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### 9. ABSTRACT
The problem of infant and preschool child malnutrition has created an ever-growing need for solutions. It has been demonstrated that a number of previously neglected sources of protein or mixtures of these and cereal grains, could support seemingly normal growth in convalescent malnourished infants or actually initiate their recovery. This report is an evaluation of new protein sources for the prevention of malnutrition. Nitrogen retentions from different sources of protein at isonitrogenous, isocaloric levels in the same child were compared, with emphasis on serum albumin levels as an adjunct to rates of weight gain and linear growth during prolonged feeding. The ability to initiate recovery in the severely malnourished child also was measured. The foods discussed and evaluated here are: isolated soy protein, toasted soy flour "milk", isolated soy protein "milk", corn-soy-milk, corn-soy-methionine, corn-soy-wheat noodles, wheat flour, wheat flour-wheat concentrate Ms-15, wheat-soy blends WSB and Mx-44, oat-soy mixture Mx-45, soy-whey mixture, rice flour, rice-cottonseed flours, casein-MCT-dextrose mixtures, and manioca-soy. Very brief reviews of parallel research studies also are given: lactose intolerance, growth studies, and hormonal adaptation.
RESEARCH TO PRODUCE NEW OR IMPROVED PRODUCTS*

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INTRODUCTION

The worldwide problem of malnutrition will not be solved through a simple increase in production and consumption of traditional foods, whether they be those of the developed societies or those characteristic of each region. Totally new foods, or improved versions of common foods, are destined to play a constantly increasing role. The great diversity in resources, actual or potential, and in the customs and food habits of different peoples, requires the formulation of a wide variety of food products. To the extent that these represent the use of new ingredients or important variations in processing, they must be subjected to critical human testing in the age groups for which they are intended. Of basic importance is the evaluation of protein quality.

Until relatively recent times, most testing in the human infant and small child involved modifications of cow's milk and estimations of milk protein requirements. The introduction of soya "milks" for the treatment of allergy brought about an expansion of techniques, with major reliance on comparative studies of nitrogen retention and weight gain in normal infants (1). On the basis of satisfactory results with one product, a number of such "milks", based on very different methods of processing the soybean, have been marketed in the United States and elsewhere, despite later clearcut evidence of their inferiority (2), and some disastrous clinical consequences (3).
The problem of infant and preschool child malnutrition created a growing need for critical testing. Major pioneering work from INCAP (4) Mexico (5), Jamaica (6), Africa (7) and India (8) demonstrated that a number of previously neglected sources of protein or mixtures of these and cereal grains, could support seemingly normal growth in convalescent malnourished infants, or actually initiate their recovery. Comparative nitrogen retentions at critical levels of intake were emphasized by INCAP (4) and have become part of the recommendations for testing of the Protein Advisory Group of the United Nations agencies (9).

Early in our work we became interested in the critical evaluation of new protein sources for the prevention of malnutrition (10–14). We compared nitrogen retentions from different sources of protein at isonitrogenous, isocaloric levels in the same child, and called attention to the importance of serum albumin levels as an adjunct to rates of weight gain and linear growth during prolonged feeding. As a further critical measure we included the ability to initiate recovery in the severely malnourished child (12, 14). It has been stated that such testing is unnecessary for dietary proteins intended for "well" children and that in the treatment of the severely malnourished child no one would use a product other than milk, or the best possible substitute when this was not available or tolerated.

There are very few "well" infants and children among the very poor. They suffer multiple episodes of protein depletion, the
result of repeated infections and periods of food withdrawal. Cow's milk proteins and others of similar biological value would permit a prompt repletion if they were the major source of dietary protein. For this reason, episodes of protein depletion in privileged children seldom become clinically apparent. The usual sources of protein for the less privileged, when consumed regularly in adequate amounts, can almost without exception support "normal" growth. Where they usually fail is in bringing a child who has suffered an episode of depletion back to his previous state. Each succeeding episode leaves a further deficit, inadequately corrected by a dietary protein which is inferior in this respect, until overt malnutrition becomes apparent. We feel that any food which is likely to become the major source of protein for infants and children should have its ability to correct a protein-deficient state tested.

The literature is full of reports on the high biological value and equivalence to milk of innumerable protein sources. Many of these are unfounded and misleading: in some studies the only criterion of adequacy has been short-term weight gain; in others, comparative nitrogen retentions have been estimated at such high levels of intake that differences in biological value have been obscured.

When thousands of infants and small children are to consume a new food, and there is the likelihood that this is to become the major source of protein in their diet, the providers of such a food have a responsibility to assure themselves of its safety and
nutritive value. A recent document (15) from the World Health Organization has reiterated the major steps to be taken prior to and in the course of human testing.

Although the recommendations list human testing of acceptability and tolerance prior to human testing of biological value, we are inclined to reverse the order. This last is carried out in sophisticated facilities, where problems of intolerance can be identified and coped with. Tolerance and acceptability studies are usually carried out in custodial institutions or ambulatory facilities where intolerance might produce significant ill health before its identification. It does not seem proper to introduce into the diets of a relatively large number of children food items whose nutritive value has not been documented. In the metabolic unit nutritional inadequacy can be recognized promptly.

The first step is the determination of digestibility and biological value, at critical levels of intake, and in direct comparison with a protein of established high quality. If such testing is satisfactory, tolerance and acceptability studies can begin elsewhere, and we can simultaneously proceed with long-term growth studies in appropriate age groups. Unfortunately such a sequence, or any other logical sequence, has often not been followed and great expectations have been created for products which later proved to be unacceptable or of very low nutritional value.

Our procedure requires preliminary estimation of the minimum calorie and high-quality protein intake required by a particular convalescent malnourished infant to maintain a steady rate of
weight gain and normal or increasing serum albumin values. The test protein is then substituted at isonitrogenous levels, maintaining the same calorie intake. Net protein utilization can be expressed as a percentage of that obtained with the control diet. With this as a basis, the protein level, as a percentage of total calories, is selected for long-term growth studies and for its use in the initial dietary management of malnourished infants, if justified. When our aim is to determine whether the biological value of a particular protein can be enhanced by amino acid or protein enrichment, a multiple comparison is made between the control protein, the test protein, and the same enriched at one or more levels. This type of testing will provide information about tolerance, but is of no value in determining acceptability in a real-life situation.

RECENT RESULTS

a. Isolated soy protein. In a series of early studies we documented the much improved digestibility of an isolated soy protein, Promine-D. Retentions of nitrogen were about 60% of those from milk in the same subjects and there was a distinct tendency for serum albumin to decrease. With methionine fortification the nitrogen retention increased to 85% of the milk values and the fall in serum albumin did not occur (16). It was possible to demonstrate that the next-limiting amino acid in this product was tryptophan.

At present we are carrying out the evaluation of a different soy protein isolate, Supro. Comparative nitrogen balance studies were first carried out using for the control diet one of the new "milks" based on a methionine-enriched soy protein isolate. To our
surprise, nitrogen absorptions and retentions from Supro were at least the equal of those from the "milk", and there was no further supplementary effect of added methionine. The studies were repeated using casein for the control diets. The results indicate nitrogen utilization which is about 80% of that from casein, and an improvement to 100% when methionine is added. It is our impression that in the first series of studies there was a strong "carry-over" effect of the methionine in the soy milk.

b. Toasted soy flour "milk". A well-known milk substitute, based on toasted soy flour as the only source of protein (Sobee) was evaluated. Net protein utilization and rates of weight gain in convalescent infants at critical levels of intake were approximately 80% of those from milk protein. The product was entirely satisfactory during prolonged feeding and it was able to initiate recovery quite effectively in two cases of Kwashiorkor. The results in the initial therapy of severe marasmus were less satisfactory: serum albumin remained at marginal levels. When appropriate amounts of DL-methionine were added to this product, net protein utilization was then equal to that from modified cow's milk or methionine-enriched soy isolate "milk". These studies demonstrate that toasted soy flour is a good source of protein, particularly so if enriched with methionine. Because of their markedly superior acceptability and versatility, however, the newer isolate based "milks" are rapidly displacing this type of product in the United States.

c. Isolated soy protein "milk". An extensive evaluation of this product (ProSobee), enriched with DL-methionine by the
manufacturer, demonstrated a protein quality the equal of the best modified cow's milk in comparative studies, prolonged feeding, and the initial dietary management of kwashiorkor and marasmus (17). Its ability to complement a diet based on poor quality protein was not measured but should prove moderately inferior to that of milk because of its lower essential/total amino acid ratio.

d. Corn-soy-milk. A detailed report of our evaluation of the corn-soy-milk blend, CSM, has been published (18). A limited acceptability trial was reported to A.I.D. Subsequent work with this product has further documented the improvement in protein quality to be expected from methionine supplementation. In a direct comparison with a corn-soy-methionine mixture (Fortifex) and a corn-soy wheat noodle, the retentions of nitrogen from CSM were moderately but definitely inferior.

e. Corn-soy-methionine. A detailed report of our evaluation of the corn-soy-methionine mixture, Fortifex, has been submitted to A.I.D. Subsequent work has been limited to the comparison mentioned above.

f. Corn-soy-wheat noodles. This product, developed by General Foods, is unique in our experience. A low-cost, high-protein mixture has been put into the highly acceptable form of a prestige food item. We have completed an extensive evaluation of its protein quality. The product was made up of approximately 50% corn flour, 30% defatted soy flour and 20% wheat flour, with or without 0.3% DL-methionine added. Nitrogen absorption was approximately 80% of intake, very similar to that from modified milk.
Apparent retention at critical levels was approximately .75% of the milk protein values, 100% of the Fortifex values, and 125% of the CSM values. The methionine-enriched version had retentions approximately 85% of the milk protein values. Prolonged feeding studies with the methionine-enriched product were generally very satisfactory. Nitrogen balance studies at the end of these were comparable to preliminary ones, with no increase in retention from milk protein during the succeeding period, indicating that protein deficits had not been created. In the initial dietary management of five infants with kwashiorkor, both versions gave results as satisfactory as those from Fortifex. Serum albumin reached satisfactory levels sooner with the methionine-enriched version. These results have been accepted for publication.

g. Wheat flour. We have reported our studies in infants of the enrichment of wheat flour with lysine (19). We showed that the equivalent of 0.12% enrichment of ordinary flour produced a significant increase in net protein utilization and that further enrichment to the equivalent of 0.2 and 0.4% produced additional enhancement, not expected on the basis of amino acid balance. We postulated that this might be the result of wastage of some of the more rapidly absorbed free lysine. In a more recent report 20) the prolonged feeding of wheat flour enriched to the same three levels has been covered. It was again apparent that further enrichment to 0.2 or 0.4% was probably beneficial. Studies are now under way to evaluate the use of lysine-enriched flour in the
initial dietary management. In two marasmic infants the results were generally satisfactory. In a third one a severe bacterial infection became evident and along with this there was a marked fall in the serum albumin: it was decided to change the infant to a milk diet. Results in kwashiorkor have been unsatisfactory.

Because the previous studies were carried out with a high protein wheat flour (of normal amino acid composition) not commonly available it was decided to validate them with ordinary white flour, with and without 0.2% lysine. In two infants the supplemental effect was as striking as in the reported studies.

h. Wheat flour-wheat concentrate Ma-15. A wheat flour-wheat concentrate mixture was evaluated and the results published (21). Digestibility was considerably below that of wheat flour alone, further limiting net protein utilization. Lysine supplementation resulted in a 50% increase in nitrogen utilization.

i. Wheat-soy blends WSB and Mx-44. A partial report on WSB, the wheat-soy blend (40.7% wheat flour, 40.5% wheat concentrate, 16.4% toasted defatted soy flour) has been made to A.I.D. Digestibility was similar to that of the wheat flour-wheat concentrate mixture. Net protein utilization was approximately 65-70% of that from milk protein. The results on prolonged feeding at 8.0% protein calories were satisfactory. In the initial dietary management of two marasmic infants weight gain was established but there was a steady fall in serum albumin levels to pre-kwashiorkor levels, necessitating discontinuance of the studies. In another convalescent infant we could not demonstrate any supplementary effect of DL-methionine fortification.
A similar wheat-soy mixture, developed by Quaker Oats Co. and submitted to us by UNICEF as Mx-44, was also evaluated. Results of comparative and long-term studies were similar to those with WSB. It was not used in the initial dietary management. In one convalescent infant we were not able to demonstrate any supplementary effect of lysine fortification.

j. Oat-soy mixture Mx-45. An oat-soy mixture, also from Quaker via UNICEF, was evaluated in a limited manner. In comparative studies, nitrogen absorption was very satisfactory, above 80% of intake. Nitrogen retention was approximately 90% of the milk protein values. In one child prolonged feeding was very satisfactory. Despite their limited nature, these studies are indicative of high biological value, one of the highest in our experience with vegetable products.

k. Soy-whey mixture. This spray-dried mixture was made by adding 25 pounds of full fat soy flour to 735 pounds of sweet Cheddar cheese whey, giving a product with 1/3 soy solids and 2/3 whey solids. It was developed at the USDA as S-12 and submitted to us by UNICEF as Mx-37. Comparative studies in five infants demonstrated absorptions and retentions of nitrogen which were 90 to 100% of those from casein in the same subjects, exceeding the relative biological value predicted from the PER in rats. In prolonged feeding the results were satisfactory although in one of two children there was evidence of intolerance to the high lactose content. Because of this, but particularly because of its high potassium content, we did not attempt its use in the initial therapy of malnourished infants.
1. **Rice flour.** A few years ago we attempted studies with flour made from ordinary rice and gave them up because of the frequent development of steatorrhea and malabsorption of nitrogen. We assumed that this problem was due to the very high rice starch content of these diets and although prolonged cooking was helpful, it did not solve the problem. Four years ago UNICEF made available to us a rice flour with approximately 15% protein made by selective milling of the outer layers of the rice kernel at the SRRL of the USDA. Because of its doubled protein content we hoped to overcome the problem of the high starch content of the final diet. Nitrogen absorptions after prolonged cooking were generally between 55 and 65% of intake, rarely as high as 75%, but without evident steatorrhea. Nitrogen retentions were 25 to 60% of the casein values. The routine addition of a starch-splitting enzyme failed to improve these results, although stool weight and stool fat were entirely normal. Lysine supplementation, in the form of "Nutricubes" from Hoffman La Roche, did produce a 50% increase in nitrogen retention. It is apparent that the inclusion of a high proportion of the external layers of the rice kernel in such a flour makes the digestibility of its protein much too low for its use in human infant nutrition.

2. **Rice-cottonseed flours.** We carried out a limited evaluation of two such mixtures, one with 82% ordinary rice flour and 18% cottonseed flour, and the other with 74 and 26% respectively. The CSF was Proflo. In three comparative studies the absorption of nitrogen from these mixtures was between 60 and 84% of intake, generally between 75 and 80%. Retention of nitrogen varied between...
60 and 90% of the values from milk or its equivalent. It was generally around 80% of these values, suggesting that net protein utilization was limited primarily, if not exclusively, by digestibility. This, however, was as good as that of CSF or most other vegetable proteins. Although these studies were very incomplete, they do support the potential value of rice-CSF mixtures. It has been suggested that Incaparina be made from these ingredients wherever rice is more available and acceptable than corn.

n. Casein-MCT-dextrose mixtures. Because of the need for disaccharide-free products with an easily absorbed fat component in the treatment of some cases of kwashiorkor and of a variety of severe malabsorption syndrome, Mead Johnson has developed and made available to us two such products. One of these, 3200-AD, is composed of casein hydrolysate, dextrose, arrowroot starch and medium chain triglycerides. The other, 3200-AH, is composed of casein, dextrose, and medium chain triglycerides. In comparative studies both products gave results which were indistinguishable from those obtained with our own casein-sucrose-cottonseed oil mixture. One of them, 3200-AH, was used in the initial dietary management of six cases of kwashiorkor. The results were probably superior to what they would have been with modified milk, which not infrequently causes lactose-induced diarrhea. In another infant with prolonged, intractable diarrhea it was also used with great success.
o. **Mandioca-soy.** We are presently carrying out studies of mandioca flour enriched with soy protein isolate. Digestibility of starch is poor but that of protein and its retention are equivalent to those of SPI.

**PARALLEL RESEARCH STUDIES**

Along with our studies of the nutritional value of different protein food sources, and supported in part by the same contracts, we have been carrying out a number of studies which are pertinent to the same subject matter.

a. **Lactose Intolerance.** The past few years have produced a wealth of documentation on this subject, of particular pertinence to nonwhite individuals. Whereas 90% of adult white individuals in the United States can tolerate a significant amount of lactose, splitting it into its easily absorbed nonosaccharides, glucose and galactose, only 20% of adult blacks can do the same, with most of the remainder developing frank clinical symptoms from the unsplit disaccharide. The figure for other nonwhite groups is even higher. We have studied elementary school children from the lower economic decentile in Baltimore: the percentage of intolerant white children is even lower than that of adults and the percentage of intolerant blacks is just under 20%, a figure much lower than that reported from Africa. We are now trying to determine the age at which intolerance becomes manifest in the other 60% and the factors responsible for the much longer persistence of lactase activity in the United States.
In Peru we have studied entire families of mestizo origin living in the peripheral slums. We find almost 100% intolerance after the age of 12, with a very rapid decline beginning at 2 years of age, as in other groups of nonwhite origin studied outside the United States. The significance of this "intolerance" to the consumption and utilization of milk and its components is now under study.

b. Growth Studies. We are well advanced in a study of the eventual growth and development of severely malnourished infants and their siblings, attempting to separate the effects of a short period of very severe malnutrition from those of the other environmental factors which are operant during the entire growth period. The findings are pertinent to the spelling out of national goals and to the evaluation of nutrition intervention programs.

c. Hormonal Adaptation. We are concerned with the way in which infants adapt their growth-regulating mechanisms to both short and long periods of dietary inadequacy and with the effect of different dietary regimes on the maintenance or correction of hormonal regulation. This is a long and tedious approach but is already being productive.
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