on enzymatic hydrolysis or determination of free ϵ-amino-groups of lysine may be a marked improvement over the unmodified chemical scoring procedure.

It would appear, however, that the CS of a protein by any method will give only a first approximation of its requirement for adequate supplementation. Its

TABLE 1
EFFECT OF LYSINE SUPPLEMENTATION OF A CORN MEAL DIET AND A DIET CONTAINING AN AMINO ACID MIXTURE PATTERNED ON CORN PROTEIN

Diet	Weight Gain 25 Days (gm)
Corn meal 96% (1.22% N)	26
Corn meal 96% (1.22% N) + 0.17% lysine HCl	25
Amino acid mixture after corn protein (1.22% N)	7
Amino acid mixture after corn protein (1.22% N) + 0.17% lysine HCl	37
Amino acid mixture after corn protein (1.22% N) + 0.95% NH_4 citrate	8

evaulation in experimental animals will give a second approximation, since human and animal requirements differ in some respects. Even so, both methods are of unquestioned value.

In table 2 are shown the amino acid profiles of the three most important cereal grains, wheat, rice, and corn, in comparison with the FAO reference pattern and the requirements for rat growth as calculated from Dr. Rose's early data. The italics indicate the deficient amino acids in each protein. It is of interest, however, that threonine and not the sulfur-containing amino acids, along with lysine, limits the growth of rats on a rice diet (table 2).

TABLE 2
AMINO ACID PROFILES EXPRESSED IN MILLIGRAMS OF AMINO ACID PER GRAM OF NITROGEN

	FAO Reference Pattern	Requirement for Rat Growth	Wheat	Rice	Corn
Isoleucine	270	185	275	295	250
Leucine	305	295	430	525	795
Lysine	270	370	*155*	*210*	*170*
Methionine + cystine	275	220	280	*180*	210
Phenylalanine + tyrosine	360	255	500	655	570
Threonine	180	185	245	225	255
Tryptophan	90	75	75	75	*45*
Valine	270	255	280	395	330

In tables 3 and 4 comparisons are made with other foodstuffs which are or may be used as major food sources. The calculations are based on the average amino acid compositions listed in The Amino Acid Handbook by Block and Weiss.[12b] It is recognized that there is great variation from sample to sample and that therefore the figures here give only an approximation of the composition of any sample. It is of interest that barley, although not widely used as a human food, has a rather good amino acid profile. Alfalfa is included only as an example of the proteins from leaf sources. The proteins from microbial sources are mentioned only as possible foods of the future. While methionine and cystine appear to be limiting in several of the protein sources, it seems likely that deficiencies of these amino acids seldom occur because the foodstuffs which show these apparent deficiencies in pattern have a high protein content. It may be recalled that Dr. Harper earlier

presented evidence that deficiencies would not occur upon feeding a protein with a BV as low as 50 if a sufficient quantity were ingested (tables 3 and 4).

The classical methods for estimating biological availability of a protein are: determination of nitrogen balance in the dog or rat, and growth and repletion determinations in the rat. Bender's NPU method[13] has definite advantages in economy of time and labor, and the more recent method of Longenecker and Hause,[14] based on variations in plasma amino acid levels before and after a test protein meal, appears to have a great potential, especially where time is a prime factor.

TABLE 3
LIMITING AMINO ACIDS IN VARIOUS PROTEINS

	FAO Reference Pattern	Peanut	Peas and Beans	Soybean Meal	Cottonseed Meal	Millet	Barley Meal	Alfalfa
Isoleucine	270	250	330	360	225	330	275	290
Leucine	305	380	505	475	355	775	420	450
Lysine	270	*220*	380	415	275	*150*	*225*	310
Methionine + cystine	275	*135*	*145*	*145*	*190*	225	245	*165*
Phenylalanine + tyrosine	360	550	610	500	475	>250	520	445
Threonine	180	170	250	245	210	240	220	320
Tryptophan	90	75	*55*	75	80	105	75	80
Valine	270	305	330	325	290	395	295	295

Need for Supplementation

Laboratory animals maintained on cereal-based diets usually are benefited by proper amino acid supplementation. For example, wheat-based diets are improved by lysine, corn by lysine and tryptophan and rice by lysine and threonine. It might be supposed, therefore, that a human population subsisting on similar diets would also benefit from amino acid supplementation. It must be remembered, however, that human requirements are not identical with those of animals; that most of our biological evaluations are made in rapidly growing animals; and that at no stage in its development does the weight of a human infant increase on a percentage basis at a rate approaching that observed with experimental animals.

The requirement of essential amino acids as determined by Rose et al.[15] for the maintenance of nitrogen balance in young adult males totals 6.35 gm. In

TABLE 4
LIMITING AMINO ACIDS IN VARIOUS PROTEINS

	FAO Reference Pattern	Oat Meal	Sorghum	Cassava	Algae	Bacteria	Fungi	Yeast
Isoleucine	270	305	325	*165*	290	280	*170*	330
Leucine	305	450	1000	*245*	480	375	550	405
Lysine	270	275	*125*	275	420	330	275	420
Methionine + cystine	275	*170*	200	*110*	*120*	215	250	*155*
Phenylalanine + tyrosine	360	540	565	*255*	525	370	510	470
Threonine	180	230	210	185	235	260	260	330
Tryptophan	90	75	70	*40*	125	*50*	95	90
Valine	270	330	350	240	390	320	395	375

addition, approximately 2.5 gm of nonspecific nitrogen is also required,[16] making a total protein requirement for maintenance of nitrogen balance in man of about 22 gm daily.

Yesterday Dr. Swendseid presented evidence that the requirement for essential amino acids increased when all are present in quantities approaching the minimum requirement. Using, then, Dr. Rose's safe values obtained by doubling the highest requirement found in any individual, the total daily protein requirement becomes approximately 28 gm. This is a surprisingly low quantity in view of the National Research Council's recommended daily allowance of 1 gm protein/kg.

On this basis it might be anticipated that very little protein malnutrition would ever be encountered in adults, at least, since all but the very poorest diets will supply this quantity of protein and the minimum requirements of the essential amino acids. The compositions of the cereal proteins, however, vary greatly from the "ideal" mixture and, as was mentioned earlier, it is with the low-protein diets that amino acid imbalances are manifest. Also, as previously discussed, poor digestibility may make these amino acids unavailable or available in such sequence that they cannot be used for tissue synthesis but are metabolized for energy. Further, the quantity of nitrogen retained as protein is a function of the nitrogen intake. This increase in body protein with increased intake is a measure of protein reserves. The optimum magnitude of reserve proteins is unknown, but they may play an important part in enabling an individual to withstand infection and other forms of stress. Certainly it is an established fact that a well nourished child withstands the effects of a contagious disease and recovers more rapidly than one whose protein intake is inadequate. Recently Truswell and associates[17] have shown that, under the natural stress of chicken pox infection, children on a maize-pea diet lost weight and plasma albumin, while those receiving maize and milk did not. Yeager and Miller[18] have reported that experimental infestation with *Trypanosoma cruzi* is less severe in rats maintained on a casein diet than in those receiving a wheat gluten diet. Addition of lysine and methionine to the gluten-bread diet again reduced the severity of the disease. Dubos and Schaedler[19] have reported that growing mice maintained on a diet containing 8% casein were much less resistant to a variety of bacterial infections than those receiving a 20% casein ration. The authors have recently expanded this work and find that mice receiving 20% wheat gluten are almost as susceptible as those receiving 5% casein and much more so than those ingesting 20% casein.[20] Animals receiving gluten, however, were not protected by lysine supplementation.[21] Whitehair and associates[22] advanced evidence that the marked value of wheat or oats in the treatment of pigs having chronic symptoms of enteritis was due in part at least to the increase in protein content of the diet.

On the other hand, it has been reported that tryptophan deficiency gives mice almost complete protection from the paralytic effects of inoculation with Theiler virus.[23] In addition, Kurek[24] reports that 6 of 9 shoats receiving a high-protein diet contracted erysipelas when inoculated and presumably all survived, whereas only 2 of 8 pigs ingesting a low-protein ration developed the disease and both died.

Scrimshaw, Taylor and Gordon[25] and Scrimshaw[26] have prepared excellent reviews of the interaction of malnutrition and infection. They show that these two factors have an additive or synergistic effect on the health of the subject much more often than they exert an antagonistic effect.

In animals it has been shown that the ratio of lysine requirement to total protein intake is greater for growth than for maintenance.[28,29] The only period in the human life span during which growth requirements are approximately equal to maintenance requirement is in infancy. This may help to explain why kwashiorkor, which is found in populations subsisting on a low-protein diet deficient in lysine, usually develops before the age of 2 years.

According to Allison, the expansion of the protein reserves is an important feature of growth and may play a significant role in the response of the individual to all types of stress. He has reported that nitrogen balance can be maintained in depleted dogs on 25% of the nitrogen intake required for balance in dogs with high protein reserves.[27] As an illustration of the value of such reserves, he showed that dogs which had been on a high-protein intake were not affected by doses of the drug, 2-aminofluorene, which produced a marked leucopenia in dogs with lower protein stores.

What is desperately needed is the establishment of the magnitude of the protein reserve for optimum performance, taking into consideration body size, health, endurance, reproduction and longevity. In addition, we need a satisfactory method for measuring this reserve. This is a very large order and not one which is likely to be filled in the near future.

Need for Supplementation in Technically Developed Countries

In industrialized countries of the Western world there is sufficient protein of high quality available to meet all requirements. It has been calculated that in the United States the per capita intake of protein is greater than 100 gm per day.[30] This figure is based on protein taken into the kitchen and not on actual consumption and is probably erroneously high.[31] In addition and more important, there are segments of the population which undoubtedly consume much less than the average. These groups include adolescent girls, pregnant women who wish to prevent weight gain, children who consume snacks consisting of bread and spreads, and elderly people with poor denture or low income. In these groups the possibility of protein deficiency is enhanced by an increase in the ratio of cereal protein to animal protein consumed. In the technically developed countries wheat is by far the most important dietary cereal. Since wheat proteins are so effectively supplemented by lysine, this amino acid is possibly the only one that need be considered at this time as a food supplement in these countries.

Since any protein deficiency existing in the industrialized countries must be marginal, it is difficult to demonstrate a beneficial effect of amino acid supplementation. Perhaps the most convincing study is that of Terry[32] at Dallas who, over the course of a year, gave 204 pregnant women 600 mg of lysine daily while 92 received only the usual prenatal treatment. At the end of the seventh month of pregnancy the hemoglobin levels of those receiving lysine were uniformly higher

than those of the control group, averaging 11.8 gm and 10.2 gm per 100 cc respectively. In the control group 7 subjects developed eclampsia, none in the treated group. Further, those receiving lysine showed subjective improvement, failing to a large extent to exhibit the lassitude and chronic fatigue so often encountered during the first trimester of pregnancy. This type of study should be repeated throughout the country.

Albanese et al.[33,34] have reported that a high percentage of infants who failed to grow satisfactorily when ingesting a standard milk-based formula developed normally when lysine was added to their diets. These experiments, however, suffer from a lack of control groups receiving no lysine supplementation. Vignec and Gasparik[35] have obtained similar results. They reported a significant increase in rate of gain of a group of infants receiving a lysine-supplemented diet, whereas the increase in rate of gain of a control group receiving no lysine was not significant. It is doubtful, however, that the difference between the test and control groups during the experimental period was a significant one. The standard milk formulas supply much more lysine than required by normal infants, but it must be remembered that the subjects of these studies who responded to lysine supplementation were not normal. It is possible that a partial failure in digestion or absorption of the milk proteins has so limited the amount of lysine available that addition of free lysine corrects this deficiency. The report of Gomez and associates[36] throws little light on the matter, since the growth of their subjects had been limited by inadequate food intake and they might not be expected to be abnormal in any other way. It is of interest, however, that, although these authors interpreted their data to mean that lysine supplementation was without effect, 2 of the 5 subjects appeared to have responded with an increase in nitrogen retention.

Tuttle et al.[37] have advanced evidence that the amino acid requirements of men increase with age. Albanese et al.[38] have conducted experiments indicating that lysine supplementation of diets of the aged may increase retention of nitrogen but, as the authors themselves realize, the results are no more than suggestive.

Many rigorously controlled experiments involving large numbers of individuals of the various suspect groups need to be conducted. In light of our present knowledge, the point of view that lysine supplementation of wheat products is valueless is equally as untenable as the opposite point of view that it has been demonstrated to be greatly beneficial. Until the evidence is in, a reasonable and sane approach is that held by an increasing number of qualified nutritionists: It is desirable to improve the quality of any foodstuff if it is economically feasible to do so.

Need for Supplementation in Technically Underdeveloped Countries

Amino acid supplementation of the diets of many of the technically underdeveloped countries whose populations subsist almost entirely on rice, corn, wheat, cassava, sorghum, millet etc. is at once more simple and more complex than such supplementation in the economically more fortunate areas. It is simpler in that the need is more clear cut and pressing, and more complex because the means are not as readily available for its implementation. As previously stated, in animal

studies wheat proteins are greatly improved by lysine. According to chemical analysis, lysine is also the limiting amino acid in rice. In rat growth experiments rice is improved by very limited addition of lysine,[39] but both lysine and threonine are required for marked improvement.[40] Corn requires lysine and tryptophan for marked improvement and is made even better by further addition of isoleucine, threonine and valine.[41] Lysine is the first limiting amino acid in millet[42] and also in sorghum,[43] with threonine probably the second most limiting in the latter grain. Thus, effective fortification in many areas of the world would require use of more than one amino acid, some of which at present are not readily available. When they do become available, the problems of distribution and incorporation into food are most formidable.

While it seems likely that judicious amino acid supplementation of these low-quality, low-protein diets would benefit large percentages of the populations subsisting on them, only limited scientific data are available to support this likelihood.

Bressani et al.[44] have shown that lysine supplementation caused a marked increase in nitrogen retention in all of 6 children who had recovered from severe protein malnutrition and were receiving 2 gm protein/kg/day in the form of wheat flour. In some of the children the retention approximated that obtained with milk protein. Addition of all the limiting amino acids as compared with the FAO reference standard produced slightly better results than lysine alone.

Rice et al.[45] have carried out nitrogen balance experiments with college students receiving 95% of their protein intake from white bread containing 4% nonfat milk solids. The authors found statistically significant increases in nitrogen retention with lysine supplementation at nitrogen intakes of both 0.7 gms and 1.0 gm of protein/kg/day. This experiment proves that lysine supplementation of the diet of an adult subsisting almost entirely on wheat flour will increase his protein reserves. Again, we cannot be absolutely certain that such an increase is beneficial.

Using 5 boys recently recovered from kwashiorkor as test subjects, Scrimshaw et al.[46] and Bressani et al.[47] studied the effect of supplementation of corn masa. At protein intakes of 1.5 to 3.0 gm/kg/day, neither lysine nor tryptophan alone affected nitrogen retention. When both were used together a marked positive effect was observed which was further increased by the inclusion of isoleucine. Addition of methionine to the level of the FAO reference pattern decreased nitrogen retention. Drs. Hansen and Brock have obtained essentially similar results and have also made the important observation that such supplementation increases plasma proteins.

Truswell and Brock[48] have reported that lysine, tryptophan and isoleucine supplementation of maize diets supplying adults with 0.5—1.0 gm protein/kg/day resulted in a statistically significant increased nitrogen retention in all of 8 subjects. Removal of the isoleucine had very little effect.

In Mexico, Gomez and associates[49] have studied the effect of supplementation of a diet consisting of corn and beans with lysine and trydtophan. In each of 4 preschool children an increased nitrogen retention was observed.

Loughlin et al.[50] have reported that lysine supplementation of the diets of growth-retarded Haitian children 6 to 16 years of age increased their rate of growth as measured by the Wetzel grid. A supplement of oxytetracycline had a similar effect.

Earlier in this conference Dr. Kaye reported that in infants the protein of rice was equally as well retained as that of milk. It should be recalled, however, that he also reported that lysine alone increased the retention of rice protein in one child, lysine and threonine in a second, while the two amino acids failed to exert any effect in a third. More experiments are required to establish the place, if any, for amino acids in the supplementation of rice in infant nutrition.

In adults, Hundley and associates[51] found that supplementation of a rice diet with lysine and threonine increased nitrogen retention to no greater extent than did an equal quantity of nonessential amino acids. In view of the findings of Rosenberg and Culik[39] previously referred to, it would appear most desirable to investigate the effect of small quantities of lysine (0.05-0.1%) in children subsisting on rice.

Hegsted and coworkers[52] observed a modest increase in nitrogen retention in young women when their all-vegetable diet was supplemented with lysine and methionine.

In the conference proceedings Dr. Nicol will report that, in adults, methionine supplementation of a diet supplying 25 gm of peanut and cassava protein daily caused an increase in nitrogen retention. When the protein in the diet was increased to 60 to 65 gm, a quantity commonly found in the Nigerian diet, supplementation was without effect.

While the assessment of the value of amino acid supplementation in technically underdeveloped countries must be based almost entirely on nitrogen retention data, it seems safe to conclude that children and pregnant women subsisting almost entirely on cereal diets would be benefited by judicious supplementation. It is also likely that an increase in the protein reserves of other adults would aid them in combatting infections and other forms of stress. Large-scale field trials are needed to determine the effect of amino acids under conditions as they actually exist. A variety of biochemical and clinical data must be obtained. The study just being completed in Haiti by Drs. Sebrell and King and their associates may serve as a small-scale model.

SUMMARY

1. The development of fermentation procedures for amino acids has made their large-scale production possible at a cost which makes supplementation of cereal grains feasible.

2. Use of amino acids to balance cereal proteins is a safe practice.

3. Methods are available for evaluating a protein and approximating the quantities of supplementary amino acids required for optimum nutritional value.

4. The magnitude of protein reserves for optimum biological performance is unknown and there is no simple method of quantitation of protein reserves.

5. Based on present knowledge, lysine is the only amino acid likely to find widespread use as a food supplement in the technically developed countries of the world. Many more studies are required to establish the value of this amino acid in the diets of children, pregnant women, adolescent girls and the aged.

6. In addition to lysine, threonine and tryptophan and possibly methionine may prove to be beneficial in the technically underdeveloped areas in which high percentages of the populations exist largely on cereals. Since at present only lysine and methionine are available at low cost, large-scale studies must be limited to use of these amino acids.

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The Present Position and Future Needs of Research on Leaf Protein

N. W. Pirie

PROTEIN PRODUCTION can be increased in two ways: by the general development of agriculture along conventional lines, or by the introduction of new sources of protein. These sources can be conveniently divided into those that already exist but are inadequately used, such as fish or the residues from production of vegetable oils, and those whose potentialities are clear in theory but have not yet been realized in practice. Leaf protein belongs to this last category. I emphasise it because, at the moment, it is underrated and very large quantities of it could be made. But I deplore any attempt to discuss whether it is better than, for example, fish meal or soy protein. The world's unsatisfied need for protein is large now and it is increasing. This need will not be met by the exclusive development of any one protein source; certain regions of the world are adapted to the production of one type of protein and other regions to the production of another. Therefore it is prudent not to rely on a single source of supplementation, and people like a mixed diet. For all these reasons I suggest that research on all reasonable sources of protein should be encouraged. The question is: Does the allocation of effort among the different protein sources bear the correct relation to their potentialities?

Rouelle made protein from hemlock leaves in 1773. That was 65 years before the word *protein* was coined by Berzelius, but Rouelle showed that he understood what he had done by referring to his substance as "la matière glutineuse ou végéto animale." Ereky proposed the use of leaf protein as a food for nonruminants in 1926, and I have been working on leaf proteins, on a gradually increasing scale, since 1935. Leaf protein is therefore no novelty and the method we use for its separation (table 1), is still, in essence, that used by Rouelle. The details of the method need not be given here for they have been published (Pirie 1952, 1955; Davys and Pirie 1960; Morrison and Pirie 1961). In the process, as much of the fibre, mineral matter and starch as possible is removed during purification. The removal of the last is wasteful and obviously unnecessary but, at this stage in the work, it seems important to get as high a protein content as possible. Later we will try the effects of less thorough processing. The primary product is a moist, compressed cake. This is perishable because it contains 60% of water and the dry matter contains 5% to 30% of fat according to the age and species of leaf used.

All the leaf fats that have been studied are highly unsaturated, so that both oxidative rancidity and the growth of microorganisms are problems in storage.

The moist protein keeps well at low temperatures and it can be canned. Here, also, there are some difficulties. The slow growth of ice crystals in a frozen block leads, as time goes on, to a product that is granular after thawing, and we have had trouble in canning because of the spores of thermophiles. These are temporary difficulties. The granular material becomes smooth again when well rubbed in a mortar or food mixer, and adequate autoclaving gives cans a satisfactory shelf life; we are working on the problem of preserving the moist protein with antiseptics. The alternative approach is to dry the protein and this has many obvious advantages. Unfortunately, if the protein is dried at a high temperature, it becomes hard and relatively indigestible. This is probably why some preparations, not made at Rothamsted, had a poor feeding value. With careful drying at low temperatures, especially if it is mixed with an extender such as flour or starch,

TABLE 1
PULPED GREEN LEAVES ARE SEPARATED INTO:

Juice, which gives after coagulation	coagulum containing	proteins, fats, starch	food for man & other non-ruminants
	fluid containing	amino acids, amides, sugars, salts etc.	medium for the growth of microorganisms
Fibrous residue containing	most of the	cellulose, hemicelluloses, lignins, pectin	still a fodder for ruminants and a substrate for microbial fermentation
	some of the	proteins, fats, starch	

the product is satisfactory. Freeze-drying gives an excellent product. Freeze-drying may not be practical on a large scale, but during the present experimental stage of the work it is the method of choice and, if done by the method we have described (Morrison and Pirie 1961), gives a light stable powder that can readily be resuspended in water. The lipids are still present so that the product, unless stored in nitrogen, slowly develops the flavour characteristic of oxidative rancidity. By solvent extraction, a dry, lipid-free powder can be made. This is troublesome and, looking further ahead, undesirable because the lipids are lost; lipids are a useful source of energy and, if even a fraction of the claims made for unsaturated fatty acids are true, may be valuable.

When this work started I assumed that leaf protein would be worth making and that, if any actual preparation should prove to be of low feeding value, the cause would be ineptitude in preparation rather than inadequacy in the original product. This seemed reasonable because many nonruminant animals, which presumably make little use of microbial synthesis of amino acids in the digestive tract, thrive well on leaves, and partly because what is loosely called leaf protein

is a mixture of an immense number of entirely different proteins. It is most unlikely that such a mixture will have an unbalanced amino acid distribution; disbalance is the prerogative of pure proteins or foods containing only a small number of separate proteins.

Amino acid determinations bear out this expectation. The early published figures were conflicting because, when whole leaf meals are analyzed, there are errors arising from humin formation and other causes, and when isolated proteins have been analyzed they have sometimes been only a small and possibly an atypical part of the whole. We can therefore get doubtful results on a relevant and defined starting material, or more precise results on a starting material of doubtful significance (c.f. Pirie, 1955, 1959). Fowden seems to achieve the happiest compromise by extracting 80% to 90% of the protein in a partly purified form. The results given in table 2 are averages of the determinations on eight lots of barley leaves taken at different ages and grown with different manurial treatments (Pleshkov and

TABLE 2
AMINO ACID COMPOSITION OF BARLEY LEAF PROTEIN
(amino acid N as a percentage of protein N)

Arginine	12.2	Alanine	7.0
Histidine	3.7	Aspartic acid	6.9
Leucines	12.7	Cystine	trace
Lysine	6.1	Glutamic acid	6.9
Methionine	2.2	Glycine	6.7
Phenylalanine	3.1	Proline	4.8
Threonine	3.4	Serine	2.6
Tryptophan	1.3	Tyrosine	3.1
Valine	6.5		

Averages from Pleshkov & Fowden (Nature, *183*: 1445. 1959)

Fowden 1959). The trustworthy figures that have been published for protein made from other species are similar, though their methionine content is generally lower. Much work remains to be done on the routine determination of the amino acid pattern of bulk preparations of protein made from different species grown under different cultural conditions.

Amino acid analysis only establishes the possibility that a protein will be valuable in nutrition; the real criterion is an experiment using the species we are interested in and using it in relevant physiological circumstances. Professor Cruickshank will describe experiments done in Jamaica showing that leaf protein is useful in the nutrition of infants. Two experiments on pigs have been completed. The first (Barber, Braude and Mitchell 1959) showed that it was as good as, or a little better than, fish meal. Fish meal is a variable commodity and some skeptics said that Braude had used a poor sample for comparison. The late Dr. J. Duckworth was one of these but was easily persuaded to repeat the experiment. The results (Duckworth, Hepburn and Woodham 1961) are in press and are summarised in table 3. This summary shows that the pigs were on a diet in which insufficient protein was limiting growth and that 7% leaf protein was as good a supplement as 8% fish meal. In that experiment, commercially freeze-dried protein made from wheat cut in April and May was used. I do not claim that these samples of leaf

protein are equivalent to the preeminent proteins, e.g. milk or egg protein, but I think that these experiments show that it is as good as most proteins and well worth making.

It is difficult, under carefully controlled conditions, to dry enough protein for pig or human experiments, but we are developing equipment for this. Duckworth et al. (1961) found no loss in feeding value for rats as long as the temperature during drying did not exceed 82° C; the presence of other materials, such as barley meal, was immaterial, although, as mentioned earlier, it may improve the texture and appearance of the product. This set of experiments also showed that barley protein dried by acetone extraction was as good as protein dried carefully with the lipids still present. The possibility that some component of the lipid fraction, choline for example, is responsible for the good results seems therefore to be unlikely. Several rat experiments by Dr. K. M. Henry (unpublished) and one by Dr. C. A. Shacklady on chickens have given similar results.

TABLE 3
COMPARISON OF LEAF PROTEIN AND FISH MEAL IN DIET OF PIGS

Supplement protein (%)		Total protein in diet (%)	Days needed to reach 100 lb.	Food eaten per lb. gain
Leaf protein	10	17.1	51	2.5
	6.9	15.5	54	2.8
	4.5	14.4	61	3.1
Fish meal	8	16.1	51	2.8
	5.5	14.8	56	2.9
	3	13.5	57	3.1

Groups of 8 weanling pigs on a 60:30 barley meal and miller's offals diet, supplementary protein replaces part of the barley meal.

(from Duckworth, Hepburn & Woodham 1961)

We have established that leaf protein can be produced in bulk and that it is worth making as a dietary supplement; presentation remains to be considered. Food preferences vary dramatically from place to place and from time to time. Though this variation is not unexpected because food preferences are largely matters of convention, it does not make them any the less real obstacles to improving the diet. Experts on various undernourished regions of the world invariably agree that it will be difficult to make any changes in the local diet because of the conservatism of the population. But conservatism only means that change cannot be made immediately; the novelty is looked at askance for a time, nibbled cautiously, and then accepted. The process may take months or years and it can be accelerated by judicious propaganda or the example of others but, almost regardless of the actual properties or merits of the novelty, acceptance can be won. A brief survey of the packaged foods and drinks now widely sold demonstrates this.

Two entirely different problems are presented by leaf protein. The first is, in the long run, unreal but it has to be solved before the second can be approached. It is to prepare dishes that will interest well fed visitors from ministries, embassies and the international organisations; also, for that matter, audiences such as this. In these circumstances anything like a plate of stew or a risotto, however acceptable

under other circumstances, would not do. We have therefore concentrated on what may not inaptly be called cocktail snacks (Morrison and Pirie 1960). These are forms of presentation for which spoons and individual plates are not needed and in which a considerable variety of styles and flavours can be tried at a time of day that is not normally considered a meal time. This form of presentation can be as simple as flavoured leaf protein paste on a wafer; fish, curry and mint flavours are suitable. The protein, mixed with spice or banana, can be encased in thin pastry or batter and baked or deep-fried. All these items are perishable and may need cooking immediately before a visit is expected. To have something readily transportable and always available, we freeze-dry spiced or curried mixtures cut into cubes. It is also possible to bake protein into biscuits with various flavouring agents but, because of association of green colour with mould in biscuits or bread, many people find the results unattractive. Although I have referred to these dishes as cocktail snacks, this form of presentation is not altogether unrealistic. No one proposes this as a sole protein source; it might reasonably be expected to give 10% supplementation to an inadequate diet—say 5 gm to 7 gm a day. Each of the snacks that we make contains 1 to 2 gm of protein; even if this form of presentation were used, only half a dozen snacks would be eaten.

So much for the short-term and somewhat artificial problem. The real problem is the introduction of leaf protein into the diet of an undernourished community. In at least two circumstances few difficulties need be expected. Where, as in much of West Africa, a complex stew containing many components is a common food, there would be no difficulty with leaf protein. Its presence in the stew would hardly be noticed. Similarly, communities accustomed to sprinkling food with powdered or crumbled substances such as spices, cheese, nuts or fish would presumably be easily persuaded to try crumbled leaf protein. In Britain it makes an attractive addition to a risotto. The various foodstuffs that are eaten in different parts of the world as boiled, baked or fried 5-gm to 10-gm balls or packets (ravioli, kromeski, bafflutes, pakodi, kabab, tikia etc.) are an obvious international extension of the forms of presentation that I have referred to as cocktail snacks. We will continue some trials on other methods of presenting the protein on the table, but this clearly is work that can be better done in places where the protein may ultimately become important. I have cooperated in a few trials in Ghana and Jamaica; surprise was shown at the appearance of the dishes but no one found them unpalatable.

Soft-textured, sappy leaves are most suitable as protein sources. For convenience we generally work with normal farm crops cut young and we have made laboratory-scale extractions from about 100 species. During a brief visit to Ghana, Byers (1961) experimented with 60 species, and work on more species is now in progress in Jamaica. The ideal source would obviously be a leaf that is a byproduct to some commercial crop such as cassava, banana, sugar cane, sweet potato, jute, ramie or kenaf; work on the laboratory scale can quickly show which will be suitable. If protein can be extracted from a leaf, we would expect, for the reasons already given, that it will have good food value. The number of tests made so far is insufficient to substantiate or refute expectation. In one experiment on chickens

(Duckworth et al. 1961), protein from mixed grasses had a Gross Protein Value of 71, kale 75, barley 77, rye 82 and tares 82. An experiment on rats (Henry, unpublished) gave protein from sugar beet or potato tops a lower value than protein from barley. Any protein made from a new species will therefore need careful assessment before it is used.

Until a unit is run simply for production on as many days of the year as possible, all estimates of cost are guesswork. We know the capital cost of machinery and housing and we can make a good guess at the amount of skilled and unskilled labour that will be needed. But the cost of the crop and the value of the residues, which, as table 1 shows, should be used, remain uncertain. A few limits can, however, be defined. In Europe and USA it is economical to grow and harvest crops and cart them to a byre to feed the cattle; this is the system of zero grazing. Though efficient, it is probably the most expensive way of getting food to a cow and, if it is an economical method of making cheese, this suggests that a crop specially grown and harvested would be an economical source of leaf protein, for the yield of protein from an acre should be at least three times as great as when cheese is made. Crop costs would be very much less if a byproduct such as pea haulm or sugar cane were used, and would be even more reduced if it should prove possible to use a pest like water hyacinth. The value of the residue as cattle fodder depends on circumstances. It is apparently economical in Florida to grow forage and then press much of the juice out of it mechanically because the drier forage is more readily eaten by cattle. This process is not intended to extract the protein but much of it is in fact extracted and wasted. This work suggests that in other wet tropical areas the residue, though depleted in protein, may have a value comparable to that of the starting material, and the extraction makes some unpalatable herbage palatable. These points are mentioned to illustrate the uncertainties.

The next stages of this work may be briefly outlined. Three questions have to be answered: What would leaf protein be made from? How would it be made? How would it be used?

In several places with dissimilar climates, laboratory-scale extractions should be made to see which of the local plants (special crops, byproducts and weeds) are suitable as protein sources. This will take 1 to 2 years, but the work we have already done makes it safe to assume that suitable plants will be found so that there need be no delay in arranging to install large-scale machinery. It is likely that, with minor modification, the type of machinery that we use will handle in the laboratory any leaf from which extraction is satisfactory. The machinery is still evolving and probably bears the same relationship to the machinery that will ultimately be used that Trevithick's steam engine bears to a modern one. But it works and we can lend the nonstandard parts of the equipment to anyone who is making arrangements to use it fully. We have already made a great many of the possible mistakes and so we suggest that people should start by using the existing machinery before they design improvements. These units would maintain a regular supply of protein from the more obvious local sources and also make experimental extractions from the species that laboratory work showed to be promising. Work on presentation need not wait for these local supplies, because we can supply 1- to 2-kg lots of freeze-

dried protein immediately and, with notice and some planning, larger amounts. I therefore suggest that, although logically the three questions should be answered in sequence, for each depends on the one before it, they could in practice be tackled simultaneously.

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Present Status of Proteins from Oilseeds

Aaron M. Altschul

WE HAVE HEARD at this Conference about specific mixtures designed to provide, particularly for children, adequate protein from plant sources. I should like to discuss principles and practices involved in applying oilseed proteins, and the like, to achieve such mixtures of high nutritive value. In so doing, I will attempt to cover the following subjects: a) the quantity of oilseeds available, or potentially available, for incorporation into mixtures for human foods; b) the problems that must be solved before these materials can be used effectively in human diets; c) the opportunities that already exist, on the basis of accumulated knowledge, for incorporating these materials into high-protein diets; and d) what we might expect as our knowledge and technology advance. While this discussion is confined to oilseeds primarily, many of the same principles are also applicable to the recovery of protein from grass and leaf sources, from seaweeds and microbial sources.

The potential for protein from some of the common plant sources is shown in table 1. Oilseeds and legumes have the potential now of furnishing almost half as much protein as do the three major cereals. Included are nonoilseed legumes because some mention will be made of their improvement through processing.

Animal vs. Vegetable Sources of Protein

The problems involved in designing satisfactory all-vegetable protein mixtures include first, production of mixtures with high protein content, and second, production of mixtures of good protein quality. But the second objective of quality remains only academic unless the first objective of quantity is achieved. One of the salient features of animal sources of proteins is their protein concentration as shown by examples given in table 2. By and large, protein represents a major proportion of the dry matter in animal foodstuffs; no one of the common sources is lower in protein concentration than 22%. The major sources of proteins of plant origin, the cereals, are low in protein concentration, no one exceeding 13% or 14%. Tapioca, which is a major foodstuff in certain areas, is as low as 1.3% in protein content. Among the legumes and oilseeds, only the soybean contains over 30% protein; and among other plant sources, only Chlorella and Torula yeast contain higher concentrations of proteins.

Satisfactory protein concentrations may be easily achieved by supplementing the cereal grains with animal products, and this is the usual practice in those parts of the world where there is adequate animal protein. But if we are forced to rely

TABLE 1
PRODUCTION OF PLANT PROTEIN SOURCES[1]

Commodity	Annual Production [1] 1000 metric tons	Average Protein Content [2, 3] %	Average Avail. Protein 1000 metric tons
	Cereals		
Wheat	180,000	13.0	23,400
Rice (paddy)	253,700	7.5	19,000
Corn (maize)	188,700	9.5	17,900
Cereal total	622,400		60,300
	Legumes		
Dry peas	4,600		
Broad beans	4,500		
Chick peas	8,000	26	
Lentils	660		
Dry beans	7,900		
Legumes total	26,060		6,770
	Oilseeds		
Soybeans	27,600	38	10,500
Groundnuts	13,900	25	3,470
Cottonseed	15,900	20	3,180
Sesame	1,750	25	437
Sunflowerseed	1,400	20	280
Oilseeds total	60,550		18,867

[1] Taken from data for 1958 in "Monthly Bulletin of Agricultural Economics and Statistics," F.A.O., Rome. Exclusive of U.S.S.R.

[2] Nitrogen x 6.25.

[3] Taken from data in "Processed Plant Protein Foodstuffs," A. M. Altschul, ed., Academic Press, New York, 1958; "Proteins in Foods," by S. Kuppuswamy, M. Sreenivasan and V. Subramanyan, Indian Medical Res. Council, New Delhi, 1958; Food Composition Tables for International Use, FAO, Washington 1949; and "Tables of the Amino Acids in Foods and Feedingstuffs," by D. Harvey, Commonwealth Agricultural Bureaux, Farnham Royal, Slough, Bucks, 1956.
There is a variation in protein content of the commodities depending on variety and growing conditions. The figures that are listed, therefore, are significant only as a basis for calculating an order of magnitude of protein supplies; the original references should be consulted for more details on composition.

only on products from plant sources, it becomes simply a matter of arithmetic to evaluate the possibilities. None of the common legumes or oilseeds contain enough protein that by themselves, even if we do not consider other factors such as taste or toxicity, they can increase the protein level of cereal foods to 25% or more, as is done in Incaparina, a supplement for children.[1] And if we take as another example the biscuits described by Dean,[1] there is still the need for including milk powder to achieve the desired protein concentration of 20%. It would seem logical to conclude that if the commonly grown plant protein sources are to supplement cereals or starchy roots effectively, there must be included a processing operation whereby their protein concentration is increased. What I am doing, of course, is restating what many of you have assumed in designing your vegetable protein mixtures.

Aside from the matter of protein concentration, animal sources of protein differ from vegetable sources in two other important respects. The quality of animal

TABLE 2
PROTEIN CONTENT OF SELECTED FOODSTUFFS [1]
(Dry Basis)

Animal Origin	% Protein (N x 6.25)	Plant Origin	% Protein (N x 6.25)
Milk			
Whole, dried	22-25	Rice, whole	7.5- 9.0
Skimmed, dried	34-38	Rice, polished	5.2-7.6
		Wheat, flour	9.8-13.5
		Corn, meal	7.0-9.4
Beef			
Dried	81-90	Chick pea	22-28
Roasted	72	Soybean	33-42
		Peanut (groundnut)	25-28
Egg		Walnut	15-21
Whole, dried	35	Potato	10-13
Whole, dried, defatted	77	Tapioca	1.3
		Alfalfa	18-23
Herring	81	Chlorella	23-44
Salmon	69	Torula yeast	38-55

[1] See comments on table 1.

protein is generally superior to that of vegetable protein. In general, the amino acid pattern of animal protein approaches more closely the ideal amino acid pattern than does the pattern for vegetable protein, which generally is deficient either in lysine or in S-amino acids. Secondly, the animal acts as an efficient filter, removing toxic and interfering materials from its own food either by detoxification or by elimination. Among the improvements achieved by the animal is the removal of fiber which, in high concentrations in vegetable sources of protein, is responsible for reduction in digestibility of the protein. The animal, therefore, may be considered as a factory for the concentration of proteins, for the production of protein of high quality and for the elimination of toxic and interfering materials. If these objectives are to be accomplished without the intervention of the animal, then processing, already required for protein concentration, must serve to improve protein quality as well. Since the animal is unavailable in many parts of the world, and is too expensive in other parts, the burden of our discussion is to determine to what extent processing will concentrate and improve the plant sources of protein.

Processing of Oilseeds[2]

Let us now consider the role of oilseeds. The unique virtue of oilseeds is their large oil content which makes possible concentration of their proteins by mechanical and chemical means as, for example, by screwpressing or other types of mechanical pressure, or by solvent extraction with non-aqueous solvents. This can be done without the addition of water—an important point, because relatively anhydrous materials are stable to storage, and the addition and subtraction of water is always a costly process. In common with other seeds, oilseeds have an outer covering; this, too, may be removed by proper hulling and screening opera-

tions. The sum of these two operations is the production from oilseeds of materials containing 50% or more of protein. Cottonseed containing 16.5% protein may be converted into cottonseed flour containing 50% to 55% protein by removal of oil and fiber.

In addition to concentrating protein, it has been possible during processing to improve the quality of the protein, particularly by removal of interfering substances, to the point where many of these concentrates have evolved as useful sources of protein for animal and man. This is demonstrated by specific examples from the cottonseed and soybean processing industry.[3]

For cottonseed the material which has been recognized as interfering with the nutritional value of the protein is gossypol, a yellow pigment located in pigment glands in the seed. There may be other toxic substances in cottonseed but, so far, the best measure of the absence of interfering materials in cottonseed products is a low level of total and free gossypol. Removal of gossypol from cottonseed takes place most easily in the screwpressing operation; the type of pressure with torque that takes place in the screwpress ruptures pigment glands and expels a large proportion of the gossypol into the oil, whence it is removed by refining. In numerous biological experiments it has been found that meals and flours prepared by operations involving screwpressing have been suitable in diets for monogastric animals and man, when their gossypol content is low enough and when they have not been overheated during processing.

There is an extensive effort in the United States to develop a strain of cottonseed which will be free of pigment glands and gossypol. Such strains have been found wild and have been bred into experimental seeds. This work is continuing apace with full support of industry and government.

The role of heat during processing in improving the quality of soybean products for monogastric animals is shown in figure 1. It is quite obvious that a slight amount of heat will improve the nutritive value immensely; the explanation offered is that the interfering materials such as soyin and trypsin inhibitor are proteins which are more sensitive to heat than are the major proteins of the seed; but there is the serious danger from overheating as shown in the same figure. Notice the effect of different kinds and degrees of heating.

Bender[1] and Mauron[1] have provided several other examples of the effect of overheating. This same effect has been demonstrated for cottonseed; it is a general phenomenon and is one of the liabilities of processing. We might consider the effect of heat on plant protein products under three different conditions of moisture content: low moisture, less than 8%; intermediate and drying moisture content, particularly the range of 10% to 20%; and soaking wet as in cooking. It is heat under drying conditions in the presence of carbohydrates that is most damaging.

Processing of oilseeds therefore turns out to be a complicated process in which a number of events take place: the protein in concentrated, toxic materials may be removed or inactivated, and protein damage may occur as a result of overheating. Oilseed processing is a compromise forced by economic circumstances and technological limitations. If oil is considered the most valuable product of the oilseed, the processing operation will be organized to obtain maximum yield and

quality of oils at lowest cost; protein will be a secondary consideration. This has been generally true in the past; that is why these seeds have been dubbed oilseeds. Now that protein value is more appreciated, protein quality is receiving greater consideration. Even so, technological limitations force a compromise. Lack of complete information on the interfering factors in soybeans prescribes heating as the best means of eliminating or minimizing their effects. In cottonseed, the lack of complete information on the chemistry of gossypol and on other toxic materials makes screwpressing or a combination of screwpressing and solvent extraction the best process for producing a satisfactory flour. Almost complete ignorance about

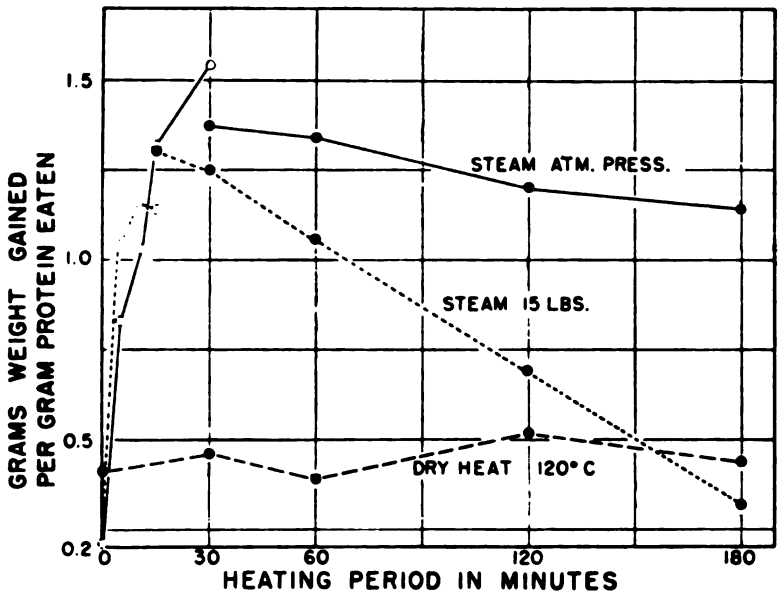

Figure 1—Effect of type and extent of heat treatment on nutritional value of soybean protein. Taken from: A. A. Klose, B. Hill, and H. L. Fevold. *Food Technology 2*, 201 (1948).
(Reproduced through the courtesy of *Food Technology*.)

the proteins of seeds prevents us from designing processing operations that would elicit the maximum nutritive response. We can safely expect that scientific and technological progress will allow the compromise to be established at higher and higher levels of quality and efficiency.

Definition of Suitable Products

The complex changes taking place during processing require understanding and adequate control; utilization of oilseed products for humans must be predicated on their adequate definition by objective tests. This is one of the serious problems involved in utilization of processed plant protein foodstuffs. The problem of measuring the effect of processing is, in a sense, no different from that of determining the nutritive value of any new protein source: it involves determination of BV or

a related parameter by one means or another. The one major difference is that these products are derived from known foodstuffs; therefore, we should be able to make assumptions about the changes that take place and hence reduce the amount of time and effort required to measure changes in protein quality.

Analysis of composition will denote whether oil and fiber removal have been adequate to increase the protein level sufficiently. There is also the analysis for interfering factors such as gossypol in cottonseed and the minor protein components of the soybean. The activity of the enzyme urease is often the measure of the adequacy of destruction of interfering materials in the soybean, since empirically it has been established that this enzyme has a similar pattern of heat destruction.[4] As far as we can gather, the urease activity aside from this empirical relationship has no *per se* interest except perhaps in rations for ruminants where urea is being added.

We have already noted that heat damage is one of the serious problems encountered in the processing of oilseeds.[5] Most of the tests that have been prescribed for measuring the extent of damage are empirical; included among them are vitamin analysis and physical-chemical tests. Thiamine is a heat-labile component. Its rate of disappearance may be related empirically to the destruction of proteins; hence, some investigators have thought to relate the amount of heat damage to the level of this vitamin. Solubility of proteins is a physical-chemical test.[6] It is not that loss of solubility is essentially a part of heat damage; it is just that the events that reduce the availability of amino acids and impair protein quality, largely sugar-amine reactions as described by Mauron,[1] also reduce solubility. Actually, pure seed proteins may be heated to some extent without serious reduction in nutritive value. Dye adsorption is another physical-chemical test.[7]

The major criticism of these empirical tests is their empiricism. They provide no information aside from their correlation with the lowering of nutritive value by heating. Moreover, any change in the conditions of processing or in the source of raw materials could render the accumulated data useless. These tests can, at best, serve as *ad hoc* tests until something better and more general becomes available.

Since the effect of heat in reducing the quality of processed oilseed proteins is related to chemical changes which render the amino acids less available to release by digestion, it has been argued that *in vitro* enzyme digestion tests could be suitable as a measure of heat damage. Tests such as digestibility by trypsin or digestion in series by a number of enzymes have, in some hands, provided valuable information. There are some rapid *in vivo* tests which might be applied. One that might recommend itself for further research and which was discussed by Longenecker,[1] is the amino acid composition of the plasma protein. There seems to be a relationship between the processing conditions of the soybeans and the amino acid compositions of the plasma in the portal vein of rats a short while after feeding the particular source of protein.[8]

The effect of processing and the availability of the limiting amino acids, lysine for processed cottonseed, is as good a method as may be necessary to determine the extent of the damage. Carpenter and others have found that the amount

of ε-amino lysine as measured by the Sanger reaction can be a measure of heat damage in some processed animal protein products.[9] Frampton and his associates at the Southern Division have extended this method to cottonseed and peanut meals;[10] they have been able to obtain a satisfactory relationship between heat damage and the ε-amino lysine content as shown in table 3. Application of the

TABLE 3
FREE ε-AMINO LYSINE IN COTTONSEED AND PEANUT PRODUCTS *

Product	Processing	Lysine, gm/16 gm Nitrogen
Peanut cotyledons	None	3.4
Peanut meal	Cooked 2 hr. at 250° F	1.9
" "	Cooked 2 hr. at 232° F	2.8
Cottonseed flour	Laboratory prepared	4.4
Cottonseed flour (commercial)	Screw pressed	3.8
Cottonseed meal (commercial)	" "	3.6
" " "	" "	2.5
" " "	Prepressed, solvent extracted	3.7
" " "	" " "	3.1

* V. L. Frampton, personal communication.

same technique to processed soybean products has failed to show serious differences in the ε-amino acid lysine content. Perhaps a study of chemical changes in the S-amino acids would provide satisfactory information. For peanuts we must assume that, parallel to loss in ε-amino lysine, is a loss in availability of the S-amino acids.

In general, we must remember that, particularly for cottonseed and soybeans, more than one property is to be measured: the elimination of toxic materials and the effect of the heat damage. One measure will usually not suffice for both properties. This is an area where additional research will enable the nutritionist to understand more clearly processing history and the suitability of any given processed plant protein.

The need for measures of changes that take place during processing is critical for the development of guidelines for flours that are suitable for feeding to humans. We cannot emphasize this point too often: the critical role of processing in improving the concentration and other properties of oilseed meals and the danger of damage from overheating or incomplete removal of toxic materials make it important to specify clearly and completely the oilseed product used in nutrition experiments or recommended for practical diets for human beings. There is no such thing as cottonseed flour *per se*, or soybean flour *per se*, or peanut flour. Neither is there a dried milk product, dried fish, or even dried tempeh. Each of these products is variable depending upon the conditions of processing.

Some of the elements that should be considered in guidelines for oilseed protein concentrates for human consumption are: 1) quality of raw material, 2) type of processing, 3) protein, oil, fiber, and moisture content, 4) maximum permissible heat damage, 5) maximum permissible level of toxic factors, 6) proper sanitation. It is quite obvious that one should start with good raw materials—such that have not suffered any storage damage. Neither should immature seeds be used.

The first and last portions of any crop, therefore, might be better used for other purposes than as source material for high-quality proteins. Since the methods of measuring heat damage or removal of interfering material are only approximate, it may be helpful to state the kind of processing that would be acceptable. For example, it is known that the screwpressed cottonseed meals or screwpressed followed by solvent extraction have a low gossypol content, but that those that have been directly solvent extracted by petroleum solvents, or which have been hydraulic pressed, an old form of cottonseed processing, have high gossypol content. These latter processes, therefore, should not be used for preparation of flours for human consumption without further investigation; they have been eliminated from similar consideration for production of protein concentrates for monogastric animals. The limit, if possible, to the maximum temperatures attained so as to avoid heat damage should be indicated, but this cannot often be done. Most of the damage that takes place in screwpressing, for example, takes place within the barrel of the press where the temperatures cannot readily be measured. However, emphasis should be given to the need for processing oilseeds at the minimum practical temperatures.

There are some who might argue that it is not necessary to achieve as high a concentration as 50% protein in order to provide material suitable for human consumption. There is, however, a hidden advantage in specifying that the materials contain more than 50% protein. Such a protein content is not produced easily. If there is, for example, a considerable amount of trash and dirt in the original material, it will be difficult to produce from it a meal containing 50% protein. Therefore, insistance on high protein content also assures that the raw materials will have the degree of cleanliness needed to make such a goal possible.

We might summarize the status of "simple" oilseed processing as follows: Protein concentration of oilseeds such as those listed in table 1 may be increased to over 50% by removal of oil and fiber. Proper control of processing conditions, which includes care to remove fiber and the known inhibitors and to minimize heat damage, yields products that have been proven highly suitable for supplementing cereal proteins in diets for monogastric animals and man. The greatest success has been achieved where there has been adequate care to describe the products by the best available objective tests. Were we to go no further, we would have already reached the point where proper utilization of available technology and information could add thousands of tons of good-quality proteins to the present world protein supply. But there are opportunities for further concentration of proteins, for removal of undesirable flavors and colors, and for improvement of protein quality by additional processing procedures. And such complex procedures might allow us to do more to solve the problems involving utilization of plant proteins as outlined in table 2.

Protein Concentrates and Isolates[11]

The elements of protein concentration from oil-free flours are: 1) isolation of the protein, 2) removal of non protein constituents. Preparations approximating

pure proteins may be produced by separating the proteins from the soluble and insoluble nonprotein constituents. This includes extraction, usually at alkaline pH, precipitation at acid pH, and drying. This involves the addition and subtraction of water, which is necessarily somewhat expensive. Such protein products might be expected to be free of many of the toxic or interfering materials that may arise in oilseed protein concentrates of lower protein concentration and should be considerably lower in fiber content. Moreover, if properly prepared, they should have no color, odor or flavor and, therefore, could be incorporated into foods without seriously affecting their traditional appearance, taste or flavor. By removing the protein from indigestible carbohydrates and fibrous materials, it is likely that the digestibility of the proteins themselves is improved. Hence, this kind of a process goes a longer way towards solving the problems involved in the utilization of plant proteins. Even though the amino acid composition cannot be improved by such a procedure, it is likely that the availability of the amino acids could be improved by the removal of the other foreign materials; certainly, the digestibility can be increased by removal of indigestible carbohydrates. Even the amino acid pattern could be improved because small amounts of amino acids could be supplemented in such an operation accurately and probably safely.

These materials might have possibilities for incorporation in human diets unavailable to the concentrates containing 50% protein. Tortillas, for example, could be improved as a source of protein if their protein contents were raised without affecting their color or taste. This cannot be done with flour, but could possibly be done with isolated oilseed protein from soybeans, peanuts and possibly cottonseed. Pasta could be improved as a source of protein in its various products and forms if its protein content were increased, but this cannot be done satisfactorily with 50% protein flours; it might possibly be done with isolated proteins. Although it is possible to supplement bread and increase its protein content with soybean flour, for example, or with cottonseed or peanut flours, there is the inevitable effect on taste, color and physical properties which limits the amount that can be incorporated. It would seem likely that much more protein could be incorporated into bread in the form of isolated proteins than in the form of 50% protein flour. To be sure, the isolated protein products are more expensive, probably now three and one-half times the cost of the proteins in the flour, but eventually they may be expected to be less than twice the raw material cost.

Gopalan and also Sreenivasan[1] described the potential uses in India for isolated proteins from peanuts in reducing the requirements for dry milk solids, and Pirie described the production of leaf protein.[1]

There are some forms of isolated proteins in between pure protein and the 50% flours. One such is produced by removing the soluble carbohydrates, either by extraction with alcohol or with acids. This process concentrates the protein to about 70%; if the solvent is alcohol, such a process succeeds in removing coloring or flavoring materials, yielding a product that would be less expensive than the fully isolated protein and yet might be more suitable for incorporation into flour and for uses for which the isolated proteins may be too expensive. Certainly this is a product deserving serious consideration.

Another possibility is that of removing the insoluble carbohydrates by sieving processes and by separation based on gravity. In our laboratory, Dr. Dieckert has been particularly interested in the subcellular components of the peanut.[12] Figure 2 shows a photomicrograph of a peanut paranchymal cell. You will notice that the cell contains a variety of subcellular particles. Many of these have been identified and separated by an anhydrous technique; among them are starch grains, aleurone grains, protein bodies, fragments of a network observed only by electron microscopy, and cell wall fragments. The chemical composition of the protein bodies and the aleurone grains is shown in table 4. Here is evidence

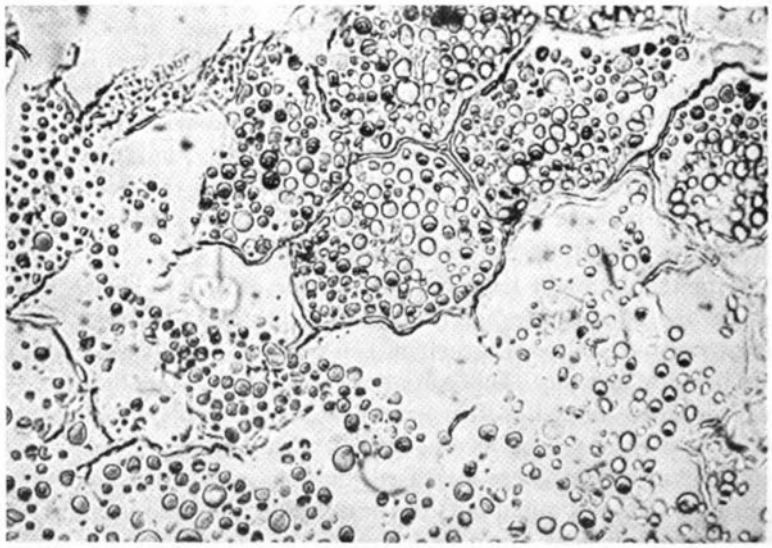

Figure 2—Photograph of aleurone grains. J. W. Dieckert, personal communication.

that by an anhydrous separation process, based entirely on gravity, fractions containing between 75% and 85% protein may be isolated. These represent the major proteins of the peanut.

While this particular separation was not devised with a view towards practicality, sound engineering might produce a practical approach. It is possible that

TABLE 4
COMPOSITION OF TWO SUBCELLULAR FRACTIONS OF THE PEANUT *

	Aleurone Grains %	Protein Bodies %
Nitrogen	11.4	13.3
Protein (Nx6.25)	71	83
Phosphorus	1.9	0.3
Sucrose	9.5	4.3
Phytic Acid	5.7	0.5

* J. W. Dieckert and J. E. Snowden, Jr., personal communication.

the cells of the soybean and cottonseed are not as prone to such separations, but the chances are that there is a high degree of compartmentalization in all seeds, and that it may be possible to achieve some sort of protein concentration in this way. This approach might be cheaper than a process which involves the addition and subtraction of water.

In table 5, we have listed examples of protein concentrates from oilseeds, ranging in concentration from 50% to 100% protein, which can supplement the

TABLE 5
OIL AND PROTEIN CONTENT OF OILSEEDS AND OILSEED PRODUCTS *

Material	Oil %	Protein % (N x 6.25)	Hulls %	Fiber %
Soybean (soybean)	20	43	8	
Soybean flour	1-2	50-52		3
Soybean protein (isolated)	0.1	93		0.7
Soybean protein (concentrate)		72-74		3.6
Tofu (soybean curd)	29	50		
Cottonseed	16.5	16.5	44.5	
Cottonseed flour	2-6	55-58		1-2
Peanut (groundnut)	46-52	25-30		3
Peanut flour	0.5-10	50-66		2-3
Peanut protein (isolated)		95		
Sesame	50	25		4
Sesame meal	7	46		5.3

* Dry basis.

protein concentration of cereal-based diets to levels where at least the protein content of the diet will be adequate, and where the quality approaches the quality that can be expected from animal proteins. Moreover, proper care in blending can produce mixtures which have adequate protein quality. Note the oil and protein composition of Tofu, a protein curd prepared from the soybeans without prior oil removal.[13]

A Note on Sesame and Legumes

Sesame and legumes are examples of two special cases. The first is that of sesame seed which is of particular interest because its protein is relatively high in methionine content; this could be used to balance out the low methionine content of legume proteins in mixtures of legume and sesame. Little success has been achieved thus far in producing a suitable sesame flour; one problem is to obtain complete removal of the hulls. Moreover, sesame seed is used directly for human consumption and this competition has, in certain areas, removed it economically from the class of oilseeds. If it were possible to make this crop more amenable to mechanical cultivation and harvesting and to dehulling, the economic position of sesame as an oilseed would be improved, and it would become a welcome addition to the repertoire of oilseed protein concentrates. Of course, the rapid advances in the production of synthetic methionine and the progress that is being made in learning how to use it, may eventually eliminate sesame from the field of special

interest. Similar arguments hold for sunflower seed, which also has an abundance of methionine.

The second special case is that of legumes. The only legumes that we have discussed thus far were the ones containing high concentrations of oil: the peanut (or groundnut) and the soybean. But most legumes have a relatively high protein content and all, with the exception of the peanut, have an abundance of lysine; the question is whether it is possible to concentrate their protein further and eliminate, where necessary, toxic material such as β-amino propionitrile and perhaps other factors that are responsible for some of the special disabilities of legumes when eaten in large amounts. Efforts to concentrate the protein by the same techniques that are used in concentrating proteins from oil-free oilseed meals, namely, removal by extraction at alkaline pH and precipitation at acid pH have been reported[14] but the results were discouraging. Large amounts of soluble carbohydrates in these seeds make the separation of the protein by a water-extraction procedure a difficult matter indeed. A more logical approach to the concentration of protein in legumes might be dry-separation which would take advantage of any compartmentilization of proteins within subcellular structures. This, indeed, may turn out to be practical and this may be one of the ways of really increasing the usefulness of legumes as protein concentrates.

Need for a Sophisticated Approach

If we are indeed to create, especially for children, all-vegetable protein mixtures that are the equivalent of animal-vegetable protein mixtures, then we must practice a higher degree of sophistication in our understanding and in the processing of the protein sources. We must consider the seed not as the end product but as the raw starting material for extensive modification of the types mentioned, modifications that are primarily or even exclusively industrial operations. We should note that the Oriental experience with soybean protein concentrates is evidence that some protein products can be manufactured on the home and village level. But the same can be done more economically on an industrial level. At this moment, perhaps, the modifications are based mostly on empirical practice, but we can look forward to fractionation based on knowledge of the seeds and their proteins. In areas where there is no previous history of the kind of technology required to manufacture the modified seed products, we cannot expect that, all at once, this will spring up. In areas where cottonseed meals, for example, go into fertilizer and fuel, one cannot expect to produce immediately a flour of the highest quality, suitable for human consumption. Where there is no background in protein chemistry, there is no basis for production of protein isolates. Need for proteins and availability of raw materials are not themselves sufficient reasons for setting up an operation which may fail for lack of knowledge and respect for proteins. This means that in those areas where the need for all-vegetable protein mixtures is critical and where the raw materials are available, vigorous efforts must be made to raise the level of technology.

Fortunately, there are areas where need and availability are combined with sufficient technological advance and sophistication in the handling of protein

materials. I saw cottonseed processing plants in Central America that were the equal in equipment and technical management of modern plants anywhere. I have no question that they can eventually produce a satisfactory flour for human consumption. We have heard at this meeting about protein sophistication in India and Japan, and I have seen such sophistication in Israel. No doubt, there are many other areas where one may consider production of one or another of the various forms of oilseed protein concentrates that were mentioned here.

In summary, the elements of a sophisticated approach to oilseed proteins for human consumption include the necessity to 1) concentrate protein, 2) preserve nutritive value by controlling heat damage, 3) eliminate interfering substances and fiber, 4) maintain chemical control over products, 5) improve amino acid pattern, 6) create forms that can be incorporated economically into accepted foods, 7) intensify research on nature and properties of oilseed proteins. We have already discussed the first five points. On the sixth point, it may be of interest to note that patents exist for modifying isolated proteins into forms that have properties similar to products from animal proteins.[15] I have not discussed the various forms of fermented products made from seeds or from various protein concentrates. These have been incorporated into foodstuffs of high acceptance as well as of high nutritive value. This is indeed an important approach to greater utilization of seed proteins, as was brought out by other speakers at this meeting.

I should like to conclude with a brief discussion of the seventh point in the list. There already exists considerable technical information which makes possible immediately the incorporation of oilseed flours or isolated proteins into practical forms for feeding to humans. I think, however, that this is only a beginning. Frightfully little information is available on seed proteins as compared with practically any other type of protein known and studied. Now a little progress is being reported in a number of laboratories. Dr. Dechary in our laboratory isolated a protein from the peanut which represents about 15% of the total protein. It is relatively homogeneous by chromatographic and ultracentrifuge standards; it is an interesting protein since it is a globulin which changes first when the peanut is germinated and might well be classified as a reserve protein.

Simple questions, such as the way pure seed proteins are split in the presence of proteolytic enzymes, remain to be answered. Many rudimentary questions, which have been asked and answered time and again for biologically active proteins and many animal proteins, remain to be asked, primarily because pure seed proteins are only beginning to become available. The trace materials in the seed which influence the quality of the proteins in these products or, for that matter, major constituents such as the insoluble carbohydrates are little known. Valuable information about the composition of the seed and its proteins can come from studies of its biochemistry, for the seed, after all, is first an organ for perpetuating the species and second a foodstuff. The seed is in a quiescent state, an extraordinarily stable state. We do not know how this stability is reflected in the architecture of the seed proteins, but we might ponder the fact that the stability in the seed might mitigate against realization of full value in nutrition without additional processing.

In full view of the limitations of our present knowedge of seed proteins, we should consider the success already achieved in applying them to nutritional ends little short of extraordinary. We may look forward to far-reaching improvements as we move toward more sophisticated approaches based on sound scientific information and better technological practice.

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Protein Problems Around The World

Protein Malnutrition from the WHO Viewpoint

R. C. Burgess

WHO Is CONCERNED with all aspects of health, and the emphasis that is placed on any one health problem is decided by the World Health Assembly. The Assembly is composed of delegates from public health departments of some one hundred governments, so that the pattern in public health programmes developed is a result of the majority opinion on the relative importance of the different hazards to health and the likelihood of applying successful control measures.

While there has been increasing recognition by public health authorities of the importance of malnutrition as a major public health problem in the last decade, this recognition is not yet wide enough to ensure that it will receive its proper share of funds that are set aside for the improvement of health.

I must make it quite clear that I do not consider that malnutrition is purely a problem for public health and medical departments. It is quite evident that the solution in the majority of countries is in improved food production, processing, distribution and marketing. Therefore agriculture, fisheries and other government departments have a great responsibility.

Nevertheless, the recognition of the magnitude and severity of malnutrition is generally a matter for public health departments. Only when the condition is placed in its proper perspective in the total field of health will a long-term policy involving the different government departments be formulated and carried out. It is sometimes not too difficult to get programmes started with an initial burst of enthusiam. However, they are not likely to be continued in countries with limited funds and limited staff unless the magnitude of the nutrition problem is clearly understood and continually presented.

There is considerable evidence to show that in some countries protein malnutrition is not recognized at all or, perhaps more often, only the frank disease is diagnosed and the significance of the minor degrees of deficiency entirely escapes attention. Annual reports of the medical departments of some of the poorer countries, which give figures for causes of admission to hospital and causes of death, most often lead one to believe that malnutrition is not a problem of any great importance. On the other hand, when WHO sends consultants to these countries they often report that many such cases of malnutrition are to be seen in hospitals but that they are not recognized.

One of the most important needs, therefore, is to reach a much wider understanding of the importance of malnutrition in the total field of disease. We are, however, confronted with a number of difficulties here. Malnutrition is more difficult to diagnose in its common mild form, and even when highly competent workers examine the same population they report different prevalences. In addition, in many countries the number of population surveys that have been done are limited and so we are dependent most often on what is seen in hospitals, which, of course, may represent only the disease pattern in a small section of the population. Severe malnutrition is often accompanied by infections and, since the medical profession in the less developed countries is still largely concerned with communicable disease, the latter is given as the cause of admission or the cause of death, and the primary nature of malnutrition is not recorded and probably not even recognized.

Perhaps the most important association is that of malnutrition with diarrhoea. This combination undoubtedly plays an important part in the production of mortality rates in the age group 1 to 4 years, which may be 50 or more per thousand children at that age. On a worldwide basis it is estimated that diseases and disorders in which diarrhoea is an outstanding manifestation account for five million deaths of infants and children each year. These diseases have received remarkably little study in relation to their enormous importance, and views regarding aetiology vary from the conviction that all diarrhoea is due to acute enteric infection to the belief that these conditions are predominantly caused by or related to preceding nutritional disease.

WHO has embarked on a long-term programme of investigation of diarrhoeal disease, and teams have already visited a number of countries. Many more such visits are planned. One of the main objectives of these visits is to sort out the part played by the infective agents and by malnutrition in the causation of disease. The composition of the team sent to a country depends somewhat on the trained personnel within the country, but the fields of epidemiology, environmental sanitation and nutrition in pediatrics are usually represented.

One such team has recently visited a tropical country with average or above average medical services for these countries, and a paragraph of the report reads as follows:

> "From the outset it soon became apparent from discussions, or observations and in part from figures and statistics available to us that confusion exists everywhere between diarrhoeal disease and malnutrition. This comparison is, of course, a general one in the world of diarrhoea in infancy and childhood and indeed in underdeveloped countries is the essence of one of the problems implicit in any investigation in these countries relative to either of the conditions."

The same confusion exists with regard to intestinal helminthic infections. In some countries more than 20% of admissions to children's hospitals are recorded as being due to ascaris infections, and a considerable number of such cases die. Would such infections occur in well nourished children? From parallel experience in animals it would appear that the answer is likely to be in the negative.

It seems to me that it is essential to look at nutritional status and disease in relation to different communicable diseases and to try to establish, on the basis of morbidity and mortality rates, their relative importance. An investigation conducted by INCAP, in which three comparable villages are the subject of study, is now in progress. * In one, communicable disease will be controlled; in another, the nutritional status will be improved; and the third will serve as a control.

This study will extend over a period of years and I believe that other similar studies are needed in order to obtain a proper perspective of nutrition in public health.

* Editor's note—See pp. 175-176.

Protein Malnutrition and the FAO Viewpoint

M. Autret

TWELVE YEARS AGO the first FAO/WHO Expert Committee on Nutrition drew attention to a syndrome described in the scientific literature after the war, and particularly in Africa, under various names. Kwashiorkor is the name which soon became prevalent in many regions of the world, and its mysterious flavour so appealed that a number of studies were carried out, both at the clinical level and in the laboratory, which probably would not have been done had the syndrome been called from the beginning "protein malnutrition."

Protein malnutrition is without doubt the main nutritional problem in the underdeveloped countries today. It is impossible to give figures of cases; there is some information on the number of severe cases here and there, but the number of incipient cases is extremely high and these are detected only where good maternal and child health services exist.

Recent information of the dietary value of proteins confirms that the No. 1 problem for FAO and for national agricultural departments is the production of protein foods of good quality. Food balance sheets and dietary surveys have always shown a deficit in protein, particularly animal protein. This deficit is much more important than was supposed. As a matter of fact, if the total intake of protein is sometimes satisfactory, it has been shown recently that the NPU is much lower than expected. It is the work of Platt and his coworkers which has drawn attention to this new notion of net protein value of the diet, which takes into account BV and digestibility as well as amino acid availability. The net utilizable proteins are much lower than requirements, even lower than the realistic requirements recommended by the FAO Expert Committee on Protein Requirements. It is the children who have the lowest protein intake as compared with their requirements.

We have known for a long time what has to be done:

1. The need to increase production of protein foods, including those of animal origin. I am convinced that, even if it is a good mixture of amino acids which counts finally, it will not be possible to supplement the basic diets of children in underdeveloped countries without a certain amount of animal protein if we want to avoid an excess of calories or a very bulky diet.

2. The need to orient selectively the production of foods. There is no sense in trying to increase all the constituents of the total production, as is generally the

tendency of farmers, who merely grow more of what they are growing. In that way, we would reach an excess of calories before covering protein requirements.

3. The need for education so as to make better use of available supplies. Of special importance is the education of the mother to ensure that the child gets its due share of the family meal.

Programs are under way to increase selectively food production and to educate the population. But there is another solution, namely the better utilization of *potential* resources. FAO started working in that direction as early as 1952 with special reference to the prevention of protein malnutrition. The problem has been clearly defined, but the solution is by no means simple. Kwashiorkor is generally the result of lack of protein in the diet, and proteins are expensive. The disease is met with in the socio-economic groups with a low purchasing power. In order to reconcile these two conflicting factors, it was necessary to find *relatively* cheap protein-rich foods. Thus the idea was conceived of utilizing the byproducts of various industries (fish, oilseeds, presscakes) and possibly, in the future, leaf proteins.

Considerable research has been done since that time, made possible through a grant of money from the Rockefeller Foundation which has been judiciously distributed and supervised by the Committee on Protein Malnutrition of the US National Research Council under the chairmanship of Dr. Sebrell. FAO has been happy to cooperate closely with the Committee, and I wish to assure Dr. Sebrell of our full cooperation in the future.

I will not discuss in detail what has been done. This has developed during the discussions at this meeting. I do want to draw certain conclusions and raise certain questions of immediate practical interest.

1. We are reasonably satisfied that we know now how to produce fish flour, peanut flour, cottonseed flour and soybean flour of good quality, and mixtures based on them which have been successfully tried. If it were merely a matter of feeding these products to animals through educating farmers—farmers with purchasing power—our task would soon be completed. But the problem is to give these foods to human beings with no or low purchasing power. Here the problem starts.

Again, if we were to give these foods to infants and very young children, we might compose a number of recipes of simple preparation that the child will accept readily. Here we have a problem of convincing the mother that the utilization of this food must be a daily one. Conditions being what they are in underdeveloped countries, it is my opinion that if we want to prevent kwashiorkor in children we have not only to educate the mothers but also to make sure that the entire family will have available such protein-rich foods. Therefore we have to face at the same time two problems: a) To prepare a commercial (ersatz) product, complete by itself, something equivalent to mixtures of wheat flour and milk. In this category, mixtures of peanut and millet, of peanut, millet and fish flour, of sorghum and cottonseed flour have been produced, but in that way mothers have to buy the full diet for the child while most of the staple foods are home produced, at least in the rural areas. The cost will limit the consumption, at least in the poorest groups. When such mixtures are commercially produced, I do not think that a study has been made of who buys them, probably not families where kwashiorkor is likely

to occur, but probably families who could afford to buy milk. b) To prepare only the protein-rich food in a form that can be added to the traditional family diet. This has been done with a mixture called *amama* found in West Africa, and with fish flour in Morocco. It requires the education of the mother in poor families; and in the underdeveloped countries ignorance and attachment to tradition go along generally with poverty.

Of these two solutions, which is the better? The problem is not solved. I do not think we have a proper answer. Probably there is room for the two types of product; from a recent survey by FAO in some West African countries, it seems that a ready-made preparation is likely to be better accepted, both by the families and by local retailers.

2. Up to now, much attention has been given to nutritional quality of the final product and very little or none at all to its commercial presentation, its appearance, taste and flavour. However, the child over 1 year or 2 years and perhaps more has already formed its food habits and preferences; in any case, the mother will have preferences. Therefore the final product must also suit the taste of the family.

It is therefore necessary to prepare a product adjusted to local tastes and habits. Technically the problem is an easy one, but up to now it has attracted very little attention (amama, Nestlé and Deans' biscuits, certain products in India). FAO has recently tackled this problem in West Africa, taking into account both the views of the consumer and those of the retailer.

3. In the same line of thought, fish flour raises problems of acceptability and commercialization. For instance, it has been decided that for reasons of digestibility in children and of keeping qualities it is necessary to have a defatted fish flour. Senecal's studies have shown that press-extracted flours with a 5% to 6% fat content are well tolerated by children above 8 months, but for a child of 2 to 4 months it is necessary to have a flour with a fat content under 3%. This necessitates solvent extraction. Moreover, a flour with 2% to 3% fat content quickly becomes rancid. It is necessary therefore to bring the fat content under 0.5%. The final product is then tasteless; it will be accepted by the young child but will not attract the interest of the family.

There will be no problem in using this product as a baby food, but for use in the family diet it will be necessary to enrich some family foods regularly consumed such as bread, pastes, flour. Unfortunately these are not foods which are very much used in underdeveloped countries, and most experiments in the field of bread enrichment have been abandoned, particularly in the Union of South Africa. The conclusion is that if we want to commercialize fish flour for family consumption, this can be done best with tasty fish flour, at least in those areas where there is no objection to fish taste. Those populations are, from what we know now, much more numerous than was thought a few years ago. The utilization of tasty fish flour raises two problems: 1) where the fat content will be above 5%, it will be necessary to add some antioxidants when permitted by the food laws (at least when the flour will not be naturally protected as is the case in Ghana where fish flours with 8% to 10% fat content keep well, thanks to a high natural content in tocopherol);

2) preparation of a defatted fish flour to which a good taste can be given by the addition of fermented fish solubles. This is a possibility which calls for further research.

4. Another problem which has not been sufficiently studied and is not solved is that of packaging. The packages must be strong enough to resist handling and transportation, thick or hard enough to prevent insect penetration, tight enough to prevent moisture and bacterial contamination, and relatively cheap. The size of each package will depend on local economy, food habits and purchasing power. FAO has been studying the question of packaging, particularly in Morocco and Senegal. We have no final solution to offer for the time being.

5. I said at the beginning that the products must be relatively cheap. I mean that the price of peanut flour or cottonseed flour, sold in packs of 5 to 10 kgs, to be later distributed in bulk at the retail level for family or community feeding, should not be higher than that of the staple foods. The protein-rich food flours mixed with cereals, flavoured if necessary and well packed in half-pound bags for child feeding, should never cost more than twice the price of staple cereals. Fish flours which are now sold at too high prices should never, both for commercial and psychological reasons, cost more than skim milk, namely 30 to 40 cents per kg. FAO is studying the question in Morocco, West Africa and Uganda. The list below indicates some prices of various products:

			Price per kg in dollars
Powdered skim milk:	Europe	Retail price	0.35—0.40
	N. Zealand	Wholesale price	0.25
	U. S. A.	Wholesale price	0.33
	Uganda	Wholesale price	0.42
Fish Flour:	Morocco	Wholesale price (per 10 kg)	0.38
		Retail price (per 100 gm)	1.00
Meat powder:	Kenya	Retail price (per ½ pound)	0.34
Flours composed of:			
Maize/cotton/yeast	Guatemala	Retail price (sold by 75 gm)	0.40
3 peanut/1 milk (Arlac)	Nigeria	Retail price (sold by pound)	0.20
Peanuts/casein/vitamins	Nigeria	Retail price	0.93
Peanut/sorghum/millet	Senegal	Retail price	0.40
Biscuits:			
Peanut/milk/maize	Uganda	Cost price	0.21
		Retail price	0.35
Ordinary biscuits *	Uganda	Retail price	1.00
Peanut flour:	Senegal	Retail price	0.08
Wheat, sorghum or Maize flour	Senegal	Retail price	0.12—0.14
Milled rice	Senegal	Retail price	0.16

* Without protein supplement.

I hope that this conference will bring some light on the various questions I have raised, which are of very great importance if we want to increase consumption and if we want the products to play a large part in the prevention of kwashiorkor.

SUMMARY

The nutritional aspects of protein-rich foods have been carefully studied both at the laboratory and at the clinical level. Some experiments on their acceptability on a small scale have been done and only in a few instances have there been any trials of commercialization.

We have now reached the stage where it is necessary to put all efforts on the promotion of sale and consumption of these products. For that it is necessary to assess the consumers' acceptability, taste, preferences and purchasing power. More studies are necessary to obtain products which can be readily commercialized. It is also time for private industry to come into the picture and, in cooperation with us, to devise suitable methods for commercialization of these nutritious products at reasonable prices.

DISCUSSION[1]

DR. COLLIS: There is just one thing I want to emphasize, and that is the urgency of this problem. I was very glad to hear Dr. Burgess say that WHO would be behind these trials. I like to call them trials rather than surveys because I think the next step is to try to put in force a great deal of what we have heard here during the last two days, to try to get to action. The urgency is tremendous in countries like Nigeria, racing forward with a huge population, and likewise in India.

I could not agree more than I do with Dr. Autret about ersatz foods, and I think we do want to get these kinds of products in these countries. In his table, prices are not altogether realistic. Take for example those I really know the details of, Product A and Product B. A is a product put on the market by a commercial firm that so far has not made any money out of it. The price includes advertisements, putting up a factory, putting the product on the market, and actually selling it to the people in the market. Whereas B is from a factory which has been built with the help of UNICEF and so far has not been sold in that way. There is a tremendous difference in price of a product produced commercially and one whose production has been aided. We have got to have it *in the market* where people will buy it, and not merely distribute it from centers. I think that is a very important point.

DR. RAO: As one who has been associated with the WHO and the UNICEF for several years, I would like to submit that my comments are in consonance with my allegiance to international organizations, including FAO.

We have moved from the era of vitamin research to the era of protein research—requirements, protein malnutrition, and other aspects. For the

[1] Editor's Note—This discussion covers the two preceding papers.

last 8 years and more, there has been intensive work which has been presented by the various international organizations. We have achieved quite a bit, but there are still many gaps. In this, I would like to point out one or two things.

There have been intensive surveys as far as protein malnutrition is concerned, but very little effort has been made to get mortality figures in the preschool children on what is due to environment or to food factors. Figures probably help to some extent, and that is one aspect which probably deserves attention.

Much has been said about fish flour, peanut flour, cottonseed flour and many more things which have been tried. I have tried them myself. There is a big gap between the rural and urban areas. While it is possible to use some of these foodstuffs in the urban areas where distribution is organized, the problem is entirely different in the rural areas where, in many parts of the state where I am working, the transportation is extremely difficult, there is poverty, there is ignornance, there is superstitution. As Dr. Griffith noted when he came to see our demonstrations, the mothers haven't got enough rupees to buy the foodstuffs, however nutritively sound the foods may be.

I am probably a little outdated as far as food technology is concerned. I still believe in natural foodstuffs. There is a very big need for the development of natural foods.

In India, many of you are aware of what are called community projects where about 60,000 people are grouped together in a project. They are trying to look after themselves. There has been intensive education. There has been intensive promotion of good will. It is these people who require attention as far as the so-called underdeveloped countries are concerned. It is this area which requires the study of the adequacy of local foods. It is very strange that certain foods which have been discarded previously as cattle fodder have now been utilized as human food. An intensive program is going on now on the adequacy of local foodstuffs.

DR. ALTSCHUL: In a complex field such as this, there is room for a number of points of view. In Dr. Autret's very interesting and provocative summary in discussing the question of ersatz products, he talked about two of the three possible alternatives. An ersatz material can be worse than the material it is substituted for, the equal, or better. We must not in our thinking exclude the third possibility. The history of society is full of examples. Take, for example, the field of textiles or the field of protective coatings or any number of fields, where ersatz materials eventually have proved to be superior. I would not want to leave out the possibility that some of these vegetable protein foods may eventually, when we know enough, prove to be superior to some of the traditional foods that some of us here are fighting so hard for.

DR. SCRIMSHAW: It is very useful to have the type of price information that Dr. Autret presented. As Dr. Collis pointed out, though, there are all sorts

of methods of expressing these, and I thought the example of Incaparina prices might make this clear.

In small packages, in small retail stores, the price does come to the 40 cents per kilo. In large packages, with advertising but with the wholesale type of distribution, it comes to about 26 cents a kilo. If you calculate in terms of bulk sales to institutions and to governments, with no advertising, it comes to about 22 cents a kilo. If you calculate the cost with the plant and machinery furnished, leaving only the operating cost, personnel and material, it would be 13 cents. The prices indicated in Dr. Autret's list are various combinations of these. It is helpful to explain which type of cost is involved.

The FAO Committee on Protein Requirements

W. R. Aykroyd

BEFORE DISCUSSING THE REPORT of the FAO Committee on Protein Requirements, I should like to say something about the FAO/WHO/UNICEF program to combat protein malnutrition. I witnessed the birth of that program and have watched it grow during the last 12 years or so. While I do not wish to imply that these organizations have been directly responsible for what has been accomplished, they have, I think, made many useful direct contributions. But more important has been their role of acting as a sort of focal point around which the activities of other organizations, institutions and, of course, individuals in many parts of the world could group themselves and develop. The Committee on Protein Malnutrition, which has supported so much research with the assistance of the Rockefeller Foundation, is not a UN body, but it has been so closely associated with the UN agencies and the UN program that it has often been hard to tell the difference.

I think we can claim that the progress achieved during the last 10 or 12 years has been striking. It seems only yesterday that I studied the draft of the Brock-Autret report on "Kwashiorkor in Africa" during stormy weather on the journey from Washington to Rome. It was half in French and half in English, which seemed in keeping with the movements of the ship. That was in 1951 and a lot has happened since then. In 1953 we had, in the Gambia, a meeting of the joint FAO/WHO/ Expert Committee on Nutrition devoted exclusively to protein malnutrition, following a CCTA meeting which concentrated on the same subject. If you look back at the reports of these meetings, you will find that many facts about protein malnutrition which we regard as obvious and take for granted today were then still pretty obscure. The program of research outlined at these meetings has been fairly closely followed and many of the problems listed have been clarified.

Increase in knowledge is one thing. But can we say that real progress has been made in applying that knowledge? That is a hard question to answer. I personally believe that the many-sided attack on protein malnutrition is now beginning to take effect and that fewer children suffer and die from it than 10 years ago. There are, of course, few figures to support such a conclusion and probably some of the people here, in direct contact with protein malnutrition in the field, would not accept it.

The convening of the Committee on Protein Requirements was part of the broad program. FAO had produced a report on calorie requirements which had become the standard work on that subject throughout the world. The Joint Expert Committee on Nutrition recommended that the problem of protein requirements should next be tackled. Some other people advised us not to touch it, saying that the problem was too complicated and that we should wait another few years until more knowledge had accumulated. They also said we would either have to repeat the simple recommendations of earlier committees on protein requirements, so many grams of total protein with such and such a proportion of animal and vegetable protein and so on, or else get into very deep water. There were times when I personally regretted not having listened to these cautious advisers.

At all events, that Committee met in October 1955 under the Chairmanship of Professor Terroine. The subject had been discussed a few months earlier at the Princeton Conference on Protein Requirements and their Fulfillment in Practice, but the Conference was not able to go deeply into the issues involved. I note that several members of the Protein Requirements Committee are present at this Conference: Professor Allison, Professor Holt and Dr. Stiebeling. Dr. Scrimshaw attended on behalf of WHO, while Dr. Autret and myself were among the FAO representatives.

The Committee sat for 8 days and at the end of that period hadn't got very far. It had produced a sort of synopsis of what it wanted to say, but there were many loose ends, some contradictory conclusions, and different parts of the synopsis didn't fit together properly. Members of the Committee, with whatever help the Secretariat could provide, then worked for 2 years to prepare a report which was considered fit for publication. We had, I think, at least 6 drafts and a whole series of further meetings between different committee members. The report was finally published in the middle of 1957, more than 2 years after the Committee as a whole had met.

The first part of the report follows to some extent the reports of earlier expert groups on the subject; that is to say, it attempts to state the protein requirements of different age and sex groups. But there were various new departures. For example, the Committee made a brave but not, I think, altogether successful attempt to define certain concepts relative to "optimum," "minimum" and "average" requirements. More important was the decision to express requirements, not in terms of total protein or of animal and vegetable protein, but in terms of a protein of high nutritive value, a "reference protein." The Committee explained what it meant by this by "putting forward examples of proteins which fall into this category. The examples are the proteins contained in milk, eggs and meat, which have long been regarded, on this basis of observation reinforced by research on infants and adults, as being of excellent value for human groups."

One result of this method of approach is that requirements so expressed are considerably lower than figures put forward by earlier groups, which are in terms of protein in general, not of proteins of high nutritive value. The Committee, after much discussion and controversy, increased its requirement figures by 50 per cent to allow a margin of safety. But even with this addition, the application of the

Committee's requirement figures to the protein consumption in prosperous countries results in a large gap between its recommendations and existing protein intake levels. The latter are, in fact, far above requirements estimated on the Committee's basis. This is probably scientifically correct, but some people have found it disconcerting.

The second part of the report deals with protein quality and requirements. It attempts to formulate requirements in terms of essential amino acids, here breaking new ground. It suggests a provisional amino acid pattern based on research data, particularly those of Professor Rose, with which the amino acid patterns of foods and diets can be compared. Such comparisons are, of course, possible only if sufficient information on the amino acid composition of foods and diets is available. The Committee felt that this point was at least being approached.

It must be admitted that the two sections of the report, relating to requirements for proteins and essential amino acids respectively, are not tied together in a fully satisfactory way. The provisional amino acid pattern resembles, but is not identical with, the pattern of the reference proteins which the Committee mentions: milk, meat and eggs. The Committee recognized that it was recommending two methods of approach rather than a single fully integrated one. The following statement illustrates this point:

"The emphasis is now passing from proteins to essential amino acids and total utilisable nitrogen, but it is not yet possible to consider requirements only in terms of the latter. For certain purposes, such as the evaluation of national diets and the food consumption of populations generally, it is still more convenient to state requirements in terms of protein. When, however, the aim is that of selecting and appraising supplements to diets poor in protein, the problem can often be considered more usefully in terms of amino acid patterns along the lines indicated in this report."

It is not easy to apply the findings of the report in the evaluation of food consumption data. Old-fashioned simple statements of protein requirements can, of course, be easily used for that purpose. However, the Committee is scarcely to blame, because the problem is too complicated to be stated in elementary terms for the benefit of student dieticians.

On the whole the report has been well received. One critic did indeed say that it ought to be burnt by the common hangman, but that has not, I think, been the general view. One notes from the papers presented at the Conference that it is now being widely used as a research tool, and certain of its recommendations have been supported by research done during the last few years.

The Committee itself remarked that it was far from satisfied with its efforts. It strongly emphasised the provisional nature of its recommendations. Its final words were as follows:

"The main contribution of the report as a whole is that it indicates the direction in which the problem of protein requirements is moving. The system of estimating requirements which it advocates should be applied carefully and cautiously. Experience in its use, the accumulation of further knowledge, and critical study on the part of nutrition workers throughout the world, will no doubt bring

to light its advantages and disadvantages, increase its value for practical purposes and open up new fields for research."

No doubt a second Committee on Protein Requirements will be arranged during the next few years, and the whole problem gone into again in the light of advancing knowledge and the experience gained with the first report.

Summary of the Conference

Summary of the Conference

DR. TEPLY: I shall simply try to summarize some of the highlights of the nonclinical aspects of the discussions, and leave the clinical aspects and any general summing up to the Chairman. I shall also try to leave him sufficient time.

I would like to say, however, as one who has been closely associated with this program for only a few months, my general impression is that the reports here have been encouraging. Indications are that progress is being made. The reports on the production of a kwashiorkor-like syndrome in monkeys and in pigs are indeed a milestone in nutrition research, and on the applied side a number of groups have carried plant protein mixtures or traditional processed foods through rather complicated laboratory analyses, animal feeding tests, clinical trials, acceptability tests and in some cases into the marketing trial stage. As Dr. Altschul mentioned, it is becoming increasingly evident that there is great need for work in the areas of food formulation, technology and production and marketing of protein-rich products, especially the development of suitable techniques for the distribution and promotion of these products.

As to laboratory testing of foods for the nutritive value of their proteins, Dr. Bender pointed out that at the present time amino acid assays by themselves are of limited value, primarily because we do not know enough about the availability of amino acids. This appears to be worked out quite well for lysine, especially in view of Dr. Mauron's report, but we need to know a great deal more about availability of other amino acids, especially methionine.

Thus, we will have to continue to depend to a great extent on biological assays. Dr. DeMaeyer gave us some reassurance that animal tests, in this case by the Bender method, do correlate quite well with tests in humans. Dr. Platt's dietary protein value approach with the use of his nomogram provides a relatively new refinement of the animal assay technique.

Laboratory results on certain products such as peanut flour and fish flour have not been uniform from group to group. This is not surprising in view of the processing and handling variables that have been involved. More than one speaker has drawn attention to the need for the careful description of products involved when reporting test results.

To consider some of the basic protein-rich foods to which the grantees of the Committee on Protein Malnutrition have given a great deal of attention—not including what Dr. Altschul has covered—cottonseed flour is one which has been subjected to rather thorough testing and which has been successfully used in protein-rich products by the INCAP group and in trials by others. The commercial pro-

duction of cottonseed flour still leaves some questions to be answered, but there is reason to hope that these will be solved in the not too distant future.

Dr. Autret discussed fish meal products that might be satisfactory in certain localities, and he mentioned some that are quite stable because of the natural high content of tocopherols. However, production on a satisfactory commercial basis of a stabilized, deodorized fish flour has not been achieved as yet, although here, too, there are some indications that we can hope for reasonably early solution of that problem.

Peanut flour is also receiving a great deal of attention. The Mysore group, which Dr. Sreenivasan represented here, has worked for some years on the development of a satisfactory edible peanut flour and its formulation into various products which Dr. Sreenivasan has described. They have also formulated peanut flour mixtures into such products as simulated rice pellets and various macaroni-like forms to facilitate introducing them into the natural diets. As Dr. Milner mentioned, UNICEF is happy to assist in the development of commercial production of peanut flour in India by providing certain equipment for plants.

The Mysore group, and also Dr. Nicol, have shown that, by the mixture of a relatively small proportion of milk powder with peanut flour, a product with good nutritive value in regard to its protein can be achieved. Madame Aubry reported on encouraging field trials with biscuits made up with milk supplement together with millet and fish flour and peanut flour in Senegal. Dr. Dean is working with a biscuit made mainly of peanuts mixed together with cottonseed oil, milk powder and certain other supplements.

There has been evidence from several speakers that satisfactory commercial production of soy flour has been achieved in a number of localities. Drs. Wei and Tung from Taiwan have gone far in formulating flakes made up of soy flour mixed with cereals.

We have also had evidence, especially from Dr. Autret's summary, that a number of satisfactory protein-rich prepartions can be prepared in bulk at low cost in comparison with the cost of skim milk powder. However, a realistic appraisal of the packaging, promotion and distribution to the family consumer on a commercial basis indicates that the final cost to the consumer may be increased substantially.

Dr. Bressani reported on tests with mixtures of a) rice and beans, b) corn and cottonseed, and c) corn, cottonseed and cowpeas, which can be made up much more easily than their more complex protein food mixture such as Incaparina No. 9. The mixtures give very good protein efficiency ratios in the rat feeding tests.

Drs. Brock and Hansen have been working on a mixture of corn and what is known locally as cowpea in South Africa.

Dr. Rao, in particular, recommended that greater attention should be given to natural foods as traditionally prepared or processed in homes or villages. He discussed fermented foods and gave some preliminary evidence indicating that the fermentation of certain foods in India results in a gain of some of the B vitamins and perhaps of methionine.

Drs. Arimoto, Nakano, Sakurai and Sano from Japan reported somewhat similar results on fermented soy products, Natto and Miso, and indicated some success in feeding trials with school children as far as acceptability was concerned.

The soy product tempeh, which is widely used in Indonesia, has been studied in Dr. György's laboratory. Rat feeding tests indicate improvement in nutritive value due to the mold treatment. Dr. György has also isolated in highly purified form an antioxidant which appears to be responsible for the stability of the dried tempeh. Results on soy milk have been somewhat variable, and studies are continuing. Dr. Hand, working with György and other groups, is preparing samples of some of these materials under controlled conditions and is studying the effects on nutritive value of some of the processing variables.

Dr. Pirie described the preparation of protein concentrates or isolates from leaves, and he and Dr. Cruickshank gave some evidence of the nutritive value of the products. Dr. Sreenivasan also described a preparation of protein isolates from plant materials. It seems that products of this type will continue to receive considerable attention; undoubtedly the economic factors will be particularly important in regard to these preparations.

Finally, it will be very interesting to follow the ecological studies described to us by Dr. Collis and by Dr. Jelliffe, and we look forward to having further reports on those investigations.

DR. SEBRELL: We now come to the end of this historic Conference. On behalf of the sponsors and the members of the Committee on Protein Malnutrition, I want to express our appreciation to each of you for your contributions to this successful Conference.

What kind of Conference has it been? We have ranged, as was planned, all the way from the most fundamental laboratory studies through clinical studies, field application, food technology, acceptability of food, economics, and even problems of commercial production.

I shall confine my remarks to a few points which seem to be of outstanding general importance. One fundamental point that has been made in this Conference is a very simple one but a very important one, namely, that the mere availability of a desirable food product is not enough. The scientist has a greater responsibility than that of just discovering a new and more desirable product. His responsibility carries over to trying to do all of the things that are necessary to make a food product into something that is beneficial and acceptable.

Another important point is the recognition that it is technically feasible and economically practicable to develop low-cost food mixtures of high protein value which must fit the agricultural economics and the cultural pattern of the people concerned. Such mixtures must be based on a limited number of foods, starting with the staple cereal crops of the world, mainly rice, wheat, millet and corn, which must be the basis for any practical answer, and there are only a few classes of foods that can be added to these to improve the protein value. The oilseed presscakes offer attractive possibilities, as has been indicated. Large amounts of these are now wasted as sources of human food and many can easily be made available. In addition to these products, the only other large and practicable sources of protein

that appear in the world at the moment in addition to animal products are in the classes of fish, seafoods and legumes.

It is also abundantly evident from this Conference that the day is fast approaching when protein isolates from vegetable sources may not only be economically feasible but may be a practical necessity.

Another important effect of this Conference is the stimulation which it will give to increased research on protein foods from vegetable sources.

Of considerable importance was the point that basic nutritional knowledge is ahead of food technology, and that the problems of large-scale commercial production, standardization and distribution of food must receive much more study and rapid development.

Some other noteworthy items were: The great importance of finding better ways of recognizing protein depletion in the individual; the possible significance of increases in serum albumin and the need for better understanding of urinary urea excretion levels; the identification and significance of unidentified nitrogen in the urine; the interpretation of nitrogen retention and nitrogen balance studies; the need for final evaluation in the human of various food products.

The presentation of the new observations on protein malnutrition in the monkey offer new and broader experimental opportunities to uncover fundamental knowledge which will help us better to understand the problems which are ahead of us.

The role of infection was emphasized in relation to severe malnutrition, and particular mention was made of tuberculosis in a very interesting way. This is something which has been long recognized by all who see serious malnutrition, and yet, while it was emphasized at this Conference, it was only to point out that the explanation and understanding of the relationship still must be found.

The possible role of synthetic amino acids was presented, together with the evidence of their availability. We heard mention of ersatz foods versus natural foods and the point was made that many nutritionists feel that there can be no objection to making a poor protein food better in any way that we can do so, if it can be done without danger and if it is truly better from a practical viewpoint.

We have been honored by having representatives of three international agencies present—the Food and Agriculture Organization, the World Health Organization and the United Nations Children's Fund, although the Conference is purely unofficial and nongovernmental. I want especially to mention their presence here and their contributions, not only to recognize the great contributions that each of these agencies has already made in this field, but also because it is only through their cooperative activities in the future that practical programs of international significance can be put into operation.

Perhaps the greatest contribution of all from this Conference is the good will, the free interchange of knowledge, experience and ideas, the new friendships that have been made, and the old ones that have become closer. If we could only have more international conferences in this atmosphere of informality and with the only motivation a sincere desire to better the lot of the underprivileged man, I feel sure

we could make more rapid progress toward the attainment of international friendship, understanding and peace.

Finally, I hope that you are leaving this Conference, as I am, with the feeling that we can attack the mountain of work ahead of us with renewed vigor and enthusiasm, stimulated by the confidence that we can now see a path through the forest of problems which will lead us toward the goal of making life better for millions of people.

Appendix

APPENDIX

NOMENCLATURE GUIDE TO PLANT PRODUCTS CITED *

Abbreviations:

Cam:	Cambodia	*Jap:*	Japan
Coch:	Cochin China	*Kan:*	Kanarese
C. Rica	Costa Rica	*Mar:*	Marathi
Ecua:	Ecuador	*Mex:*	Mexico
El Salv:	El Salvadore	*Nicar:*	Nicaragua
Ethio:	Ethiopia	*Pak:*	Pakistan
Guat:	Guatemala	*Pan:*	Panama
Guj:	Gujarati	*Phil:*	Philippines (Republic)
Hond:	Honduras	*Port:*	Portugal
Hung:	Hungary	*P.R.:*	Puerto Rico
Ital:	Italy	*Turk:*	Turkey
		Viet:	Vietnam

Bengali, Gujarati, Hindi, Kanarese, Marathi, Oriya, Tamil, Telugu—One of the 14 languages of the Constitution of India.

Khmer—One of the native races of Cambodia.

Parentheses are used in Part I where uncertainty exists in the spelling of local plant names. In Part II parentheses indicate synonyms or unapproved names.

Part I: Listing by common English names.

Part II: Listing by Latin names.

PART I: Listing by common English names

African oil palm, red palm. *Elaeis guineensis Jacq.*, *E. melanococca. Bengali:* Khejur Tail. *Coch:* Dau dau. *Dutch:* Olispalm. *French:* Palme a huile. *German:* Olpalm. *Hindi:* Surkh Khajur ka'tel. *Spanish:* Palma de aceite (Venezuela). *Turkish:* Yaghurmasi. Seeds source of an oil which when refined is suitable for margerine, vegetable shortenings. A substitute for cacao butter. E. melanococca—Used to make toddy.

* From Selected Edible Plants and Plant Products. 2nd Provisional Draft, 15 April 1960, prepared by D. B. Hand for ICNND.

Alfalfa, burclover. *Medicago sativa. Chinese:* Mu-su. *Dutch:* Luzerne. *French:* Luzerne commune. *German:* Luzerne. *Hindi:* Lasunghas. *Ital:* Erba Medica, Luzerna. *Mex:* Alfalfa. *Peru:* Alfalfa. *Port:* Luzerna. *Spanish:* Trevol, Alfalfa, Mielga. *Turkish:* Kaba yonca. A forage plant grown principally for hay; also made into meal.

Amaranth. *Amaranthus chlorostachys, A. hydridus L., A. spinosus, A. viridis, A. tricolor* (*gangeticus; melancholicus*). *Bengali:* Banopata nate, Kanta nate, Cholai ka sag. *Cam:* P'ti bania. *Ceylon:* Tampala. *Coch:* Rau den tia *Ecua:* Bledo. *El Salv:* Bledo. *French:* Amaranthe, epinards Vietnamiens *Guj:* Dant, Rajagaro, Kantemedant. *Hindi:* Lal choalai, Lal sag. *Hond:* Bledo. *Kan:* Yele Dantu, Mulla Dantu. *Khmer:* Phti banla. *Lao:* Phek hon mam. *Mar:* Mathi, Kate Math. *Mex:* Quelite. *Oriya:* Khada Saga, Kanta Neutia Saga. *Peru:* Yuyo. *Phil:* Halon, Kolitis, uray. *Spanish:* Bledo. *Tamil:* Mulaikeerai. *Telugu:* Thota Koora, Mulla Thota Koora. *Tonkin:* Dau den tia. Leafy vegetable—Cooked and eaten like spinach. May be collected as it grows wild; some varieties cultivated by Indians and Chinese.

Barley. *Hordeum vulgare, H. sativum, H. distichon* (*distichum*). *Bengali:* Job. *Cam:* Spou. *Chinese:* Ta-mai, No-mai, Kung-mai. *Dutch:* Gerst. *Ecua:* Cebada. *Ethio:* Gebs, Gubs, Gueba. *French:* Orge. *German:* Gerste. *Guj:* Jau. *Hindi:* Jau, Jave, Jawa, Yava. *Ital:* Orzo nudo. *Mar:* Juv. *Oriya:* Jaba Dhana. *Pak:* Jau. *Persian:* Jao. *Peru:* Cebada. *Port:* Cevada das cuatro carreras. *Spanish:* Cebada. *Tamil:* Barliarisi. *Telugu:* Barli Blyyam. *Tonkin:* Lua mach nha. Cereal—Source of flour for bread, cakes, etc. Unleavened barley cakes esteemed in some northern parts of Europe. Used as breakfast food. Pearled barley used in soups. H. distichon—grains when roasted are a substitute for coffee.

Bengal Gram, see Chick Pea

Black Gram, see Mung Bean; Mungo Bean

Broad Bean, see Horse Bean

Buckwheat. *Fagopyrum sagittatum* (*esculentum*). *Cam:* Srau barang. *Chinese:* Chi'iao-mia, Hsin-ch'iao, T'ien'ch-iao-mai. *Coch:* Lua mach. *Dutch:* Boekweit. *French:* Sarrasin. *German:* Buckweizen. *Hindi:* Kaspat. *Ital:* Fagoptro. *Mar:* Kutu. *Russian:* Grechevnaya. *Spanish:* Trigo negro, T. sarraceno, Grano turco, Alforyon. *Tonkin:* Lua mach. Cereal—Source of buckwheat flour. Groats are kernels with hulls removed, used as breakfast food, thickening for soups, gravies, dressing, porridge. Flowers source of commercial honey.

Cassava, tapioca, manioc. *Manihot esculenta* (*utilissima*), *M. dulces. Arabic:* Dandenahri. *Cam:* Kduoch sras, Kduoch svet ru, Msau kduoch. *Chinese:*

Pai-fu-tzu. *Coch:* Khoai mi, Bot khoai mi. *Cuba:* Yuca. *Dutch:* Braziliaans Arrow root, Maniok. *Ecua:* Yuca. *French:* Manioc, cassave amere. *German:* Brasilianisches Arrow root. *Haiti:* Manioc. *Hindi:* Maravali, Simla Alu, Bagderenda, Maravuli. *Ital:* Manioc. *Kan:* Mar Genasu. *Khmer:* Kduoch sra. *Lao:* Man ton hong, ken ko. *Mex:* Yuca. *Oriya:* Katha Kanda. *Persian:* Dandenahri. *Peru:* Yuca. *Phil:* Kamoteng-kahoy. *Spanish:* Yuca. *Tamil:* Maravalli Kizhangu. *Telugu:* Karrapendalam. *Tonkin:* Cu san tau. *Turkish:* Manihot. *Viet:* Khoai mi, cu san tau. Starchy root—Starch made into tapioca, used in soups, puddings, etc. Also boiled as a leafy vegetable.

Chick pea, Bengal gram. *Cicer arietinum L. Bengali:* Chola Chola Sag (leaves), Gota. *Ecua:* Garbanzo. *Ethio:* Shimbra. *Guj:* Chana, Chanana pan (leaves). *Hindi:* Chana, Sag Chana (leaves). *Kan:* Kadale, Kadale Soppu (leaves). *Mar:* Hurbura, Hurbhura Pan (leaves). *Mex:* Garbanzo breve. *Oriya:* Buta, Chana Saga (leaves). *Pak:* Chana. *Peru:* Garbanzo. *Phil:* Garbanzo. *Spanish:* Garbanzo. *Tamil:* Muzhu Kadalai, Kadali Ilaigal (leaves). *Telugu:* Sanagalu, Sanaga Aku (leaves). Seeds are consumed fresh or dried in various dishes and soups; also made into a flour for bread making. Used as substitute for coffee. Leaves also consumed as a vegetable.

Corn, Indian corn, maize. *Zea mays Linn. Arabic:* Durah shami. *Bengali:* Kacha Bhutta, Bhutta churna. *Cam:* Put kraham. *Chinese:* Yu-shu-shu, Yu-kao-liang, Pa-lu, Liu-su. *Cuba:* Maiz, maiz tierno (green corn). *Dutch:* Mais, Turkse tarwe. *Ecua:* Canguil, Chulpi, maiz. *Ethio:* Bakalo. *French:* Mais, Ble de Turquie. *German:* Mais. *Guj:* Makai. *Haiti:* Mais. *Hindi:* Makka, Bhutta, Makai. *Ital:* Granturco, Freementone, Formentone, Melica. *Kan:* Yele Musukinu Jolu. *Khmer:* Paut. *Lao:* Khao phot, Khotsoli. *Mar:* Muka. *Mex:* Maiz blanco, M. amarillo, M. cachuatzintle, M. pepitilla, M. reventador, Amarillo, Elote amarillo, Elote blanco. *Oriya:* Kancha Maka. *Pak:* Makki, Sitta, Atta makki (maize flour). *Persian:* Khoshahe-makki, Zorrat. *Per:* Maiz. *Phil:* Mais. *Port:* Milho. *Spanish:* Maiz, Panizo de Indias, Borona. *Tamil:* Makkacholam. *Telugu:* Mokka Jonnalu. *Tonkin:* Lua ngo. *Turkish:* Misir. *Viet:* Bap, bap ngo, Lua ngo. Eaten as a vegetable; as fried cakes after scraping from the cob; and as corn meal made from the parched grains.

Cowpea, blackeyed pea, string beans, yard-long beans. *Vigna catjang, V. sinensis, V. unguiculata, V. sesquipedalis, V. cylindrica. Africa:* Labia adoug-gouari (Abyss.). *Bengali:* Barbati sim, Barbati. *Cam:* Sandek Sar, Sandek chen. *Chinese:* Tau kok. *Coch:* Dau trang, Dau dua. *Cuba:* Frijol carita, F. precioso. *French:* Dolique chinois, Dolique de Chine, Dolique mongette, Pois a vache, Pois chique, Voeme, Bannette, Dolique asperge, Dolique de Cuba, Dolique geant, Pois ficelle, Pois demi-aune, Haricot baguette (Saigon), Catjang a gousee courte. *Guj:* Chola. *Haiti:* Pois inconnu (Blackeye peas). *Hindi:* Lobia, Lobia bada, Lobhia, Chowli, Paythenkai, Thattapayru, Boberlu. *Kan:* Thadaguni. *Khmer:* Sandek sar, Sandek chrok, Sandek

kraham. *Lao:* (Mak) thoua do, thoua nhao. *Mar:* Kuleeth, Chawali. *Oriya:* Chani, Suji. *Pak:* Rawanh, Lobia. *Peru:* Chiclayo tresmesino, Frijol de vaca. *Phil:* Sitaw, Paayap. *Spanish:* Frijol carita, F. precioso. *Tamil:* Karamani. *Telugu:* Alachandalu. *Tonkin:* Dau trang, Dau dua. *Viet:* Dau trang, dau dua, dau do, dau tia. Pulse—In Cuba prepared in the form of small cakes called "bollos." V. sesquipedalis eaten also as a leafy vegetable. V. cylindrica is similar to the common cowpea and may even be a race of it, but seeds lack the black "eye."

Dolichos lablab beans, bonavista beans, Egyptian kidney beans, field beans, hyacinth beans, tonga beans. *Doliches lablab L., D. sinensis. Bengali:* Makhan Sim, Sukna, Mug. *Cam:* Sandek kuor. *Chinese:* Pin tou. *Coch:* Dau dua. *C. Rica:* Chimbolo. *Ecua:* Frijol ornamental. *French:* Dolique lablab, dolique d'Egypte, Antaque, Pois d'un sou, Pois indien (Antilles), pois boucoussou (Guyane), Macape (Madagascar), Dolique noir, Dolique blanc. *Guj:* Mag. *Hindi:* Sim, Val. *Hond:* Lablab. *Khmer:* Dok pep. *Lao:* Mac pep pa, mac pep eo, mac thoua cao, mac pep ba buk. *Mar:* Mug. *Nicar:* Frijol de vaca. *Oriya:* Muga (field beans). *Pak:* Lobia (white beans), Rajmash (red beans). *Peru:* Frijol bocon chileno. *Phil:* Bataw (hyacinth bean). *Tamil:* Mochachi. *Telugu:* Advaichikkudu. *Tonkin:* Dau dua. *Viet:* Dau van, dau mong chim (Saigon), Qua tao do. Pulse—Pods are eaten as snap beans; also seeds are consumed as food.

Fenugreek, see Trigonella

Foxtail millet, Italian millet. *Setaria italica. Bengali:* Syamadhan, Kangni. *Cam:* Kuor thpon. *Coch:* Ke. *French:* Millet (farine). *Guj:* Ral, Kang. *Hindi:* Kangni. *Lao:* Khaopong. *Mar:* Rala. *Pak:* Kangni. *Persian:* Arzan. *Peru:* Mijo italiano. *Tamil:* Thenai. *Telugu:* Korralu. *Tonkin:* Ke. An annual grass cultivated as a cereal.

Gourd, calabash cucumber, bottle gourd, calabash gourd. *Lagenaria sinceraria (lucantha; vulgaris). Bengali:* Lau. *Chinese:* Hu-lu, P'ao. *Dutch:* Fleskalebas. *Ecua:* Proru o puro. *French:* Courge bouteille calebasse, gourde. *German:* Flashchenkurbis, Calebasse. *Hindi:* Lauki Safed kaddu, Kaula, Lowki, Ghia Kadu. *Kan:* Sorekai. *Khmer:* Khluk. *Lao:* (mak) nam, (mak) nam tao. *Mar:* Pandhara, Bhopala. *Oriya:* Lau. *Pak:* Loki. *Peru:* Calabaza no comestible. *Phil:* Upo. *Port:* Colondro, Colombro, Cabaca. *Spanish:* Camasa Calabaza vinatera, C. de peregrino, C. de San Roque, Mazo, Trompeta, Pierna de pobre (fruit). *Tam:* Soraikkai. *Telugu:* Sorakaya. *Turkish:* Asmakabagi. *Viet:* Bau, Bau sao. Young fruits are eaten boiled in some parts of Africa and Asia. See also Malabargourd; Pumpkins; Winter Squash.

Green Gram, see Mung bean; Mungo Bean

Horse bean, broad bean. *Vicia faba var. equina. Chinese:* T'san-tou, Hu-tou. *Dutch:* Tuinboon, Duivenboon, Paardenboon. *Ecua:* Haba. *Ethio:* Bakella.

French: Feve de marais, Feve des champs. *German:* Buffbohne. *Hindi:* Bakla. *Ital:* Fava. *Mex:* Haba seca, Haba verde. *Persian:* Bakila. *Peru:* Haba. *Port:* Fava, Faveiro. *Spanish:* Haba panosa, Haba, Habichuela. *Turkish:* Bakla. Beans eaten as vegetable; seeds sometimes eaten roasted; also made into flour and mixed with wheat.

Jak fruit, jack fruit. *Artocarpus heterophyllus* (*integer; integrifolius*). *Bengali:* Echore, Kathal (seeds), Kanthal. *Cam:* Khno (p'le), Khno (krab). *Coch:* Mit, hot mit. *French:* Jacquier. *Guj:* Kawla Phanas (seeds). *Hindi:* Kathal. *Kan:* Yele Halasu (seeds). *Khmer:* Khnor. *Lao:* Mak mi, Mak sida. *Mar:* Phunas. *Oriya:* Panasa Katha (seeds). *Peru:* Jaca. *Phil:* Buto ng nangka (seeds), Nangka. *Tamil:* Pila. Pinchu (seeds). *Telugu:* Letha, Panasa Pandu (seeds). Fruit—the pulp and seeds are eaten.

Lentil. *Lens culinaris Medic.* (*esculenta*). *Arabic:* Adasa. *Bengali:* Musuri. *Cam:* Sandek Lentille. *Dutch:* Linze. *Ecua:* Lenteja. *Ethio:* Miser. *French:* Lentille. *German:* Linse. *Guj:* Masur. *Hindi:* Masur. *Ital:* Lente. *Kan:* Masur Bele. *Mar:* Masur. *Mex:* Lenteja. *Oriya:* Masura. *Pak:* Masur. *Persian:* Adas, Mirajumaka. *Peru:* Lentejas. *Port:* Lentilha. *Spanish:* Lentejas. *Tamil:* Misur Paruppu. *Telugu:* Misur Pappu. *Turkish:* Mercimek. *Viet:* Dau lang-ti. Seeds eaten boiled, in soups, made into flour for bread.

Maize, see Corn

Manioc, see Cassava

Mung bean, green gram. *Phaseolus aureus*, *P. radiatus*, *P. roxburghii*. *Afri:* Dochirokko, Posue, Bokwa, Ntoya. *Bengali:* Mug. *Cam:* Sandek bay, S. bandos. *Chinese:* Loke tau, Nga-choi, Ch'ih-hsio-tou, Hung-tou. *Coch:* Dau xanh. *French:* Amberique verte, amberique, emberique, mungo, haricot a graine verte, pois chiche annamite, haricot dore, boubour, haricot radie. *German:* Strahlfruchtige Bohne. *Hindi:* Mung, Urithulu, Muneta, Payaru, Urid. Ulundu. *Ital:* Fagiolo. *Jap:* Moyashi. *Khmer:* Sandek bay. *Lao:* Mac thousa lai khieu. *Pak:* Mung. *Peru:* Frijol mungo. *Phil:* Munggo, togue (mung sprouts). *Tonkin:* Dau xanh, Gia. *Viet:* Dau xanh. Seeds are used as food.

Mungo bean, black gram, urd. *Phaseolus mungo*. *Bengali:* Mashkalai, Chhata. *Chinese:* Lu-tou. *French:* Haricot mungo. *German:* Rauhhaarige Bohne. *Hindi:* Urd, Ulandu, Minimula, Patcheypyre, Mash kalai, Moong, Mung. *Ital:* Fagiolo verde, Fagiolo peloso, Pelosino. *Kan:* Bili Uddu. *Oriya:* Biri. *Pak:* Mash Urd. *Spanish:* Frijol chino, Poroto Ura. *Tamil:* Ulutham paruppu. Often confused with P. aureus. Green pods used as a vegetable. Eaten whole, boiled or parched.

Mustard: White (yellow), India mustard, brown (black), Malay sawisawi. *Brassica hirta* (*alba*), *B. juncea var. foliosa Bailey* (*B. rugosa*) (*Sinapis juncea*), *B. nigra*,

B. integri-folia. Bengali: Sarisa. *Cam:* Kailat (slek). *Chinese:* Gai choi. *Coch:* Cai be trang. *Dutch:* Zwarte Mosterd. *Ecua:* Mostaza. *Ethio:* Gommon. *Guj:* Rai. *Hindi:* Rai. *Jap:* Ohgarashi. *Kan:* Sasave. *Mar:* Mohori. *Oriya:* Sorisa. *Pak:* Rai. *Peru:* Mostaza. *Phil:* Mostaza. *Port:* Mostarda ordinario, M. negra. *Tamil:* Kadugu. *Telugu:* Avalu. *Tonkin:* Cai sen. *Viet:* Cai xanh.

Mustard: Brown (black). *Chinese:* Ch'ing-chieh, Tzu-chieh. *French:* Moutarde noire (grise). *German:* Schwarzer Senf, Senfkohl. *Hindi:* Kalorai. *Hung:* Fekete mustar. *Ital:* Senape nera. *Persian:* Sar-shaf. *Spanish:* Mostaza negra. *Turkish:* Siyah Hardal.

Panicum, Sanwa millet, proso millet, broomcorn millet, samai. *Panicrum crusgalli var. frumantaceum, P. miliaceum, P. miliare, P. psilopodium. Bengali:* China. *Chinese:* Chi. *Dutch:* Pluimgierst. *French:* Millet commun, Millet panicule, Millet d'Inde. *German:* Echter Hirse, Gemeiner Hirse. *Guj:* Sawo. *Hindi:* Kutki, sanwali (Samai), Meneri, Panivaragu (Bajra), Sawan (Sanwa millet), China. *Hung:* Koles. *Ital:* Miglio, Miglio nostrale. *Mar:* Ghotisanja, Shamulu. *Oriya:* Suan. *Peru:* Proso. *Port:* Milho miudo. *Spanish:* Mijo comun, Mijo mayor, Borona de Filipinas. *Tamil:* Pani Varagu. *Telugu:* Pedda Wundu. *Turkish:* Akdari. Cereal—Used in Japan as a cereal, porridge, macaroni and in dumplings. P. miliaceum is source of a flour, used in bread. Sanwa millet is sometimes used in India as a famine food.

Pearl millet, bulrush millet, spiked millet, Kaffir manna corn, cattail millet. *Pennisteum glaucum* (*spicatum; typhoideum*). *Africa:* Heruah (Abyss.), dokhn, matesi, gero. *Bengali:* Bajra. *Guj:* Bajri. *Haiti:* Petit mil. *Hindi:* Bajra, Gantelu, Cambu. *Mar:* Bajri. *Oriya:* Bajra. *Pak:* Bajra. *Peru:* Mijo perla. *Tam:* Cambu. *Telugu:* Gantelu. Cereal—Smaller seeds than such cereals as wheat and rice. In some parts of the temperate zone they are familiar only as bird seed.

Peas, sugar peas, green peas, sweet peas. *Pisum sativum var. macrocarpon* (*P. sativum var. saccharatum Hort.*). *P. maritimum. Bengali:* Sukna Matar, Lal Sim. *Cam:* Sandek sras, S. snguot. *Chinese:* Hoh lang dau, Wan-tou, Jung-shu, Ch'ing-Hsiao-tou. *Coch:* Dau hoa lan. *Dutch:* Erwt, Doperut. *Ecua:* Arveja. *Ethio:* Atter, Dau tay. *French:* Petit pois, Pois vert. *German:* Saat-Erbse, Schal-Erbse. *Guj:* Vatana. *Haiti:* Petit pois. *Hindi:* Bada Mattar (dried), Matar, Mattar. *Ital:* Pisello. *Jap:* Chabo-endo. *Kan:* Batani, Kempu Huruli. *Lao:* Mac thoua nhat, Mac thoua nhat heng. *Mar:* Vatana. *Mex:* Alverjon, Chicharo, Guisantes. *Oriya:* Matara, Nali Simba. *Pak:* Matar. *Persian:* Nokhod. *Peru:* Arvejas. *Port:* Ervilha. *Spanish:* Guisante, Pesol, Chicharo, Arveja. *Tamil:* Pattani, Pachai. *Telugu:* Pattani, Battani, Pachi. *Tonkin:* Dau hoa lan. *Turkish:* Bezelye. Seeds are consumed as a vegetable, also in soups. Some varieties the entire pods are eaten.

Phaseolus vulgaris beans; bountiful beans; bush beans; common beans; dwarf beans; French beans; haricot beans; navy beans; pink beans; pinto beans;

pole beans; red kidney beans; snap beans; white kidney beans. *Phaseolus vulgaris L. Bengali:* Mocha. *Cam:* Sandek barang kriem, Sandek k'chiey. *Chinese:* Lu-tou. *Coch:* Dau tay. *Cuba:* Frijol comun (common beans), Frijol colorada (red kidney beans), Habichuela catalana (snap beans). *Dutch:* Stamboom, Pronkboon, Stokboon. *Ecua:* Frijol comun (green beans), vainita. *French:* Haricot commun, haricot verte, haricot beurre, petite feve, haricot nain. *German:* Gartenbohne, Buschbohne. *Haiti:* Pois rouge (dry beans), Pois blanc, Pois noir, Pois navet. *Hindi:* Bakla, Babri, Khurdya, Moongi, Bakla, Bunchi, Bonche, Bunchi kai, Avarai. *Ital:* Fagiolo nano. *Khmer:* Sandek barang kriem, Sandek k'chiey. *Lao:* Mac thoua frang heng, Mac thousa khiem. *Mex:* Alubia chica, frijol amarillo, azufrado, bayo gordo, blanco, canelo, cocona, garbancillo, mexicano, negro, ojo de liebre, palacio, parraleno, rosita, xinacalteca, ejotes, ejotes de alubia, ejotes de cachibache, ejotes de espelon, frijol espelon fresco, frijol neuvo. *Pak:* Frasbeen. *Persian:* Bando Mash. *Peru:* Agusho, amarillo, comun, plomo, california, canario, caraotas, chiclayo, dosmesino, cocacho, frijolito chino, negro, panamitos. *Phil:* Habichuelas (snap beans). *Port:* Feioerio. *Spanish:* Habichuela enana, judia, alubia, frijol negro, frijol colorado, frijol chino, habichuela. *Tonkin:* Dau tay. *Turkish:* Fasulye. *Viet:* Dau tay, a-ri-co-ve, qua dau. Many varieties grown for their pods (snap beans), green-shelled beans, and those used in dry condition (dry-shell beans).

Pigeon pea, red gram, cajan. *Cajanus cajan* (*indicus*), *C. bicolor. Bengali:* Arhar Dal. *Coch:* Dau sang. *French:* Pois d'Agnale, ambrevade, pois cajan, pois pigeon, pois en arbre (Antilles). *Guj:* Tur. *Haiti:* Pois congo. *Hindi:* Arhar. *Kan:* Thugare Bele. *Khmer:* Sandek day. *Lao:* Co thua he. *Mar:* Toor. *Mex:* Frijol de arbol. *Oriya:* Harada. *Pak:* Gandul, Arhar. *Peru:* Palo. *Phil:* Kadios. *Spanish:* Gandul. *Tamil:* Tuvaram Paruppu. *Telugu:* Kandi Pappu. *Tonkin:* Dau chieu. *Viet:* Dau sang (So. Vietnam), dau chieu (No. Vietnam). Very young seeds are eaten as green peas, when dry and ripe used in soups, eaten with rice, etc.

Plantain. *Musa paradisiaca L., M. coccinea. Bengali:* Kanch Kola. *C. Rica:* Platano, P. curare. *Cuba:* Platano. *Dutch:* Pisang. *Ecua:* Platano verde y maduro. *El Salv:* Platano, Guinea criollo, morado, jajonch. *Guat:* Platano, Guineo blanco, morado. *Haiti:* Banane. *Hindi:* Kele ka. *Hond:* Platano, macho, cuadrado, tres filos, butuco, majoncho. *Mex:* Platano macho, P. roatan o barbaro. *Pak:* Kela. *Pan:* Guineo chino, Platano. *Peru:* Platano, P. maduro. *Phil:* Saguing, saba. *Spanish:* Platano burro, P. fongo, P. macho. *Viet:* Cay ma de. Similar to bananas but they have to be cooked in order to be edible. May be baked, boiled or fried depending upon degree of ripeness. Also may be cut up, dried and made into a flour. The large purple plantain buds are also boiled as a vegetable. Buds from species of wild Musa are also used.

Plantain. *Plantago major L. Arabic:* Lasana-el-hamala. *Dutch:* Grote Weegbree. *French:* Grand Plantain, Plantain, Plantain des oiseaux. *German:* Breit-

wegerich, Grosser Wegerich, Wegbreit. *Hindi:* Lahuriya. *Ital:* Piantaggine. *Khmer:* Slap chravea. *Lao:* (Phak) phay. *Persian:* Bartang. *Port:* Tanchagem. *Spanish:* Llanten mayor. *Turkish:* Buyuk sinirliot. *Viet:* Ma de. In India the roots, leaves, and seeds are used in medicine and in Indochina the leaves are eaten as a seasoning for meat and fish. In China and Malay they are eaten as a vegetable.

Ragi millet, red millet, African millet, South Indian millet, finger millet. *Eleusine coracana. Africa:* Talabun (Sudan), dakussa, tocusso, (Abyss.), murwa. *Ethio:* Dagusa. *Guj:* Ragi, Bhav. *Hindi:* Ragi, tanudelu, kurakkan, mandal, okra. *Kan:* Ragi. *Mar:* Nachni. *Oriya:* Mandia. *Pak:* Mandal. *Peru:* Mijo Africano. *Tamil:* Ragi. *Telugu:* Ragulu, Chollu. Cereal—Source of ragi flour used in cakes, puddings; also made into an alcoholic beverage.

Red Gram, see Pigeon Pea

Red Palm, see African Oil Palm

Red pepper, chillies, capsicum, cayenne pepper, tobasco pepper, paprika. *Capsicum frutescens* (*annum; baccatum*), *C. frutescens var. conoides*, *C. frutescens var. longum* (*C. longum*), *C. frutescens var. grossum* (*L*) *Sendt.*, *C. frutescens var. acuminatum*, *C. frutescens var. cerasiformec*, *C. tetragonum. Arabic:* Filfile-ahmer. *Bengali:* Sukna Lanka. *Chinese:* La-chiae, Pi-po-li. *Cuba:* Aji. *Dutch:* Cayenne Peper, Lombokpeper, Spaanse Peper. *Ecua:* Aji. *Ethio:* Berbera. *French:* Poivron, pimet du Chili, paprika, piment doux d'Espagne, pimet rouge long, pimet doux. *German:* Spanischer Pfeffer, Beissbeere. *Guj:* Lavang. *Hindi:* Mirch, Lal, Mirchi, Marchu. *Ital:* Peperone, Pepe cornuto. *Kan:* Vona Menasinakayi. *Khmer:* Motis phluk. *Lao:* (Mak) phet. *Mar:* Mirchi Lal. *Mex:* Chile ancho, C. bravo, C. carricillo, C. chilaca, C. cascabel, C. chipotle, C. dulce, C. cristalino, C. guajillo, C. jalapeno, C. largo, C. pasilla, C. poblano, C. serrano, C. trompito, C. valenciano. *Oriya:* Sukhila Lanka. *Pak:* Mirch. *Pan:* Aji. *Persian:* Tiffile, surkh. *Peru:* Pimiento, Aji amarillo, A. dulce, A. panca, A. verde, Rocote. *Phil:* Siling laburjo (leaves), Siling laburjo (fruit). *Port:* Pimentao de cheiro, Pimentao de Caiena. *Spanish:* Guindilla, Pimiento chiles, Cerecilla, Ajidulce, (Venezuela), Aji, Pimenta. *Tamil:* Milagai Vethal. *Telugu:* Endu Mirapakayi. *Tonkin:* Ot. *Turkish:* Kirmizi biber, Hint biberi. *Viet:* Ot Da lat (Saigon), ot tau. A vegetable fruit used for salads, vegetables, condiments, and in sauces. It is the main condiment in Indian curries and sambols; is used as seasoning and in the preparation of sauces. Some varieties are not very hot and can be eaten as a green vegetable. Red pepper is the ground up dried fruit; it is used in sauces, pickles and chutneys. In some species the leaves are also eaten.

Sesame seeds, gingelly seeds, benniseed. *Sesamun indicum Linn.*, (*S. orientale*), *S. radiatum. Arabic:* Simsim. *Bengali:* Til. *Cam:* Longo. *Chinese:* Hu-ma, Ch'ing-jang. *Coch:* Me. *Cuba:* Ajonjoli. *Dutch:* Sesamstruik. *Ethio:*

Saliff. *French:* Sesame. *German:* Sesamstrauch. *Guj:* Tal. *Hindi:* Til, Teel. *Ital:* Sesamo, Ginggiolena. *Kan:* Acchellu. *Khmer:* Longo. *Lao:* Mac nga. *Mar:* Til. *Oriya:* Rasi. *Pak:* Til. *Persian:* Kunjad. *Peru:* Sesamo, Ajonjoli. *Spanish:* Ajonjoli. *Tamil:* Ellu. *Telugu:* Nuvvulu. *Tonkin:* Vung. *Turkish:* Susam. *Viet:* Me. Seeds source of an essential oil used for edible purposes.

Sorghum, great millet, Kaffir corn, millo maize, American broom corn, Guinea corn. *Sorghum vulgare Pers. Africa:* Dhurra, dawa, matama, michelli. *Bengali:* Juar. *Cam:* Srau srok, Sa-cu. *Chinese:* Kao-liang, Lu-su, Ti-liang, Ti-che. *Coch:* Lua mien. *Ethio:* Mashila. *French:* Grand millet, sorgho. *German:* Sorghohirse. *Guj:* Kuar. *Hindi:* Juar, Cholam. Jowar. Jovaree, Pyoung (Burma). *Ital:* Miglio saggina. *Jap:* Shu-shu. *Kan:* Jola. *Mar:* Jwari. *Mex:* Sorgo. *Oriya:* Janha. *Peru:* Sorgo. *Spanish:* Sorgo. *Tamil:* Cholam. *Telugu:* Jonnalu. *Tonkin:* Lua mien. *Turkish:* Dari. Cereal—Used for human consumption, also for pasture grass, hay.

Squash, see Gourd; Winter Squash

Sunflower, Jerusalem artichoke, *Helianthus annus L., H. tuberosus. Chinese:* Hsiang-jih-j'uei, Chao-jih-k'uei. *Dutch:* Zonnebloem, Aardpeer, Jeruzalem-Artisjok. *Ecua:* Girasol. *French:* Helianthe, Fleur de Soleil, Tournesol, Grand Soleil, Topinambour, Artichaut de Jerusalem, Artichaut common, Poire de terre. *German:* Sonnenblume, Sonnenrose, Erdbrine, Topinambur, Knollen-Sonnenblume, Echte Artischoke. *Hindi:* Suraj mukhi, Hurduja, Sarajmaki, Hatichuk Kunjor. *Ital:* Elianto, Girasole, Tartufo di canna, Pero di terra, Artichoccio, Cariofo, Girasole tuberoso, Topinambour. *Mex:* Maiz de Texas, Girasol. *Pak:* Suraj mukhi. *Persian:* Aftab gardan, Sibzamini torshi, Guli-aftab. *Peru:* Topinambour, Girasol. *Port:* Girassol, Tupinambo, Batata, Girassol batateiro. *Spanish:* Girasol, Mirasol, Copa de Juniper, Giganta, Sol de las Indias, Mirabel, Peranton, Pataca, Girasol tuberoso, Batata de cana, Aguaturma. *Turkish:* Gundondu, Aycicegi, Yerelmasi. Grown for its oil which is used in foods, salads, butter substitutes.

Tapioca, see Cassava

Trigonella, fenugreek. *Trigonella foenum-graecum. Arabic:* Hulabaha, Hulba. *Bengali:* Methi Sag. *Chinese:* Hu-lu-pa, K'u-tou. *Dutch:* Fenegriek, Grieks Graszaad. *Ethio:* Abish. *French:* Fenugrec. *German:* Bockshorn, Griechische Heusamen, Bockshornklee, Hornklee. *Guj:* Methi. *Hindi:* Methi. *Hung:* Gorog Lepkeszeg. *Ital:* Fieno greco. *Mar:* Methi. *Oriya:* Methi Saga, Methi (seeds). *Pak:* Methi. *Persian:* Shamlita. *Peru:* Fenogreco, Alhova. *Port:* Ervinha, Alforvas, Fenacho, Feno grego. *Spanish:* Alholva, Heno griego. *Tamil:* Venthiam, Venthiyam (seeds). *Telugu:* Mentulu, Menthulu (seeds). *Turkish:* Boyotu. Seeds used as a condiment. The extract, together with other aromatics, gives the flavor of maple and is used in confectionery.

Turnip. *Brassica rapa. Bengali:* Shalgom. *Chinese:* Choy sam. *Ecua:* Papanabo. *Haiti:* Navet. *Hindi:* Shalgham. *Pak:* Shaljam, Sarson. *Persian:* Chalgham. *Peru:* Nabo, N. silvestre. *Spanish:* Nabo. *Viet:* Cu cai tay. Broad thick roots are consumed as a vegetable.

Watermelon. *Citrullus vulgaris Schrad. Arabic:* Belik Zichi. *Bengali:* Kamala, Lebu. *Chinese:* Hsi-Kua, Han-kua. *Cuba:* Melon de agua. *Dutch:* Grote Water-Meloen. *Ecua:* Melon de agua. *French:* Pasteque, Melon d'eau. *German:* Grosse Wasser-Melone. *Haiti:* Melon d'eau. *Hindi:* Tarbuz. *Khmer:* Oulek. *Lao:* (Mak) mo. *Mex:* Sandia. *Pak:* Tarbooz. *Persian:* Hendvaneh. *Phil:* Pakuan, buto (seeds). *Port:* Melancia. *Spanish:* Melon de agua, Sandia. *Viet:* Dua hau, Qua dua hau. Fruits eaten ripe; rinds of fruits sometimes preserved in sugar. Some grown mainly for their seeds which are chewed as snacks.

Winter squash, Hubbard, marblehead squash. *Cucurbita maxima Duchesne. Bengal:* Kumra. *Ecua:* Zapallo. *Ethio:* Dubba. *French:* Potiron. *Guj:* Kohlu. *Hindi:* Kaddu. *Kan:* Kumbala. *Khmer:* Rapou trach. *Lao:* (Mak) u, (mak) fak. *Mar:* Lal Bhopla. *Mex:* Calabaza amarilla. *Nicar:* Ayote. *Oriya:* Kakharu. *Pak:* Kaddu. *Persian:* Kadon tambal. *Phil:* Dahong kalabasa (tops), kalabasa. *Spanish:* Calabaza. *Tamil:* Parangikkai. *Telugu:* Gummadi Kayi. *Viet:* Bi ngo, bi ro, Giong bi ngo. Vegetable-fruit. Consumed boiled, made into pies. Also tops and flowers are sometimes eaten as a leafy vegetable. See also Pumpkins.

PART II: Listing by Latin Names

LATIN NAMES	ENGLISH NAMES
Artocarpus heterophyllus (interger; intergrifolius)	Jak fruit
Capsicum frutescens (annum; baccatum), C. frutescens var. conoides, C. frutescens var. longum (C. longum), C. frutescens var. grossum (L) Sendt., C. frutescens var. cerasiforme, C. frutescens var. acuminatum, C. tetragonum	Red pepper
Cicer arietinum L.	Chick pea
Citrullus vulgaris Schrad	Watermelon
Dolichos lablab, D. sinensis	Dolichos lablab beans
Panicum crusgalli var. frumantaceum, P. miliaceum, P. miliare, P. psilopdium	Panicum

Pennisetum glaucum (specatum; typhoideum)	Pearl millet
Phaseolus mungo	Mungo bean
Phaseolus vulgaris	Phaseolus vulgaris beans
Pisum sativum var. macrocarpon (P. sativum var. saccharatum Hort.) P. maritimum	Peas
Sesamum indicum Linn. (orientale), S. radiatum	Sesame
Setaria italica	Foxtail millet
Sorghum vulgare	Sorghum
Vigna catjang, V. cylindrica, V. sinensis, V. unguiculata, V. sesquipedalis	Cowpea
Zea mays Linn.	Corn

THE FOOD AND NUTRITION BOARD

The Food and Nutrition Board, established in 1940 under the Division of Biology and Agriculture of the National Academy of Sciences—National Research Council, serves as an advisory body in the field of food and nutrition. It promotes needed research in the broad field and helps interpret nutritional science in the interests of the public welfare. The Board may act on its own initiative or on request from public or private agencies.

The members of the Board are appointed from among leaders in the sciences related to food and nutrition on the basis of their qualifications of experience and judgment to deal with the broad problems that come before the Board. Appropriate contact with Federal agencies, scientific societies, and other associations is maintained through liaison representatives appointed from their respective organizations. Specific activities of the Board are carried on by committees composed of experts in each field. Members of the Board and its committees serve without compensation beyond their actual expenses.

Financial support for publications and meetings of the Board is primarily provided by foundations such as the Milbank Memorial Fund, the Nutrition Foundation, and the Williams-Waterman Fund of the Research Corporation. Funds for specific purposes may come through government contract or grants from industry.

NATIONAL ACADEMY OF SCIENCES
NATIONAL RESEARCH COUNCIL

The National Academy of Sciences—National Research Council is a private, nonprofit organization of scientists, dedicated to the furtherance of science and to its use for the general welfare. The Academy itself was established in 1863 by the terms of a Congressional charter under which it is empowered to provide for all activities appropriate to academies of science and is required to act as an adviser to the Federal Government in scientific matters. The National Research Council was established by the Academy in 1916, at the request of the President of the United States, to enable scientists generally to associate their efforts with those of the limited membership of the Academy. With funds contributed from both public and private sources, the Academy and its Research Council work to stimulate research and its applications, to survey the broad possibilities of science, to promote effective utilization of the scientific and technical resources of the country, to serve the Government, and to further the general interests of science.