All-Vegetable Protein Mixtures for Human Feeding

V. Clinical Trials with INCAP Mixtures 8 and 9 and with Corn and Beans

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PROTEIN DEFICIENCY is widely recognized to be the major cause of the high rates of morbidity and mortality of infants in the immediate postweaning period in most technically underdeveloped areas. Despite all measures that can be taken to accelerate the production of animals, in many of these areas, there is little prospect that enough animal protein will be available soon enough and at a sufficiently low cost to solve the entire problem.

Previous papers in this series have described the laboratory studies and biologic trials involved in the development of a product containing only vegetables which has high nutritive value and is identified as INCAP Mixture 8.[1,2] Detailed descriptions of similar work with INCAP Mixture 9, an even less expensive mixture, have since been prepared.[3,4] While designed initially for the supplementary and mixed feeding of infants and young children, both preparations are suitable for consumption by persons of all ages and are relatively inexpensive. In dry form, they contain over 25 per cent protein of a quality comparable to that of some proteins of animal origin. They also supply desirable amounts of other essential nutrients except ascorbic acid. The generic name INCAPARINA has been given to cereal mixtures of this type which are developed by the Institute of Nutrition of Central America and Panama.

The biologic trials of these two preparations and variations of them were so favorable that the metabolic and therapeutic studies described in this paper were carried out with children. As a result, these mixtures were recommended for field acceptability trials in human populations. The satisfactory outcome of both types of studies has led in turn to marketing trials of Mixture 9B.

MATERIAL AND METHODS

Children with acute kwashiorkor were admitted to the INCAP metabolic unit for experimental treatments, and these, or similar children in a late stage of convalescence, were used in the studies comparing nitrogen balances in patients receiving milk and the vegetable mixtures. All patients were between thirteen months and five years of age, except for one child who was seven years old. To simplify collection of specimens of urine and feces, only male children were selected. The administration of milk as our standard treatment of patients with kwashiorkor has already been described[5] as have been the procedures followed in metabolic balance studies.[6] Intakes

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TABLE I
Formulas for INCAP Vegetable Mixtures for Human Feeding

Ingredients	Mixture (per cent)				
	8	8A	9	9A	9B
Corn					
Lime-treated	50	50	28	...	...
Cooked	...	...	...	29	...
Uncooked	...	...	...	...	29
Sesame flour with					
33 per cent fat	35	...	...	...	...
18 per cent fat	...	35	...	...	...
Sorghum					
Lime-treated	...	...	28	...	...
Cooked	...	...	...	29	...
Uncooked	...	...	...	...	29
Cottonseed	9	9	38	38	38
Torula yeast	3	3	3	3	3
Dehydrated leaf meal	3	3	3	...	...
$CaCO_3$	...	...	...	1	1
Vitamin A (in I.U.)	...	...	...	4,500	4,500

only in using sesame flour with a lower content of fat.* Before grinding, the corn and sorghum in Mixture 9A were cooked for fifteen minutes at 5 pounds pressure† with the material being soaked to a moisture content of 25 to 30 per cent. The specifications for the Torula yeast were based on Type 200‡ which contained 0.6 mg. thiamine, 0.2 mg. riboflavin and 1.0 mg. niacin. After the completion of the trials described herein, Torula yeast Type CF2‡ was specified. This type is identical in all respects to Type 200 except for a doubled content of riboflavin.

The nutrient content of vegetable Mixture 8 has already been published;[7] that of Mixture 9B, made with the Type CF2 Torula yeast, is given in Table II. The compositions of proteins and amino acids of Mixtures 9, 9A and 9B do not differ significantly. All of the mixtures were boiled for ten to fifteen minutes with sufficient water to make a gruel known locally as "atole" and fed in this form seasoned with sugar and cinnamon or other flavor. Five children were treated with a precooked form of Mixture 9B which was prepared by the same process of cooking, drying and flaking

TABLE II
Content of Mixture 9B (per 100 gm.)

Protein	27.5 gm.	Calcium	656 mg.	Leucine	2.08 gm.
Fat	4.2 gm.	Iron	8.4 mg.	Lysine	1.09 gm.
Carbohydrate	53.8 gm.	Phosphorus	698 mg.	Methionine and cystine	0.82 gm.
Calories	370	Sodium	3.7 mEq.		
Thiamine	2.3 mg.	Potassium	27.9 mEq.	Phenylalanine and tyrosine	2.11 gm.
Riboflavin	1.1 mg.	Arginine	2.57 gm.	Threonine	1.18 gm.
Niacin	7.8 mg.	Histidine	0.53 gm.	Tryptophan	0.29 gm.
Vitamin A	4,500 I.U.	Isoleucine	1.11 gm.	Valine	1.44 gm.

of proteins and calories were based on estimated requirements per kg. of body weight. Supplementary ascorbic acid was given with all diets and, when frank anemia was present, ferrous sulfate was given orally after initial recovery. Intakes of dietary proteins were determined by actual analyses of the diets. Urine and feces were collected for three- or five-day periods. Nitrogen was measured by the Kjeldahl procedure.

The formulas for the various INCAP vegetable mixtures tested are given in Table I. Specifications for the ingredients in Mixture 8 were given previously[7] and Mixture 8A differs

* Both lots of sesame flour were obtained from American Sesame Products Inc., Paris, Texas, through the courtesy of Mr. John H. Kraft.

† These substances were prepared through the courtesy of the Instituto Centro Americano de Investigaciones y Tecnología Industrial (ICAITI), Guatemala, C.A.

‡ This type was supplied by the Lakes States Yeast Corporation, Rhinelander, Wisconsin.

TABLE III

Comparison of Vegetable Mixture 8 and Milk in Young Children Recovered from Kwashiorkor

No. of Children	Age	Weight (kg.)	Calories per Kilogram	Milk				Vegetable Mixture 8			
				Days	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)	Days	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)
48	1 yr. 9 mo.	7.5	101	5	2.5	87	0	5	2.3	73	11.3
23A	3 yr.	11.2	106	5	2.6	74	23.2	5	2.6	78	23.2
48	1 yr. 9 mo.	9.9	110	5	3.8	90	14.8	5	3.8	71	12.0
56	3 yr. 6 mo.	9.5	101	5	2.8	74	11.0	5	3.0	71	13.5
57	4 yr. 3 mo.	11.6	108	5	2.9	80	33.6	5	2.8	68	22.5
73	1 yr. 1 mo.	7.4	100	5	3.0	83	17.6	10	3.5	78	31.3
75	1 yr. 3 mo.	7.5	95	10	2.6	83	38.2	5	2.9	70	23.8
88	3 yr.	10.7	108	5	2.0	78	25.9	10	3.3	67	15.2
			(104)*		(2.8)	(81)	(20.5)		(3.0)	(72)	(19.1)

* Numbers in parentheses are averages.

employed in the manufacture of commercial "instant" baby cereals.*

Efforts were also made to treat three children with kwashiorkor with a diet containing lime-treated corn in the form of atole and tortillas and cooked black beans (Phaseolus vulgaris) in which 43 per cent of the protein came from the former and 57 per cent from the latter. Biologic values of this corn and bean mixture, of milk and of Mixture 9B were each measured in a single child by the method of Mitchell.[8]† Also included in this report are preliminary data obtained from a series of trials in which either Mixture 8 or 9 was fed, both with and without fat, and nitrogen absorption and retention compared.

RESULTS

Tolerance and Acceptability

Before prolonged trials of feeding Mixture 8 *per se*, it was added to the mixed diets of eight children who had recovered from the acute phase of kwashiorkor.[9] No gastrointestinal disturbances were observed. It was then given as the sole source of protein to two children, who were completely recovered from kwashiorkor, and later to five children, who had passed the acute phase but were not yet ready for discharge from the hospital.[7,9] These children received Mixture 8 for periods of 12, 33, 41, 59 and 50 days, respectively. In every instance, the clinical response was excellent, and the mixture seemed more effective than milk in reducing the tendency of diarrhea by these subjects. Therefore, it was deemed safe to proceed with therapeutic trials of Mixture 8 in cases of patients with acute kwashiorkor.[7,9]

The tolerance of patients to Mixture 9 was established in the nitrogen balance studies in three children and by administration of the mixture to two children who had partially recovered from kwashiorkor. The employment of this formula in the treatment of children with acute kwashiorkor followed promptly. Subsequent to the studies of nitrogen balance and treatments reported in this paper, the long term tolerance to and acceptability of INCAP Mixtures 9A and 9B were confirmed through field studies in four Guatemalan communities.‡ The children in these com-

* Furnished by the "Junket" Brand Foods Division of Christian Hansen's Laboratory, Little Falls, New York, through the courtesy of Theodore F. Irmiter, M.D.

‡ Supervised by Dr. Romeo de León, Nutrition Section, Public Health Department, Guatemala.

$$\dagger \text{ Biologic value} = \frac{\text{N intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{fecal N} - \text{metabolic N})}.$$

TABLE IV
Comparison of Vegetable Mixture 9B and Milk in Young Children Recovered from Kwashiorkor

Gram Protein per Kilogram	No. of Children	No. of Balance Periods	Milk			Vegetable Mixtures 9A or 9B		
			Protein Intake (gm./kg).	Absorption (% of intake)	Retention (% of intake)	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)
>4.0	1	2	4.0	84.4	22.0	4	66.1	24.1
3.0–3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0–2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0–1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
<1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

munities consumed one of these formulas daily for periods of 15 to 19 weeks. During any given week, an average of 78 per cent of all children scheduled to receive the mixture, consumed two or more glasses daily. No instances of intolerance to the mixture were encountered. Ninety-seven per cent of all offerings were accepted and only 3 per cent rejected. Subsequently, similar results were obtained in a study of fifty-three children given Mixture 9B for four weeks in El Salvador.* From March 22 to July 22, 1960, a total of 485,000 packages of 75 gm. of INCAPARINA, made according to formula 9B, have been consumed in a marketing trial in Guatemala with a very good acceptability and no reports of intolerance.

Nitrogen Balance Studies

All results of the nitrogen balance studies carried out during intake of INCAP vegetable Mixture 8 are summarized in Table III, including results obtained from five patients which have been published previously.[7] Although there are variations in the relative retention of nitrogen during intake of either milk or Mixture 8, they seem to be random; thus there is no significant difference between the average retention with milk and that with Mixture 8. However, a consistently higher percentage of the protein intake is absorbed during the periods of milk intake, so that the percentage of absorbed nitrogen retained by the child is higher with vegetable Mixture 8 than with milk. Three patients also received Mixtures 8 and 8A at about the same levels of protein intake. In two of these, PC-83 and PC-88, results were comparable, i.e., 16.8 and 15.2 per cent retention for Mixture 8 and 13.9 and 14.1 per cent for Mixture 8A. For unknown reasons, the apparent retention in the third patient (PC-85) was 21.3 per cent with Mixture 8 and 4.7 per cent with Mixture 8A.

Many more balance studies were carried out with Mixtures 9, 9A and 9B than with Mixtures 8 and 8A because of increasing indications of the practical value of the former in Central America. The results of nitrogen balance tests are summarized in Table IV by level of protein intake, making no distinction among the three variations of Mixture 9. At adequate levels of protein intake, i.e., 2 gm. per kg. and above, no significant differences between the amounts of nitrogen retained were observed during intake of milk and that of INCAPARINA. At lower levels of intake, retentions with milk were significantly better, although the retentions with Mixture 9 were still positive.

Therapeutic Trials

Mixture 8. The case histories of the first three children with kwashiorkor, PC-65 (E.P.L.), PC-66 (J.A.Q.) and PC-67 (C.A.P.), who were treated with Mixture 8 from the time of admission to the hospital, have already been published.[7] In all three cases, the mixture was well accepted, and recovery was prompt

* Supervised by Dr. Amanda E. Castillo de López, Nutrition Service, Public Health Department, El Salvador.

and complete. The results were indistinguishable from those obtained from comparable patients who were treated with milk, except for a slightly slower regeneration of serum protein.[10]

The case histories of two additional children with acute kwashiorkor are cited below. The first of these, PC-73, was treated from the beginning with Mixture 8, while PC-88 was treated alternately with Mixtures 8A and 8; both recovered uneventfully. A sixth patient, PC-85, who was treated from the beginning with Mixture 8A, recovered normally from kwashiorkor but died subsequently of aspiration pneumonia during recovery from measles. No intolerance to Mixture 8 was noted at any time. However, in two instances, children PC-83 and PC-84 who were initially treated with Mixture 8A for ten days and subsequently treated with Mixture 8 for one month, the treatment with Mixture 8A was discontinued because of excessive vomiting.

PC-73, a boy one year of age, was admitted with severe kwashiorkor; he weighed 7.9 kg. and measured 68 cm. The level of total proteins in his serum at the time of admittance was 3.6 gm. per 100 ml. He was given Mixture 8 at a level of about 2 gm. protein per kg. body weight per day. This was rapidly increased in order to provide an intake of 3 gm. protein and 100 calories per kg. per day by the third day of treatment. Two weeks after admission, the edema had disappeared and he weighed 6.4 kg. After two months on the vegetable mixture, he was considered clinically recovered. His weight at this time was 7.3 kg., and the level of total proteins in his serum was 6.4 gm. per 100 ml.

PC-88, a boy two years and eight months of age, was admitted with moderate kwashiorkor; he weighed 8.4 kg. and measured 76 cm. The level of total proteins in his serum at the time of admittance was 3.4 gm. per 100 ml. Treatment was initiated with Mixture 8A at 2 gm. protein and 60 calories per kg. body weight per day. This amount was increased gradually to a level of 4 gm. protein and 110 calories per kg. per day by the ninth day. Edema began to disappear by the fourth day and the skin lesions were definitely improved by the sixth day. After two weeks, the edema had disappeared completely. The child's minimum weight at this time was 7.4 kg. and there was good improvement of all the other signs and symptoms. In view of the fact that he vomited frequently, he was changed to Mixture 8 which he accepted well without further vomiting. He was kept on this diet for two weeks and then again given Mixture 8A during which time there was no further vomiting. He was kept on Mixture 8A for eight additional weeks by which time complete clinical recovery and a weight of 10 kg. had been attained. The level of total proteins in his serum ten weeks after admission was 6.6 gm. per 100 ml. During treatment he developed measles and recovered uneventfully.

PC-85, a boy one year and eight months of age, was admitted with moderate kwashiorkor; he weighed 7.4 kg. and measured 68 cm. The level of total proteins in his serum was 3.5 gm. per 100 ml. Treatment was initiated with vegetable Mixture 8A at an intake of 2 gm. protein and 60 calories per kg. body weight per day, which was gradually increased to 3.5 gm. protein and 100 calories per kg. by the end of the second week. After seven weeks, edema and deficiency signs had disappeared and satisfactory clinical recovery had been attained. His weight was then 7.1 kg. and the level of total proteins in his serum was 5.5 gm. per 100 ml.

Mixture 9. In addition to the satisfactory results of the metabolic studies, beneficial experience was gained from our treatment of PC-88 and PC-94 before the mixture was used to treat patients with acute kwashiorkor.

PC-88, a boy two years and eleven months of age, was clinically and biochemically completely recovered from kwashiorkor. He weighed 10 kg. Balance studies were begun using other sources of protein. As a result of an accidental fall three months later, he suffered a fracture of the right femur. At this time his weight was 11.3 kg. and his general condition was satisfactory. He was then placed on a diet in which the only source of protein was Mixture 9, with an intake of 3 gm. and later of 3.5 gm. protein and 100 calories per kg. body weight per day. While on this diet he remained in a cast for a period of ten weeks. Satisfactory healing of the fracture occurred. His general condition remained satisfactory, and he gained 1.2 kg. of weight.

PC-94, a boy one year and eleven months of age, was admitted with severe kwashiorkor; he weighed 7.4 kg. and measured 77 cm. The level of total proteins in his serum was 3.4 gm. per 100 ml. He was treated with a diet based on milk and given other therapy as necessary. Three weeks after admission when he was placed on a diet of Mixture 9, he had no edema and the level of proteins in his serum was 5.7 gm. per 100 ml. His skin lesions and other manifestations of deficiency had improved considerably. He was thin, however, weighing only 6.8 kg. and in addition had a decubitus ulcer measuring 8 by 9 cm. which exposed the periosteum of the sacrum. His initial intake was 3 gm. protein and 100 calories per kg. body weight per day which was progressively increased in view of his good appetite and acceptance to 5 gm. protein and 150 calories per kg. A multivitamin-mineral preparation was administered as a dietary supplement. The level of total proteins in his serum continued to increase after Mixture 9 was introduced, and reached 6.6 gm. per 100 ml. two months after admission. At the end of three months he was a happy active child with no clinical signs of nutritional deficiency. He weighed 10 kg., a gain of 3.2 kg. over his minimum weight in nine and a half weeks with Mixture 9B. His decubitus ulcer healed spontaneously without need of grafting or other surgical procedures.

Two children with kwashiorkor, PC-95 and PC-101, were subsequently treated with precooked Mixture 9B in the INCAP metabolic unit. Results were satisfactory although recovery was complicated by an upper respiratory infection in the former and by vomiting in the latter. In order to obtain information more rapidly than would have been possible if the therapeutic trials had been limited to the INCAP metabolic unit, three children with kwashiorkor, EM9-1, EM9-2 and EM9-3, were treated with satisfactory results in the Guatemala General Hospital for one month.* Since the conditions varied, the recovery of these three patients could not be compared directly with those treated in our metabolic unit with either milk or mixtures of vegetables. The case histories of all five children are the following.

PC-95, a boy two years and nine months of age, was admitted with moderate kwashiorkor; he weighed 9.1 kg. and measured 80 cm. The level of total proteins in his serum was 3.6 gm. per 100 ml. During the first week of hospitalization, the child suffered from an upper respiratory infection. He was given precooked Mixture 9B for three weeks, at an intake that varied from 2 to 3.5 gm. protein and 80 to 120 calories per kg. body weight per day. At the end of two weeks, the edema had disappeared and his weight was 8.0 kg. Three weeks after admission, he was changed to regular Mixture 9B at a protein intake that varied from 3.5 to 5 gm. and 120 to 150 calories per kg. per day. One month after admission, the level of total proteins in his serum had reached 5.7 gm. per 100 ml. and his weight 8.6 kg. At this time, signs and symptoms of kwashiorkor had almost completely disappeared. Two months after admission, his weight had reached 9.9 kg. and the level of total proteins in his serum was 7.0 gm. per 100 ml.

* The cooperation of Dr. Ernesto Cofiño and Dr. Carlos Manuel Monzón Malice of the Pediatrics Service, General Hospital, Guatemala, is gratefully acknowledged.

PC-101, a boy five years and two months of age, was admitted with moderate kwashiorkor; he weighed 12.6 kg. and measured 92 cm. The level of total proteins in his serum was 3.0 gm. per 100 ml. He was given Mixture 9 at an intake of 2 gm. protein and 80 calories per kg. body weight per day, which was promptly increased to 3 gm. protein and 90 calories. His protein intake was reduced a week later to 2.5 gm. per kg. because of vomiting, and kept at this level until the fifth week of hospitalization when it was gradually increased to 4.0 gm. per kg. On the tenth day, he was changed to precooked Mixture 9B. His edema began to disappear by the fourth day and was gone by the seventeenth day at which time his weight was 10.8 kg. At the end of the sixth week, the level of total proteins in his serum was 6.3 gm. per 100 ml. and at two months his weight was 13.8 kg.

EM9-1, a boy five years old, was admitted with moderate kwashiorkor; he weighed 13.0 kg. and measured 92 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. He was started on precooked Mixture 9B at an intake that increased from 1.7 gm. protein and 95 calories to 2.96 gm. protein and 129 calories per kg. body weight per day. On the third day of hospitalization this child had serious diarrhea with severe dehydration which required parenteral rehydration and other measures. He was continued on Mixture 9, however, and recovered satisfactorily. His weight after ten days of treatment and loss of edema was 10.4 kg. At the end of one month, the child's level of total proteins in his serum was 6.2 gm. per 100 ml., and his weight at the end of five weeks was 12.1 kg.

EM9-2, a boy two years and two months old, was admitted with severe kwashiorkor; he weighed 7.8 kg. and measured 72 cm. The level of total proteins in his serum was 2.9 gm. per 100 ml. He was started on precooked Mixture 9B at an intake of 1.3 gm. protein and 75 calories per kg. body weight. This was increased gradually to 5 gm. protein and 150 calories per kg. body weight. After eight days of treatment, although his edema had practically disappeared, the hyperpigmentation had increased and the skin lesions had become more extensive. On the eleventh day of hospitalization, cheilosis and cheilitis were more evident than at the time of admission. At the end of two weeks, his skin was clear and the oral lesions had disappeared. His dry weight after sixteen days of treatment was 6.1 kg., remaining practically stationary for the remainder of the period of observation. On the sixteenth day of hospitalization, an abscess developed on his right arm. During virtually the entire period of treatment, he had a chronic upper respiratory infection and elevations in temperature varying up to 40°c. At the end of one month, he weighed 6.4 kg. and the level of total proteins in his serum was 4.7 gm. per 100 ml. The child had progressed satisfactorily but because of the numerous complications he did not achieve the same state of health as the preceding patient.

EM9-3, a boy three years old, was admitted with edema and moderate kwashiorkor, weighed 9 kg. and measured 78 cm. The level of total proteins in his serum was 3.5 gm. per 100 ml. Precooked Mixture 9B was administered at an intake of 1.8 gm. protein and 100 calories per kg. body weight per day which was gradually increased to 4 gm. protein and 120 calories per kg. Although his edema began to disappear after two days of treatment, it had not completely disappeared in eleven days; he was still apathetic and anorexic and had to be fed by gavage. He also had a chronic upper respiratory infection, and after three weeks of hospitalization developed styes, first in the left eye and later in the right eye. His weight after twenty-eight days of treatment was 7.8 kg., 0.5 kg. over his minimum weight. The level of total proteins in his serum was 4.9 gm. His recovery at the end of thirty days was still incomplete but not inconsistent with that which might have occurred with milk in a case with similar complications.

Corn and Beans. In order to determine whether or not mixtures, known to be definitely inferior to milk in the quality of protein, would give a satisfactory therapeutic response in patients with kwashiorkor if a sufficient quantity of protein could be administered, attempts were made to treat three children, PC-89, PC-90 and PC-91, with a mixture in which approximately half of the protein was furnished by corn and the rest by beans. As the following case histories indicate, this failed completely and such experiments were discontinued.

PC-89, a boy one year and five months of age, was admitted with moderate kwashiorkor; he weighed 7.7 kg. and measured 70 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. After one day on electrolytes, he was placed on a diet of corn and beans (corn protein:bean protein ratio [43:57]) at an intake of 2 gm. protein and 90 calories per kg. body weight per day. A month after admission his diet was changed to skim milk at the same level of protein and calorie intake. At this time the level of total proteins in his serum was 4.3 gm. per 100 ml. and his weight was 7.1 kg. Some edema was still present. His skin lesions had not cleared completely and he still showed irritability and some apathy. During this time, cheilosis and cheilitis appeared. It was found impossible to cure this patient with a diet of corn and beans.

PC-90, a boy three years and five months of age was admitted with moderate kwashiorkor; he weighed 9.4 kg. and measured 80 cm. The level of total proteins in his serum was 5.3 gm. per 100 ml. After one day on electrolytes, he was given an ad libitum diet of corn and beans (43:57) at a level up to 2 gm. protein and 90 calories per kg. body weight per day. Because of failure to improve after twelve days, he was given a milk diet. At this time the level of total proteins in his serum was 5.0 gm. per 100 ml. and his weight was 9.1 kg. No improvement in mental symptoms had occurred; skin and mucous lesions and edema were also present.

PC-91, a boy five years and six months old, was admitted with moderate kwashiorkor; he weighed 11.4 kg. and measured 86 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. After one day of electrolytes, he was placed on a diet of corn and beans (43:57) at an intake of 2 gm. of protein and 70 calories per kg. body weight per day. Because of failure to improve, he was changed to milk one week later.

Biologic Value Determinations

Studies have also been initiated to determine the biologic value of Mixture 9B, milk and the combination of corn and beans. The experimental design required a nitrogen-free diet to be fed for four days, and the test mixture at an intake of 0.5 gm. protein per kg. per day for four additional days following a four day adaptation period. Caloric intake was held constant at 90 calories per kg. per day. During the "nitrogen-free period," actual intake of nitrogen by the three children studied to date varied between 16 and 21 mg. per day, and the nitrogen absorbed varied from 2 to minus 6 per cent. Under these conditions, the calculated biologic value for milk in child PC-101 was 92 per cent, for Mixture 9B, in PC-95 it was 88 per cent and for the combination of corn and beans in PC-91, it was 51 per cent.

Effect of Fat on Nitrogen Retention

Systematic investigations of the effect of fat on nitrogen balance are still in progress in the INCAP metabolic unit, but preliminary results seem to indicate that nitrogen retention is not influenced by the presence or absence of fat at the levels of protein and caloric intake maintained in these studies. In eleven pairs of trials of vegetable mixtures, both with and without fat, average nitrogen retentions were 12.9 per cent and 13.8 per cent of intake, respectively; the difference was not statistically significant. Protein intakes in these comparisons varied from 1.9 to 4.3 and averaged 2.7 gm. per kg. body weight.

COMMENTS

The foregoing data demonstrate that, when the protein intake is adequate for the needs of the child, the body is able to retain as much nitrogen from the vegetable mixtures tested as from an equivalent amount of protein in milk. The results obtained in the treatment of patients with kwashiorkor also indicate that the protein of the vegetable mixtures is of relatively good quality. Nevertheless, the absorption of nitrogen as per cent of intake is always significantly less with the vegetable mixtures than with milk. Data are also presented which suggest that during inadequate intakes of protein proportionately more nitrogen is retained from protein in milk.

Since biochemical analyses have shown the vegetable mixtures tested to be deficient in several amino acids in comparison to milk or the amino acid pattern of the FAO reference protein,[10] it is not immediately clear why they should give such good results both in previous trials with rats and chicks and in the present studies with children. It would appear that a more favorable pattern of amino acids is absorbed by the child or experimental animal than is measured by microbiologic analysis following acid hydrolysis. It is perhaps significant in this regard that more of the nitrogen consumed in vegetable mixtures remains unabsorbed than when milk is the source of protein.

Direct evidence that Mixture 9B is comparable to milk in the pattern of amino acids absorbed from the gastrointestinal tract is provided by the studies of Arroyave et al.[11] They found that the pattern of amino acids in serum after a standard test meal was similar, whether milk or Mixture 9B furnished the protein in the diet, and that it was quantitatively and qualitatively different when a combination of corn and beans furnished the dietary protein.

Since the proportions of ingredients in INCAP Mixtures 8 and 9 were determined after extensive biologic trials with chicks, rats and pigs, it is not surprising that they represent efficient combinations of the ingredients. It should be noted, however, that vegetable proteins can be combined in such a way that their protein value as fed is superior to that predicted from their contents of amino acids alone.

It is theoretically possible that the protein-depleted child is less selective in his needs for nitrogen, and perhaps can use efficiently, patterns of amino acids which would not give as good results in well nourished persons. We do not know of any evidence to support such a hypothesis, and the present studies indicate that regardless of the total protein intake even the relatively good pattern of amino acids of corn and beans, compared to that of corn alone, was not sufficient for the treatment of children with kwashiorkor. Brock and Hansen report a similar outcome of their efforts to treat patients with kwashiorkor with a mixture of corn and cowpeas; satisfactory "initiation of cure" process was obtained only when corn germ was added to the combination.[12] Furthermore, experimental studies with animals have established firmly that differences in the quality of proteins are best detected through the repletion thereof in an animal which has been depleted of proteins.[13]

As previous studies of children fed corn diets supplemented with amino acids demonstrate, at low levels of protein intake deficiencies and imbalances of amino acids have a more pronounced adverse effect on nitrogen retention.[14] Therefore, it is to be expected that differences in the quality of protein between corn and Mixture 9 and its variations, which would not be apparent at relatively high levels of intake, may be detectable at low levels. This is confirmed in the data presented.

For the practical purpose of preventing protein malnutrition, all that is required of a vegetable mixture is a sufficient protein quality and concentration to be effective in the form in which it is to be consumed.

It should be stressed that all of the clinical trials have been with children over one year of age and that the mixtures have not yet been tested as milk substitutes for the artificial feeding of infants. Although they may prove suitable for this purpose, they have been developed for the supplementary and mixed feeding of infants and young children and as a low cost source of good quality protein for persons of all ages in areas where protein of animal origin is expensive or in short supply.

The data presented indicate that both Mixture 8 and Mixture 9 and its variations meet these criteria and can be highly recommended for the intended purpose. For reasons previously reported, Mixture 9B is the most economical of the formulas for the Central American area.[15] It is already in widespread use in Guatemala under the generic name INCAPARINA, and should prove useful wherever cottonseed flour suitable for human consumption can be made available at low cost.

SUMMARY

Low cost vegetable mixtures containing over 25 per cent protein, intended for use in the prevention of protein malnutrition in technically underdeveloped areas, were fed experimentally to young children. These mixtures had been previously demonstrated to have a relatively high protein value by biologic trials with experimental animals. Mixture 8 contained 50 per cent lime-treated corn, 35 per cent sesame flour, 9 per cent cottonseed flour, 3 per cent Torula yeast and 3 per cent dehydrated leaf meal. Mixture 9B, the more practical for Central America, included 29 per cent each of uncooked ground corn and sorghum, 38 per cent cottonseed flour, 3 per cent Torula yeast, 1 per cent $CaCO_3$ and 4,500 I.U. vitamin A acetate.

In eight children, each fed at an average protein intake of 2.8 gm. per kg. per day for five day balance periods, 19.1 per cent of the nitrogen intake was retained with Mixture 8 and 20.5 per cent with milk. In nine children, forty-eight balance periods on each diet at a protein intake between 2.0 and 3.0 gm. per kg. per day gave an average retention of 16.3 per cent for milk and 17.8 per cent for Mixture 9B. In thirteen balance periods at higher levels of intake, nitrogen retentions were essentially the same for milk and Mixture 9B; but in sixteen periods at levels of 1 gm. per kg. per day and below, retentions with milk were higher. No effect of fat on nitrogen retention was detected when it was added to either of the mixtures.

Six children with kwashiorkor were treated with Mixture 8 or a lower fat variation, Mixture 8A, and five children were treated with Mixture 9B. Results were similar to those previously obtained with milk. In field trials, involving 115 children in Guatemala for fifteen to nineteen weeks and fifty-three children in El Salvador for four weeks, approximately 97 per cent of the offerings were accepted and only 3 per cent rejected. On the basis of the extensive and favorable biologic and clinical findings, both mixtures are recommended for the supplementary and mixed feeding of infants and young children and as low cost protein-rich foods of good quality for persons of all ages in any area where the basic ingredients are available and where protein of animal origin is expensive or in short supply.

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