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Vegetable protein mixtures for human consumption[1]

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THE WIDE-SPREAD PREVALENCE and great public health significance of protein malnutrition among young children in most technically underdeveloped areas of the world is now well recognized. Educational measures are generally of value, but successful prevention often depends on the availability of sources of good quality protein. While it is frequently possible to increase production and consumption of protein-containing foods of animal origin such as milk, cheese, meat, eggs and fish, these foods are often beyond the economic reach of the population segments needing them most. In many countries, it is impracticable to provide all the foods of animal origin necessary to furnish the major part of protein requirements. Furthermore, this situation tends to become worse as populations increase. Better use of vegetable protein is the only practical solution to the problem of protein malnutrition for many areas.

Since individual plant proteins are relatively poor in biological value because their amino acid content does not suit mammalian needs, they must either be supplemented with animal protein or mixed with other plant proteins which have a complementary amino acid content. The other alternative of supplementing with synthetic amino acids may some day be practicable, but costs are still prohibitive. The first mentioned possibility, combining animal and vegetable protein sources, is the basis of the ordinary good mixed diet. When skim milk, fish flour and certain other animal products are available at relatively low cost, they may, of course, also be of major importance in technically underdeveloped areas. The primary purpose of this paper, however, is to describe the progress which has been made in devising vegetable combinations of low cost and high protein value.

BASIC PRINCIPLES INVOLVED

In recommending the supplementation of predominately vegetable diets with proteins of animal origin, the greater the proportion of animal to vegetable protein, the higher the nutritive value, although beyond a certain point there is no practical advantage. When adding protein-rich foods of vegetable origin to a diet, however, there is an optimum proportion above or below which biological value is decreased. For this reason, the combination of proteins of vegetable origin for child feeding, where protein quality may be critical, requires a degree of precision which implies the elaboration of a mixture available as a single protein source as nearly comparable as possible in quality to protein-rich foods of animal origin.

It is obvious that in addition to having a high protein quality, such a mixture should be a relatively concentrated source of protein, at least before preparation as a beverage or mixing into other components of the diet. Preliminary estimates of the protein quality of a vegetable mixture may be made from a comparison of the amino acid composition with a theoretical amino acid pattern such as that of the FAO Reference Protein (21), or a good animal protein such as supplied by egg or milk.

There are several reasons, however, why a protein mixture may be better or worse than such a comparison would suggest. One reason is that current amino acid assay procedures give the amount present but not the amount available to the human organism. Furthermore, the amino acid proportions given in the FAO Reference Protein appear too high for methionine and tryptophan, and too low for lysine (7, 8, 33), and no other theoretical pattern has been proved to be any better. A relative amino acid imbalance may also interfere with the utilization of individual essential amino acids, rendering the protein score unreliable. The final test thus becomes the protein value as determined in suitable experimental animals together with effectiveness in child feeding trials. In INCAP, we find the comparison of the nitrogen retention of children fed various vegetable mixtures with that of children receiving isoproteic amounts of milk, a particularly useful means of evaluation.

In devising protein-rich vegetable mixtures, it is important to specify the objective and form of use. Protein malnutrition is most acute in children 1–5 years of age who, after weaning, are not given a diet with adequate protein. This age group needs a supplement to their customary diet which will make up the protein deficit and also correct any other nutrient deficiencies. This means that for most areas, the mixture should be a good source of vitamin A, riboflavin and calcium, and preferably that it also supply supplementary niacin. Iron may also be needed. In Latin America, at least, adequate amounts of thiamin and vitamin C are usually supplied by the cereals and fruits common in the diets. The desirable caloric content of the supplementary

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food depends upon the adequacy of the habitual diet, and is usually of minor concern.

When they are fed as a semisolid, vegetable mixtures for the mixed feeding of infants 6–12 months of age, who are still receiving mothers' milk, do not present special problems. If the vegetable mixture is prepared as a liquid, care should be taken, as with supplementary milk feeding, not to displace breast feeding prematurely.

A vegetable mixture formula employed for infant feeding when neither breast milk nor cow's milk is available, carries with it additional problems. It must be recognized that a vegetable mixture may be entirely satisfactory for the prevention of protein malnutrition in older children and quite unsatisfactory as the basis for the formula feeding of young infants. The suitability of a vegetable mixture for feeding premature infants has still less practical importance if its primary purpose is the prevention of protein malnutrition in preschool children. The use of vegetable mixtures as supplementary sources of protein is, of course, not limited to children. It is also beneficial for pregnant and lactating women, and acceptance as a staple in the diet of all members of the family, increases the probability that the mixture will be made available to the family members who need it most.

In developing vegetable mixtures it is important to consider agricultural, economic and cultural factors simultaneously with nutritional value and palatability. There are probably many vegetable mixtures of high nutritive value which can be produced from the vegetable resources of most areas, but most of these will prove to be unacceptable or too expensive if nutritional value is the only major consideration.

PRESENT SCOPE OF EFFORTS TO DEVELOP VEGETABLE PROTEIN MIXTURES

Under the stimulus of WHO and FAO, numerous international nutrition and health conferences in the last decade have stressed the need for developing new sources of protein-rich foods for human feeding. The joint WHO-FAO-Macy Foundation Conference on Human Protein Requirements held in Princeton in 1955 (44) led to the designation by WHO of a Protein Advisory Group to give international guidance and encouragement to programs designed to develop new protein sources. It also resulted in a substantial research grant from the Rockefeller Foundation to be administered by a committee of the National Research Council for the same purpose. Personnel of FAO and UNICEF, as well as WHO, have participated actively in these programs. Projects sponsored by the NRC Committee are now underway in Mexico, Guatemala, French West Africa, Nigeria, Belgian Congo, Uganda, Union of South Africa, India, Japan, United States, United Kingdom, Taiwan and Brazil, and many additional centers are engaged in this work with other support. Not all of these projects have as yet produced results, and some deal only with the use of animal protein to enrich plant protein mixtures or are basic biochemical and biological studies of plant proteins. It is obvious, however, that with such interest in the problem and so many centers actively engaged, rapid progress can be expected in this field.

VEGETABLE INGREDIENTS FOR PROTEIN-RICH FOODS

From a practical point of view the elaboration of protein-rich vegetable mixtures involves the supplementation of the cereal grains which form the staple food in most technically underdeveloped areas. Unfortunately, all cereal grains have the double disadvantage of a relatively low protein content which often decreases further with processing, and a poor protein quality due to deficiencies of several of the essential amino acids. These disadvantages can be corrected by combining cereal grains with foods of higher protein concentration which supply essential amino acids in such proportions as to correct the deficiencies in the cereal grain.

Five large groups of vegetable products which could be used for this purpose are: *1*) legume seeds, *2*) oil seed cakes, *3*) nuts, *4*) palm kernels, and *5*) leaf proteins. Of these, some have received attention as protein supplements, but many have been neglected or require further study. Although algae and yeast have frequently been suggested as protein sources, thus far, they have not proved sufficiently palatable to constitute more than a small percentage of the total diet.

The legume seeds form a vast group with a protein content of around 25%. In general, their quality as single protein sources is not much better than the cereal grains. They are, however, relatively good sources of lysine, the first limiting amino acid in cereal grain protein, but are limiting in methionine, which is adequate in cereal grains. Legume seeds must be cooked for maximum utilization, and improper processing may decrease their protein value. Actual protein value varies widely within the group which includes the peanut, *Arachis hypogaea*, the soybean, *Glycine max*, the common bean, *Phaseolus vulgaris*, the cow pea, *Vigna sinensis*, the chick pea (Bengal gram), *Cicer avientinum*, and beans of the genera *Vicia*, *Pism*, *Lens*, *Dolichos* and *Canavalia*. *Phaseolus vulgaris*, *Glycine max*, *Pism sativum*, and *Arachis hypogaea* seeds have been studied, but more research is needed on these and the other genera if full advantage is to be taken of their potential contribution to the prevention of human protein malnutrition.

Since soybean has been a human food for centuries in certain parts of the world, and because it is now an important commercial source of oil and of press cake for animal feeding, this legume has received the greatest attention. It has been prepared in a variety of forms for human consumption and the literature is voluminous (15, 38). Cooked soybean meal contains approximately 44% protein of a quality superior to most vegetable proteins but inferior to most proteins of animal origin. Its strong, slightly bitter flavor has limited its acceptability in some populations.

Of major current interest are the efforts to develop soy protein isolates of more concentrated protein content

(90%) (36), and to adapt fermentation procedures used on a home-scale in the Far East (2, 16) to commercial production. The peanut or groundnut has been widely investigated because the commercial value of its oil contributes to the low cost of the meal. Like other legume seeds, peanut is limiting in methionine and relatively low in arginine, lysine and isoleucine compared to animal protein (31).

The second group of important protein sources is that of oil seeds such as cotton, sesame, sunflower, rape, mustard, poppy, linseed, safflower, hemp and several others. Many of these are produced in such small quantities that they have no practical importance at the present time, and several have specific disadvantages of flavors or toxic substances which limit their usefulness. Because cotton is produced extensively for fiber, cottonseed is readily available for oil extraction, and cottonseed presscake is produced at low cost in such large quantities that it is of major importance as a protein concentrate in animal feeds. It has the disadvantage of containing gossypol which is toxic to some animals including the guinea pig, hampster, pig and rabbit, and which reacts to render lysine unavailable to the animal organism (1). Technological developments, however, have resulted in the production of cottonseed flours with protein of high nutritive value and sufficiently low free and total gossypol concentration that they can be fed liberally even to the most sensitive animals (1). It is probable that nearly gossypol-free flours of higher protein concentration and better protein quality will be developed. At present, good cottonseed flours for human consumption are low in both total and free gossypol and have a protein content in excess of 50% with a biological value of about 65%. As will be discussed in another section, they have a very favorable amino acid pattern for complementing cereal proteins.

Sesame has a nutritive value similar to cottonseed. It has no known toxic principle, but the removal of the hard hull is necessary to reduce the crude fiber content and to eliminate a slight bitter aftertaste. It is not popular with agriculturists because the seed pod of most varieties complicates harvesting operations by shattering readily. New varieties are being developed to overcome this difficulty. Sunflower seed is another good source of both oil and protein concentrate, but so far its large-scale production is limited to Argentina, Chile and Russia. The possibility of using the other oil seeds has not yet been investigated.

The true nuts are not sufficiently plentiful or low in cost to offer important practical advantages for large scale protein supplementation of cereal diets. Of the palm kernel proteins, only copra has been studied. A review on copra protein by Curtin (12) discusses production, composition, processing and properties. Lyman (29) has indicated that lysine is probably the first limiting amino acid, while work on protein isolates of coconut has shown that some of these fractions are good sources of lysine but low in tryptophan (9). Copra protein has been used in low cost protein foods in combinations with peanut flour and Bengal Gram (28). The results in experimental animals were satisfactory and acceptability trials gave excellent results. A disadvantage of copra protein is its high fiber content. Little is known of the other palm nut meals, but their crude fiber content is equally unfavorable. If the demand were great and the nutritive value of the protein sufficiently high, procedures for the removal of part of the crude fiber and preparation for human consumption could be developed.

Methods for the separation and concentration of leaf protein have recently received considerable attention (16, 30). At the present time, however, the cost is high and the amount which is acceptable in human diets limited. While these difficulties can probably be overcome, the quality of the protein is no better than that of the legumes, and leaf proteins are not as effective in improving the protein quality of cereal diets because of a relative deficiency in methionine-cystine. Studies of Hughes and Eyles (24–26), and Ellinger (18) in poultry have shown that the gross protein values of leaf protein are generally lower than expected from their amino acid composition, probably due to low digestibility. Nevertheless, improvement of the nutritive value of rice by adding a cooked leaf meal has been reported (40).

Many other basic plant materials might also serve as sources of protein concentrates. The by-products of the cereal industry should be mentioned among these, particularly the germ of the seeds. They are rich in oil and the protein is of good quality. Wheat germ is already an accepted human food; similar uses could be found for the maize germ left from the production of corn starch and for the rice germ which is a by-product of rice milling. Maize germ has been used in South Africa (23) to enrich vegetable mixtures.

SOY BASED PROTEIN-RICH FOODS

Dean (13, 14) was one of the first to propose a soybean-banana mixture for the treatment of kwashiorkor; this he later abandoned in favor of better balanced mixtures. Following Dean's suggestion, Thompson (41, 42) attempted therapy with such a mixture in 42 cases, but only 55% gave satisfactory results. It was difficult to achieve sufficiently high protein intakes because the fluid-soy mixture for tube feeding had 2½ times the volume of skim milk with an equivalent protein content. INCAP abandoned this type of regimen in 1955 when only 1 of 3 kwashiorkor patients so treated responded satisfactorily.

Soybeans must be soaked, skinned, minced and cooked before being fed, which limits their use for infant feeding in the home. Dean (15) reviewed the few reported trials where comparison with milk was adequate and found the results inconclusive, due partly to failure to neutralize the trypsin inhibitor. Moreover, several of the preparations seemed to cause gastrointestinal upsets. The value of soybean protein, even for mixed feeding, is thus limited by the difficulties of preparation. Subrahmanyan and co-workers (38) reviewed additional evidence that soybean milk can be made an adequate food for infants, but they also concluded that more studies are needed. Work with a fungus treated soybean product tempeh, and with manufactured preparations such as the Indonesian 'saridele' and with U.S. proprietary 'soy milks,' hold promise that the problems inherent in the use of soybean for infant feeding will eventually be solved (2).

Soybean can be processed to increase its percentage of protein and its nutritive value can be raised by adding synthetic amino acids. A product with 14.2% protein supplemented with 240 mg of methionine and 16 mg of cystine per 100 g plus vitamins and carbohydrate has been tested for acceptability by Barnes (3), and for nitrogen retention by Fomon and May (20). No difference was found in the rate of growth or nitrogen retention of infants, whether the entire protein intake was derived from human milk or 41–45% from the soy preparation. In a subsequent study, Fomon (19) gave to 4 infant girls for 36–72 days a formula with 1.14 g of soy protein per 100 ml without amino-acid fortification as the sole source of protein; he obtained growth and nitrogen retention results as good as those with human milk. It should be noted that this was a specially processed multi-ingredient formula, quite different from cooked crushed beans, and that the daily protein intakes averaged 1.7 g/kg of body weight.

Bassir (4) gave 30 g of soy flour daily for 3 months to nursing mothers during the first 6 months of lactation and reported a significantly higher milk output with no change in quality as compared with an equal number of controls. The soy flour supplemented ad libitum diets of meat, bread, rice, beans, cassava and yams.

In an extensive series of studies in rats, Gilbert and Gillman (22) have examined the effectiveness of soy as a supplement to maize protein at levels from 2 to 20% of the protein in the diet by measuring growth, survival and gross organ pathology. Although 'skimmed milk powder complemented in every way the various ingredients,' growth was improved very little by added soybean up to 10% of the protein in the diet even when vitamins A, E and D, yeast and salts were added; with 20% of soy protein the results still fell far short of these with the control diet containing 20% protein from skim milk powder and the remainder from maize. DeMaeyer and Vanderborght (17) found soy flour less effective in increasing nitrogen retention in children when added to a diet of rice, bread, banana flour, butter, palm oil, sugar and fruits, than isonitrogenous amounts of milk, beans and peanuts or peanut flour. The supplements supplied 75% of the nitrogen and 50% by weight of the total diet.

For countries with a large soybean production, soy protein is undoubtedly a valuable addition to the diet. There has been, however, a tendency to focus too much attention on the possibilities of soy for countries not producing it, to the neglect of more immediately available local protein sources. On the other hand, where soy is grown, research should be intensified to assure its most practical and effective utilization for human consumption.

MIXTURES USING CHICK PEA, COW PEA, BEANS, PEANUT FLOUR OR SESAME

The Central Food Technological Institute, Mysore, India, has developed several formulas for an Indian multipurpose food (39). Two of these known as Mysore Food A and B, have undergone extensive biological trials in the Nutrition Research Laboratories in Hyderabad, India. Mysore Food A apparently contains 25% Bengal gram (chick pea) 'dhal' and 75% peanut meal, and Mysore Food B, 25% Bengal gram, 65% peanut flour and 10% sesame meal. Bengal gram was previously shown by Venkatachalam *et al.* (43), to give good results in the treatment of 56 cases of kwashiorkor compared with 49 given skim milk.

In more recent studies in 53 children (27) with kwashiorkor, the administration of Mysore Food A and B gave results as good but no better than previously obtained with Bengal gram alone. Serum albumin regeneration was notably slower with these 3 than with milk. Peanut protein alone did not promote good serum albumin regeneration although it was of some clinical benefit. In these studies the addition of peanut flour to Bengal gram, with or without sesame flour, did not improve serum protein regeneration. When the treatment was sufficiently prolonged or the daily protein intake increased enough, satisfactory levels of serum albumin were achieved with any of these regimens.

Venkatachalam *et al.* (43) have also compared isoproteic diets of Bengal gram and banana, Bengal gram and rice, and wheat bread and skim milk in the treatment of 124 cases of nutritional edema syndrome. The response to the 2 exclusively vegetable protein diets was on the whole satisfactory, and there was no difference among the 3 treatments as regards disappearance of edema, attainment of minimum weight or subsequent weight gain.

The group for research on infantile malnutrition of the Children's Hospital in Mexico City, have investigated the nutritive value of a mixture of lime-treated maize (as tortillas) and black beans in the 4:5 proportions by weight common in some Mexican diets. The slope of the weight gain of rats fed this mixture was only 0.93 ± 0.037 as compared with 1.84 ± 0.040 when 22% of the protein came from milk (1). Similarly, the absorption and retention of nitrogen by malnourished children was variable from child to child and much lower than with isocaloric diets containing isonitrogenous quantities of milk (10). The addition of tryptophan and lysine to the maize-bean diet greatly improved nitrogen absorption and retention in all 4 cases. The obvious challenge is to find an inexpensive local protein source which will supply additional protein and also help correct the tryptophan and lysine deficiency of maize (7, 33), or a mixture of maize and beans as above.

In common with other investigators, Hansen and co-workers (23) found that milk initiated cure of kwashiorkor without difficulty; a 2-component mixture of 66% maize meal and 33% cow pea meal, however, did not initiate cure in the 3 cases treated with it. A 3-component mixture made up of equal parts of maize meal, maize germ and cow pea, did bring about satisfactory recovery in the one case in which it was employed. It should be noted that it would require 238 g of the dry 3-component mixture and 267 g of the 2-component mixture to supply the essential amino acids contained in 100 g of skim milk. Since the vegetable formulas also required relatively greater dilution, it was difficult to feed enough of the vegetable formulas to supply protein needs. We consider excessive bulk for protein content to be one reason for our lack of success in initiating cure in kwashiorkor with mixtures of maize and beans, and this is the same problem encountered with ordinary soy preparations. The differences in biological value of the protein are clearly reflected in the nitrogen retentions which averaged 13–14% for milk, 8.8% for the 2-component mixture and only 5.7% for the 3-component mixture. The percentage of nitrogen absorbed was also much lower with the vegetable mixtures. It was concluded that either the 2 or the 3-component mixture is satisfactory to promote consolidation of cure and to prevent kwashiorkor, but only the 3-component mixture is of sufficiently good concentration and protein quality to be satisfactory for use in treatment.

Sénécal (35) has reported on the use of 4 kinds of supplementation of the diets of children with incipient kwashiorkor seen in an out-patient clinic in Kakar. These were *1*) 60 g of millet flour, *2*) 24.6 g millet flour, 24.6 g peanut press cake, *3*) 23.0 g millet, 23.0 peanut press cake and 4.2 g fish flour, *4*) 46 g of skim milk powder. The first contained 5 g of sugar and the remainder 10 g each. The total protein provided was 4.7, 8.8, 11.9 and 16.6 g, respectively, with slightly over 200 calories in each supplement. Results with *supplements 2* and *3* were much better than with *supplement 1*, but the results with the skim milk were poorer than with *supplements 2* and *3*. Even the millet supplement, *no. 1*, caused some improvement in growth.

The nitrogen balance data furnished by DeMaeyer and Vanderborght (17) on the supplementation of a mixed diet with either skim milk, a combination of beans and peanuts, peanut flour or soybean fed to children convalescing from kwashiorkor, merit further mention. Their basal diet supplied from 11 to 47% of the nitrogen intake from rice, bread and banana flour, and the remainder from the test supplement. Nitrogen absorption and retention were measured for each supplement at intakes varying at intervals of 100 mg of N from 2.0 to 5.8 g protein per kg. Skim milk had the highest supplementary value by a considerable margin and the combination of beans and peanuts came next, followed by soybean flour and peanut flour.

Of great practical importance was the constant relationship between both the nitrogen absorbed and ingested, and the nitrogen retained over the entire range of protein intakes. The slope of the linear regression line for milk of absorbed nitrogen plotted against that retained was 0.78 and had not begun to decrease at the highest level tested. The slopes for beans and peanuts, peanut flour, and soybean were 0.72, 0.65 and 0.62, respectively. These data mean that in terms of protein, somewhat higher levels of the vegetable supplement gave the same total amount of protein retention as lower levels of milk. If vegetable supplements are cheap and available, they could thus be used in larger quantities to get the same protein effect as milk if they were of relatively good quality to begin with and providing they were in a form which permitted consumption in sufficiently large amounts.

INCAP VEGETABLE MIXTURES 8 AND 9

As a result of 7 years of cooperative work between the Instituto Agropecuario Nacional of Guatemala (IAN) and the Institute of Nutrition of Central America and Panama (INCAP), preliminary descriptions were published in 1957 (5, 6, 32, 34) of INCAP Vegetable Mixture 8. Mixture 8 has the following formula: dried maize masa 50%, sesame flour 35%, cottonseed flour 9%, Torula yeast 3% and Kikuyu grass leaf meal 3%. It has 25.1% protein, 13.7% fat and 503 calories per 100 grams. Comparing the amino-acid pattern of Mixture 8 with the FAO Reference Protein, the limiting amino acids in decreasing order are lysine, tryptophan and methionine with scores of 83, 86, and 91, respectively.

The lime-treated maize (34) is the cereal base for the mixture and a source of calcium. The sesame flour, as supplied (American Sesame Products, Inc.), was pressed from the de-hulled seeds and contained 33% fat. The cottonseed flour was a high protein, low gossypol product supplied under the name of 'Pro-flo' (Traders Oil Mill Co.). The Torula yeast provides B-complex vitamins and the carotene-rich leaf meal is added as a source of vitamin A activity.

Basic animal studies leading to this development have been recently reported (6, 37). Extensive trials with rats and chicks showed that the mixture sustained good growth although it was improved by the addition of lysine. It proved to be well tolerated by children and,

TABLE 1. *Formulas for INCAP Vegetable Mixture 9*

Ingredients g/100 g	9	9′	9A	B9
Whole maize				
Lime treated	28			
Uncooked		28		29
Cooked			29	
Sorghum				
Uncooked	28	28		29
Cooked			29	
Cottonseed flour	38	38	38	38
Torula yeast	3	3	3	3
Dehydrated leaf meal	3	3		
$CaCO_3$			1	1
Vitamin A IU			4500	4500

surprisingly, to give a nitrogen retention equivalent to milk in a series of 5-day balance trials carried out in 5 children fed at levels of 2.4–3.8 g protein per kg of body weight. After children recovering from kwashiorkor had done well on the mixture as the sole source of protein for periods exceeding 2 months, it was used for the treatment of 7 children with acute kwashiorkor. The results were similar to those customarily obtained with milk except for a slightly slower regeneration of serum albumin. From this work there was no doubt that a relatively simple vegetable mixture could be prepared which would be an excellent source of protein as well as other essential nutrients.

When the time came to plan its possible commercial production at low cost, it was discovered that the supply of sesame seed in Central America had decreased and the price had risen. This, coupled with difficulties in harvesting and in separating the husk, make it desirable to search further for an equally nutritious formula which would eliminate the necessity of using sesame at this time. All of the experience with the cottonseed flour in Mixture 8 and in a long series of biological trials in rats and chicks previously carried out in IAN and INCAP had proved favorable. Accordingly, in 1957 a new formula was developed based on cottonseed flour as the principal concentrated protein source. In it, sorghum was optionally substituted for part of the maize to reduce the cost. Preliminary calculations suggested that on the basis of an optimal combination of cottonseed flour and cereal grain, this should give a mixture of slightly higher protein content and slightly lower protein score if the Torula yeast and leaf meal were held at the same concentration.

As shown in table 1, the formula for INCAP Vegetable Mixture 9 went through a number of variations, the original formula used lime-treated maize and sorghum grain along with Torula yeast and dehydrated leaf meal. Mixture 9′ was prepared with whole ground maize and sorghum to facilitate animal feeding experiments. Mixture 9A used maize and sorghum, cooked without the addition of lime, substituted synthetic vitamin A acetate for dehydrated leaf meal, and had 1% calcium carbonate added as a source of calcium.

The formula at present employed for commercial distribution is 9B, which uses uncooked maize and sorghum. It consists of 29% ground maize, 29% ground sorghum, 38% cottonseed flour, 3% Torula yeast, 1% calcium carbonate and 4500 IU of vitamin A per 100 g. Type CF-2 Torula supplied by the Lakes States Yeast Corporation or its equivalent is specified. All ingredients must pass an 80-mesh screen. For all practical purposes, the protein quality of the variations in table 1 is identical. Formula 9B avoids the expense of cooking the maize and sorghum, eliminates the unstable and relatively costly leaf meal as an ingredient, and still supplies suitable amounts of calcium and vitamin A. It represents the minimum cost which could be achieved with the available raw materials.

The proximate composition and vitamin and mineral content of INCAP Vegetable Mixture 9B are given in table 2, and the amino acid composition in table 3. By comparison with the FAO Reference Protein, it has a tryptophan score of 61, a methionine-cystine score of 77, and lysine should not be limiting. These figures are markedly lower than those indicated by the results of

TABLE 2. *Composition of INCAP Vegetable Mixture 9B*

Nutrient	Per 100 g	Nutrient	Per 100 g
Moisture	7.6 g	Thiamine	2.1 mg
Protein	27.5 g	Riboflavin	1.1 mg
Ether extract	4.2 g	Nicotinic acid	7.8 mg
Ash	3.5 g	Vitamin A acetate	4500 IU
Crude fiber	2.4 g	Calcium	0.5 g
Carbohydrate	54.8 g	Phosphorus	0.8 g
Calories	370	Iron	6.2 mg

TABLE 3. *Essential Amino Acid Composition of INCAP Vegetable Mixture 9B*

Amino Acid	g/100 g	% FAO Pattern	Amino Acid	g/100 g	% FAO Pattern
Arginine	2.34		Phenylalanine	1.52	192
Histidine	1.00		Total sulphur A.A	0.92	77
Isoleucine	1.12	94	Threonine	0.87	110
Leucine	2.08	154	Tryptophan	0.24	61
Lysine	1.53	129	Valine	1.14	96

TABLE 4. *Representative Rat Growth Experiment with INCAP Vegetable Mixture 9′ (21 Days—5 Rats/Group)*

Protein in Diet, %	Average Weight Gain, g		Protein Efficiency*	
	V.M. 9′	Casein	V.M.9′	Casein
5	22	22	1.88	2.14
10	66	67	2.30	2.38
15	104	105	2.11	2.31
20	114	117	1.75	2.00
25	115	121	1.47	1.65

* Av. Wt. Gain/Av. Protein Consumed.

TABLE 5. *Representative Rat Repletion Study: Amino Acid Supplementation of INCAP Vegetable Mixture 9′ (10% Protein in Diet, 6 Rats/Group)*

A.A. Added	g % of Diet	Repletion Gain in 14 Days, g
None	0	51
L-Lysine HCl	0.1	60
L-Lysine HCl	0.2	71
L-Lysine HCl	0.3	62
DL-Methionine	0.1	53
DL-Methionine	0.2	51
DL-Methionine	0.3	53
L-Lysine HCl + DL-Methionine	0.1 / 0.1	64
L-Lysine HCl + DL-Methionine	0.2 / 0.2	69
L-Lysine HCl + DL-Methionine	0.3 / 0.3	67

subsequent biological and clinical trials. Up to the present time, 10 biological trials have been completed in rats, using some 600 animals; 20 have involved some 1000 baby chicks, and 96 nitrogen balance periods in 9 children have been studied.

These biological trials will be reported in detail elsewhere, but the results of a representative rat growth experiment are shown in table 4. The average weight gains of rats fed diets varying from 5 to 25% protein, are essentially the same whether the protein is provided by Mixture 9 or casein. Protein efficiencies, however, tend to be slightly higher when casein is the protein source. When Formula 9′ is varied by using either 56% maize or 56% sorghum as compared with an equal mixture of 28% of each, identical results are obtained. Moreover, anyone of these 3 variations gives the same result at the 10% level of protein in the diet as when skim milk is the protein source.

Table 5 gives the results of a representative rat repletion study in which the effect of adding lysine and methionine to INCAP Vegetable Mixture 9 is measured in rats fed at the 10% protein level. There is no effect of methionine, but 0.2% L-lysine HCl produces a significant improvement in growth which is not increased by doubling or tripling the amount of lysine added. There is no further increase in growth when lysine and methionine are added together, compared with the effect of lysine alone. At higher levels of protein in the diet, this effect of added lysine disappears.

Representative data obtained from chick growth trials are shown in table 6. As with rats, it is possible to use either 56% maize or 56% sorghum instead of the equal mixture of maize and sorghum, without any detectable difference in growth. With chicks, however, the results with these variations of Mixture 9 are not as good as those obtained with commercial poultry food. The second half of table 6 indicates that this is due to a deficiency of lysine and methionine for optimum chick growth. The addition of 0.3% DL-methionine and 0.2% L-lysine HCl together gives much better growth than the unsupplemented mixture. Similarly, the feed efficiency, expressed as grams of feed consumed per gram of weight gained, is improved by the amino acid supplementation, to the point where it is the same as obtained with a good commercial poultry ration.

TABLE 6. *Representative Chick Growth Trials with INCAP Vegetable Mixture 9′ (35 Days—20 Chicks/Group)*

Diet	Protein in Diet, %	Final Weight, g	Feed Efficiency
V.M. 9′	23.8	460*	2.25
V.M. 9′ with 56% maize	23.5	479*	2.31
V.M. 9′ with 56% sorghum	24.1	479*	2.27
'Ace-Hi'	23.9	587*	2.01
V.M. 9′	23.0	310†	2.45
V.M. 9′ + 0.3% DL-met	23.0	361†	2.26
V.M. 9′ + 0.2% L-lys HCl	23.0	472†	2.14
V.M. 9′ + 0.3 L-lys HCl	23.0	494†	2.07
V.M. 9′ + 0.3 DL-met + 0.2 L-lys HCl	23.0	490†	2.04

* 55 g initial weight. † 45 g initial weight.

TABLE 7. *Comparison of Nitrogen Balance in Preschool Children*

	Milk	INCAP VM-9
No. of children	9	9
Balance periods	48	48
Average protein intake g/kg/day	2.3	2.3
Average % absorbed	82.6	68.9
Average % retained*	16.3	17.8

* Difference in retention is not significant.

Nitrogen balance studies carried out in children are summarized in table 7. At an average protein intake of 2.3 g/kg, there is no significant difference in nitrogen retention whether the protein is furnished by cow's milk or by Mixture 9, even though the percentage of nitrogen absorbed is consistently less with the latter. Five children with acute kwashiorkor have been treated with Mixture 9 from the time of admission, with results fully equivalent to those previously obtained with Mixture 8 and similar to those with milk in all respects except for the slightly slower rate of serum protein regeneration previously mentioned. One such child before and after treatment with Mixture 9 is illustrated in figure 1.

The next step was the determination of its acceptability under field conditions. Seventy-six needy families in 4 Guatemalan communities were selected by local health centers and sufficient Mixture 9 provided for each preschool child to drink 3 glasses daily for 17–19 weeks. Initial acceptance was extremely good and tended to improve further during the trial period. 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 consumed 2 or more glasses daily.

On the basis of these encouraging results, an experimental sales trial was begun in the village of Palin with a predominantly Indian population of about 4000. The mixture was marketed under the name of Incaparina at 3 cents for a 75 g bag, sufficient for 3 glasses of a drink with the same protein quantity and quality as an equivalent amount of milk. During the 5-month trial period, sales early stabilized at 1200 bags a week; the product was recommended by the health center and school teachers but not was commercially advertised. The name Incaparina has been retained and the product is on commercial sale in Guatemala with wide and enthusiastic acceptance. Arrangements have been made for its manufacture and sale in the INCAP member countries of El Salvador, Honduras and Nicaragua and any country with the need and necessary raw materials will be encouraged to make use of the formula. A group has already begun to produce and market it for needy individuals and institutions in the southern United States.

In Central America, Incaparina is most commonly consumed as an 'atole,' a thin gruel made by adding 1

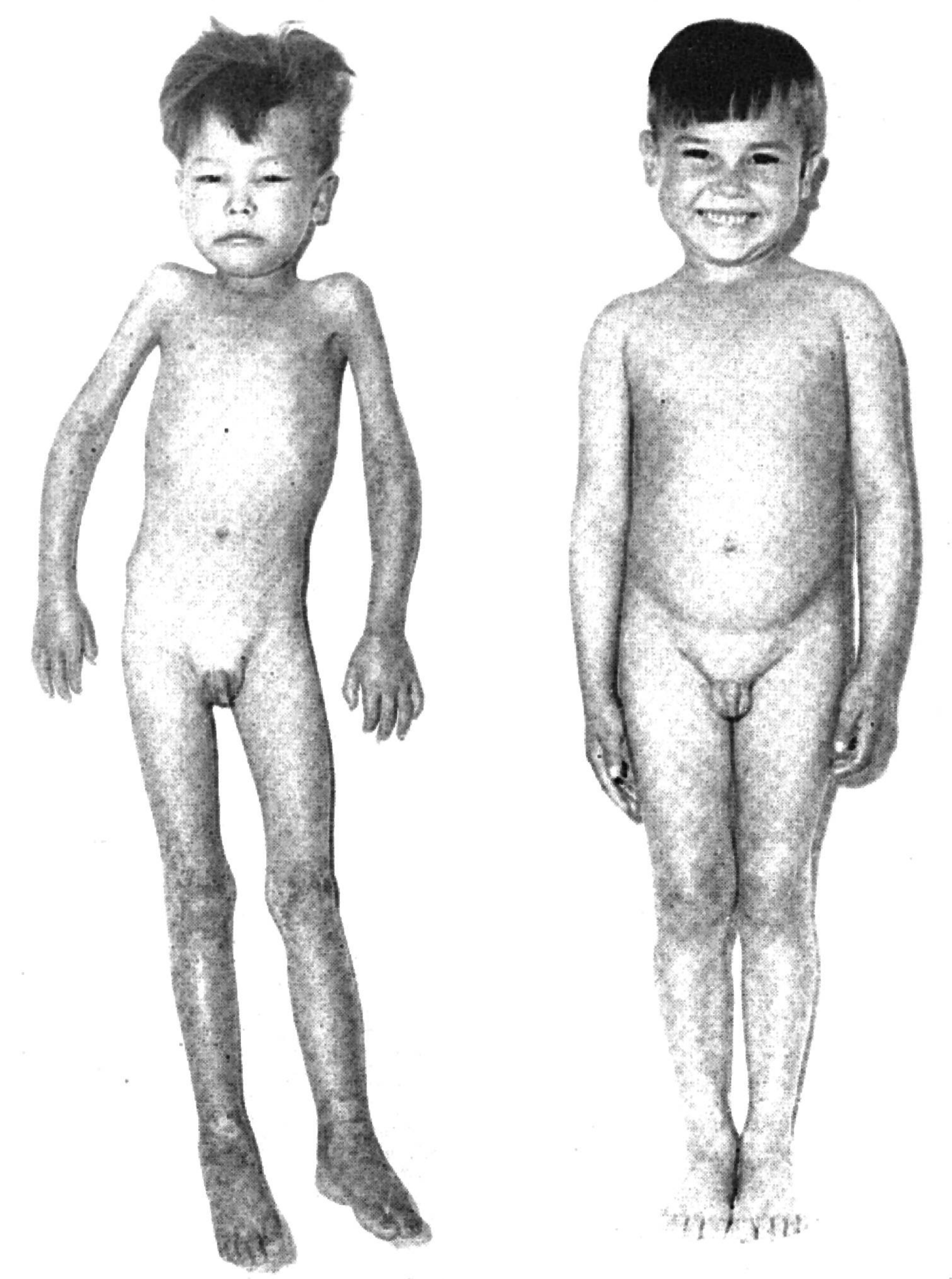

FIG. 1. Child with kwashiorkor treated with INCAP vegetable mixture 9.

glass of water for each 25 g of mixture and cooking it for 15 minutes. It is flavored to taste with sugar and cinnamon, vanilla, anise or chocolate and served either hot or cold. Incaparina can also be substituted for ⅔ of the flour in most nonbread recipes calling for wheat flour and can be made into puddings as well as used for enriching soups. It has a high acceptability for mass feeding and is a means of stretching limited institutional food budgets without lowering the nutritional value of the diet. Whether or not it is found to be useful outside the INCAP area, the results to date prove conclusively that a highly acceptable low-cost vegetable mixture of high nutritive value can be formulated and can find a useful place in national efforts to prevent protein malnutrition. It is certain that products similar to Incaparina can be developed in other areas by taking advantage of those local food sources most readily available at low cost.

SUMMARY

From a practical point of view the elaboration of vegetable protein mixture for human feeding involves the combination of cereal grains with other foods which increase the quantity and improve the quality of protein in the diet. Among the possible vegetable foods which could be used are legume seeds, oil seeds, nuts, palm kernels and leaf proteins. The concentrated protein sources which have received the most attention in this regard are the common bean, cow pea, Bengal gram (chick pea), soybean flour, peanut flour, cottonseed flour and copra proteins.

INCAP Vegetable Mixture 8 made up of 50% lime-treated maize (masa) flour, 35% sesame flour, 9% cottonseed flour, 3% Torula yeast and 3% Kikuyu leaf meal, has a protein content of 25.1% which in growth experiments in rats and chicks, and metabolic balance trials in children recovering from protein malnutrition, closely approximates the quality of milk protein. INCAP Vegetable Mixture 9B, containing 29% whole ground maize, 29% whole ground sorghum grain, 38% cottonseed flour, 3% Torula yeast, 1% calcium carbonate and 4500 units of added vitamin A/100 g, has a protein content of 27.5% and is similar in protein quality to Mixture 8 and milk. It can be produced at very low cost, and in the form of a thin gruel (atole), is highly acceptable in Central America. Its favorable initial commercial acceptance under the name 'Incaparina' suggests that it will make a significant contribution to the problem of preventing protein malnutrition in this area. It is hoped that it will also prove useful for other technically underdeveloped areas with similar economic, agricultural and health problems.

The development and testing of INCAP Vegetable Mixture 9 has at one time or another involved most of the INCAP professional staff. Dr. Edgar Braham, Mr. Alvaro Aguirre and Mr. Luiz Elías were particularly helpful in the biological trials in rats and chicks. The therapeutic and metabolic studies in children were begun by Dr. Moisés Béhar and continued by Dr. Dorothy Wilson. Dr. Miguel Guzmán planned the field and stability trials and Dr. Romeo de León supervised the field trials. Miss Susana Icaza has been responsible for cooking tests and the development of recipes and nutrition education material. Miss Raquel Flores assisted in preparation of the references for this manuscript.

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