Use of Isolated Vegetable Proteins in the Treatment of Protein Malnutrition (Kwashiorkor)

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It is well recognized that the incidence of protein malnutrition is high among weaned infants and young children in many tropical and subtropical countries.[1] The foodstuffs which have been extensively used in the treatment of protein malnutrition are half cream milk, skim milk powder and calcium caseinate.[2] In view of the shortage in the supply of these foods in several technically underdeveloped countries, several workers have investigated the possible use of protein-rich foods of vegetable origin, e.g, oil seeds, oil seed meals and legumes, in the treatment of kwashiorkor.[3,4] Soy bean, sesame and cottonseed meals and blends of chick pea (*Cicer arietinum*), peanut, peanut flour and skim milk powder have been found by some workers to be effective in the treatment of patients with mild and moderately severe cases of kwashiorkor.[4–6] For the effective treatment of protein malnutrition, large amounts of the protein-rich foods (about 120 to 250 gm.) providing about 50 to 60 gm. of protein have to be administered. For example, Venkatachalam et al.[5] gave each child 250 gm. of chick pea dhal, 100 gm. ripe banana and 70 gm. powdered jaggery daily. Dean[6] used 300 gm. of cooked soy bean paste, 300 to 400 gm. mashed ripe banana and 25 gm. sugar for the successful treatment of kwashiorkor in children. Because of the severe anorexia and impaired digestion commonly observed in patients with kwashiorkor, it is generally difficult to administer large quantities of protein-rich foods. Consequently, it may be necessary to resort to tube feeding during the first week of treatment in severe cases.[7] Further, in contrast to milk and casein, oil seed meals and legumes contain indigestible polysaccharides which may cause digestive upsets. Therefore, it was thought that isolated oil seed proteins, which are two to four times as rich in protein as oil seed meals and legumes, may prove more suitable for the treatment of kwashiorkor than the parent materials. These can be administered along with readily assimilable carbohydrates such as glucose, cane sugar and dextri-maltose. In addition, protein isolates have a bland flavor and are free from indigestible carbohydrates and odoriferous or bitter principles which are present in several legumes and oil seed meals. Proteins of nuts, oil seeds and legumes are partially deficient in one or more of the essential amino acids and hence have a lower nutritive value than animal proteins. It has been shown, however, by a number of workers that the nutritive value of oil seed and legume proteins can be improved by judicious blending of different proteins or by fortification with the amino acids they lack.[8]

The present report relates to studies on the efficacy of the following protein blends in the treatment of kwashiorkor. Blend 1, a mix-

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TABLE I
Formulas of Protein Blends*

Ingredient	Protein Blend		
	I	II	III
Peanut protein (%)	30	52	66.6
Soy bean protein (%)	30	...	...
Casein (%)	40	...	...
Skim milk powder (%)	...	48	33.3
l-Lysine hydrochloride (%)	0.75	...	...
dl-Methionine (%)	1.96	...	...
Protein content (nitrogen × 6.25) (%)	78.8	61.2	68.2

* Mineral salts per 100 gm. of protein blend: dipotassium hydrogen phosphate, 3.5 gm.; disodium hydrogen phosphate, 2.0 gm.; potassium bicarbonate, 1.0 gm.; tricalcium phosphate, 0.5 gm.; calcium carbonate, 0.5 gm.

TABLE II
Essential Amino Acid Content of Protein Blends as Compared to Milk Protein
(Calculated to 16 gm. Nitrogen)

Amino Acid	Protein Blend			Milk Protein	FAO Reference Protein Pattern
	I	II	III		
Lysine	7.0	4.5	4.3	7.4	4.2
Tryptophan	1.3	1.2	1.1	1.4	1.4
Methionine	4.3	1.4	1.2	2.8	2.2
Methionine plus cystine	5.3	2.8	2.6	3.9	4.2
Threonine	3.8	3.3	3.1	4.6	2.8
Phenylalanine	5.3	5.3	5.1	5.5	2.8
Leucine	8.3	7.9	6.7	12.1	4.8
Isoleucine	5.6	4.7	4.5	6.7	4.2
Valine	5.9	5.3	5.1	7.1	4.2

TABLE III
Age and Sex Incidence of Kwashiorkor
(Forty-Five Patients)

Age (mo.)	Boys (no.)	Girls (no.)
9–12	1	1
13–24	6	5
25–36	10	11
Over 36	6	5

ture of peanut and soy bean protein isolates and casein fortified with l-lysine and dl-methionine. Blend II, a mixture of 52 parts of peanut protein isolate and 48 parts of skim milk powder. Blend III, a 2:1 mixture of peanut protein isolate and skim milk powder, as compared with skim milk powder.

EXPERIMENTAL

Materials

The composition of protein blends I, II and III used in this experiment is given in Table I. Peanut and soy bean protein isolates were obtained from edible quality peanut and soy bean meals according to Anantharaman et al.[9] Skim milk powder (spray dried) and lactic casein were obtained locally. The protein samples were powdered to pass through a 60 mesh sieve. The protein blends I, II and III were prepared by mixing the different ingredients in suitable proportions as indicated in Table I. The protein content of the blends determined by the Kjeldahl method is also given in the same table. The amino acid composition of the proteins of blends I, II and III calculated using values given in

TABLE IV
Clinical Signs and Symptoms Observed in Forty-Five Patients with Kwashiorkor

Symptoms and Signs	No.
Diarrhea	45
Edema of the limbs	45
Generalized edema	2
Skin changes	
Dry scaly skin with crazy pavement	44
Pigmentation	37
Ulceration	5
Desquamation	15
Hair changes	
Discoloration	15
Brittle and sparse	12
Eye changes	
Keratomalacia	3
Bitot's spots	4
Moderate xerosis	18
Mild xerosis	20
Angular stomatitis	
Mild	10
Severe	15
Glossitis	
Mild	15
Severe	10
Bleeding gums	
Mild	10
Severe	20

the literature,[10] as compared with that of milk protein and FAO reference protein pattern,[11] is given in Table II. Since mineral and vitamin deficiencies have also been found in children suffering from kwashiorkor, the subjects were given supplements of mineral salts and vitamins. The different mineral salts added to the protein blends are given in Table I. Each subject received the following quantities of different vitamins per day: vitamin A, 3000 I.U.; vitamin D, 400 I.U.; thiamine, 0.5 mg.; riboflavin, 0.9 mg.; niacin, 5 mg.; pyridoxine, 0.5 mg.; and calcium pantothenate, 2.5 mg.

Subjects

In all, forty-five patients with kwashiorkor (one to five year old) were treated with the different protein blends and skim milk powder. The age and sex incidence of kwashiorkor are given in Table III. The patients were clinically examined immediately after admission. Data regarding the clinical signs and symptoms observed in the subjects are given in Table IV. Except for traces of albumin, the urine in all patients was normal. No parasitic ameba or cysts (except round worm ova in some subjects) were found in the stools. Anemia was present to varying degrees in all patients. Blood samples were analyzed for the following constituents soon after admission; red blood cell count and hemoglobin according to Reddy et al.[12] serum proteins (total protein, albumin and globulin), total cholesterol,

TABLE V
Biochemical Findings in Forty-Five Patients with Kwashiorkor

Findings	Range	Mean
Red blood cell count (10^6/cu. mm.)	1.6–3.5	2.6
Hemoglobin (%)	4.6–8.5	6.8
Serum (%)		
Total protein	3.4–4.9	3.9
Albumin	1.3–2.1	1.7
Globulin	1.4–3.2	2.2
Blood		
Urea (mg./100 ml.)	15–43	26.5
Total cholesterol (mg./100 ml.)	110–145	120
Thymol turbidity	+ – ++	

TABLE VI
Changes in the Body Weight and Time Taken for the Disappearance of Edema and Diarrhea in Patients with Kwashiorkor Treated with Different Protein Blends*

Data	Blend I	Blend II	Blend III	Skim Milk Powder
No. of patients treated	10	10	10	15
Age (yr.)	2½–5	2–5	1–5	1–5
Body weight (kg.)				
Range	7.2–11.9	6.9–12.0	5.9–11.5	5.8–12.2
Mean	8.8	8.6	8.2	8.5
Protein administered (gm./child/day)				
From protein blend	30	30	30	30
From cereals	8–10	8–10	8–10	8–10
No. of days taken for clinical disappearance of edema				
Range	3–10	8–16	7–18	8–19
Mean	5.8	12.4	14.5	12.3
No. of days taken for disappearance of diarrhea				
Range	2–8	4–15	5–12	4–16
Mean	3.5	6.5	6.2	9.8
No. of days taken for attaining minimum body weight				
Range	4–12	8–17	8–19	10–20
Mean	6.2	13.5	14.6	12.8
No. of days taken for an increase of 1 pound body weight calculated from 1st day of reaching minimum weight				
Range	3–6	7–11	6–15	6–14
Mean	4.2	8.9	10.0	7.5

* Composition of blends given in Table I.

TABLE
Increase in Total Serum Protein, Albumin and Globulin in Patients Blends and Skim

Group	Protein Source	Total Protein (%)				
		Initial	10th Day		30th Day	
			Total	Increase*	Total	Increase*
A	Protein blend I	3.88	5.56	1.68 ±0.06	6.82	2.94 ±0.08
B	Protein blend II	4.01	5.36	1.35 ±0.06	6.20	2.19 ±0.08
C	Protein blend III	3.84	5.03	1.19 ±0.06	5.80	1.96 ±0.08
D	Skim milk powder	4.02	5.53	1.51 ±0.05	6.90	2.88 ±0.08
	Test of difference of mean:					
	A∽B			t = 3.88†		t = 7.08†
	A∽C			t = 5.77†		t = 9.25†
	A∽D			t = 2.21‡		t = 0.62§
	B∽C			t = 1.88§		t = 2.17‡
	B∽D			t = 2.08‡		t = 7.11†
	C∽D			t = 4.16†		t = 9.49†

* Mean with standard error based on 41 degrees of freedom from the analysis of variance from the data for all the groups.
† Significant at 0.1 per cent level.

urea and thymol turbidity test according to King and Wootton.[13] The results are summarized in Table V.

Treatment

The children, as and when admitted, were assigned to one of four groups (identified as A, B, C and D) so that as far as possible each group consisted of cases of equal degree of severity. The protein blends I, II and III were reconstituted in nine times their weight of boiling water and sweetened with cane sugar and glucose. Skim milk powder was reconstituted with six times its weight of water and sweetened with cane sugar. The supplements were administered orally in four or five doses daily. The quantity of protein feeds was gradually increased until each child in groups A, B, C and D received 30 gm. protein from blends I, II, III and skim milk powder, respectively, by the end of the first week of treatment. This was established in most of the cases within the first week of treatment. The vitamin supplements were given along with one feed. Additional calories were provided in the form of cooked rice, bread and vegetable soup. The protein derived from these sources was about 8 to 10 gm. daily. The protein intake by the patients was approximately 4 to 5 gm. per kg. body weight. The calories intake per day was gradually increased to about 120 calories per kg. body weight; this was achieved in the majority of cases in about seven to ten days from the day of admission. The children were weighed daily in the morning at a fixed time before administering protein food. Specimens of blood were taken on admission and on the tenth and thirtieth day of treatment for the estimation of serum proteins.

RESULTS

The results of treatment of kwashiorkor with protein blends I, II and III and skim milk powder are given in Tables VI and VII and briefly described in the following paragraphs. One of the subjects before and after treatment with blend III is shown in Figures 1 and 2.

Disappearance of Edema

After a few days of treatment, the edema began to decrease and disappeared completely as treatment was continued. The average time taken for the disappearance of the edema

VII
with Kwashiorkor Treated with Different Protein Milk Powder

Albumin (%)					Globulin (%)				
Initial	10th Day		30th Day		Initial	10th Day		30th Day	
	Total	Increase*	Total	Increase*		Total	Increase	Total	Increase
1.66	2.76	1.10 ±0.06	3.76	2.10 ±0.12	2.22	2.80	0.58	3.06	0.84
1.72	2.61	0.89 ±0.06	3.32	1.60 ±0.12	2.29	2.75	0.46	2.88	0.59
1.62	2.37	0.75 ±0.06	3.02	1.40 ±0.12	2.22	2.66	0.44	2.78	0.56
1.71	2.71	1.00 ±0.05	3.91	2.20 ±0.10	2.31	2.82	0.51	2.99	0.68
		t = 2.39‡		t = 2.98‡					
		t = 3.98†		t = 4.17†					
		t = 1.24§		t = 0.65§					
		t = 1.59§		t = 1.19§					
		t = 1.36§		t = 3.92†					
		t = 3.09‖		t = 5.23‖					

‡ Significant at 5 per cent level.
§ Not significant.
‖ Significant at 1 per cent level.

was 5.8 days with blend I, 12.4 days with blend II, 14.5 days with blend III and 12.3 days with skim milk powder. It is evident that blend I was superior to the others in this respect.

Disappearance of Diarrhea

Diarrhea began to subside within a week of the institution of protein therapy in a large number of cases. The average time taken for the stoppage of diarrhea was 3.5 days with blend I, 6.5 days with blend II, 6.2 days with blend III and 9.8 days with skim milk powder. The results indicate that blends I, II and III were superior to skim milk powder in this respect.

Minimum Body Weight

The time taken to attain minimum body weight also varied with the different protein supplements. The average time taken for the attainment of minimum body weight was 6.2 days with blend I, 13.5 days with blend II, 14.6 days with blend III and 12.8 days with skim milk powder.

Increase in Body Weight

The time taken for a 1 pound increase in body weight after reaching the minimum weight also varied with the different protein foods. The average time taken was 4.2 days with protein blend I, 8.9 days with protein blend II, 10.0 days with protein blend III and 7.5 days with skim milk powder. It is evident that protein blend I was superior to the other blends and skim milk powder.

Increase in Serum Proteins

The rate of increase of serum proteins and serum albumin varied with different protein blends. Both protein blend I and skim milk powder promoted regeneration of total serum proteins and serum albumin to a significantly greater extent than protein blends II and III.

COMMENTS

The results indicate that the three protein blends are almost as effective as skim milk powder in the treatment of kwashiorkor.

FIG. 1. Boy, three years and six months of age with kwashiorkor, showing edema of legs, hands, crazy pavement dermatosis and hyperpigmented areas.

FIG. 2. Same boy after treatment with protein blend III for thirty days.

The rate of regeneration of serum proteins and serum albumin varied with the different protein blends. Both protein blend I and skim milk powder promoted regeneration of total serum proteins and albumin to a greater extent than protein blends II and III. This may be due to the fact that the amounts of lysine and methionine are less in blends II and III than in blend I and skim milk powder. It is of interest to note that diarrhea stopped earlier in children receiving protein blends I, II and III as compared with those given the skim milk powder. The results obtained in the present study are in conformity with those of Dean[14] who found that the administration of 30 to 40 gm. of protein daily in the form of a biscuit containing 20 per cent protein (15 per cent from peanut and 5 per cent from skim milk powder) was as effective as skim milk powder in the treatment of kwashiorkor. The biscuit used by Dean[14] has the disadvantage of being low in protein, thus necessitating the administration of 150 to 200 gm. of biscuit daily per child.

The results obtained in the present study have been independently confirmed by Gopalan,* of the Nutrition Research laboratories, Hyderabad, India, and also by Drs. Webb and Dumm* of the Christian Medical College and Hospital, Vellore, India. It may be concluded from our results and from those obtained by Gopalan, Webb and Dumm, that a 2:1 blend of groundnut protein isolate and skim milk powder could be used with advantage in the treatment of kwashiorkor. The cost of blend

* Personal communications.

III is estimated to be approximately one rupee per pound and is of the same order as that of skim milk powder. It has the added advantage of being twice as rich in protein as skim milk powder. The other important advantages of protein blends based on protein isolates are the small bulk, ease of administration and ready digestibility. There is considerable scope for the production and use of vegetable protein isolates in the treatment of protein malnutrition in many tropical and subtropical countries.

SUMMARY

Forty-five children with kwashiorkor aged one to five years were treated with three protein blends: Blend I, a mixture of isolated peanut and soy bean proteins and casein fortified with dl-methionine and l-lysine. Blend II, a mixture of 52 parts of peanut protein and 48 parts of skim milk powder. Blend III, a mixture of 66.6 parts of peanut protein and 33.3 parts of skim milk powder.

Each child in different groups received 30 gm. of protein daily from blends I, II, III or skim milk powder. The protein blends were readily digested by the children; diarrhea stopped in a shorter time in children receiving the protein blends than in those receiving skim milk powder.

The different protein blends compared favorably with skim milk powder in their effectiveness in the treatment of kwashiorkor. The rate of regeneration of serum albumin with protein blend I and skim milk powder was significantly greater than that observed with protein blends II and III.

The serum protein contents and albumin: globulin ratio were brought to normal levels after treatment with the different protein blends for a period of thirty days.

The results indicate that a 2:1 blend of peanut protein isolate and skim milk powder can be used with considerable advantage in the treatment of kwashiorkor.

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REFERENCES

1. Jelliffe, D. B. Infant nutrition in sub-tropics and tropics. WHO Monograph, No. 29, Geneva 1955.
2. Trowell, H. C., Davies, J. N. P. and Dean, R. F. A. Kwashiorkor. London, 1954. Edward Arnold Publishers Ltd.
3. Narayana Rao, M. and Swaminathan, M. Processed protein foods of vegetable origin. *Ann. Rev. Food Technol.*, 1: 73, 1960.
4. Behar, M., Bressani, R. and Scrimshaw, N. S. Treatment and prevention of kwashiorkor. In: World Reviews of Nutrition and Dietetics, p. 77. London, 1959. Pitman Medical Publishing Co.
5. Venkatachalam, P. S., Srikantia, S. G., Mehta, G. and Gopalan, C. Treatment of nutritional oedema syndrome (kwashiorkor) with vegetable protein blends. *Indian J. M. Res.*, 44: 539, 1956.
6. Dean, R. F. A. The treatment of kwashiorkor with milk and vegetable proteins. *Brit. M. J.*, 2: 791, 1952.
7. Jelliffe, D. B. and Dean, R. F. A. Protein-calorie malnutrition in early childhood. *J. Trop. Pediat.*, 5: 96, 1959.
8. Joseph, K., Narayana Rao, M., Swaminathan, M. and Subrahmanyan, V. The nutritive value of protein blends having amino acid composition similar to that of FAO reference protein pattern. *Ann. Biochem. & Exper. Med.*, 20: 243, 1960.
9. Anantharaman, K., Subramanian, N., Bhatia, D. S. and Subrahmanyan, V. Processing of groundnut cake for edible protein isolate. *Indian Oil Seeds J.*, 3: 85, 1959.
10. Altschul, A. M. Processed plant protein food stuffs. New York, 1958. Academic Press Inc.
11. Food and Agriculture Organization, Nutritional Studies No. 16. Protein Requirements. Report of the FAO Committee, Rome, 1957.
12. Reddy, S. K., Doraiswamy, T. R., Sankaran, A. N., Swaminathan, M. and Subrahmanyan, V. Effects on the general health and nutritional status of children of partial replacement of rice in a poor vegetarian diet by tapioca flour. *Brit. J. Nutrition*, 8: 17, 1954.
13. King, E. J. and Wootton, I. D. P. Micro-analysis in Medical Biochemistry. London, 1956. J. & A. Churchill, Ltd.
14. Dean, R. F. A. Treatment of kwashiorkor with moderate amounts of protein. *J. Pediat.*, 56: 675, 1960.