It is probably fair to say, therefore, that the co-ordinated approach in the Philippines has made some headway and is one step towards making our community nutrition programmes more effective. Ultimately, we hope, it will lead not only to increased nutrition-consciousness among the people but also to the elevation of their nutritional and health status necessary for socio-economic development and the political security of this country and of the world.

**Summary and conclusion**

To achieve functional co-operation and co-ordination in nutrition work the following concepts and principles may be used as guide lines :—

1. Co-operation and co-ordination must start from the hearts and minds of the people involved, since these terms imply a highly " interpersonal " relationship. Furthermore, there must be sincerity of purpose among those who will co-operate or co-ordinate.
2. Integrated thinking and planning of projects should be done by the co-ordinating body, but the actual implementation should be left to the appropriate agency. In this way the implementing agency rightly feels that the project is its very own.
3. The composition of the co-ordinating body should be of the intermediate (supervisory) level rather than the highest level as suggested in the Hot Springs Conference in 1943. In this way more effective implementation of the programme is better assured, because the individuals concerned would be near enough to the top-level personnel and at the same time close enough to the personnel who will carry out the programme.

Let me conclude by saying that what we are doing in the Philippines, in so far as achieving co-operation and co-ordination is concerned, is an experiment. It is an experiment in living and working together for the fulfilment of everyone concerned, in the sense that those who participate in the progress of nation building through proper nutrition have the satisfaction of having tried to make their little contribution toward the welfare of their fellow-men; fulfilment, in the sense that peace and security cannot be attained by weapons or warfare, but through love between neighbours.

# THE USE OF PLANT PROTEIN FOODS IN PREVENTING MALNUTRITION [1]

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## Introduction

International and national organizations are conscious now of the fact that large areas of the world do not produce enough food to satisfy the minimum requirements of their populations. Sources of proteins of high biological value, particularly those of animal origin, are limited and in recent years the rate of population growth has aggravated this problem. This situation has been analysed and discussed in detail in many recent publications (Altschul, 1962; Anson, 1962; Autret and Van Veen, 1955; Scrimshaw and Béhar, 1961;

[1] I.N.C.A.P. Publication, I-281.

Sebrell, 1961; Teply and György, 1962; Voris, 1961; Waterlow and Stephen, 1957). The following comments will be concerned with one of its aspects, namely the need for and means of utilizing proteins of vegetable origin more efficiently.

The areas in which the problem already exists are, in general, those in which population growth is rapid, so that the situation is becoming more serious every day. It can be argued that the scarcity of proteins in these areas can be made good by the surplus of other areas. But the problem of food shortage in any given country is not permanently solved until that country is able to produce or obtain independently the foods it needs. As Thompson (1960) has commented: " From the practical standpoint there is no such thing as a world supply of food that can be distributed to different peoples of the world according to the need."

The economy of countries where proteins are already scarce, and may become even more so in the future, is based on agriculture. Most of these countries cannot feasibly at present develop another type of economic structure which would permit them to buy the food they do not produce. It is important therefore in these countries to consider as potential sources of dietary protein all products, both of animal and of vegetable origin, which can be locally produced. To-day non-industrialized countries in the tropical and sub-tropical regions, because of climatic characteristics, soil composition, available facilities for preservation, transportation and processing of foods, economical structure and cultural factors, could more easily develop their agriculture for the production of the needed protein from vegetable sources rather than develop, to a sufficient extent, animal husbandry and the facilities required for an adequate utilization of its products. In fact, in many regions where sources of animal protein for human consumption are scarce, products of vegetable origin are already available and can, if properly utilized, go a long way in fulfilling protein needs. Many of these products are sub-products of industries already established which use locally produced raw materials, oil seed cakes being an example. This has obvious economic advantages.

Can proteins of vegetable origin replace those of animal origin in human nutrition? From the nutritional point of view animal or vegetable proteins should not be differentiated. It is known to-day that the relative concentration of the amino acids, particularly of the essential ones, is the most important factor determining the biological value of a protein. Although most proteins of animal origin have a satisfactory amino acid pattern and, therefore, a high biological value, this is not true in every case. On the other hand, most proteins of vegetable origin have a low biological value because they are deficient in one or more essential amino acids. If, however, such deficiencies are corrected, either by adding the amino acids present in low concentration, or by combining different proteins in appropriate ways, vegetable proteins cannot be distinguished nutritionally from those of animal origin. The amino acids and not the proteins should be considered as the nutritional units. Animal foods are, of course, more likely to be adequate sources of other essential nutrients, such as vitamin $B_{12}$ or vitamin A.

Another important difference between natural products of vegetable and animal origin is the amount of protein they contain. In general, the concentration of protein in most foods of vegetable origin is too low to make them, in their natural form, adequate sources of proteins, particularly for the feeding of young children.

All these problems can now be solved through advances in nutritional knowledge and food technology. There are other factors, however, which are frequently even more important in determining whether a given product is acceptable or not as human food. Among these are cultural beliefs and practices.

Marked cultural differences exist regarding food habits. For example, worms and ants are considered delicacies by some groups and will not be consumed by others. Vegetable products may, in general, be more readily accepted than those of animal origin by certain groups, yet the reverse may be true of others. When a new product containing substances that have not been recognized as human foods is introduced, these problems assume particular importance. For example, I.N.C.A.P. has encouraged the use of mixtures based on cotton-seed flour and cereal grains as a protein supplement for populations without adequate supplies of other good sources of protein, particularly for children during the post-weaning period. Some people have, however, objected to the use of such a product instead of the " natural " food for children after weaning —cow's milk. Why should cow's milk be acceptable, and a product based on cereal grains and cotton-seed flour or any other combination of vegetable proteins be regarded as unsuitable for children ?

From the economic standpoint vegetable proteins have advantages over animal proteins. The cost of the raw materials needed to make vegetable protein products or mixtures is usually low and the same applies to methods of preservation, storage and transport. This is of vital importance in poor countries.

## I. Vegetable protein sources for human feeding

*(A) Raw or processed products and concentrates.*—Sources of vegetable proteins can be classified into seven main groups : (1) cereal grains ; (2) legume seeds ; (3) oleaginous seeds and their industrial by-products the oil-seed cakes ; (4) nuts ; (5) palm kernels ; (6) leaf and algae proteins ; and (7) microbial proteins such as plankton, yeasts and others.

Cereals are an important source of protein for the majority of the world's population. In general, their protein value is low with respect both to quantity and quality, but can be improved through supplementation by other proteins. Cereal grains contain from 6 to 14 % protein. The amino acids in which their proteins are deficient are lysine, tryptophan and, in some cases, threonine and methionine.

The vast group of legume seeds form a large source of protein. The legumes contain approximately 25 % of protein, deficient in methionine but with a high lysine content. The digestibility of legume protein is rather low and the value of some of them is inferior to that of cereal proteins (Bressani and Valiente, 1962 ; Bressani, Valiente and Tejada, 1962). The variation in the value of the proteins of different legumes is, however, large. For proper utilization, most legume seeds must be well cooked, but drastic processing may reduce their value. This important group of foods rich in vegetable protein should receive more intensive study. Legumes are available in abundance throughout the world and could serve as cheap sources of protein isolates and concentrates.

Oil seeds and press-cakes constitute another large group of sources of vegetable proteins of great economic importance. They contain a large amount of protein which is already being used for various purposes and could be more widely used for human consumption. World production figures, as estimated by F.A.O. for 1958, are shown in Table I. Soya beans are the oil seed produced in greatest quantity, although cotton-seed and peanuts are also produced in large amounts. The oil-containing seeds have several advantages over the two previous groups. When the oil and crude fibre is removed their content of protein is approximately 50 % of a quality which is in general superior to that of cereal and legume proteins. Table II shows the essential amino acid content of several oil-seed meals. Soya bean is low in sulphur-containing amino acids, while cotton-seed, peanut and sesame flour are deficient mainly in lysine. Soya bean

**Table I.** *World production of some oil seeds* (1958).

| | Million tons |
|---|---|
| Soya bean (*Glycine max.*) | 28 |
| Peanut (ground nut) (*Arachis hypogaea*) | 14 |
| Cotton-seed (*Gossypium hirsutum*) | 19 |
| Sesame (*Sesamum indicum*) | 2 |
| Sunflower (*Helianthus annuus*) | 2 |
| Copra (*Cocos nucifera*) | 3 |

**Table II.** *Essential amino acid content of cotton-seed, sesame, peanut and soya bean flours.*

| Amino acid | Cotton-seed | Sesame | Peanut [1] | Soya bean [1] |
|---|---|---|---|---|
| | mg./g. N | mg./g. N | mg./g. N | mg./g. N |
| Arginine | 702 | 486 | 669 | 452 |
| Histidine | 225 | 90 | 152 | 149 |
| Isoleucine | 409 | 288 | 257 | 336 |
| Leucine | 367 | 480 | 380 | 482 |
| Lysine | 235 | 151 | 223 | 395 |
| Methionine [2] | 210 | 305 | 149 | 195 |
| Phenylalanine [3] | 468 | 677 | 540 | 508 |
| Threonine | 221 | 175 | 168 | 246 |
| Tryptophan | 63 | 90 | 69 | 86 |
| Valine | 322 | 207 | 311 | 328 |
| Nitrogen % | 8·30 | 6·16 | 9·38 | 7·82 |

[1] Orr and Watt (1957).
[2] Includes cystine.
[3] Includes tyrosine.

**Table III.** *Essential amino acid content of some palm nut oil meals.*[1]

| Amino acid | African palm | Corozo | Mbocaya |
|---|---|---|---|
| | mg./g. N | mg./g. N | mg./g. N |
| Arginine | 659 | 468 | 1,120 |
| Histidine | 214 | 280 | 297 |
| Isoleucine | 309 | 226 | 329 |
| Leucine | 395 | 314 | 396 |
| Lysine | 332 | 294 | 381 |
| Methionine | 218 | 146 | 15 |
| Cystine | 50 | 63 | 75 |
| Phenylalanine | 245 | 274 | 219 |
| Tyrosine | 123 | 100 | 157 |
| Tryptophan | 41 | 40 | 58 |
| Valine | 341 | 297 | 331 |
| Nitrogen % | 2·20 | 3·50 | 4·65 |

[1] Squibb *et al.* (1958).

protein is superior in quality to that of other oil seeds, although if proper care is taken during processing, cotton-seed protein can be of high quality also. Peanut flour (McOsker, 1962; Milner, 1962) is also a vegetable protein concentrate of high potential value.

Nuts and palm kernels are not as plentiful as the items in the previous groups. Oil meals from these two sources have the disadvantage that their crude fibre content, particularly that of palm kernels, is extremely high, which significantly reduces their protein value and makes them less suitable for human consumption. Table III shows the essential amino acid content of three palm kernel meals (Squibb, Aguirre and Bressani, 1958), while Table IV gives the essential amino acid content of nut-meals (Bressani and Arroyave, 1963; I.N.C.A.P., unpublished data). Because of the factors mentioned, it is doubtful that either group will contribute substantially to world supplies of protein.

**Table IV.** *Essential amino acid content of Brazil nut, jicara nut and pepitoria nut flours.*[1]

| Amino acid | Brazil nut | Jicara nut | Pepitoria nut |
|---|---|---|---|
| | mg./g. N | mg./g. N | mg./g. N |
| Arginine | 1,174 | 678 | 899 |
| Histidine | ... | 181 | 150 |
| Isoleucine | 405 | 284 | 313 |
| Leucine | 457 | 367 | 492 |
| Lysine | 340 | 266 | 482 |
| Methionine | 412 | 158 [2] | 188 [2] |
| Phenylalanine | 245 | 242 | 270 |
| Threonine | ... | 190 | 219 |
| Tryptophan | 79 | 69 | 94 |
| Valine | 299 | 310 | 515 |
| Nitrogen % | 7·17 | 10·38 | 9·47 |

[1] I.N.C.A.P. Unpublished data.
[2] Includes methionine + cystine.

The next group includes leaf and algae proteins. Recent work carried out on these two products (Leveille, Sauberlich and Shockley, 1962; Pirie, 1960; Powell, Nevels and McDowell, 1961; Sur, 1961; Waterlow, 1962) has resulted in improved materials for human feeding. More work is, however, needed, not only on the preparation of proteins from these sources but on the evaluation of their nutritive value as single proteins and as supplements to other proteins.

Except for food yeasts, very little attention has been given to microbial proteins. Food yeasts contain 50 % protein and have been reported to be deficient in methionine (Bender and Doell, 1960; Goyco, 1956). They have an advantage over other plant proteins in that they are good sources of vitamins of the B-complex. Microbial proteins should receive more attention as possible sources of food protein. They can be grown in nutrient media of low cost and on by-products or residues which are often discarded.

There are other products which could be more widely used as human foods (Goering, Thomas, Beardsley and Curran, 1960). The germ of cereal grains and the by-products of the cereal grain industry have been reported to be as high in protein quality as meat protein (Mitchell and Beadles, 1944). Since tremendous quantities of wheat are milled, large quantities of germ are produced. Attention should be given to this product with the aim of converting it into a food of good quality for human feeding.

(*B*) *Vegetable protein isolates.*—Recent technological advances have enabled vegetable protein isolates to be prepared (Smith, 1958). These products are isolated proteins, and when corrections for other factors are made contain 100 % protein. The usual sources of raw material are the oil seeds, which are already rich in protein. Protein isolates have also been prepared from cereal grains, *e.g.*, zein from corn and gluten from wheat. Legumes have not been used to any extent in the preparation of isolates on a large scale. However, they offer good possibilities, and attention should be given to legumes which contain toxic compounds even after cooking. The isolation of the protein not only concentrates the protein itself, but also eliminates the insoluble undigestible carbohydrate and removes toxic factors and bad-tasting materials.

The use of isolated protein and protein concentrates for food is in an early stage of development and progress depends primarily on basic and technological research. Dietary and nutritional grades of isolated proteins, particularly soya-bean proteins isolates, have become available commercially. In India (Bhagavan, Doraiswamy, Subramanian, Narayama Rao, Swaminathan, Bhatia, Sreenivasan and Subrahmanyan, 1962; Subrahmanyan, Bhagavan and Swaminathan, 1958; Subrahmanyan, Sreenivasan, Bhatia, Swaminathan, Bains, Subramanian, Narayano Rao, Bhagavan and Doraiswamy, 1961) protein isolates from peanuts and coconut are being produced on a commercial scale, and are already being used in the preparation of special human foods.

## II. Factors affecting protein value

(*A*) *Amino acid composition.*—The nutritive value of a protein is determined by its amino acid balance, that is, by the relative proportions of the amino acids it contains. In general, animal proteins contain amino acids in quantities and proportions more in accord with human needs than vegetable proteins. The majority of plant proteins are deficient in one or more of the essential amino acids. In corn, for example, three of these are deficient. Further, some vegetable proteins are characterized by an excess of certain amino acids which can also reduce the value of the protein. Examples include the large amounts of leucine in most cereal proteins, of phenylalanine and tyrosine in cereal proteins and a few other plant proteins, and methionine in the protein of Brazil nut. The significance of these excesses has still to be evaluated, but it may be as important as amino acid deficiencies in the efficient utilization of plant proteins.

The effective amino acid balance of a protein for the animal organism is not always indicated by a simple determination of its amino acid content. There are several factors which affect this balance, the principal one being the digestibility and availability of the amino acids. In general, the digestibility of vegetable proteins is lower than that of animal proteins, so that the contribution of vegetable proteins to needs is less than is indicated by its essential amino acid composition. This is illustrated in Table V. For example, the absorption of nitrogen is less with vegetable proteins than with milk protein (De Maeyer and Vanderborght, 1958). Again, millet proteins (Kurien, Swaminathan and Subrahmanyan, 1961) have an apparent coefficient of digestibility lower than that of rice proteins. Table V gives evidence that faecal nitrogen increases as the amount of milk protein decreases. Several other investigators have reported similar results (Bray, 1953; Gómez, Ramos-Galvan, Cravioto, Frenk, Peña, Moreno and Villa, 1957). The digestibility of vegetable proteins is not improved even when the deficiencies are corrected by appropriate amino acid supplementation (Bressani, Elías and Valiente, 1963; Bressani, Scrimshaw, Béhar and Viteri, 1958) suggesting that many of them contain enzyme-resisting substances, about which little is known.

Several excellent reviews dealing with the effect of processing on the nutritive value of vegetable proteins (Kuiken, 1958; Liener, 1958) have been published. In general, vegetable proteins are not consumed in their " natural " state, but after being subjected to some degree of heat treatment. Since the product of primary interest from the industrial standpoint is not the protein, the treatments applied are such that they leave a product of little nutritive value. Oil-seed flours are the products of processes designed to remove the oil from the seed, and the application of heat is a feature of such processes. Cereals and legumes intended for human feeding are cooked or processed to enhance

**Table V.** *Protein digestibility and faecal nitrogen of children fed vegetable proteins.*

| Diet [1] | Absorption | | Diet [2] | Apparent coefficient of digest. |
|---|---|---|---|---|
| | Apparent intake of N | Corrected intake of N | | |
| | % | % | | % |
| Milk . . | 81·89 | 89·28 | Rice | 75·3 |
| Peanuts + beans . | 72·82 | 85·52 | 25 % pearl millet | 73·1 |
| Peanut flour . | 75·50 | 84·38 | 50 % pearl millet | 64·4 |
| Soya-bean flour . | 77·74 | 85·86 | Pearl millet | 52·9 |

Endogenous faecal N: 42 mg./kg./24 hours.

| Diet [3] | Nitrogen | |
|---|---|---|
| | Intake | Faecal |
| | mg./kg./day | mg./kg./day |
| Milk . . . | 387 | 70 |
| 25 % milk . . / 75 % corn + beans . | 358 | 98 |
| 10 % milk . . / 90 % corn + beans . | 353 | 134 |
| Corn + beans . . | 347 | 107 |

[1] De Maeyer and Vanderborght (1958).
[2] Kurien, Swaminathan and Subrahmanyan (1961).
[3] I.N.C.A.P. Unpublished data.

their palatability and acceptance. As a result of such treatment, the nutritive value of the protein may be affected to an extent which varies according to the protein components in the food, and depends on factors such as the temperature, duration of heating and the presence or absence of moisture. More attention should be given to the effect of processes, conditions or chemical and physical properties of the protein, since a knowledge of the physical-chemical changes induced in the proteins may indicate the effect of heat on the nutritive value of the proteins and the processing conditions which produce minimum damage. In general, proper heat treatment results in beneficial effects on nutritive value.

The decrease in nutritive value resulting from the excessive application of heat is associated with significant changes in the protein molecule itself, or changes resulting from the interaction of the protein with carbohydrate or other compounds. These modified proteins become more resistant to enzymatic hydrolysis, which results in a retardation in the rate of release of the essential amino acids during digestion. The formation of peptide linkages which cannot be split by enzymatic hydrolysis may arise in two ways : first, by the interaction of non-peptide amino groups of lysine or arginine, giving rise to abnormal peptide linkages, and, second, by the interaction of the non-peptide amino group of lysine and arginine with reducing sugars. These reactions modify the substrate in such a way that it no longer conforms to the requirement of specific enzymes. Excessive heat treatment may cause the amino group-sugar reaction to proceed to a point at which the basic amino acids can no longer be completely recovered even after more drastic hydrolysis such as acid hydrolysis.

(*B*) *Other factors affecting protein quality.*—The nutritive value of vegetable proteins is also influenced by the presence in the seed of different and complex compounds which provoke deleterious physiological responses in animals (Liener, 1958 ; Moudgal, Raghupathy and Sarma, 1958). In most cases the treatment applied to the seed results in the destruction, elimination, inactivation or decrease of these toxic compounds. Legumes seeds contain a relatively large number of factors with adverse physiological action. Table VI summarizes

**Table VI.** *Anti-nutritional factors in some vegetable protein sources.*

| Vegetable protein source | Anti-nutritional factor |
|---|---|
| Soya bean . . . . | Trypsin inhibitor |
| | Haemagglutinin |
| | Saponin |
| | Goitrogenic factor |
| | Anticoagulant factor |
| | Lipoxidase |
| Cotton-seed . . . | Gossypol |
| Legumes . . . . | Trypsin inhibitor |
| | Haemagglutinin |
| Linseed . . . . | Antipyridoxine factor |
| | Cyanogenetic glycoside |
| Castor . . . . | Ricin |
| Pumpkin-nut meal . . | Toxic factor |
| Brazil-nut meal . . . | Toxic factor |
| Ground nut . . . | Goitrogenic factor |

the anti-nutritional factors in some vegetable protein sources. The most important and best-known toxic factors in legumes are the trypsin inhibitors. Haemagglutinins are also present in some legumes ; these have the ability of agglutinating the red blood cells of various species.

The pigment gossypol has limited the more extensive use of cotton-seed flour. Gossypol not only reduces the nutritive value of cotton-seed protein by reacting with lysine upon processing but also exerts a toxic effect which varies with the species of animal. Animals sensitive to this compound include guinea-pigs, rabbits and swine. There are other species which are not affected unless the quantities consumed are relatively large.

Recent studies have indicated also that certain vegetable proteins such as those of sesame flour increase zinc requirements (Lease, Barnett, Lease and Turk, 1960) and that soya-bean protein increases the requirement of certain minor elements (Davis, Norris and Kratzer, 1962).

Vegetable protein concentrates are often excellent natural media for the growth of micro-organisms which may produce compounds toxic to animals. An example of this is the growth of *Aspergillus flavus* in peanut flour. This organism produces a compound which is extremely toxic to poultry (Allcroft, Carnaghan, Sargeant and O'Kelly, 1961 ; Sargeant, Sheridan, O'Kelly and Carnaghan, 1961 ; Lancaster, Jenkins and Philp, 1961).

## III. Utilization of vegetable proteins

There are at least three approaches to the more effective utilisation of plant proteins. One is to find the optimum proportions in which the proteins from common staple foods complement each other efficiently. The results of Bressani, Valiente and Tejada (1962), shown in Figure 1, indicate that the maximum Protein Efficiency Ratio (P.E.R.) of a mixture of corn and bean is found when 50 % of the total protein of the mixture comes from corn and 50 % from beans. With beans and rice the corresponding proportions were 60 % and 40 % (Bressani and Valiente, 1962). These results were confirmed by De Groot and Van Stratum (1963), using N.P.U. values as a measuring technique. On a

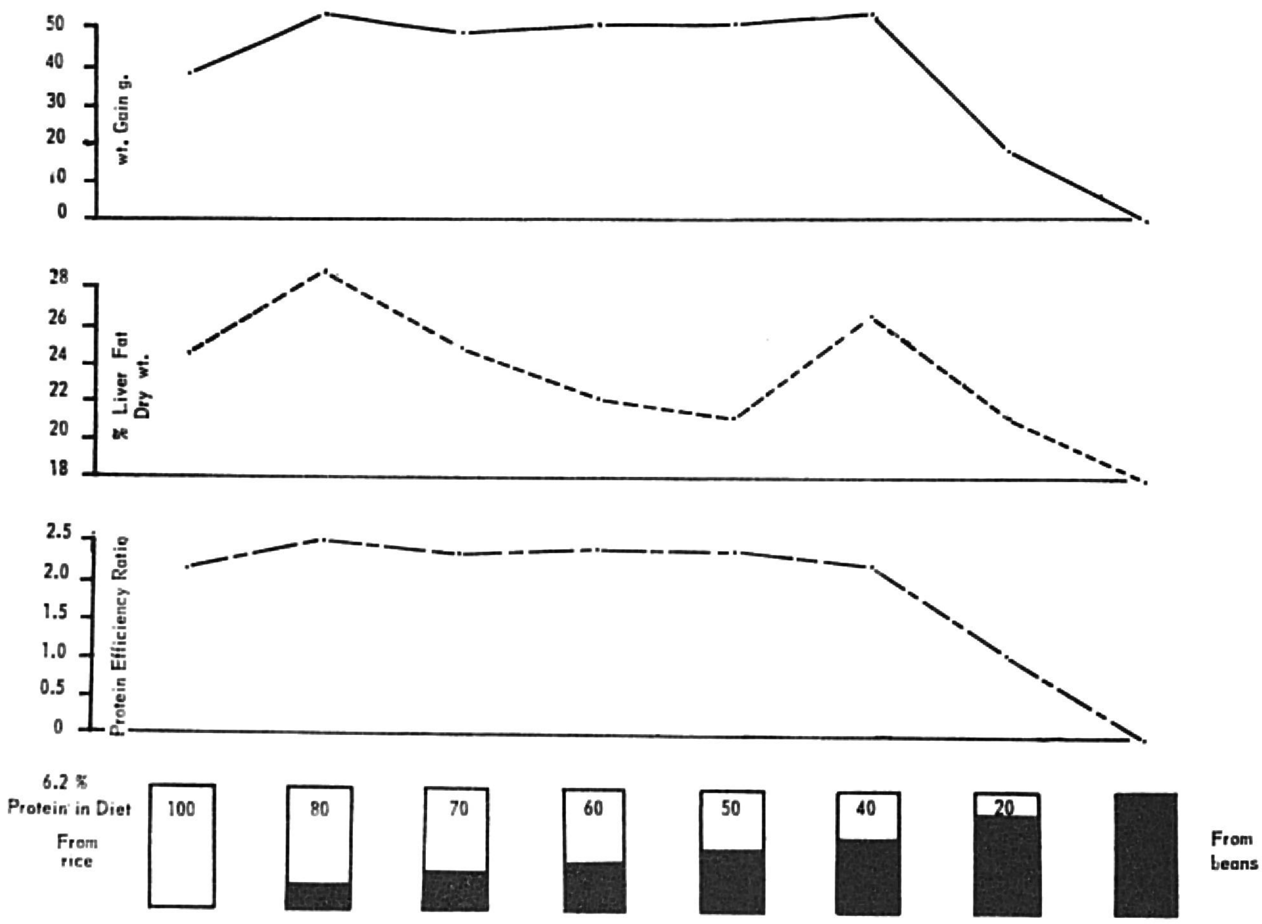


**Fig. 1.** Combinations between lime-treated corn and cooked black-bean proteins. The bars in the lower part of the figure indicate the proportions of protein from lime-treated corn and beans respectively. The curves from top to bottom represent the average gain of rats in 28 days, the protein efficiency ratio (P.E.R.) and the liver fat percentage of the rats fed the different combinations. (Bressani and Scrimshaw, 1961.)

weight basis the best combination of corn and beans consists of a mixture containing 72 % of corn and 28 % of beans, which has a total protein content on a crude weight basis of about 12 %. Such mixtures, however, contain after cooking between 40 % to 60 % of moisture. They are therefore bulky, and, taken as the sole source of protein, could scarcely be consumed in sufficient quantities to meet protein needs, particularly by small children.

A second approach is the supplementation of staple foods such as cereal grains with protein concentrates or isolates. The objective here is to find the minimum quantities of protein concentrate or isolate which when added to the cereal grains gives maximum efficiency of protein utilization. The studies of Bressani and Marenco (1963) summarized in Table VII, indicate that the P.E.R. of lime-treated corn can be increased from about 1 to about 2·50 by adding the appropriate percentages of the following: cotton-seed flour, 11 %; soya bean flour, 9 %; soya bean protein isolate, 8 %; yeast, 3 %; and pepitoria (*Cucurbita farinosa*), 11 %. Gilbert and Gillman (1959) showed that the nutritive value of corn proteins can be improved significantly by adding soya-bean flour. Similar results have been reported by others (Sure, 1946). These new foods, if they can be called this, contain about 12 % protein. An advantage of this approach is that the " protein-enriched " food can be converted into the form in which it is usually consumed. For example, in Mexico, Central America and other Latin American countries, corn is consumed in the form of " tortillas." The physical, culinary and organoleptic characteristics of the enriched product remain essentially the same or are improved.

**Table VII.** *Quantities of vegetable protein concentrates giving maximum P.E.R. as supplements to lime-treated corn.*[1]

| Vegetable protein concentrate | Amount % diet | P.E.R. |
|---|---|---|
| Lime-treated corn . | ... | 1·00 |
| Soya-bean protein . | 8·0 | 2·46 |
| Soya-bean flour . | 9·0 | 2·37 |
| Cotton-seed flour . | 11·0 | 1·98 |
| Torula yeast . . | 3·0 | 2·06 |
| Pumpkin-seed flour . | 11·0 | 1·84 |

[1] Bressani and Marenco (1963).

**Table VIII.** *Complementation between corn and soya-bean flour proteins.*[1]

| Corn % of diet | Soya bean % of diet | % Protein diet | Soya protein. Corn protein | P.E.R |
|---|---|---|---|---|
| 90 | 0·0 | 7·8 | 0/1 | 1·60 |
| 72 | 3·2 | 8·0 | 0·25/1 | 2·29 |
| 54 | 6·3 | 7·8 | 0·66/1 | 2·72 |
| 36 | 9·5 | 8·0 | 1·50/1 | 2·91 |
| 18 | 12·7 | 8·2 | 3·30/1 | 2·67 |
| 0 | 16·0 | 8·1 | 10/1 | 2·56 |

[1] Bressani and Elias. (Manuscript in preparation.)

A third approach is the development of suitable vegetable protein mixtures, based on the principles of protein complementation as outlined above. There are at least two possibilities. In one, an admixture is made of a basic staple food of low-protein quality and content and a vegetable protein concentrate to achieve a good amino acid combination. In another, two protein concentrates are combined. Examples of complementation between corn and soya bean flour are shown in Table VIII (Bressani and Elias, 1963). Maximum complementation took place at a protein ratio of soya-bean flour to corn flour of 1·5 to 1. With corn and peanut flour complementation cannot be detected at a protein level of 10 % in the diet, as is shown in Table IX; this happens when the protein foods concerned have a common amino acid deficiency, but are of a different magnitude. In these cases tests should be carried out at higher levels of protein intake to enable the best combination to be chosen as is illustrated in Table X.

Complementation between two protein concentrates is illustrated in Table XI. Soya bean flour and cotton-seed flour each contributed equal percentages of protein to the diet, while with sesame flour and cotton-seed flour,

**Table IX.** *Nutritive value of combinations between lime-treated corn and peanut flour.*[1]

| Protein distribution in diet | | Weight distribution in diet | | P.E.R. |
|---|---|---|---|---|
| Lime-treated corn | Peanut flour | Lime-treated corn | Peanut flour | |
| % | % | % | % | |
| 100·0 | 0·0 | 90 | 0·0 | 1·55 |
| 77·3 | 22·7 | 70 | 3·31 | 1·48 |
| 55·5 | 44·5 | 50 | 6·50 | 1·52 |
| 44·5 | 55·5 | 40 | 8·09 | 1·50 |
| 33·6 | 66·4 | 30 | 9·68 | 1·61 |
| 21·9 | 78·1 | 20 | 11·40 | 1·46 |
| 0·0 | 100·0 | 0 | 14·58 | 1·48 |

[1] I.N.C.A.P. Unpublished data.

**Table X.** *Protein efficiency of combinations between lime-treated corn and peanut flour at different levels of protein in diet.*[1]

| Protein in diet | Experiment 1 | | | Experiment 2 | | |
|---|---|---|---|---|---|---|
| | Peanut flour | Lime-treated corn flour | P.E.R. | Peanut flour | Lime-treated corn flour | P.E.R. |
| % | g. | g. | | g. | g. | |
| 7·82 | 9·7 | 30·0 | 1·74 | 11·4 | 20·0 | 1·83 |
| 11·44 | 14·5 | 45·0 | 1·89 | 17·1 | 30·0 | 1·97 |
| 14·03 | 19·4 | 60·0 | 2·06 | 22·8 | 40·0 | 2·06 |
| 15·82 | 21·7 | 67·3 | 1·98 | 25·7 | 45·0 | 2·00 |

[1] I.N.C.A.P. Unpublished data.

**Table XI.** *Effect of replacing cotton-seed flour by soya bean flour on P.E.R. and biological value.*[1]

| Protein distribution in diet | | Protein efficiency ratio | | |
|---|---|---|---|---|
| Cotton-seed flour | Soya bean flour | 10 % protein in diet | 19 % protein in diet | Biological value in dogs |
| % | % | | | |
| 100 | 0 | 1·91 | 1·74 | 71·0 |
| 80 | 20 | 2·31 | 1·82 | ... |
| 60 | 40 | 2·50 | 1·84 | ... |
| 50 | 50 | ... | ... | 75·5 |
| 40 | 60 | 2·46 | 1·92 | ... |
| 20 | 80 | 2·60 | 1·93 | ... |
| 0 | 100 | 2·65 | 1·93 | 73·1 |

[1] Bressani and Elías. (Manuscript in preparation.)

complementation took place at a ratio by weight of about 6 to 1 of cotton-seed to sesame (Bressani and Scrimshaw, 1961). These combinations have served for the development of vegetable protein mixtures at the Institute of Nutrition of Central America and Panama (I.N.C.A.P.).

While adding small amounts of oil-seed meals to corn improves protein quality, the mixture still does not contain sufficient protein to become an important source of protein in diets poor in this nutrient. For this reason, vegetable protein mixtures of higher protein content, which have wider applicability in human nutrition, must be devised. The evolution of I.N.C.A.P. Vegetable Mixture 9 will now be described as an example.

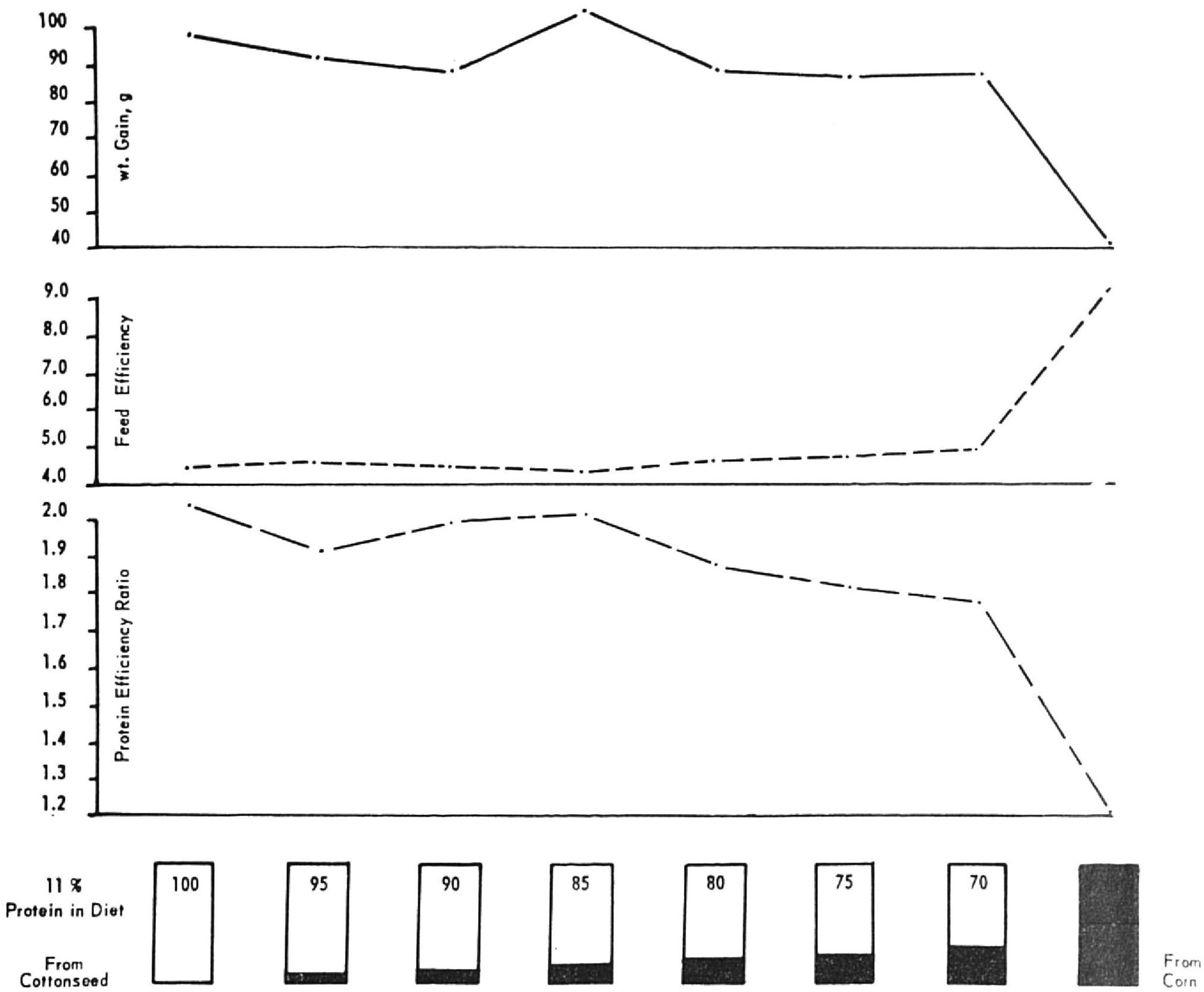


**Fig. 2.** Combinations between cotton-seed flour and corn proteins. (Bressani and Scrimshaw, 1961.)

(*A*) *Basic studies*.—In this mixture the staple food was corn, because of its high availability and consumption in Latin America and elsewhere. The protein concentrate tested was cotton-seed flour, which is abundant in the area where the mixture was to be used. In experiments with chicks and rats, given diets in which the protein from corn was isoproteically replaced by cotton-seed flour protein, it was demonstrated that the amino acid pattern of high quality cotton-seed flour can complement that of corn protein. Figure 2 gives the results of such complementation in experiments with rats. The bars in the lower part of the figure represent the distribution of dietary protein, and the curves from top to bottom indicate that better weight gains and higher protein efficiency were obtained when corn and cotton-seed flour provided 15 % and 85 % of the protein of the diet. When cotton-seed flour contributed less than 70 % gain in weight, protein efficiency and feed efficiency decreased. The results with baby chicks indicated that better weight gains and improved feed efficiencies were obtained when 15 % to 25 % of the protein of the diets was from corn and 85 % to

75 % from cotton-seed flour. Table XII shows the calculated amino-acid pattern of the 15 % to 85 % corn protein-cotton-seed flour mixture. Comparison of this pattern with the F.A.O. Provisional Amino acid Pattern (F.A.O. Committee on Protein Requirements, 1957) reveals deficiencies in isoleucine, lysine, total sulphur amino acids and tryptophan. Biological tests with chicks and rats indicated that lysine is the only limiting amino acid at intake levels of 15 % of the dietary protein, but that at higher levels of intake this deficiency disappears. The amino-acid proportions in the mixture, with tryptophan as the reference amino acid, are similar to those of F.A.O. Pattern.

On the basis of these experiments and calculations of the final concentration of other substances such as gossypol in the mixture, I.N.C.A.P. Vegetable Mixture 9, a corn-cotton-seed protein combination of close to 20 % from corn and approximately 80 % from cotton-seed flour, was formulated (Bressani, Elías, Aguirre and Scrimshaw, 1961). Since corn contains around 9 % protein and

**Table XII.** *Essential amino acid patterns.*[1]

| Amino acid | Corn | Cotton-seed flour | 15 to 85 protein combination corn-cotton-seed | F.A.O. reference protein |
|---|---|---|---|---|
| | mg./g. N | mg./g. N | mg./g. N | mg./g. N |
| Arginine | 262 | 719 | 647 | ... |
| Histidine | 231 | 113 | 132 | ... |
| Isoleucine | 213 [2] | 231 [2] | 228 [2] | 270 |
| Leucine | 572 | 413 | 437 | 306 |
| Lysine | 126 [2] | 256 [2] | 235 [2] | 270 |
| Methionine, Cystine | 189 [2] | 169 [2] | 172 [2] | 270 |
| Phenylalanine | 276 | 294 | 291 | 180 |
| Threonine | 214 | 294 | 281 | 180 |
| Tryptophan | 32 [2] | 75 [2] | 68 [2] | 90 |
| Valine | 281 | 331 | 323 | 270 |

[1] Bressani and Scrimshaw, 1961.
[2] Limiting amino acids.

cotton-seed flour 50 %, the mixture chosen contained from 26 % to 28 % protein. The initial formula was : 50 % whole ground corn, 38 % cotton-seed flour, 3 % torula yeast, and 3 % dehydrated leaf meal. The yeast provides vitamins of the B-complex and the leaf meal vitamin A activity, and both additional protein. These additions were considered necessary so that the mixture, although primarily designed as a protein supplement, could be used to correct other prevalent deficiencies. Further experiments indicated that sorghum grain, as well as other cereal grains, could replace all or part of the corn without changing the nutritive value (Bressani, Aguirre and Scrimshaw, 1959). Since sorghum costs less than most cereal grains, at least in Central America, the experimental formula for I.N.C.A.P. Vegetable Mixture 9 became : 28 % whole ground sorghum, 28 % ground corn, 38 % cotton-seed flour, 3 % torula yeast, and 3 % leaf meal (Bressani, Elías, Aguirre and Scrimshaw, 1961). Table XIII shows the chemical composition, vitamin and essential amino acid content of Mixture 9. This formula was extensively tested on chicks, rats, dogs and swine before it was fed to children (Bressani, Aguirre, Elías, Arroyave, Jarquín and Scrimshaw, 1961 ; Bressani, Aguirre and Scrimshaw, 1959 ; Bressani, Braham, Elías and Zaghi, 1963 ; Bressani, Braham, Jarquín and Elías, 1963 ; Bressani, Elías, Aguirre and Scrimshaw, 1961 ; Bressani, Elías and Scrimshaw, 1962).

Results of testing in chicks, shown in Table XIV, indicate that growth and feed efficiency are similar when the mixture is made either with 28 % corn and 28 % sorghum with 56 % corn or 56 % sorghum. The response is 82 % of that obtained from a commercial chick feed containing animal protein (Bressani, Aguirre, Elías, Arroyave, Jarquín and Scrimshaw, 1961). Other cereal grains, such as whole wheat, rice and oats, can replace corn in the mixture without decreasing nutritive value (Bressani, Aguirre, Elías, Arroyave, Jarquín and Scrimshaw, 1961 ; Bressani, Elías and Scrimshaw, 1962).

Other studies indicated that torula yeast contributed to the protein quality of the mixture, while the leaf meal could be eliminated as it does not so contribute.

**Table XIII.** *Nutrient content of Mixture 9B per* 100 *g.*[1]

| | | | | | |
|---|---|---|---|---|---|
| Protein | 27·5 g. | Calcium | 656·0 mg. | Leucine | 2·08 g. |
| Fat | 4·2 g. | Iron | 8·4 mg. | Lysine | 1·09 g. |
| Carbohydrate | 53·8 g. | Phosphorus | 698·0 mg. | Methionine and cystine | 0·82 g. |
| Calories | 370 | Sodium | 3·7 mEq. | | |
| Thiamine | 2·3 mg. | Potassium | 27·9 mEq. | Phenylalanine and tyrosine | 2·11 g. |
| Riboflavin | 1·1 mg. | Arginine | 2·57 g. | | |
| Niacin | 7·8 mg. | Histidine | 0·53 g. | Threonine | 1·18 g. |
| Vitamin A activity | 4,500 i.u. | Isoleucine | 1·11 g. | Tryptophan | 0·29 g. |
| | | | | Valine | 1·44 g. |

[1] Scrimshaw *et al.* (1961).

**Table XIV.** *Representative studies with baby chicks.*

| Diet | Protein in diet | Final weight gain | Feed efficiency |
|---|---|---|---|
| Vegetable Mixture 9 | 23·5 | 479 [1] | 2·31 |
| Vegetable Mixture 9 with 56 % corn | 23·8 | 469 [1] | 2·25 |
| Vegetable Mixture 9 with 56 % sorghum | 24·1 | 479 [1] | 2·27 |
| Commercial chick feed | 23·9 | 587 | 2·01 |
| Vegetable Mixture 9 | 20·8 | 300 [1] | 2·54 |
| Vegetable Mixture 9 without torula | 19·7 | 250 | 2·79 |
| Vegetable Mixture 9 | 20·8 | 344 [2] | 2·73 |
| Vegetable Mixture 9 without leaf meal | 20·8 | 331 [2] | 2·56 |

[1] 55 g. initial weight.
[2] 50 g. initial weight.

The vitamin A activity of the mixture can be more easily assured in practice by the inclusion of synthetic vitamin A. Since the mixture was intended for use in an area where enough milk is not available, the addition of calcium was considered necessary, and calcium carbonate at the level of 1 % was added.

In Figure 3 compare the protein value of Mixture 9 as compared with that of milk, casein and meat flour at several levels of protein intake (Bressani, Braham, Elías and Zaghi, 1963). The growth of rats fed the vegetable mixture at all protein levels was slightly less than for those fed the animal proteins. At low levels of protein intake the animal proteins produced higher protein efficiency ratios than the vegetable mixture ; at higher levels the protein efficiency ratios were similar.

Growth curves of rats fed Mixture 9 and milk at 20 % and 10 % protein levels and of Vegetable Mixture 9 and casein, both containing 24 % protein

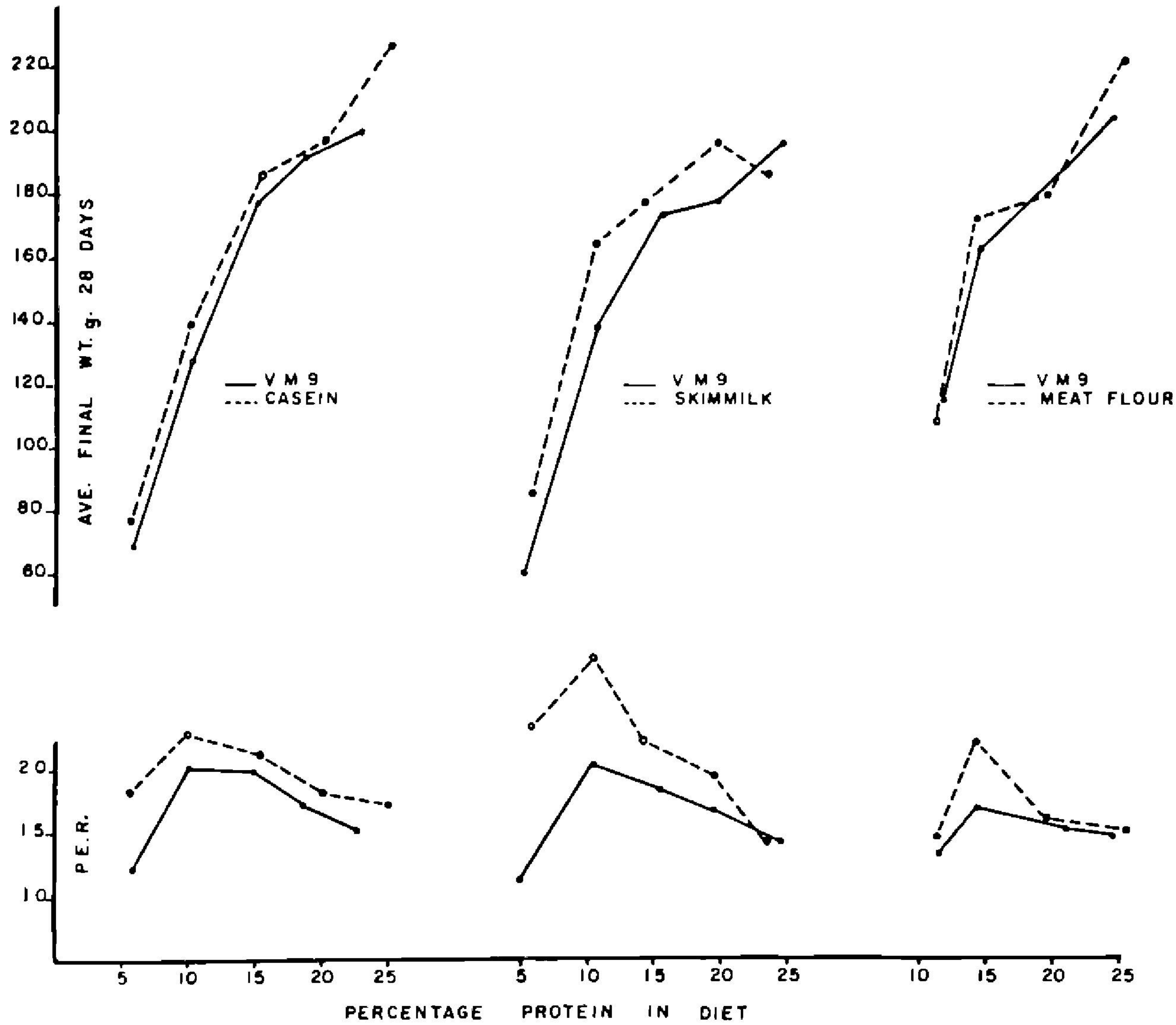


**Fig. 3.** Nutritive value of Vegetable Mixture 9, casein, skim milk and meat flour. (Bressani, 1962.)

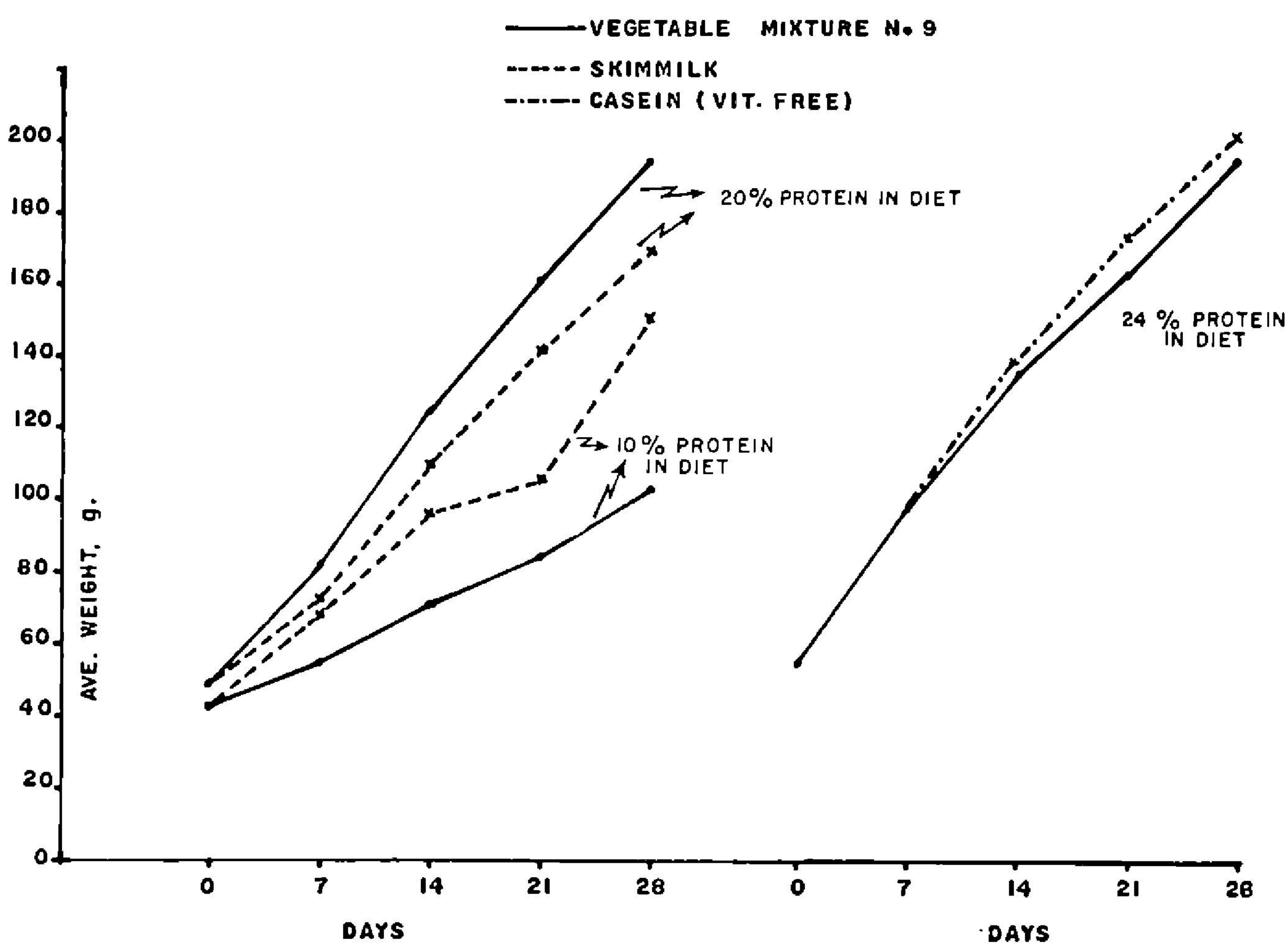


**Fig. 4.** Growth of rats fed I.N.C.A.P. Vegetable Mixture 9, skim milk and casein. (Bressani, 1962.)

in the diet, are shown in Figure 4. No differences in growth were noted, although the P.E.R. was slightly higher for casein (Bressani, Braham, Elías and Zaghi, 1963).

Several other kinds of tests were carried out with Mixture 9. In chicks N.P.U. values averaged 64 % (Braham, Bressani and Guzmán, 1963); in young dogs the biological value averaged 71·0 % with Mixture 9 as compared to 78 % with casein. Figure 5 shows the results of nitrogen balance tests with growing swine fed cooked Vegetable Mixture 9 *ad libitum* and with the nitrogen intake varying from 1·46 to 2·60 g. kg. day (Bressani, Braham, Jarquín and

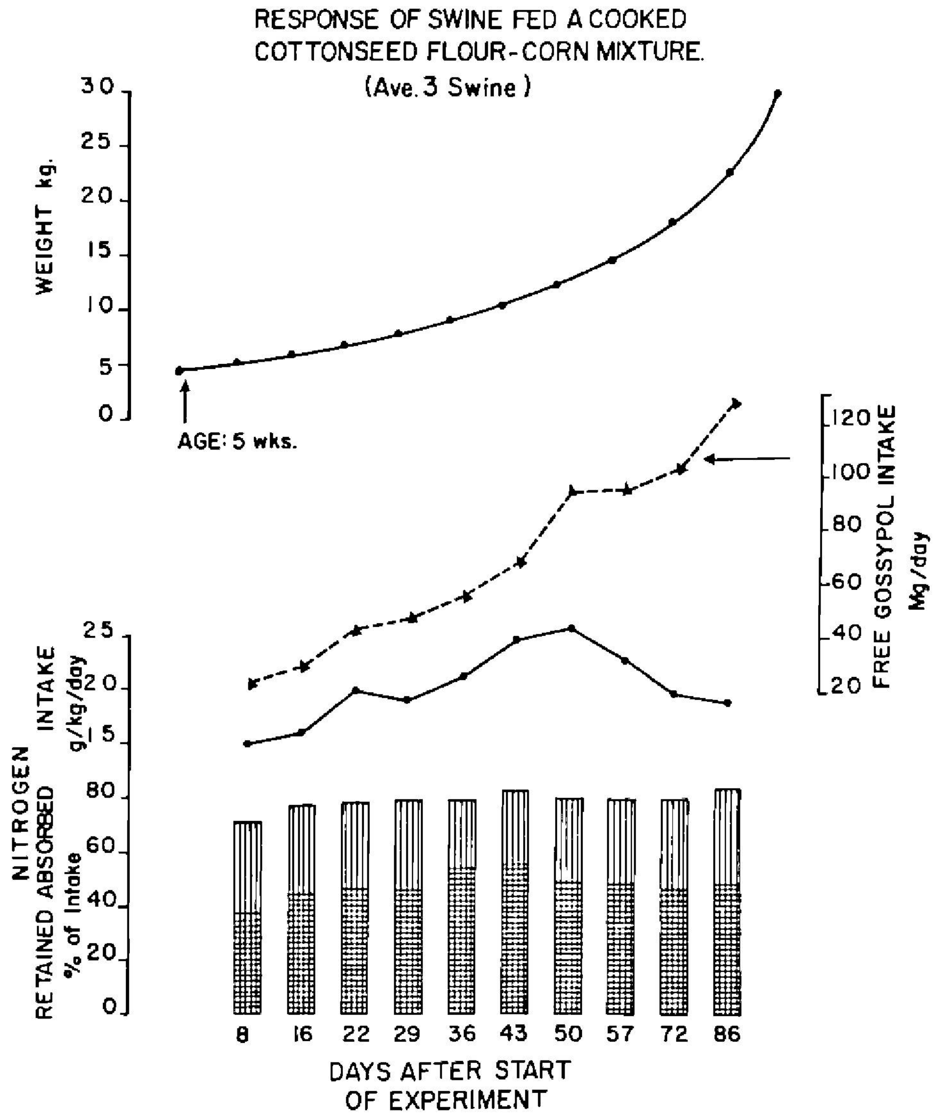


**Fig. 5.** Response of swine fed a cooked cotton-seed flour-corn mixture. (Average, three swine.)

Elías, 1963). Nitrogen retention was relatively high, varying from 37·4 % to 45·4 % of the nitrogen intake. The swine confined in metabolism cages gained an average of 18 kg. and showed no adverse effects during the experiment.

Table XV gives the chemical composition of the carcass, liver and bones of rats fed either Vegetable Mixture 9 or milk (Bressani, Braham, Elías and Zaghi, 1963). The carcass and bone fat of the animals fed Vegetable Mixture 9 appears in some cases to be higher than that of animals fed milk. Other chemical constituents were present in similar concentrations, except for the slightly lower ash content of the bones of rats fed Vegetable Mixture 9.

Table XVI shows the blood chemistry of rats fed Mixture 9 and milk at two levels of protein in the diet. At low levels, total serum proteins and albumin were lower in the rats fed the Vegetable Mixture; they were slightly higher, however, in the rats fed this mixture, with higher levels of protein intake. Additional constituents studied, such as serum globulins, blood urea, red blood

**Table XV.** *Chemical composition of carcass, liver and bone of rats fed Vegetable Mixture 9 or milk.*

| Diet | Moisture | Fat [1] | Nitrogen [1] | Ash [1] |
|---|---|---|---|---|
| | % | % | % | % |
| Carcass— | | | | |
| Vegetable Mixture 9 . | 67·1 | 27·9 | 11·8 | ... |
| Milk . . . | 68·0 | 18·9 | 11·9 | ... |
| Liver— | | | | |
| Vegetable Mixture 9 . | 67·6 | 3·3 | 9·9 | ... |
| Milk . . . | 67·8 | 3·0 | 9·9 | ... |
| Bone— | | | | |
| Vegetable Mixture 9 . | 47·9 | 4·8 | 3·0 | 25·5 |
| Milk . . . | 46·0 | 2·6 | 3·1 | 26·8 |

[1] Dry weight basis.

**Table XVI.** *Blood chemistry of rats fed Vegetable Mixture 9 and milk.*

| Serum | 10 % protein | | 20 % protein | |
|---|---|---|---|---|
| | Vegetable Mixture 9 | Milk | Vegetable Mixture 9 | Milk |
| Protein . . | 4·36 | 5·02 | 5·32 | 5·18 |
| Albumin . . | 2·28 | 2·70 | 2·96 | 2·63 |
| Globulin . . | 2·08 | 2·31 | 2·35 | 2·54 |
| A./G. ratio . . | 1·10 | 1·17 | 1·28 | 1·09 |
| Urea N. . . | 19·5 | 18·9 | 18·5 | 25·0 |
| Whole blood— | | | | |
| R.B.C. . . | 5·93 | 5·33 | 5·34 | 4·75 |
| W.B.C. . . | 5,041 | 5,708 | 6,320 | 5,360 |
| Haemoglobin % . | 13·0 | 13·6 | 14·4 | 14·2 |

**Table XVII.** *Effect of cooking on several substances in Vegetable Mixture 9.*

| Cooking time | Thiamine | Riboflavin | Gossypol | | Free ε-$NH_2$ groups of lysine |
|---|---|---|---|---|---|
| | | | Free | Total | |
| minutes | mg./100 g. | mg./100 g. | mg./100 g. | % | g. % |
| Raw | 2·18 | 1·21 | 15·8 | 0·41 | 1·24 |
| 0 | 2·25 | 1·12 | 7·1 | 0·43 | 1·16 |
| 5 | 1·96 | 1·07 | 5·3 | 0·35 | 1·06 |
| 10 | 2·01 | 1·05 | 6·4 | 0·43 | 0·95 |
| 15 | 1·99 | 1·14 | 8·4 | 0·41 | 1·18 |
| 20 | 1·75 | 0·86 | 5·3 | 0·41 | 1·19 |
| 25 | 1·54 | 1·18 | 2·7 | 0·42 | 1·09 |

cells, white blood cells and haemoglobin were similar at the same levels of protein intake.

Since the mixture was intended for consumption in the form of a gruel, studies were made of possible changes during cooking. Table XVII shows that cooking decreased the thiamin concentration and that small changes in riboflavin concentration also occurred. The most important observation, however, was the significant decrease in free gossypol with no change in total gossypol, and in available lysine as determined by free epsilon amino groups of lysine (Bressani and Elías, 1963). Table XVIII shows that cooking up to 24 minutes did not change the percentage of nitrogen absorbed or the percentage of nitrogen retained, expressed on the basis of the nitrogen intake in nitrogen balance studies with dogs (Bressani and Elías, 1963).

Figure 6 shows the supplementary effect, determined experimentally, of Vegetable Mixture 9 when given in association with a diet similar to the one consumed by a group of pre-school children in rural Guatemala. When the

**Table XVIII.** *Nitrogen balance on dogs fed Vegetable Mixture 9 cooked for different periods of time.*[1]

| Nitrogen | Cooking time, minutes | | | | |
|---|---|---|---|---|---|
| | 0 | 8 | 16 | 24 | 0 |
| Intake [2] | 844 | 862 | 862 | 865 | 854 |
| Faeces [2] | 234 | 241 | 249 | 253 | 257 |
| Urine [2] | 462 | 481 | 472 | 471 | 481 |
| Absorbed [2] | 610 | 621 | 613 | 612 | 597 |
| Retained [2] | 148 | 140 | 141 | 141 | 116 |
| Absorbed [3] | 72 | 72 | 71 | 71 | 70 |
| Retained [3] | 17 | 16 | 16 | 16 | 14 |

[1] Average three dogs for each experiment.
[2] mg. N. kg./day.
[3] % of N intake.

rats consumed the diet alone *ad libitum* they gained 116 g. in eight weeks, while, with a daily supplement of 3·5 g. of Mixture 9, the weight gain for this period was 194 g. A daily supplement of 3·5 g. of Mixture 9-milk (50 50) gave a weight gain of 220 g., while a daily skim milk supplement of 3 g. gave in eight weeks a weight gain of 227 g.

Extensive animal and chemical studies showed that Mixture 9 is free of toxic effects, has a high nutritive value, contains protein of good quality, supplements the low-protein rural diets, and thus would be suitable for human feeding.

(*B*) *Biological trials in human beings.*—When a mixture designed for human use has been shown to be adequate by chemical analyses and biological tests in animals, the next step should be trials on human beings. In such trials small children make the best subjects, for they are easy to handle, particularly when new foods and monotonous diets are being given. Furthermore, being rapidly growing organisms, they are sensitive to differences in nutritive value which can be measured easily and in a relatively short period of time.

With the vegetable mixture developed by I.N.C.A.P., the following procedures were adopted :

1. Test of acceptability and tolerance. For this purpose small children between 1 and 5 years of age were observed, because this was the age group for which the mixture was primarily targeted. The subjects were children

hospitalized for malnutrition, but who had already recovered, had no other complications and were eating a mixed complete diet. The vegetable mixture, prepared in the form to be recommended for popular use (*i.e.*, as a gruel) was administered to these children, replacing the milk in one of their meals. This was done for a number of days and with as many children as possible to be sure that the product was acceptable to the children as regards taste and other organoleptic characteristics and that it did not cause vomiting, diarrhoea or any other disturbances. After a satisfactory outcome of this test, the children were given a diet in which the mixture, now prepared in different ways, provided the majority of the protein. Here the purpose was to test tolerance and acceptability.

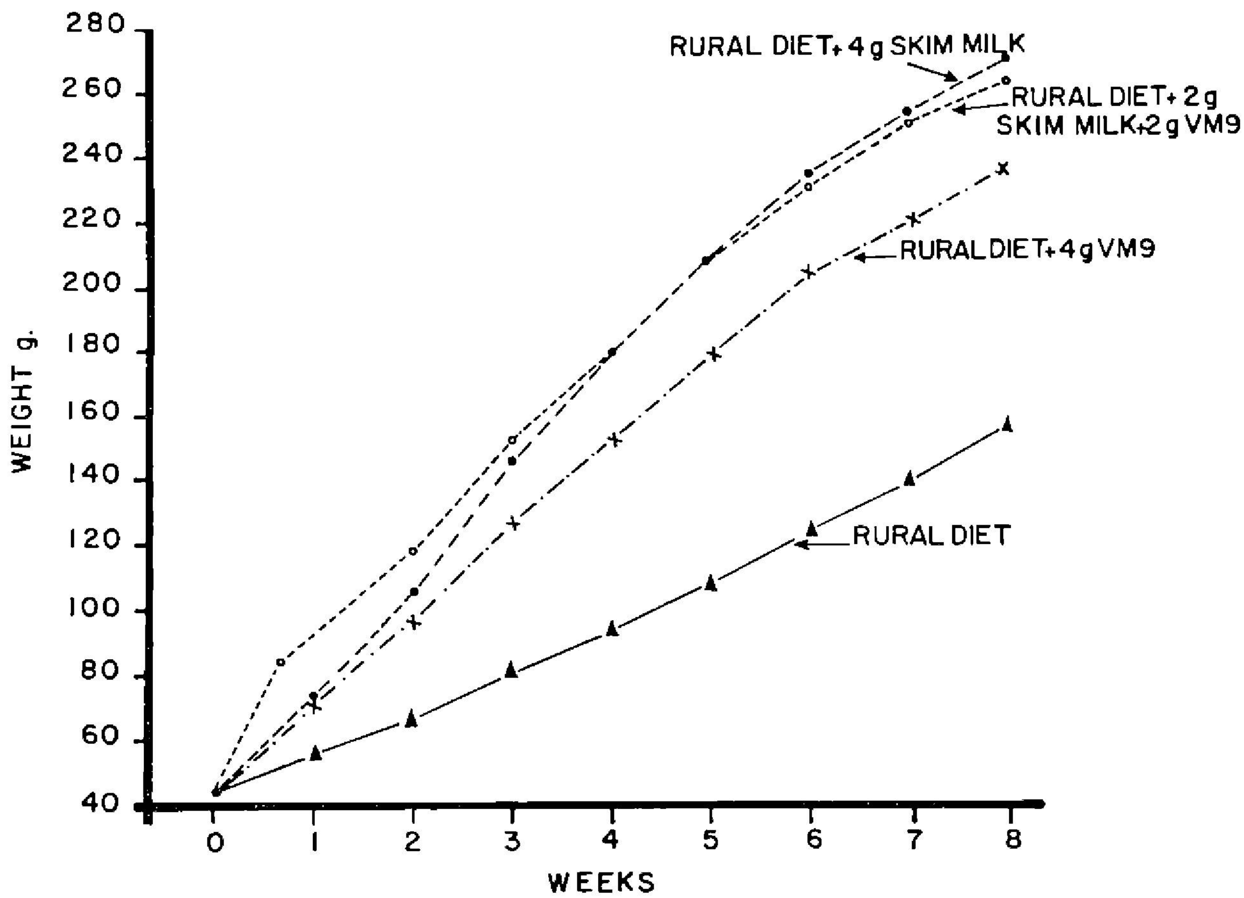


**Fig. 6.** Effect of skim milk and Vegetable Mixture 9 supplementation to a rural diet on the weight of rats.

2. Evaluation of nutritive value. Vegetable mixtures, such as those developed and tested by I.N.C.A.P., are designed primarily as protein supplements. Testing must therefore be oriented towards the evaluation of their protein value. For this purpose the following were included during the testing of I.N.C.A.P. mixtures: (*a*) measure of nitrogen absorption and retention by metabolic balance technique, comparing the protein in the mixture with that of milk; (*b*) administration of the mixture as the main source of protein during a period of time long enough to evaluate its effect on the growth and general health of the child; (*c*) the treatment of cases of protein malnutrition with the mixture as the source of protein, comparing recovery with that obtained when milk or other adequate sources of animal protein were given.

The results of the nitrogen balance studies with one of the formulas tested (Vegetable Mixture 9) (Scrimshaw, Béhar, Wilson, Viteri, Arroyave and Bressani, 1961) are summarized in Table XIX. The children were between 1 and 4 years. The diets were isocaloric and isonitrogenous; in one, all the protein was provided by milk and in the other by the vegetable mixture. The diets were also similar in content of fat and other nutrients, and in both instances a daily supplement of vitamins and minerals was provided to ensure that the difference in protein was the only variable.

The children were all well adapted to the balance technique in a special bed. The balance tests lasted for five days. The same children were used to test both diets, and the order in which these were tested in each child varied in order to eliminate the possible effect of one period upon the other. Experienced personnel were available to handle carefully controlled techniques for the preparation of the diets, its administration to the children, the collection of urine and faeces and other details. Analytical data were used to determine the intake.

It is interesting to note from the results summarized in Table XIX, that although the absorption of nitrogen was lower for the vegetable mixture, there was no significant difference in retention at adequate levels of protein intake. Only when this was below 2 g./kg./day, which was considered insufficient for the children under observation, was higher retention observed on the milk diet.

**Table XIX.** *Comparison of Vegetable Mixture 9 and milk in young children recovered from kwashiorkor.*

| G. protein per kg./day | No. of children | No. of balance periods | Milk | | | Vegetable mixture | | |
|---|---|---|---|---|---|---|---|---|
| | | | Protein intake (g./kg.) | Absorption (% of intake) | Retention (% of intake) | Protein intake (g./kg.) | Absorption (% of intake) | Retention (% of intake) |
| >4 . | 1 | 2 | 4·0 | 84·4 | 22·0 | 4·0 | 66·1 | 24·1 |
| 3 to 3·9 . | 4 | 11 | 3·0 | 83·9 | 17·1 | 3·0 | 70·2 | 16·8 |
| 2 to 2·9 . | 9 | 48 | 2·3 | 82·6 | 16·3 | 2·3 | 68·9 | 17·8 |
| 1 to 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 |

In another series of tests pre-school children were given diets in which all the protein was provided by one of these mixtures for periods as long as three months. The gain in height and weight and the general health and behaviour of the children were normal.

Although the mixtures were not designed to be used in the treatment of malnourished children but rather to prevent malnutrition, it was considered useful to assess the biological value of the protein of the mixtures by treating some cases of protein malnutrition with a diet in which the mixture was the only source of protein. Children with kwashiorkor who had already passed, on a milk dietary treatment, into the period of initial recovery or " initiation of cure," as described by Brock, Hansen, Howe, Pretorious, Davel and Hendrickse (1955), were observed. " Consolidation of cure " was obtained with the mixture instead of milk as the protein source. After the adequacy of the mixture for this purpose had been proved, kwashiorkor cases were treated with the mixture from the time of admission to the hospital.

With children treated thus far with either Mixture 8 or 9, it has been observed that recovery, as indicated by clinical and biochemical changes, is parallel to recovery when milk is used, the only exception being that the return of the serum proteins to normal values is somewhat slower when the vegetable mixtures are given, as can be seen in Figure 7.

(*C*) *Acceptability and market trials.*—Trials were carried out in which 76 needy families with pre-school children in four Guatemalan communities were selected by local health centres. Sufficient amounts of the product were provided so that each pre-school child could drink three glasses a day. The trials varied in duration from 17 to 19 weeks. Acceptance was good at the beginning and tended to increase progressively during the trial period. Ninety-nine children out of a total of 129 consumed an average of two or more glasses daily throughout the entire period; during the final two weeks 110 children consumed two or more glasses daily. The majority of these children said they liked the drink very much and most of the parents indicated a desire to buy it if it were available at a reasonable price.

I.N.C.A.P. Vegetable Mixture 9 has been commercially produced and distributed successfully in Guatemala. It is sold in small bags containing 75 g.,

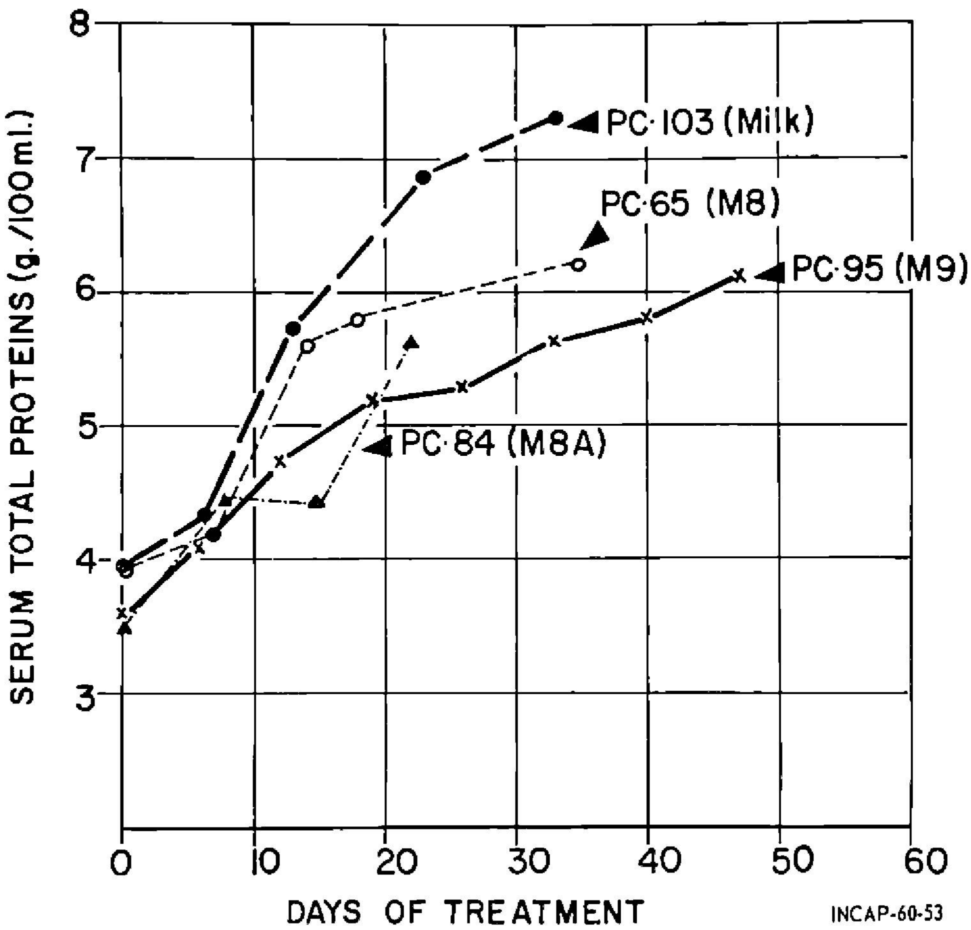


**Fig. 7.** Serum protein changes in kwashiorkor patients treated with milk (patient P.C.-103) or vegetable mixtures (patients P.C.-65, P.C.-95 and P.C.-84).

estimated to be the ideal amount as a daily supplement to the usual diet of the majority of the population. At this price it competes favourably with any other adequate source of protein, particularly food of animal origin. At present it is in different stages of industrial development in other Latin American countries. In some the acceptability or market trials have shown a need for changes in certain of the organoleptic characteristics of the mixture to adapt it more fully to local food preferences.

This experience has given I.N.C.A.P. a sense of confidence, not only in the possibility of utilizing vegetable proteins to replace those of animal origin in human feeding, but also in its practicability of their use in areas where protein deficiency is a serious problem.

## V. Other vegetable protein mixtures

During the last few years several similar food preparations have been developed with apparently good results. One of the laboratories engaged in the development of vegetable protein mixtures is the Central Food Technological Research

Institute, Mysore, India. Several formulas have been developed by this Institute (Subrahmanyan, Rama Rao and Swaminathan, 1959; Subrahmanyan, Sreenivasan, Bhatia, Swaminathan, Bains, Subramanian, Norayana Rao, Bhagavan and Doraiswamy, 1961). 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 contains 25 % Bengal gram (chickpea), and 75 % peanut meal, and Mysore Food B 25 % Bengal gram, 65 % peanut flour and 10 % sesame meal.

The same Institute has been making intensive efforts to introduce peanut flour into Indian diets in other forms. Some examples, cited in a review by Milner (1962), are a " tapioca macaroni " consisting of a blend of tapioca flour, peanut flour and wheat semolina in the ratio of 60 : 15·25. This product contains about 11 % protein.

Another mixture is a combination of whole wheat flour with peanut flour and some additional tapioca. This food, known as " Panshtik atta," is used for the preparation of unleavened bread. Protein-rich biscuits known as " Nutro " biscuits are made from a 2 : 3 blend of peanut and wheat flour. They contain sugar, salt, leavening and vitamins and about 17 % protein.

The Mysore Institute has also devoted considerable time and effort to the production of a peanut isolate. This contains 90 % protein and has been reported to be bland and stable. Several formulas containing this protein isolate with and without milk powder and other supplements have been devised. They can be utilized in the same way as milk. The isolates have been used also for the preparation of " weaning foods " containing 20 % or more of protein. These have been used for child-feeding programmes with successful results.

Other foods containing peanut flour have been developed and tested. Dean, in a study in Uganda (Dean, 1951,1958), fed children a biscuit containing 20 % protein, consisting of whole peanuts, providing 60 % of the total protein, with corn meal, sugar and some dry skim milk. The biscuit was fed to children and to infants as young as 6 months. Their weight gains and general growth were compared with another group fed a diet based on milk. The group receiving the biscuit showed gains in weight about equal to those receiving the milk diet.

Sénécal (1958) fed young children peanut flour over long periods of time at levels of 75 to 150 g. per child daily and found good nitrogen retention. He reported that peanut flour-millet mixtures were essentially equal in value to milk-millet mixtures in the treatment of protein malnutrition.

In Nigeria, Nicol and Phillips (1961) fed boys and adults peanut flour and cassava with good acceptance. De Mayer and Vanderborght (1958) gave a peanut flour preparation to children and used the nitrogen balance technique to measure its protein value. The results suggested that the peanut flour used has a value 75 % of that of skim milk. Peanut flour alone and in combination with pulses was used by Gopalan (1961) in the treatment of protein malnutrition in India. The response to the mixture was apparently satisfactory, but not to each ingredient fed alone. Tung and co-workers (Chen, Wei, Huang and Tung, 1961) prepared food mixtures of peanut and soya-bean flour protein which, when fed to children, were well tolerated and gave satisfactory nitrogen retention values.

Legumes have been used to some extent in the preparation of vegetable mixtures. Venkatachalam and co-workers (Venkatachalam, Srikantia, Mehta and Gopalan, 1956) have tried isoproteic diets of Bengal gram and banana, and Bengal gram with rice and wheat bread and skim milk, in the treatment of cases of kwashiorkor. The results were satisfactory.

Mixtures of lime-treated corn and black beans, in the proportions of 4 : 5, were studied by investigators in the Children's Hospital in Mexico City. The absorption and retention of nitrogen by malnourished children varied from child to child and were much lower than with isocaloric diets containing isonitrogenous quantities of milk (Gómez, Ramos-Galván, Cravioto, Frenk, Peña, Moreno and Villa, 1957). In Mexico a mixture of chickpeas and soya bean flour supplemented with methionine is also being marketed, but no information on its value is yet available.

Soya bean flour and soya-bean products have also been used in developing protein mixtures. Dean (1951) proposed a soya-bean-banana mixture for the treatment of protein malnutrition. Subrahmanyan, Bhagavan and Swaminathan (1958) have reviewed the evidence on the use of soya-bean milk as infant food, but more information is needed. There are other products derived from soya bean which look promising, such as " saridele " and soya milk (Voris, 1961).

Sesame flour has been used to some extent. I.N.C.A.P. Vegetable Mixture 8 (Squibb, Wyld, Scrimshaw and Bressani, 1959) consists of dried lime-treated corn 50 % flour, sesame flour (full fat) 35 %, cotton-seed flour 9 %, torula yeast 3 % and kikuyu leaf meal 3 %. This mixture, containing 25·1 % protein and 13·7 % fat, proved to be well tolerated by children and gave a nitrogen retention equivalent to that of milk in a series of five-day balance trials, carried out in five children fed at levels of 2·4 to 3·8 g. protein per kg. body-weight. It also gave good results when fed to children with kwashiorkor.

Other mixtures developed recently are " Argentarina " in the Argentine (Argentarina, 1962), containing 4 % ground nut flour, 20 % sorghum flour, 10 % wheat flour, 17 % millet flour, 10 % white bean flour, 2 % yeast, 1 % calcium carbonate, and 4,500 i.u. vitamin A per 100 g. This mixture contains 27·8 % protein and has a good pattern of essential amino acids. Acceptability trials have been satisfactory. A mixture called " $V^{10}$ Protein " (Prier and Derse, 1957), containing wheat germ, the aleurone layer of the wheat kernel and processed defatted soya-bean meal, was found to compare favourably in protein value with casein and other animal proteins. Tasker *et al.* (Tasker, Narayanarao, Swaminathan, Sankaran, Tayaraj and Subrahmanyan, 1961) reported on the supplementary value of a blend of peanut, coconut and chickpea flours. Chaves *et al.* in Brazil (Chaves, Rego Barros, Madruga, Lapa, Freitas, De Lima and Da Casta, 1962) tested mixtures of cashew-nut flour (*Anacardium occidentale*, L.) and cowpea flour (*Vigna sinensis*) in the protein proportions of 3 to 1, and reported good results.

## Summary

1. The relative concentration of the essential amino acids is the most important factor determining the biological value of a protein. From the nutritional point of view, proteins should not be differentiated according to their animal or vegetable origin, but in regard to their biological value.

2. Vegetable proteins, in general, contain one or more essential amino acids in low concentration. It is possible, however, to combine them so that their individual amino acids complement each other. As a result, protein food mixtures of relatively high biological value can be obtained, which can and will help in solving the problem of protein malnutrition to-day and the problem of the expanding world population to-morrow.

3. Although important advances have been made, there are several problems still to be solved. Attention should be given to processing factors affecting the nutritive value of vegetable proteins, such as temperature, moisture, presence of organic substances such as free sugars, pigments and fat. Study of the factors which limit vegetable protein digestibility is also of importance.

3. Vegetable proteins in general, besides being deficient in some amino

acids, have excessive amounts of others. The significance of this imbalance has still to be evaluated, but it may have a bearing on the efficient utilization of plant proteins.

4. Several practical ways of making better use of vegetable protein foods are available. One is to enrich staple foods with small amounts of vegetable protein concentrates. A second method is to combine two staples in balanced proportions so that their proteins complement each other. A third is to combine vegetable protein concentrates with or without other vegetable products to obtain a mixture of high protein concentration and adequate biological value which can be used as a protein dietary supplement; I.N.C.A.P. vegetable mixtures are examples of this procedure.

5. As a result of the work of many laboratories, several vegetable protein mixtures have been developed and in some cases have been applied with success. Examples are given of such mixtures, with more details on the development and application of I.N.C.A.P. Vegetable Mixture 9, commonly known as "Incaparina." This experience has demonstrated not only the possibility of utilizing vegetable proteins as a replacement for those of animal origin in human feeding, but also that suitable mixtures can be commercially successful and culturally acceptable in areas where protein deficiency is a critical problem.

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