Reprinted from *Amino Acid Malnutrition, XIII Annual Protein Conference.*
New Brunswick, N.J., Rutgers Univ. Press 1957. p. 28-46.

3

VEGETABLE PROTEIN MIXTURES FOR THE FEEDING OF INFANTS AND YOUNG CHILDREN[1]

Nevin S. Scrimshaw, Institute of Nutrition of Central America and Panama (INCAP)[2], Robert L. Squibb, Instituto Agropecuario Nacional (IAN)[3], Ricardo Bressani, Moisés Béhar, Fernando Viteri, and Guillermo Arroyave (INCAP)

Protein deficiency, or more specifically, the lack of the right amounts and proportions of the essential amino acids, is now widely understood to be the major nutritional problem, particularly among young children, in most of the world's underdeveloped areas. The word *kwashiorkor,* as used for a syndrome which includes retarded growth and maturation, edema, alterations in the skin and hair, apathy, anorexia, diarrhea, and a variety of accompanying biochemical and physiological changes, has become part of our professional vocabulary. In Latin America it is known as *Sindrome Pluricarencial Infantil.* Studies are in progress in many parts of the world, not only to understand the nature of kwashiorkor, but also to find practical means of preventing it in the countries in which it is now prevalent.

So much has recently been written about the characteristics and treatment of kwashiorkor *(1-5)* that no attempt is made to review these aspects of the problem in the present paper. The purpose is rather to discuss the suitability of different foods for the prevention of kwashiorkor with special reference to products of vegetable origin. This approach must contend with the problem of the poor quality of vegetable protein from simple sources and the degree to which it can be improved through suitable combinations or even through direct supplementation with synthetic amino acids.

There is no doubt that sufficient milk or other protein of animal origin, given to young children whenever the supply of mother's milk becomes inadequate for their needs, would effectively prevent the occurrence of kwashiorkor. Unfortunately, animal protein is costly and

[1] Various parts of this work have been assisted by Grant No. 981 from the National Institutes of Health, and by funds from the National Research Council, E. I. Du Pont de Nemours and Company, and by the World Health Organization of the United Nations.

[2] INCAP is a cooperative Institute for the study of human nutrition, supported by the Governments of Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama, and administered by the Pan American Sanitary Bureau, Regional Office of the World Health Organization.

[3] IAN is the National Agricultural Institute of Guatemala.

in short supply in most areas where the syndrome is a problem. Despite the great amount that can be done to improve animal production in many underdeveloped areas, there is no reasonable prospect that this will furnish sufficient quality protein soon enough or at a sufficiently low cost to provide a satisfactory solution.

As has been recently pointed out *(6)*, there are a number of contributing factors, such as intercurrent infection and particularly diarrheal disease, which serve to precipitate kwashiorkor in children who are already basically malnourished. Environmental sanitation and other measures aimed at the control of these precipitating causes can do much to reduce the incidence of acute kwashiorkor but cannot in themselves solve the basic problem which is protein malnutrition.

For these reasons research workers in a number of countries and the specialized agencies of the United Nations concerned with nutrition – the World Health Organization (WHO), the Food and Agricultural Organization (FAO), and the United Nations Children Emergency Fund (UNICEF) – have turned to the problem of making suitable combinations of vegetable protein available for infant feeding in underdeveloped areas in which protein malnutrition is a serious problem.

While such a mixture is theoretically possible, the task is not simple. Much more information is needed about the amounts of amino acids required and the adverse effects of possible amino acid imbalances as well as further data on the biological availability of the amino acids in many of the most likely mixtures. New vegetable-protein combinations should not be tested in human beings until after painstaking animal testing. Frequently these facilities are not available in the area where the greatest need and interest in the development exists. There must be assurance that the mixture contains no toxic factors inherent in the ingredients or resulting from the proposed method of processing. In addition, it is axiomatic that practical mixtures for human feeding in underdeveloped areas must be inexpensive, made principally from locally available ingredients, palatable, capable of easy storage and transportation, and acceptable as a food for infants and young children to the parents of those for whom it is intended.

Succeeding sections discuss the initial experiences of the Institute of Nutrition of Central America and Panama (INCAP) in its efforts to develop such a mixture and present some preliminary observations on the use of the amino acid pattern of the provisional reference protein recently proposed by the Committee on Protein Requirements of FAO *(7)*.

BASIS FOR THE DEVELOPMENT OF AN INCAP VEGETABLE MIXTURE

As the result of nine years of work in the Instituto Agropecuario Nacional of Guatemala (IAN) by one of the authors (R.L.S.) in developing animal rations based on local plant resources *(8-12)* and seven years of study of the nutritive value of the foods of Central America and Panama by INCAP *(13-15)*, considerable basic information was available as to potential ingredients of an all-vegetable mixture for human feeding. Because of the very large part which corn already plays in the Central American diet, and because a practical commercial procedure existed for preparing lime-treated corn in the form of tortilla flour, it was decided to use this flour as the basis for the mixture and to look for a combination of other products of plant origin which would be locally available and which would improve sufficiently its nutritive value.

Soybean, which would otherwise have been a major ingredient of the mixture is not at present grown in Central America in significant quantities. The background of successful use of sesame meal in improving the protein quantity and quality of animal rations and its local availability indicated that this meal should be a key ingredient. The large quantity of cottonseed meal available as a by-product and its known value as a protein concentrate for animal feeding, together with the availability of a process for preparing a safe and palatable cottonseed meal for human consumption led to the use of this product.

Since the objective was to develop a food mixture which did not require supplementation with manufactured nutrients, a food source of vitamin-A activity was necessary. Experience showed that a number of the leafy forage plants such as kikuyu grass *(Pennicetum clandestinum)*, desmodium *(Desmodium intortum)*, and ramie *(Boehmeria nivea)* contained high vitamin-A activity and were locally available in Central America *(16, 17)*.

No combination of these proposed ingredients would, however, supply sufficient riboflavin, niacin, or ascorbic acid. Because the raw materials for the manufacture of yeast are available at low cost in Central America, the incorporation of a small amount of this ingredient was chosen as the means of introducing an adequate content of B-complex vitamins. Since ascorbic-acid-rich fruits and fruit juices are readily available, no attempt was made to include this vitamin in the mixture.

Although this mixture was not intended to serve as the sole food of the child, and in theory need not serve as anything more than a protein supplement, it was highly desirable to have it as complete nutritionally as practical. Even with the acceptance of this protein-rich mixture by people in underdeveloped areas as appropriate food for their young children, the other dietary components are likely to be primarily carbohydrate in nature. In such cases neglect of the vitamin content of the mixture could result in clinical deficiencies.

FORMULA FOR INCAP VEGETABLE MIXTURE 8

All available data were assembled about the various potential ingredients and on the basis of calculated nutritive value, economic feasibility, and palatability, formulas were developed which seemed progressively more promising. A combination identified only as INCAP

Vegetable Mixture 8, while undoubtedly capable of further improvement and of variation to meet the current availability and price of its ingredients, was considered sufficiently promising to be tested in rats and chicks. The animal trials were so successful as to justify human-feeding experiments, and these have also proved highly satisfactory.

The composition of INCAP Vegetable Mixture 8 is as follows:

	%
Dried corn masa	50
Sesame meal	35
Cottonseed press cake	9
Torula yeast	3
Kikuyu-leaf meal	3

The source of each of these ingredients is as follows:

Dried Corn Masa *(Zea mays)*

Corn masa is made by heating whole corn to boiling in a solution of 0,5 to 1 per cent calcium hydroxide and holding it at this temperature for 30 to 45 minutes. After it has cooled, the water is poured off and the whole kernels ground in a mechanical mill *(18)*. It is then dried to make a flour. In the initial rat, chick, and human trials reported, corn masa made locally was dried for the purpose. For the latter trials, including all the metabolic balance studies, tortilla flour was purchased in Mexico.[4] The composition of this product is given in Tables I-III. It should be pointed out that the amino acid composition *(19, 20)* and the carotene content *(21)* will vary significantly with the variety of corn employed.

TABLE I

PROXIMATE COMPOSITION OF INCAP VEGETABLE MIXTURE 8

	Moisture %	Protein %	Fat %	CHO %	Calories / 100 g.	Crude Fiber %	Ash %
Corn Masa	9.6	8.1	3.2	77.7	372	1.2	1.4
Sesame Meal	7.0	41.0	33.0	5.5	483	5.2	13.5
Cottonseed Press Cake	5.4	54.0	4.8	29.1	376	2.6	6.7
Kikuyu Leaf Meal	10.5	18.0	4.0	57.8	339	19.0	9.7
Torula Yeast	7.0	50.0	-	35.5	342	0.5	7.5
Mixture 8	8.3	25.1	13.7	46.3	503	3.2	6.6

[4] Minsa produced by license from the Banco Nacional de México.

TABLE II

VITAMIN AND MINERAL CONTENT OF INGREDIENTS AND OF INCAP VEGETABLE MIXTURE 8[1]

Ingredient	Carotene mg/100 g	Thiamin mg/100 g	Riboflavin mg/100 g	Nicotinic Acid mg/100 g	Calcium mg/100 g	Phosphorus mg/100 g	Iron mg/100 g
Corn masa	0.15[2]	0.40	0.12	2.31	131	145	0.8
Sesame meal	-	1.02	0.47	10.60	2154	1324	107.0
Cottonseed press cake	-	0.44	0.36	4.24	149	1535	10.6
Kikuyu-leaf meal	23.80[3]	0.35	1.89	4.62	231	188	21.0
Torula yeast	-	27.00	10.00	100.00	900	2000	18.0
Mixture 8	0.78	1.41	0.61	8.39	867	740	40.0

[1] Ascorbic acid content negligible.
[2] Varies with type of corn used.
[3] Based on average value of other leaf meals.

TABLE III

AMINO ACID CONTENT OF INGREDIENTS OF INCAP VEGETABLE MIXTURE 8

Amino Acid	Corn %	Sesame %	Cottonseed %	Torula %	Alfalfa[1] %	Calculated Total mg Amino Acid	mg Amino Acid/ g N	Provisional Protein mg Amino Acid/ g N	Score
Arginine	0.42	3.19	5.64	1.82	0.83	1910	478	-	-
Histidine	0.23	0.59	1.32	1.00	0.25	490	122	-	-
Isoleucine	0.40	1.89	1.88	1.83	0.61	1100	276	270	100
Leucine	0.86	3.15	2.94	2.68	1.03	1900	475	306	100
Lysine	0.26	1.00	2.15	2.62	1.06	780	195	270	72 (83)[2]
Methionine	0.16	1.14	0.70	0.67	0.04	560	245	270	91
Cystine	0.08	0.86	0.80	0.34	-	420			
Phenylalanine	0.40	2.90	2.60	1.51	0.68	1520	603	180	100
Tyrosine	0.36	1.54	1.31	1.64	-	890			
Threonine	0.28	1.15	1.76	1.82	0.54	770	193	180	100
Tryptophan	0.04	0.59	0.59	0.51	0.22	310	78	90	86
Valine	0.44	1.36	2.46	1.98	0.87	1010	253	360	70 (100)[2]

[1] Figures are for alfalfa since no data are yet available for kikuyu.
[2] When score is based on analysis of complete mixture rather than calculation from ingredients.

Sesame Meal (*Sesamum orientale*)

Locally produced sesame oil meal was used in the initial animal trials, but in later experiments a flour specially prepared for human consumption was obtained from the American Sesame Products, Inc. of Paris, Texas.[5] In making this product the grain was dehulled, cleaned to maximum purity, steamed, roasted, and then crushed before being ground. The composition of this product is given in Tables I-III. The temperature of the material entering the press did not exceed 230°F. The sesame from which the meal was prepared is the variety known as Renner No. 1, and was grown in western Texas, and the irrigated areas around Lubbock and Artisia, New Mexico.

Cottonseed Meal *(Gossypium hirsutum)*

The initial animal trials were made with a cottonseed press cake prepared locally. For all the later work, a cottonseed flour, made for human consumption, was obtained through UNICEF from the Traders Oil Mill Company of Fort Worth, Texas. To prepare it, prime cottonseed was delinted and dehulled and the hull-free meats flaked in conventional equipment. The fresh, flaked meats were not heated above 275°F. prior to screw pressing. The free gossypol content did not exceed 0.045 per cent. Other analytical values are given in Tables I-III.

Torula Yeast *(Torulopsis utilis)*

Torula yeast, designated as Type 100, manufactured by the Lake States Yeast Corporation of Rhinelander, Wisconsin, was obtained through the courtesy of Charles Bowman and Company, New York. In addition to 50 per cent protein, this product contained 0.27 mg of thiamin, 0.1 mg of riboflavin, and 1.0 mg of niacin per gram, according to the manufacturers' specifications.

Kikuyu-leaf Meal *(Pennicetum clandestinum)*

Fresh young kikuyu grass averaging 45 cm in height, grown in the grounds of the Instituto Agropecuario Nacional in Guatemala City, was collected and dried for approximately 48 hours in warm moving air at a temperature not exceeding 140°F. The dry grass was then ground as finely as possible in a Wiley mill. Its average β-carotene content was 23.8 mg per cent. Analytical values for its content of other nutrients are given in Tables I-III.

COMPOSITION OF THE MIXTURE

The approximate composition of the mixture and its individual components is given in Table I and the values for the vitamin and mineral

[5] Through the courtesy of Mr. John Kraft and Mr. Roy H. Anderson.

content of these ingredients are listed in Table II. Except for the torula yeast and the kikuyu grass, the values are based on INCAP analyses. In the quantities ordinarily consumed by young children when the vegetable mixture is the sole source of protein, it contains satisfactory amounts of all known essential nutrients except ascorbic acid.

The amino acid content of the mixture as compared to the amino acid pattern for a provisional reference protein is shown in Table III *(7)*. It will be apparent that the mixture contains as much of all the essential amino acids as the reference protein per gram of nitrogen, except for lysine, valine, and possibly tryptophan. Since the final factor determining protein value is the availability of the essential amino acids to the animal organism, and not their content as determined microbiologically on the hydrolyzed protein, the mixture was tested in rats before attempting to improve its theoretical value when compared with the reference protein. Its protein score by calculation would appear to be approximately 70, if lysine is considered to be limiting, and 85 if valine is taken to be the limiting amino acid.

All the ingredients except the yeast and the leaf meal are, of course, heat treated in the course of their preparation, but for feeding to children further cooking before serving is desirable to assure a completely sanitary product and to improve palatability. Most of the mixture fed to children has been cooked with hot water in the same manner as oatmeal, using a double boiler for convenience. It was served as a hot gruel flavored with sugar, similar to the *atoles* which are commonly used in Central America. The mixture was also made into a very palatable dessert by cooking it with oleomargarine, sugar, vanilla, and a small amount of water in a double boiler; it can be served either hot or cold. Although only these two methods of preparation have been used thus far in the feeding trials with children, there are obviously a wide variety of recipes which can be developed for its use, and there is the possibility of preparing the mixture in a completely precooked form.

ANIMAL TRIALS

Work with Rats

Rats fed the vegetable mixture as the sole source of protein with added vitamins and minerals showed very satisfactory growth which was not improved by the addition of lysine, under the conditions of the experiments. The results in Table IV are representative of six trials employing 272 rats in 26 groups; the complete animal tests of the mixture will be reported elsewhere.

It will be seen that the mixture as fed supplied approximately 25 per cent protein and that the growth was good. There was an apparent improvement in feed efficiency, however, when lysine was added. When the percentage of protein in the experimental diet was reduced by the addition of sucrose to 19.2 and 12.8 per cent respectively, final weights of 151 and 120 grams were observed in three weeks. In the latter case, a subsequent trial has shown that lysine does result in some further growth improvement as well as greater feed efficiency.

TABLE IV

EXAMPLES OF GROWTH IN RATS FED INCAP VEGETABLE MIXTURE 8[1]

Trial	Mixture	Feed Efficiency[2]	Initial Weight g	Final Weight g
1A	8	2.55	54	238
1B	Modified 8[3]	2.58	54	250
1C	8 with 9% skim milk[4]	2.55	54	237
1D	8 with 14.3% skim milk[4]	2.50	54	264
2A	8	2.71	44	223
2B	8 + 0.45% lysine	2.14	44	230
2C	Modified 8[3]	2.08	44	233
2D	Modified 8[3] + 0.45% lysine	1.90	44	232
2E	Modified 8[3] with 9% skim milk[4]	2.29	44	236
2F	Modified 8[3] with 9% skim milk[4] + 0.45% lysine	1.98	44	235

[1] Trials 1A to 1D and 2A to 2F were carried out with 12 rats per group, 8 weeks with 25.2% protein.
[2] Feed efficiency grams feed consumed per gram of weight gained.
[3] Cottonseed meal replaced by additional sesame.
[4] Substituted for corn masa.

Work with Chicks

Baby chicks, three days of age, were fed the mixture as the sole source of protein in eight trials employing 664 chicks in 40 groups. Representative data from these trials are shown in Table V. Fair growth was obtained with the mixture alone and this was improved slightly by substituting whole ground corn, which increased palatability for the chick over the relatively sticky lime-treated corn (Tortilla flour). It is obvious that due to the greater requirement of the chick for lysine, this amino acid must be added if good growth is to be expected. When 0.2 per cent lysine was added, the growth response of chicks improved, and when 0.4 per cent was given, the growth was excellent. Higher levels of lysine did not improve the growth of the chicks fed the mixture.

Generalization from the Animal Trials

It was concluded from the rat and baby chick feeding experiments that the protein quality of INCAP Vegetable Mixture 8 was adequate for

TABLE V

EXAMPLES OF GROWTH IN CHICKS FED INCAP VEGETABLE MIXTURE 8[1]

Trial		Feed Efficiency[4]	Initial Weight g	Final Weight g
1A	8	2.80	42	200
1B	Modified 8[3]	2.84	42	217
1C	8 + 0.2% lysine	2.19	42	298
1D	8 + 0.4[4] lysine	2.05	42	369
2A	8 + 0.5% lysine	2.40	42	405
2B	Modified 8[3] + 0.5% lysine	2.39	42	431

[1] 24 chicks per group, 5 weeks with 25% protein.
[2] Mixture was supplemented with minerals and vitamins to meet chick requirements.
[3] Whole ground corn substituted for lime-treated corn (masa).
[4] Grams fed per grams gained.

good growth when the added lysine needed by the chick was provided. Although the tests were qualitative in nature only, it was reassuring that excellent growth was obtained in rats even when the level of protein in the diet was reduced to 12 per cent. Furthermore, the chick growth observed was comparable in some trials to that expected with the commercial rations. The mixture proved palatable and no signs of toxicity were encountered. Although long-term trials in rats extending over more than one generation and trials in baby pigs are contemplated before the mixture is recommended for commercial production, the data available appeared to justify carefully supervised human trials with hospitalized children.

HUMAN TRIALS

The initial trials consisted of giving small quantities of the mixture to children receiving a mixed diet to determine its palatability, and to make sure that it would not produce diarrhea or other gastrointestinal disturbances. The children accepted the mixture well and it was then given as the sole source of protein to two children who had completely recovered from kwashiorkor and later to three children who had recovered from the acute phase but were not yet ready for discharge from the hospital. The case reports on these five children have recently been published *(6)*, and can be summarized by saying that the results were highly satisfactory even in a child given the mixture as the sole source of protein for a period of thirty days. This child has since received the mixture for an additional seventy days with excellent results.

We are now able to report on five additional children who have been treated with the mixture, including three who were given it from the time of their entrance to the hospital with acute kwashiorkor. In each case the clinical response has been excellent and the mixture appears to be more effective than milk in reducing the tendency to diarrhea in the acute phase. There is some indication that the biochemical response, at least in serum protein and pseudocholinesterase was slightly slower in the three children given the mixture from the time of admission, but this was not reflected in the clinical progress.

The photographs of one of these children (E.P.L.) taken at the time of admission with acute kwashiorkor and following seven weeks of treatment with only the vegetable mixture as a protein source are shown in Figure 1. The detailed case histories of the five additional children to receive the mixture are as follows:

A.R.S., a 14-month-old boy, 65 cm in height, was admitted with moderate kwashiorkor and weighed 5.85 kg when his edema was lost. He was placed on a mixture of 18 amino acids, starch and sugar which supplied 3.0 g of protein and 100 calories per kg, and the results will be described in more detail elsewhere. At the end of 18 days the edema and skin lesions had cleared but apathy, pallor, cheilitis, angular stomatitis, and atrophy of the papillae of the tongue were still present. Treatment with the vegetable mixture was begun and the amount increased progressively to 7.0 g of protein and 140 calories per kg. The mixture furnished the only source of protein, vitamins, and minerals except for 120 cc of orange juice daily. The child showed a dramatic further improvement during the next week on the vegetable mixture and was ready for discharge after receiving the vegetable mixture for 70 days. At this time he appeared healthy and his weight had increased to 8.5 kg.

L.P.L., a 5-year-old boy, 96 cm in height, was admitted with severe kwashiorkor and weighed 11.5 kg when his edema was lost. He was given half-skimmed milk for five days and then changed to a diet in which the vegetable mixture furnished the only source of protein. This diet provided 4 g of protein and 100 calories per kilo for 13 additional days. It was then increased gradually to 5.6 g of protein and 150 calories per kilo by the addition of tortillas, beans, and other vegetables and fruits. The child did very well and weighed 17.0 kg at the end of 61 days of hospitalization.

E.P.L., an 8-year-old, 106 cm tall, older brother of L.P.L., was admitted at the same time with severe kwashiorkor and also weighed 11.5 kg when his edema was lost. He was placed on the vegetable mixture as the sole source of protein, vitamins, and minerals until the fifth day when 120 cc of orange juice and 2 bananas daily were added to the diet. By the fourth day after admission, he was consuming 4 grams of protein and 100 calories per kilo. The "initiation of cure" was judged complete 17 days after admission when tortillas, beans, and other vegetables and fruits were gradually added to his diet to give a maximum of 6.0 g of protein and 180 calories per kg. At this time half the protein was obtained from the vegetable mixture and half from other vegetable sources in his diet. At the end of 64 days his condition was excellent and his weight had risen to 18.6 kg.

J.A.Q., an 18-month-old boy, 68 cm in height, weighed 7.8 kg when admitted with moderate kwashiorkor. He was placed on the vegetable mixture as the sole source of protein, vitamins, and minerals and was receiving 5 g of protein and 150 calories per kg by the 12th. hospital day. Apathy, anorexia, skin lesions, and diarrhea disappeared in three weeks, but a trace of edema persisted. The child has now received the mixture for a total of 28 days and is continuing to make a good clinical recovery.

C.A.P., a 15-month-old boy, 70 cm in height, weighed 6.8 kg when admitted with moderate kwashiorkor. He was placed on the vegetable mixture as the sole source of protein, vitamins, and minerals and was receiving 5 g of protein and 150 calories per kg by his eleventh hospital day. "Initiation of cure" was complete at the end of 3 weeks. The child has now received the mixture for a total of 26 days and is making a good clinical recovery.

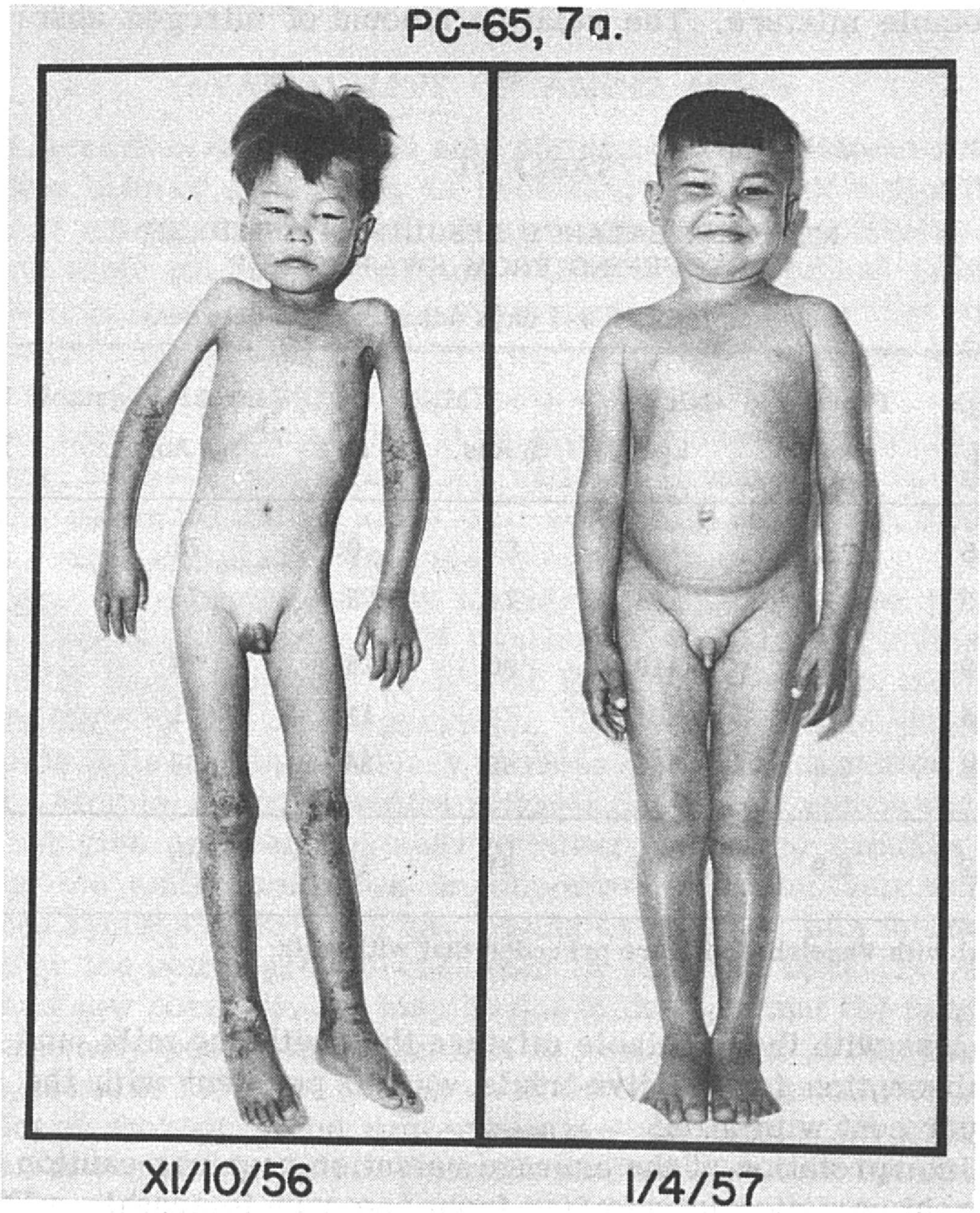

Figure 1. The photograph on the left was taken at the time of admission of E.P., an 8-year-old boy with acute kwashiorkor, and the one on the right following 7 weeks of a diet in which INCAP Vegetable Mixture 8 was the sole source of protein.

Metabolic Balance Studies in Children

Studies of the absorption and retention of nitrogen in children recovering from kwashiorkor on a diet in which milk was the sole or major source of protein, have been previously reported and the technique described *(22)*. Following the last two of these trials, the children were gradually changed over to a diet in which the vegetable mixture furnished the sole source of protein at an intake of protein and calories per kilo equivalent to that of the previous milk diet. They were then studied metabolically for an additional five-day period. The results are shown in the first two cases in Table VI together with the results of three additional trials in which the procedure differed in that the metabolic period on a milk diet followed rather than preceded the trial with the vegetable mixture. The relative amount of nitrogen absorbed was

TABLE VI

NITROGEN BALANCE RESULTS IN CHILDREN RECOVERING FROM KWASHIORKOR

(5-day trials with 3-7 days adjustment in between)

	Weight	Protein/	Calcium/	Milk		INCAP Vegetable Mixture 8	
	kg	kg	kg	% Abs.	% Ret.	% Abs.	% Ret.
	7.5	2.4	102	87	0	73	11
	11.2	2.6	106	74	23	78	23
*	9.9	3.8	110	90	15	74	12
*	9.5	2.9	101	74	11	71	14
*	11.6	2.8	108	80	34	68	23
Aver.	9.9	2.9	105	81	17	73	17

* Trial with Vegetable Mixture preceded that with milk.

usually less with the vegetable mixture than with the milk, and the average absorption for the five trials was 73 per cent with the mixture and 81 per cent with milk.

The interpretation of the average retention requires caution because of the great variation in retention from one case to another. This is an inevitable biological consequence of the differing degree of recovery and hence of repletion of depleted nitrogen stores. When individual cases are examined, the retention was approximately equal in three trials, better with milk in one and with vegetable protein in the other. From these initial trials it appears that the protein of the vegetable mixture once absorbed is as well utilized as that of milk. When the percentage of the nitrogen absorbed which was retained by the body was calculated, the resulting values were generally higher for the vegetable mixture.

GENERAL EVALUATION OF VEGETABLE MIXTURES

The compounding of suitable vegetable mixtures is at present made difficult by two factors: the uncertainty of arriving at an optimum amino acid content and balance from analytical figures alone, and the possibility that inhibitory or toxic factors may be contained in the plant ingredients selected or result from processing.

AVAILABILITY OF AMINO ACIDS

The description of the amino acid pattern of a provisional reference protein has been of great help in providing a means of comparing the amino acid combinations of individual foods and food mixtures and in estimating their probable biological value. As Allison has pointed out in this Symposium *(23)*, there is every reason to believe that this reference pattern constitutes a useful tool, and the estimations calculated from it have thus far agreed extraordinarily well with the results of biological assays. It is still a first approximation subject to further refinement, however, and it does not indicate at what point an excess of any of the essential amino acids will adversely affect the theoretical biological value calculated from the limiting amino acid.

Still greater difficulty arises in extrapolating from the microbiologically determined amino acid content of vegetable proteins which have usually been subjected to drastic hydrolysis to make their amino acids available to the test organisms. The availability of these same amino acids following the ordinary process of *in vivo* digestion may be different. Simple *in vitro* studies following complete enzymatic digestion do not give an adequate answer either, since the relative rate of release of the amino acids has an important effect on their utilization for protein synthesis *(24)*. An interesting example of this is the recent finding that the better growth observed in rats fed lime-treated corn rather than raw corn *(25, 26)* may be due to the fact that the zein, which is an extremely poor quality protein in comparison to the other corn proteins, is made selectively less available by the lime treatment *(27)*. The effect of cooking on the easier release of amino acids with *in vivo* digestions is a more familiar illustration. Furthermore, food processing may either decrease or increase protein value.

It is apparent from even this brief discussion that, however useful calculations of the potential nutritive value of vegetable mixtures may be, their validity can be determined only by careful biological trials which are relatively costly and time consuming. The importance of support for this vital phase of the world-wide development of suitable vegetable mixtures for human feeding is now beginning to be recognized.

PROBLEM OF TOXIC FACTORS

As the search is pursued for suitable ingredients for the locally produced vegetable protein combinations, it is inevitable that many plants will be suggested for which there is no long background of either laboratory study or human use. There are so many examples of toxic or interfering substances in plants that each ingredient must be carefully tested before it is assumed that it can be safely fed to human beings. The necessity of destroying the enzymes soyan and trypsin inhibitor in soybean is well known, but the violent toxicity of meal made from aceituno *(Simarouba glauca)*, which would otherwise be an excellent protein source, is an equally striking example *(28)*. The binding of phosphorus by phytins, calcium by oxalic acid, the cyanide-containing goïtrogenic substances in plants of the family Brassicae, and the toxic effect of the gossypol in cottonseed-oil meal, all illustrate the need for caution in recommending any new food mixture for human consumption. To the natural hazards must be added the danger of using toxic solvents for fat extraction and the alteration of protein quality that can come about with improper processing. Persons wishing to develop mixtures for human feeding along the lines of the vegetable mixture described in this report are urged to study the discussion and statement of principles laid down by the Conference on Protein Requirements and their Fulfillment in Practice held in Princeton, New Jersey, in June 1955 *(29)*.

AMINO ACID SUPPLEMENTATION

As the large-scale commercial preparation of certain of the key essential amino acids at a low cost becomes practical, the question is increasingly raised as to the value for underdeveloped areas of supplementing predominantly vegetable protein diets, deficient in several of the amino acids, in a manner analogous to the now well-accepted enrichment program for wheat flour, corn meal, and rice. Much has already been written on this subject and tentative proposals have been made *(30, 31)*. The situation, however, is not truly comparable to the enrichment of food with vitamins. Within the maximum conceivable range of enrichment with vitamins, an excess of one or more vitamins has no significant effect on the utilization of the others. In the case of the amino acids, however, it is abundantly clear that an excess of an amino acid may in some circumstances have as devastating an effect on the biological value of the protein as a relative deficiency *(32)*.

This would indicate that a great deal more must be known about the relative interaction of individual amino acids before the amino acid enrichment of foods can be seriously considered. Furthermore, in using the provisional amino acid pattern to determine how much of a given amino acid may be required to provide an optimum level, the uncertainty of extrapolating from analytical values to biological availability in the human being again becomes a formidable obstacle. With the increasing evidence that the proportion of amino acids in a diet, i.e., the amino acid pattern, is more important than the absolute amounts, it would appear that amino acid supplementation on the basis of our present knowledge is still a hazardous procedure.

On the other hand it is entirely possible that, given sufficient knowledge, it will be found practical to improve the protein quality of the diets of underdeveloped areas with suitable combinations of those essential amino acids in which they are deficient. Studies of the practicality of such a procedure are urgently needed. INCAP has recently begun a series of studies in which vegetable proteins and vegetable-protein mixtures are to be brought to the theoretically desirable amino acid proportions suggested by the amino acid pattern of the provisional reference protein. This is done by systematic addition of synthetic amino acids, and the nitrogen retention with each combination is observed and compared with that of an equal quantity of milk protein. By adding a small amount of glycine or glutamic acid to the basal diet, the amino acid substitutions can be made without changing the proportions of other ingredients or altering the total amount of nitrogen fed. Since differences in the amino acid supplements did not affect the appetite of the children in the study and the great bulk of the diet remained unchanged, a one-day adjustment period between three-day trials was considered sufficient.

Because of its importance in the basal diet of Central America and because the biological value of its protein is known to be relatively poor, lime-treated corn, fed as corn *masa* or as *tortilla*, was employed in the first two such trials. The relation between the amino acid content of corn masa as compared with the provisional amino acid pattern is shown in Figure 2. It will be seen that the limiting amino acid de-

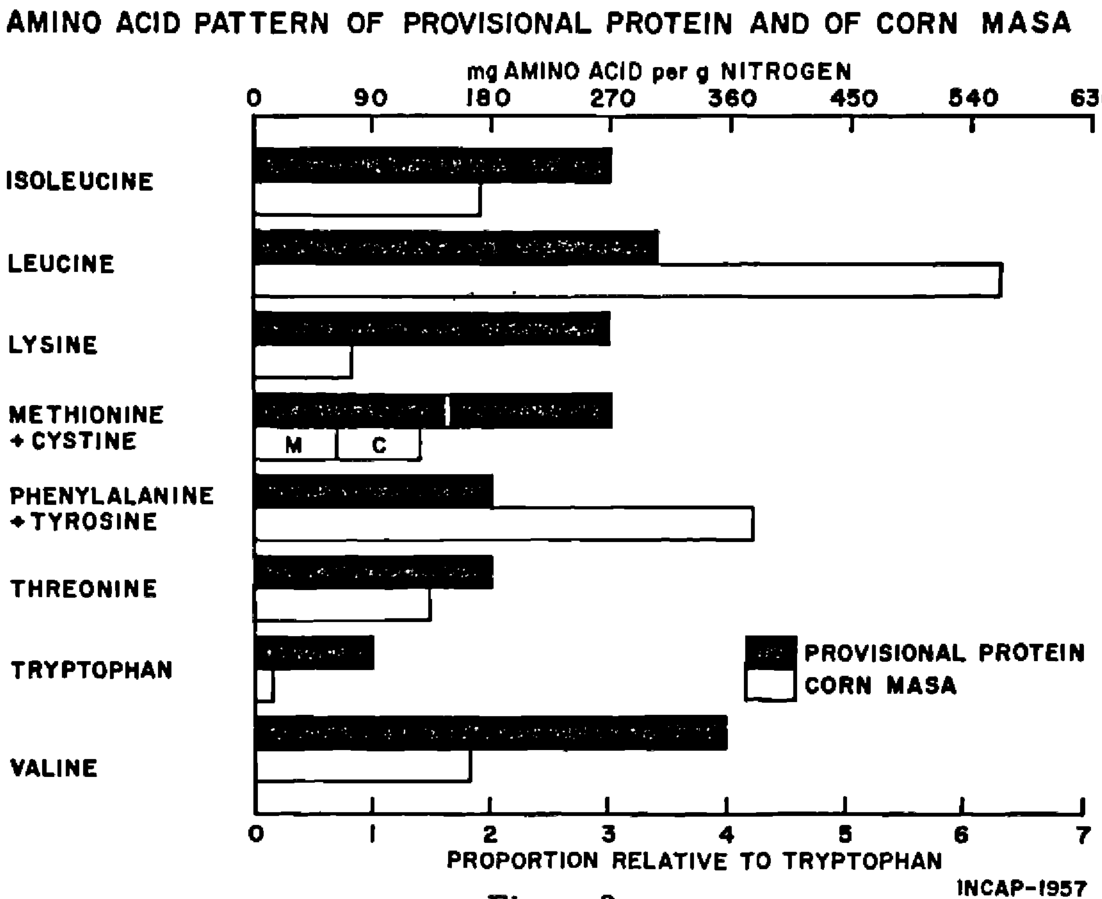

Figure 2.

ficiencies are tryptophan, lysine, methionine, valine, and isoleucine in this order.

The results of adding the first three of these amino acids to corn masa are shown in Figure 3. When the children were switched from milk to an isoproteic diet consisting entirely of lime-treated corn supplemented by vitamins and minerals and with added calories in the form

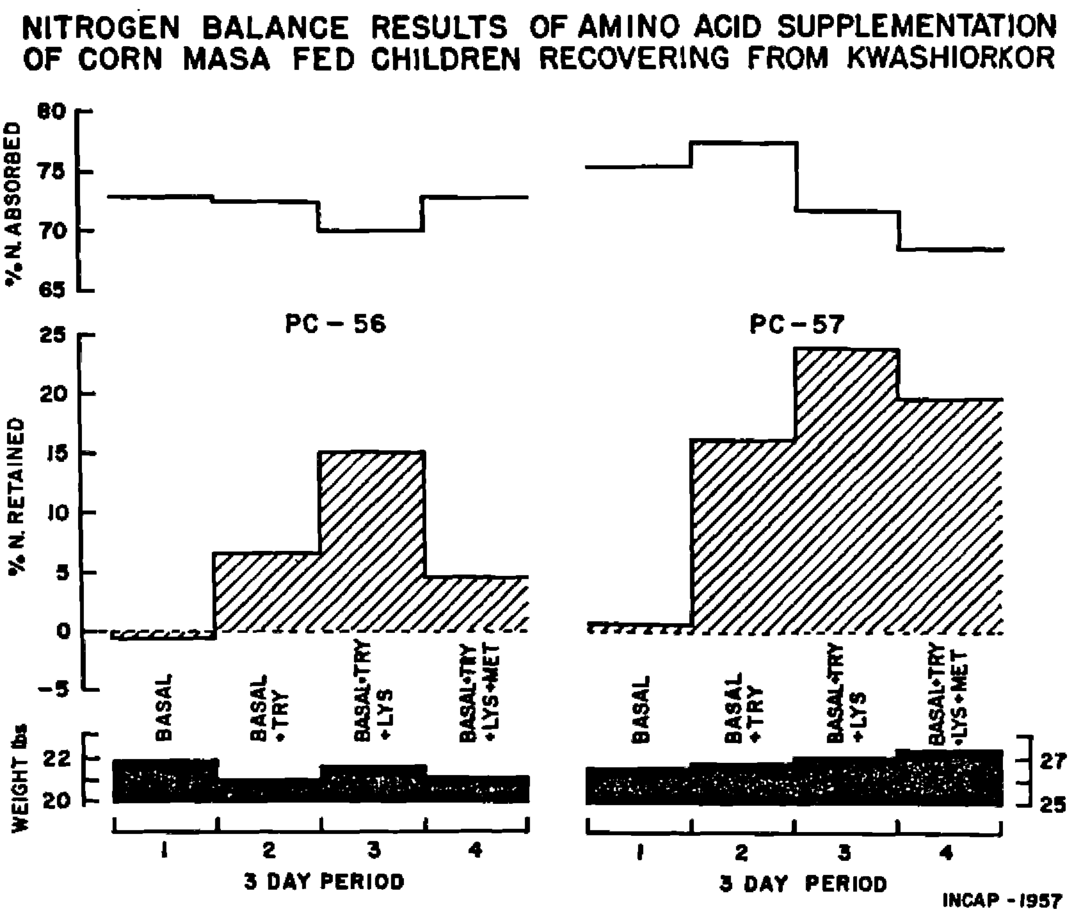

Figure 3.

of sugar, starch, and margarine, the nitrogen retention dropped sharply as expected. When tryptophan and lysine were added successively to bring the total amino acid content to that of the provisional protein, significant increments in the nitrogen retention were observed. When methionine was added, however, the nitrogen retention dropped instead of rising further.

At this point, one of the children developed chicken-pox and the balance studies had to be suspended, but the other showed no improvement with valine; not until isoleucine was added and a more favorable leucine-isoleucine ratio restored did recovery occur. It seems possible that the amount of added methionine had an adverse effect of some kind on the leucine/isoleucine ratio and that if isoleucine had been added first, the results might have been different. Only further trials can elucidate this point.

These very preliminary results with amino acid supplementation are cited only to point out that the nitrogen balance method of evaluating amino acid additions seems to be an effective one to apply to the problem at hand and also to illustrate the problems of imbalance which arise as soon as amino acid supplementation is undertaken. Regardless of the explanation, methionine addition in both cases had the unexpected effect of decreasing rather than increasing nitrogen retention.

CONCLUSIONS

The results obtained thus far in the development and testing of INCAP Vegetable Mixture 8 show that it is practical to develop a low-cost all-vegetable mixture from locally available ingredients for the supplementary and mixed feeding of infants and children in underdeveloped areas. It is a happy circumstance that the biological value of the protein of this Mixture 8 appears to be so closely equivalent to that of milk.

Nevertheless, such a mixture need not be equivalent in biological value to milk to have an important place in the prevention of protein malnutrition, nor need it be the best possible formula from a nutritional viewpoint, but it must be effective as well as inexpensive, palatable, and easily transported and stored.

Further improvements in the formula are obviously possible from both a nutritional and an economic point of view, and these will be worked out as soon as trials of the present mixture are completed. A number of obvious modifications should be tested to adapt this formula to other underdeveloped areas where the availability and cost of local ingredients are different. Substitution of all or part of the corn for sorghum or rice may be envisaged. The use of rice instead of corn would theoretically improve its protein value and make it more palatable to rice-eating people. With the availability of low-gossypol cottonseed meal, the proportions of this ingredient can probably be greatly increased or if necessary it can be left out entirely. Whether or not the supplementation of diets with synthetic amino acids will prove practical and desirable can only be determined by fundamental studies to obtain data not now available.

All these developments may be expected in the future in those areas which are in greatest need of them. As described elsewhere in this *Symposium (33)* the current programs of WHO, FAO, and UNICEF, assisted by funds from the Rockefeller Foundation and the National Research Council, will play a decisive part in stimulating the necessary scientific investigations and practical applications.

REFERENCES

1. Trowell, H. C., Davies, J. N. P., and Dean, R. F. A., *Kwashiorkor*, 1st ed. (London, Edward Arnold & Co., 1954).
2. Autret, M., and Behâr, M., *FAO Nutritional Studies No. 13*, Food and Agriculture Organization of the United Nations, Rome, Italy (1954).
3. Waterlow, J. C., and Scrimshaw, N. S., *Bull. Wld. Health Org.* (in press).
4. Scrimshaw, N. S., Behâr, M., Arroyave, G., Viteri, F., and Tejada, C., *Federation Proc.*, 15:977 (1956).
5. Scrimshaw, N. S., Behâr, M., Arroyave, G., Tejada, C., and Viteri, F., *J. Am. Med. Assoc.* (in press).

6. Scrimshaw, N. S., Behár, M., Viteri, F., Arroyave, G., and Tejada, C., *Am. J. Pub. Health*, 47:53 (1957).
7. Report of the Committee on Protein Requirements. FAO, Rome, Italy (in press).
8. Squibb, R. L., Falla, A., Fuentes, J. A., and Love, H. T., *Poultry Sci.*, 29:482 (1950).
9. Squibb, R. L., and Wyld, M. K., *Poultry Sci.*, 29:586 (1950).
10. Squibb, R. L., and Salazar, E., *J. Anat. Sci.*, 10:545 (1951).
11. Squibb, R. L., and Wyld, M. K., *Poultry Sci.*, 31:118 (1952).
12. Squibb, R. L., Guzmán, M., and Scrimshaw, N. S., *Poultry Sci.*, 32:1078 (1953).
13. Instituto de Nutrición de Centro América y Panamá, *Suplemento No. 1 del Boletín de la Oficina Sanitaria Panamericana*, "Publicaciones Científicas del Instituto de Nutricion de Centro América y Panama," (1953) p. 129.
14. Instituto de Nutrición de Centro América y Panamá, *Suplemento No. 2 del Boletín de la Oficina Sanitaria Panamericana*, "Publicaciones Científicas del Instituto de Nutrición de Centro América y Panama," (1955) p. 232.
15. Instituto de Nutrición de Centro América y Panamá and Instituto Agropecuario Nacional de Guatemala, *Suplemento No. 2 del Boletín de la Oficina Sanitaria Panamericana*, "Publicaciones Científicas del Instituto de Nutrición de Centro América y Panamá." (1955) p. 227.
16. Squibb, R. L., Mendez, J., and Scrimshaw, N. S., *Turrialba*, 3:163 (1953).
17. Squibb, R. L., Guzmán, M., and Scrimshaw, N. S., *Turrialba*, 3:91 (1953).
18. Illescas, R., *Soc. Mex. Hist. Nat.*, 4:129 (1943).
19. Aguirre, F., Robles, C. E., and Scrimshaw, N. S., *Food Research*, 18:268 (1953).
20. Aguirre, F., Bressani, R., and Scrimshaw, N. S., *Food Research*, 18:273 (1953).
21. Bressani, R., Campos, A. A., Squibb, R. L., and Scrimshaw, N. S., *Food Research*, 18:618 (1953).
22. Robinson, U., Behár, M., Viteri, F., Arroyave, G., and Scrimshaw, N. S., *J. Trop. Pediatrics* (in press).
23. Allison, J. B., In *Amino Acid Malnutrition, XIII Annual Protein Conference* (New Brunswick, N.J., Rutgers University Press, 1957) p. 1 .
24. Allison, J. B., *J. Agri. and Food Chem.*, 1:7; (1953).
25. Laguna, J., and Carpenter, K. J., *J. Nutr.*, 45:21 (1951).
26. Cravioto, R. O., Anderson, R. K., Lockhart, E. E., Miranda, F. de P., and Harris, R. S., *Science*, 102:91 (1945).
27. Bressani, R., *Federation Proc.*, Vol. 16 (1957) (in press).
28. Squibb, R. L., unpublished data.
29. *Protein Requirements and their Fulfillment in Practice* (FAO, WHO, and the Josiah Macy Jr. Foundation) (in press).
30. Sure, B., *J. Agri. and Food Chem.*, 3:789 (1955).
31. Flodin, N. W., *J. Agri. and Food Chem.*, 1:222 (1953).
32. Elvehjem, C. A., In *Some Aspects of Amino Acid Supplementation, XII Annual Protein Conference* (New Brunswick, N. J., Rutgers University Press, 1956) p. 22.
33. Sebrell Jr., W. H., In "Amino Acid Malnutrition," *XIII Annual Protein Conference* (New Brunswick, N. J., Rutgers University Press, 1957) p. 47.