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Subject: Report on Isolated Soy Protein Milk
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SYNOPSIS - ABSTRACT

Milks based on isolated soy protein enriched with DL-methionine are rapidly coming into common use in pediatric practice. The biological value of its protein has been measured at critical levels in the initial therapy of malnourished infants and children and during convalescence. Absorption and retention of nitrogen, as well as growth rates, were equivalent to those from modified cow's milk. Normal serum albumin levels were achieved and maintained, as were levels of fasting plasma amino acids, both indicative of dietary protein adequacy. These results confirm the enormous potential of isolated soy protein in human nutrition, particularly in the diet of those intolerant of milk proteins or lactose.

The successful production of isolated soy protein (ISP) of high biological value (1) has opened up numerous possibilities in human nutrition. Although soy "milks" have been used for many years in the management of infants allergic to cow's milk proteins, their application has been restricted by their obvious soy taste and odor, unpleasant gastro-intestinal side effects, inferior digestibility, and often questionable biological value (2).

Within a few years of their introduction, milks based on ISP enriched with DL-methionine have found wide acceptability, not only in the prolonged management of allergy, but also in that of the transient lactase deficiency which follows many severe diarrheal episodes in general pediatric practice. ISP should find wide application in the nutrition of those children, particularly the non-white, who are genetically predisposed to lose most of their intestinal lactose activity in childhood (3) and will not tolerate more than small amounts of cow's milk.

As the use of ISP is broadened and its unit cost decreases, it is likely to play an important part in efforts to prevent the widespread malnutrition which plagues the world. Cow's milk and its derivatives are not only expensive and perishable but also inappropriate for mass application in populations where the majority of children lose their lactase activity as a matter of course (4). In the treatment of severe malnutrition lactose intolerance is a

serious problem, making necessary the use of lactose-free formulas put together from simple ingredients and often defective in their micro-nutrient content (5). In those who can tolerate lactose, modified cow's milk has proven far superior to other products in the initial management of the severely malnourished (6, 7, 8, 9), suggesting that in this state differences in protein quality are brought out which are not apparent in healthy infants.

Although animal studies (1, 10) have precisely documented the relative biological value of complete milks based on ISP, there have been few critical studies in the human infant. It has been shown that prolonged feeding at generous protein to calorie ratios in a mixed diet supported "normal" growth (11). At much more critical ratios, just below the minimum level we have found to be consistently satisfactory with modified cow's milk, a moderate fall in serum albumin levels was reported (12).

The present report details our evaluation of an ISP milk[1] in the diet of convalescent malnourished infants and its use in their initial dietary management. Comparisons were made with milk protein at critical protein to calorie ratios.

MATERIALS AND METHODS

The methods used have been described in previous publications (13). Although the ISP milk has a full complement of accepted micro-nutrients, these were diluted when sugar and oil were added

to reduce the protein/calorie ratio. Consequently, appropriate amounts of vitamins and minerals were added to satisfy the recommended daily intakes. The same was done when milk was the source of protein.

The nitrogen (N) balance studies usually correspond to the entire dietary period reported. When not, they were carried out during the last 6-9 days of the diet period.

Changes in body weight of the convalescent children usually parallel the N retentions. The comparative studies were carried out when "weight age" had at least equaled "height age" and the initially abnormal body water content and distribution had been corrected (14). In the acutely malnourished, particularly those with hypoalbuminemia and visible or inapparent edema, or those with dehydration, changes in body weight must of course be interpreted differently. Weight loss may be accompanied by excellent N retentions and a rising serum albumin, while weight gain may be associated with sodium and water retention, poor or no N retention, and a falling serum albumin.

Although discordant changes are occasionally seen, we attach considerable significance to serum albumin levels, measured by paper electrophoresis (15). When these rise or are maintained at normal levels (more than 4.0 g/100 ml), in association with steady gain in weight, we consider that dietary protein is adequate for the level of caloric intake. If during periods of steady weight

gain there is a fall in serum albumin levels, or these are maintained at subnormal values, we assume that body composition is deteriorating or not being corrected, and that dietary protein is inadequate.

Interpretation of long-term growth must be made with caution in convalescent malnourished children. Some resume linear growth immediately, others after a lag of some weeks, regardless of the diet. The period of accelerated linear growth is variable in duration and efficiency. Consequently, the timing of such studies may influence the extent of the growth observed. If gains in "height age" equal or exceed those in chronologic age, satisfactory "catch-up" growth is occurring. Rapid gains in "weight age" occur more promptly and may continue to the point of visible obesity (14). If during these, serum albumin remains normal we feel confident that the weight gain is not water retention (15).

The methods for plasma amino acid determination, as well as our rationale in reporting the results, have been reported (16, 17).

RESULTS

Table 1 summarizes comparisons made in four convalescent children between ISP milk and a modified cow's milk[2] as the sole source of protein at isonitrogenous and isocaloric levels, with 6.4 to 9.4% of calories as protein. In all four the apparent retention of N, as a percentage of intake, was moderately, though not significantly superior on the ISP milk. The same was true for

the rates of weight gain. These differences were not due to differences in rates of N absorption, nor can they be claimed to represent differences in biological value. Stool weights were consistently higher on the modified milk, suggesting a greater loss of calories, probably as stool fat. Although limited measurements suggest this to be true, they were not complete enough to be included in this report. In all 4 cases serum albumin remained at normal levels.

In the fifth child, in whom the comparison was made with calcium caseinate[3] as the source of protein, an error of calculation caused the diets to be different in calorie content. The more efficient absorption of N from casein, and the higher calorie intakes, probably account for most of the differences in apparent N retention. Serum albumin also remained normal.

In one child (table 2) the ISP milk was the sole source of protein for 6.2 months. During the first 2.5 months, 8% of calories were provided as protein. Gains in "height age" matched the advance in chronologic age, gains in "weight age" far exceeded it, but serum albumin remained normal. During the next 1.7 months, protein was reduced to 6% of the same calorie intake: progress in "height age" actually accelerated, as did that in "weight age", but serum albumin remained normal. A further reduction in protein, to 5% of calories, did not slow the accelerated growth and did not reduce serum albumin. Calorie intake was at all times high for

this child and he became markedly obese. There was never any indication of protein inadequacy. Liver biopsy revealed normal architecture at the end of the 6.2 months.

Table 3 summarizes the results in the initial management of 5 severely malnourished infants. The first, admitted with severe marasmus and a serum albumin of 4.59 g/100 ml, first experienced significant weight loss and a marked fall in serum albumin, at least partly dilutional. Subsequently both trends were reversed and very satisfactory "catch-up" growth was established. In the second infant, a not quite so severe case of marasmus, satisfactory response was more prompt.

The third child, admitted with severe marasmic kwashiorkor and acute dehydration, responded promptly and dramatically after initial rehydration. The fourth, another case of moderately severe marasmus, also responded promptly. The fifth, a case of marasmic kwashiorkor in a 9-month old infant, also responded well, though not as promptly.

Table 4 summarizes our experience in the initial management of severely malnourished twin boys, 9 months of age, with marasmic kwashiorkor and acute dehydration. Intravenous rehydration resulted in marked weight gain and a further dilutional fall in serum albumin. After this, one was given modified cow's milk and the other the ISP milk. Because of individual differences, adjustments in protein and calorie intakes, though similar, were not exactly

parallel. Both had a satisfactory diuresis, a gradual but satisfactory correction of the hypoalbuminemia, complete disappearance of hepatic steatosis on biopsy, and establishment of steady weight gain. The fact that both were extremely sick initially, as well as their young age, probably accounts for responses that were not as rapid and steady as those usually seen in older children with uncomplicated kwashiorkor.

Measurements of fasting plasma amino acids were made in four infants receiving ISP milk at 6.4 to 6.7% of calories as protein, a generally very critical level. In one, a single determination was made; in the others, three separate determinations, at least 15 days apart, were made. The individual results are reported in table 5; a comparison with the results obtained in similar children receiving other sources of protein at similar, though not quite so critical levels, is reported in table 6. Total free amino acids were not significantly different from those of infants receiving cow's milk protein (17) or wheat flour enriched with lysine (16). They were moderately higher than those of infants on a corn-soy-milk blend, a significantly less well absorbed protein mixture (18). Essential plus semi-essential amino acids, both in μM/ml and as a fraction of the total, were moderately but not significantly lower than on the milk diets. They were significantly ($P<0.05$) higher than on the other two sources of protein. We interpret these values, particularly the molar fraction, as indicative of dietary protein adequacy, and to a lesser extent, of the amino acid composition of

the dietary protein. Methionine concentration, as a fraction of the essentials, was not different from that found on milk or CSM diets.

DISCUSSION

Of the many sources of protein which we have evaluated in a similar manner in human infants (6, 7, 13, 16), this is the first which we have found to match cow's milk protein in nutritional value in all the situations tested. It is possible that if the comparative studies had been carried out at a slightly more critical level, below 6.4% of calories as protein, a modest inferiority might have been apparent, as in the studies of Fomon et al (12). In the single instance of prolonged feeding, even at 5% of calories as protein, an accelerated linear growth rate was maintained. Because of a calorie intake that was excessive for the child's age and stage of recovery, weight gain proceeded to the point of obvious obesity. Serum albumin remained normal and there was no accumulation of liver fat, probably because of the lower protein requirement of the child's age. In the most critical situation, that of the initial rehabilitation of severely malnourished infants, the response was as complete as it might have been with modified cow's milk. The results in equally malnourished twins were as satisfactory with ISP milk as with modified cow's milk. Although in the past we have obtained good results with 8% or less of milk protein calories (15), we are now more inclined to increase this to 10%, at least for a few days. The risks of sodium and water retention are seemingly less at such levels.

The fact that ISP has only 1/3 of its nitrogen as essential and semi-essential amino acids, while modified cow's milk has nearly 50% in the same form, would suggest that inferior nutritive value should consistently be demonstrated at critical levels. That it is not, supports the observation that the first-limiting factor in cow's milk protein is total nitrogen and that considerable dilution with "unessential" N is possible before a shortage of the next-limiting factor, methionine, can be demonstrated (19). In this "excess" of essential amino acids lies one of the main reasons for its excellent supplementary value to most diets of poor protein quality. This quality was not challenged in our studies.

We have previously demonstrated in the human infant that methionine is first-limiting in soy proteins (20). Its addition to the ISP milk makes the balance between the essential amino acids nearly ideal. The slight excess of lysine in the final product is of no concern and if anything permits significant supplementation of the lysine-poor cereals typically given to infants and small children as an important part of the diet.

The critical levels at which most of these studies were carried out, and the very satisfactory results in severely malnourished infants, both indicate that "milks" based on a high-quality isolated soy protein appropriately enriched with methionine are the practical equivalent of the best modified cow's milk products as the main or only source of protein in the diet of rapidly growing infants and small children.

On the basis of the respective amino acid compositions it is probable that cow's milk protein would prove superior as a supplement to a diet in which the main source of energy was a cereal or tuber deficient in protein quality and content. This assumption was not tested in these studies.

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TRADE NAMES OF PRODUCTS USED IN THIS STUDY

1. Isolated soy protein milk - ProSobee.
2. Modfied cow's milk - Similac with Iron.
3. Calcium caseinate - Casec

Table I. Nitrogen balances, rates of weight gain, and serum albumin levels of convalescent malnourished infants receiving modified milk (M), casein (C), or the isolated soy protein milk (S) as the sole source of dietary protein.

Age in Months*			Diet Protein, g/kg/day and Kcal/kg/day			No. of days	Nitrogen Balances** mg/kg/day		% of intake		Δ wt g/kg day	Ser. albumin g/100 ml	
CA	HA	WA					int	ret	abs	ret		Δ	Final
31	9	9	M	2.0	100	20	320	64	84	20	2.9	-0.07	4.57
			S	2.0	100	14	320	78	82	24	3.3	0.02	4.59
32	11	11	S	2.0	100	9	320	96	83	30	5.5	-0.06	4.24
			M	2.0	100	11	320	66	84	21	3.0	0.25	4.49
26	12	13	M	2.0	125	10	304	58	78	19	3.7	0.46	4.43
			S	2.0	100	8	320	66	80	21	2.1	-0.15	4.28
			S	2.0	125	7	291	77	80	26	6.4	-0.11	4.17
28	16	18	S	2.0	85	8	320	45	86	14	1.4	0.08	4.36
			M	2.0	85	6	320	36	81	11	-2.6	0.05	4.41
11	4	4	S	2.5	138	8	400	79	74	20	9.6	-0.10	4.20
			C	3.0	150	9	480	140	91	29	10.7	0.36	4.56
			C	2.5	150	6	400	119	85	30	6.5	-0.41	4.15

* Chronologic (CA), height (HA) and weight (WA) ages at time of study.

** Intake (int), retention (ret) and absorption (abs).

Table 2. Prolonged feeding of isolated soy protein milk as the only source of dietary protein to a convalescent malnourished infant.

Initial age in months*			Protein g/kg per day	Kcal per Kg per day	Kcal % Protein	Progress in months*			Ser.albumin g/100 ml	
CA	HA	WA				CA	HA	WA	Δ	Final
31.5	10.0	10.5	2.0	100	8.0	2.5	2.5	6.5	-0.24	4.33
34.0	12.5	17.0	1.5	100	6.0	1.7	2.5	6.0	0.04	4.37
35.7	15.0	23.0	1.25	100	5.0	2.0	3.5	7.0	-0.13	4.24

* Chronologic (CA), height (HA), and weight (WA) ages

Table 3. Isolated soy protein milk in the initial dietary management of severely malnourished infants.

Age, Height, Weight	Protein g/kg per day	Kcal Per kg per day	Kcal % Protein	No. of days	Δ wt g/kg per day	Serum albumin g/100 ml Δ	Serum albumin g/100 ml Final
13 months	2.0	100	8.0	7	-10.0	-0.41	4.18
66 cm	2.5	125	8.0	4	-7.2	-0.80	3.38
4.9 Kg	3.0	150	8.0	12	6.2	-0.95	2.43
	4.0	150	10.3	23	6.5	1.42	3.85
	3.0	150	8.0	17	3.9	0.59	4.44
7 months	1.7	71	9.6	9	8.0	-0.23	3.54
64 cm	2.4	93	10.3	14	-1.8	0.28	3.82
4.9 Kg	2.5	125	8.0	11	3.8	0.23	4.05
	2.5	150	6.7	28	6.5	0.17	4.22
22 months	I. V.	f l u i d s		2	10.0	-1.13	2.28
71 cm	1.7	46	12.6	4	0.4	0.44	2.72
5.9 Kg	2.4	73	13.1	9	-2.2	0.71	3.43
	2.5	100	10.0	5	6.7	0.15	3.58
	3.0	125	9.6	26	6.3	0.79	4.37
15 months	2.1	83	10.1	9	1.8	0.15	3.73
70 cm	2.5	125	8.0	20	0.7	0.44	4.17
5.9 Kg	2.5	150	6.7	22	5.7	0.42	4.59
9 months	1.75	47	14.9	5	-10.0	0.34	3.02
64 cm	2.5	75	13.3	4	3.9	0.18	3.20
4.6 Kg	2.5	112	9.0	6	5.0	-0.15	3.05
	3.0	140	8.6	10	7.9	0.24	3.29
	4.0	150	10.7	35	6.7	0.95	4.24

Table 4. Isolated soy protein milk or modified cow's milk as the sole source of protein in the initial dietary management of severely malnourished twin boys.

Protein g/kg per day	Kcal per kg per day	Kcal % protein	No. of days	Δ wt g/kg per day	Serum albumin g/100 ml Δ	Serum albumin g/100 ml Final
Twin "A" - 9 months - 58.5 cm - 3.91 kg - Modified milk						
I. V. fluids only			2	40.0	-0.64	2.15
0.45	18	10.0	2	-22.9	0.15	2.30
1.6	62	10.0	2	18.3	-0.18	2.12
3.0	78	15.4	6	-6.6	0.92	3.04
3.0	100	12.0	4	12.2	0.44	3.48
3.0	125	9.6	10	3.0	0.26	3.74
3.0	150	8.0	30	7.0	0.52	4.26
Twin "B" - 9 months - 57.5 cm - 3.61 kg - Soy milk						
I. V. fluids only			2	50.0	-0.35	2.55
1.2	47	10.0	4	-6.2	-0.42	2.13
3.0	81	14.8	6	-2.9	0.97	3.10
3.0	100	12.0	4	10.8	0.37	3.47
3.0	125	9.6	10	2.5	-0.19	3.28
3.0	150	8.0	5	-3.5	-0.18	3.10
3.0	175	6.8	27	9.2	0.91	4.01
2.5	138	7.3	32	6.1	0.19	4.20

Table 5. Fasting plasma free amino acids of infants receiving the isolated soy protein milk as the sole source of dietary protein for a minimum of 15 days. Total amino acids (AA), total essential amino acids (EAA), the ratio of essentials to total (EAA/AA), and methionine as the molar fraction of essentials (met/EAA).

Case No.	Dietary Protein g/kg /day	Dietary Protein % of Kcal	Plasma Amino Acids AA µM/ml	Plasma Amino Acids EAA µM/ml	Plasma Amino Acids EAA/AA	Plasma Amino Acids met/EAA
171	2.5	6.7	2.556	0.537	0.210	0.050
215	2.0	6.4	2.771	0.617	0.223	0.042
	2.0	6.4	3.211	0.634	0.197	0.036
	2.0	6.4	3.274	1.001	0.306	0.012
208	2.0	6.4	1.996	0.593	0.297	0.035
	1.65	6.6	2.016	0.611	0.303	0.029
	1.65	6.6	2.195	0.655	0.298	0.026
229	1.5	6.7	2.205	0.627	0.284	0.038
	1.5	6.7	2.273	0.599	0.264	0.042
	1.5	6.7	2.279	0.692	0.304	0.045
	Mean		2.478	0.657	0.269	0.036
	S. D.		0.465	0.128	0.043	0.011

Table 6. Comparison of plasma free amino acids of infants receiving the isolated soy protein (ISP) milk with those of similar infants receiving milk protein, wheat flour with added lysine (WL), or a corn-soy-milk blend (CSM) as the source of dietary protein.

Protein in the Diet			Plasma Amino Acids			
Source (n)	g/Kg/day Mean (Range)	% of Kcal Mean (Range)	AA µM/ml	EAA µM/ml	EAA/ AA	met/ EAA
ISP (10)	1.8 (1.5-2.5)	6.6 (6.4-6.7)	2.478 ±0.465	0.657 ±0.128	0.269 ±0.043	0.036 ±0.011
MILK (17)	1.9 (1.5-2.5)	6.7 (6.4-6.9)	2.310 ±0.291	0.692 ±0.103	0.295 ±0.034	0.040 ±0.008
WL (24)	1.9 (1.5-2.0)	6.9 (6.4-8.0)	2.205 ±0.274	0.552 ±0.104	0.244 ±0.025	
CSM (11)	2.4 (2.0-3.0)	6.9 (6.4-8.0)	1.931 ±0.450	0.483 ±0.190	0.247 ±0.057	0.042 ±0.013