The Development of INCAP Vegetable Mixtures

II. Biochemical Testing[1]

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THE RESULTS of basic animal studies leading to the development of vegetable mixtures for human feeding in the Institute of Nutrition of Central America and Panama (INCAP) have been presented by Dr. Bressani.[1] The formulas for three of these, Mixtures 8, 8A and 9, which have been extensively tested in clinical trials in young children, are shown in figure 1. As Dr. Scrimshaw will report,[2] these mixtures have given results similar to those obtained with milk as a protein source in comparative metabolic studies in children who have recovered from severe protein malnutrition, and in therapeutic trials in children with the acute syndrome.

In planning feeding mixtures for human consumption, however, as many criteria as are available should be applied to evaluate the quality and safety of such foods. In addition to the biological studies in experimental animals reported by Dr. Bressani,[1] and to the nitrogen balance and therapeutic trials in children, other biochemical tests to which INCAP Vegetable Mixtures 8, 8A and 9 have been submitted include their effect in promoting the regeneration of serum proteins in the therapeutic trials, and the study of the plasma amino acid pattern before and after a test feeding in children who have been receiving either milk or one of the vegetable mixtures.

REGENERATION OF SERUM TOTAL PROTEINS IN KWASHIORKOR

Serum proteins are a useful and simple biochemical measure of the extent of initial recovery of a child who is being treated for kwashiorkor. For practical purposes, their measurement in these cases has the same significance as that of albumin, and a marked increase in their concentration is a good prognostic sign. The density gradient method of Lowry and Hunter[3] can give the results within less than an hour after the blood sample collection, employing only 5-10 μl of

[1]INCAP Publication I-174

En: "Progress in Meeting Protein Needs of Infants and Preschool Children". Proceedings of an International Conference held in Washington, D. C. August 21-24, 1960. Washington, D. C., National Academy of Sciences-National Research Council, 1961, p. 49-55. Publication 843.

FORMULAS FOR INCAP VEGETABLE MIXTURES FOR HUMAN FEEDING

		8	8A	9	9A	9B
Corn	Lime-treated	50	50	28	---	---
	Cooked	---	---	---	29	---
	Uncooked	---	---	---	---	29
Sesame flour	33% fat	35	---	---	---	---
	18% fat	---	35	---	---	---
Sorghum	Lime-treated	---	---	28	---	---
	Cooked	---	---	---	29	---
	Uncooked	---	---	---	---	29
Cottonseed flour		9	9	38	38	38
Torula yeast		3	3	3	3	3
Dehydrated leaf meal		3	3	3	---	---
$CaCO_3$		---	---	---	1	1
Vitamin A		---	---	---	4500	4500

Figure 1

serum. Using this procedure, we have compared serum protein regeneration in a number of patients with kwashiorkor who were given therapeutic diets of either Mixture 8, 8A or 9, at levels of nitrogen intake varying in each child from 2 to 5 gm /kg/day. These patients were compared with patients receiving milk at levels of intake not exceeding those of the children given the vegetable mixtures, usually 3 to 4 gm/kg/day. The results of this comparison are represented graphically in figure 2.

The milk treatment resulted in consistent increases in serum total protein (fig. 2A). All three children gained around 3 gm/100 ml in a period of about 4 weeks. The children treated with Mixture 8, 8A or 9 (figs. 2B, 2C and 2D), attained a level of gain around 6 gm/100 ml in 4 to 5 weeks of treatment; it appears that this level was reached regardless of the initial serum protein concentration; PC-67, for example, starting with the highest value, showed the least increment.

From these results, it may be concluded that although higher serum protein values are attained in the same period of treatment with milk, INCAP Vegetable Mixtures 8 and 9 have sufficiently high protein quality to meet the high nitrogen demands of a severely protein-depleted child for plasma protein synthesis.

Plasma Amino Acid Levels After a Protein Test Feeding

The comparative effect of milk, Mixture 9 and corn-bean diets on the plasma amino acid increases of children after a milk feeding is also of interest.

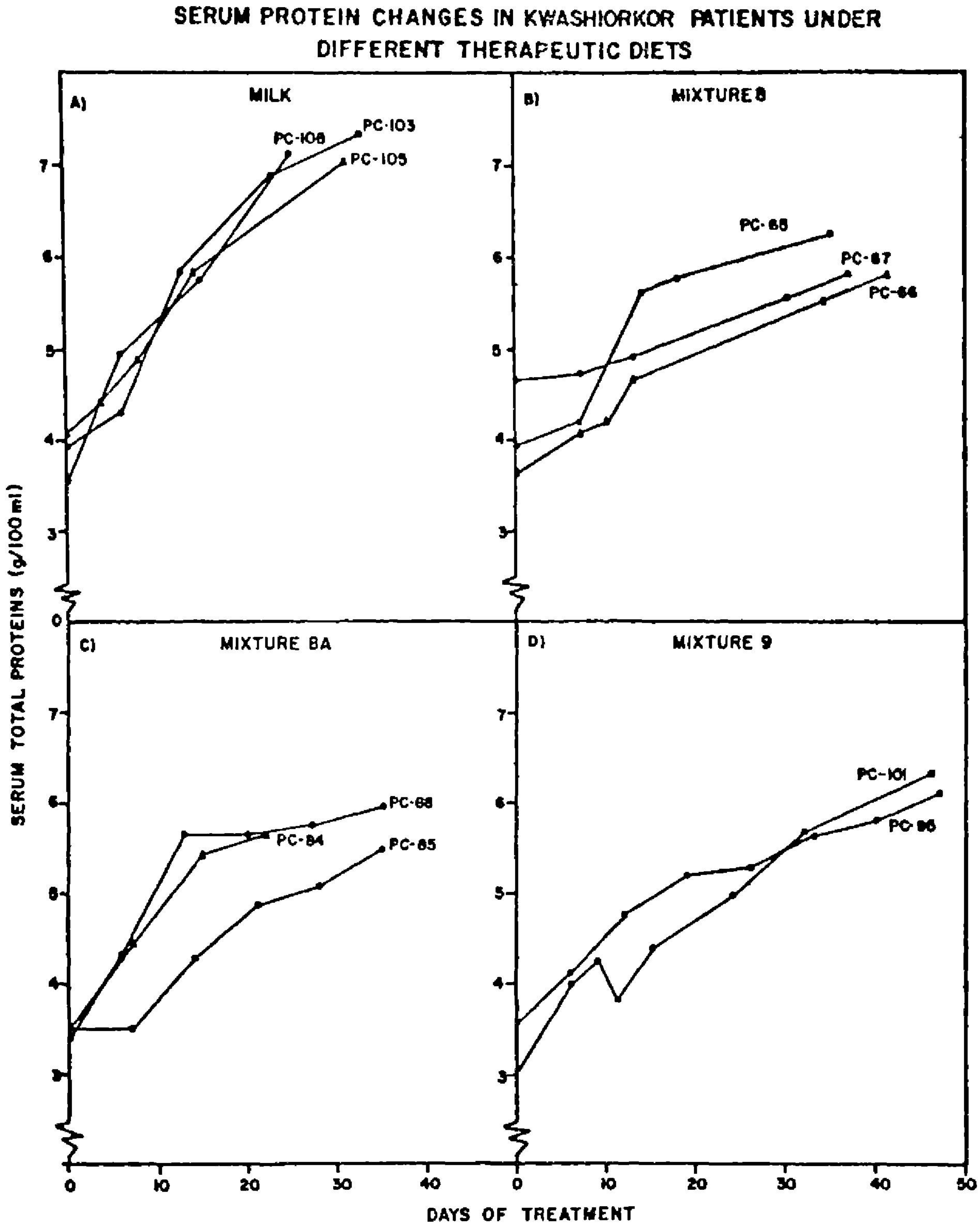

Figure 2

Observations in dogs and children indicate that the plasma amino acid increases after a standard protein feeding are influenced by the physiological state of the subject as determined by the previous diet (INCAP unpublished data). A decreased response seems to occur after a protein of poor nutritional value has been fed for some time before the test.

In view of these preliminary observations, 2 experiments were designed with 3 children each, for the specific objective of comparing the influence of milk, Mixture 9 or a corn-bean combination on the plasma amino acid responses to a milk feeding.

Experiment 1. Child PC-97, a boy 3 years and 8 months old, was maintained on milk at 2 gm protein and 90 cal/kg/day for 6 weeks, followed by 3 days on a nitrogen-free diet immediately preceding the test. Child PC-95, a boy 3 years and 7 months of age, received INCAP Vegetable Mixture 9 at the same protein and calorie intake for 3 months, followed also by 3 days on a nitrogen-free diet just before the test. Child PC-92, a boy 3 years and 6 months old, received a diet in which 50% of the protein was from corn and 50% from beans, also at 2 gm protein and 90 cal/kg/day for 7 weeks, followed by 4 days on a nitrogen-free diet immediately before the test. The period of nitrogen-free diet was included to determine endogenous nitrogen excretion for other purposes.

After a 16-hour overnight fasting a blood sample was taken and the test meal of skim milk at 2 gm protein/kg was administered to the 3 children. A postprandial blood sample was taken 2½ hours later. The results of plasma amino acid analysis are shown graphically in figure 3. In each block, representing one essential amino acid, the increases observed in the child fed milk are shown in the

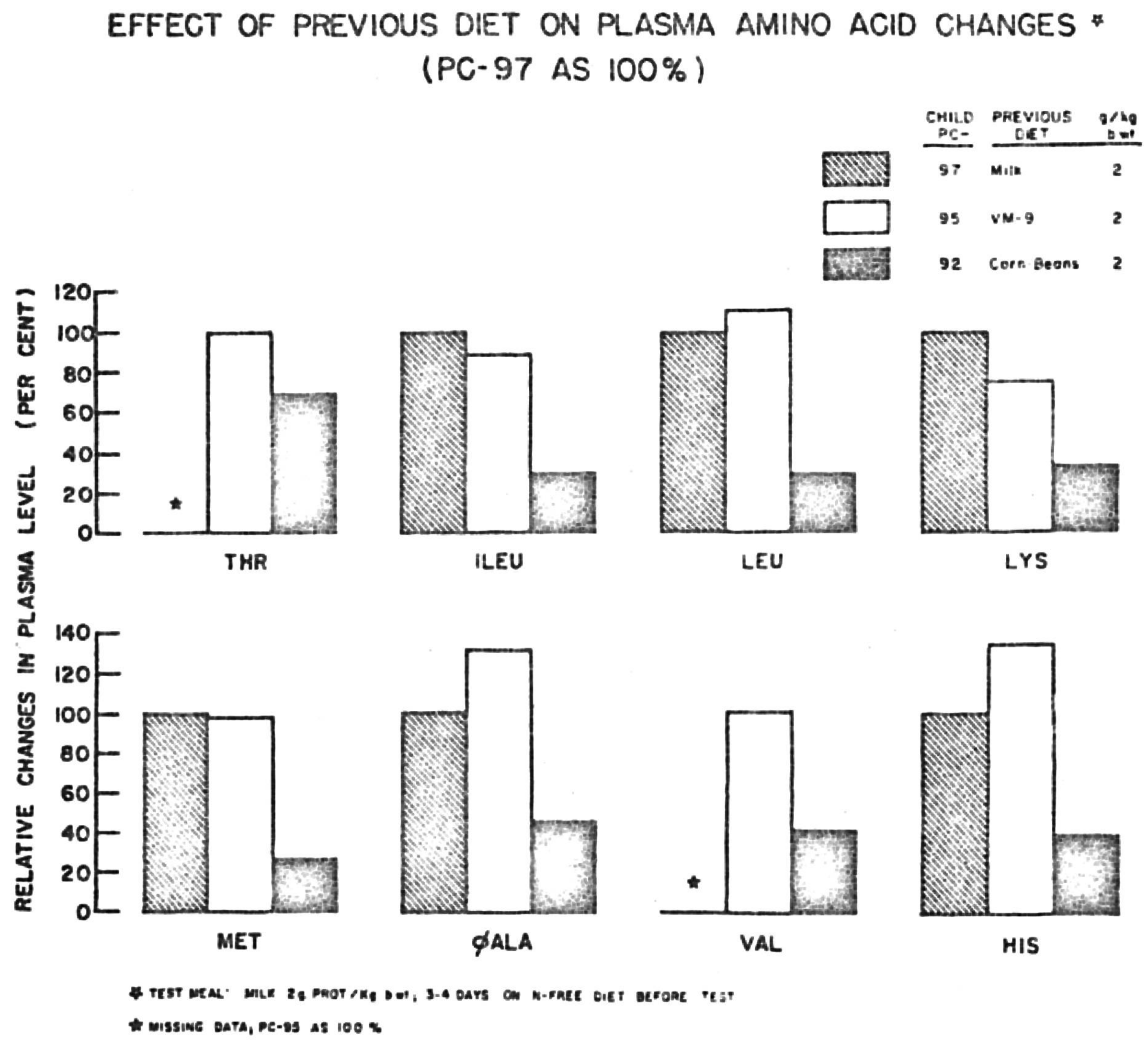

Figure 3

first column as 100%. The second and third columns show the plasma amino acid changes observed in the child fed INCAP Vegetable Mixture 9 and in the child fed corn and beans. The last two are both expressed as percentage of the change observed in the milk-fed subject. Exceptions to this are threonine and valine, for which changes in PC-95 were taken as 100%.

The child fed corn and beans showed consistently lower increases than either the child fed milk or the one fed Vegetable Mixture 9. It is interesting that the children fed milk and Vegetable Mixture 9 responded so similarly.

Experiment 2. In order to confirm the above observations, another group of 3 children was studied according to a very similar plan of investigation, but no period of nitrogen-free diet was included. Figure 4 illustrates the results of this experiment: PC-103, a boy 3 years and 4 months of age, was given a diet in which Mixture 9 was the sole source of protein, for a period of 6 weeks at a level of intake of 2 gm protein and 90 cal/kg/day. PC-101, a boy of 6 years and 2 months, received a milk diet at the same level of protein and calorie intake for 7 weeks, and PC-102, a boy of 5 years and 10 months, was given corn and beans as the only source of protein, which provided 2 gm protein and 100 cal/kg/day.

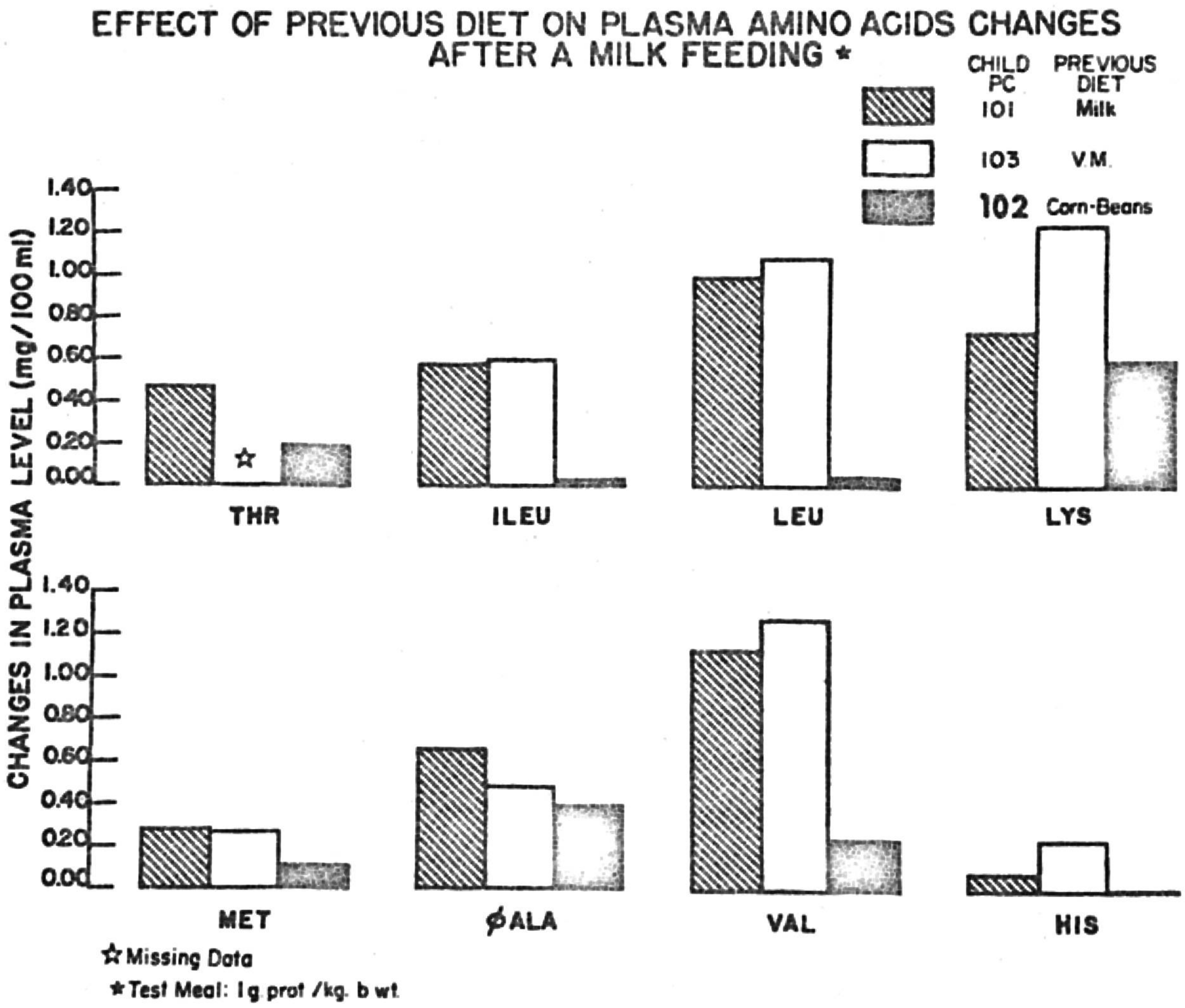

Figure 4

The 3 children received a test meal of milk calculated to provide 1 gm protein/kg/day in one feeding. Child PC-102 refused a small portion of this feeding, so that his actual intake was 0.86 gm protein/kg. The first column represents the plasma amino acid increases in the child whose previous diet was milk, the second represents the Mixture 9-fed child and the third corresponds to the child fed corn and beans. The results clearly confirm those of the previous experiment. Again, the increases in child PC-102 (previous diet of corn-beans) were markedly inferior to both the Mixture 9- and the milk-fed children. The agreement between results of experiments 1 and 2 indicates also that the period of nitrogen-free diet in experiment 1 did not interfere appreciably with the results.

To assist in interpreting the observed differences in the increase of plasma essential amino acids, some known facts must be considered. The 3 diets used, milk, INCAP Vegetable Mixture 9 and corn and beans, have their own characteristic protein quality as indicated by promotion of growth and nitrogen retention. Milk and Mixture 9 rate as very similar in quality by the above criteria, while the corn-bean diet appears quite inferior. Whether this is due to differences in digestibility, to lower amino acid utilization because of imbalance, or to some other cause, the important fact remains that the relative value of the 3 diets fed to children seems to be well reflected by the magnitude of the plasma amino acid increase after a common test meal of milk protein. Both independent experiments reported here are consistent in giving this result.

In the discussion of the biochemical significance of the relationship between plasma amino acids and the composition of the ingested protein in dogs, Longenecker and Hause[4] stated that one important factor in determining postprandial amino acid levels was the rates at which these compounds were removed from the plasma by the tissues and that these rates were proportional to the specific amino acid requirements. It may be speculated, therefore, that the long-term feeding of corn and beans produces a nutritional stress on the organism which results in an increased tissue demand for essential amino acids.

SUMMARY

The regeneration of total serum proteins was compared in 11 children suffering from kwashiorkor and treated with Vegetable Mixtures 8, 8A, or 9 or with milk. It was apparent that the vegetable mixtures, although not producing as high protein values as milk in the period of treatment, were of adequate quality for satisfactory regeneration of serum proteins. Plasma amino acid changes following a protein test meal were also studied in 6 children who had been given either milk, INCAP Vegetable Mixture 9 or a corn-bean diet for several weeks. The increase in plasma amino acid levels following a test meal of milk was essentially the same when milk or Mixture 9 constituted the previous diet and was much higher than that following the period of corn-bean feeding. Regardless of the specific mechanism or mechanisms responsible, this difference in response to the milk test meal seems to bear a direct relationship to the protein value of the previous diet and illustrates again the similarity of INCAP Vegetable Mixture 9 and milk in this respect.

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