APPENDIX V-n

PN-AAS-569

Nutritional Value of Normal, Opaque-2, and Sugary-2
Opaque-2 Maize Hybrids for Infants and Children.
II. Plasma Free Amino Acids

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ABSTRACT

Plasma free amino acids of convalescent malnourished infants and children were determined before and 3 and 4 hours after the first meal of the 27th day of maize endosperm (E) or whole kernel (WK) meal consumption. Three varieties of E: Normal, opaque-2 opaque-2 (O_2-O_2), or sugary-2 opaque-2 (Su_2-O_2), had been consumed in six different sequences during consecutive nine-day periods by six subjects; the same protocol was followed for WK meals. Plasma aminogram was expected to reflect the effects of 26 days of maize consumption and eight days of the last variety consumed, particularly the latter. The significantly poorer absorptions of nitrogen from the E meals were reflected in lower fasting molar ratios of total essentials to total amino acids (TEAA/TAA = 0.192 vs. 0.229 for WK). Fasting leucine/TEAA molar ratio was much elevated, notably after Normal maize, being three or more standard deviations above the mean for milk protein diets. With the exception of one very low (0.067) and one moderately low (0.120) lysine/TEAA ratio, the first-limiting amino acid was not identified by fasting values. Striking postprandial falls singled lysine out as first-limiting in five, possibly six, studies (both O_2-O_2 E, one Su_2-O_2 E, one Normal WK, one Su_2-O_2 WK, and one O_2-O_2 WK study), tryptophan in two studies (Normal E and Su_2-O_2 WK), and probably isoleucine in one O_2-O_2 WK study. In the remaining two studies (Su_2-O_2 E and Normal WK) lysine and tryptophan seemed equally limiting. These two amino acids are known to be almost equally limiting in Normal maize. The improved lysine and tryptophan contents of O_2-O_2 and Su_2-O_2 maize still leave one or both of these, and possibly isoleucine, as potentially first-limiting for small children.

The opaque-2 gene changes the distribution of endosperm proteins in maize, resulting in an increase in lysine and tryptophan, and a decrease in leucine contents (1). The sugary-2 gene lowers lysine content but improves kernel vitreousness and density. The double-mutant sugary-2 opaque-2 (Su_2-O_2) has favorable kernel characteristics, a higher lysine content, and a PER in rats which is slightly higher than that of opaque-2 opaque-2 (O_2-O_2) in isogenic triple hybrids (2).

In a comparison paper[2] we reported an evaluation, in eight convalescent malnourished infants and children, of the energy and protein digestibilities and protein quality of the above two hybrids, and an isogenic normal triple hybrid, as the endosperm (E) and the whole kernel (WK) meals of each. Digestibility of energy from E meals was seemingly lower than that of WK meals, probably due to the greater percentage of calories from E meals (83.2, 91.2 and 82.1% for Normal, O_2-O_2 and Su_2-O_2) than from WK meals (73.2, 68.2 and 67.2%) needed to make maize supply the 6.4% protein calories in each diet. Protein digestibility from WK meals (72.6±3.4% of intake) was significantly higher than that from E meals (66.8±7.6%). Apparent nitrogen retention from WK meals (29.5±7.5% of intake) was significantly higher than from E meals (20.9±7.5%). When related to presumed amounts of lysine absorbed, nitrogen retention from the WK meals was higher, suggesting that better energy absorption, a better balance of amino acids absorbed, or more available tryptophan may have been responsible. Nitrogen retention from Normal E meal (15.1±8.9%) was distinctly inferior to that from O_2-O_2 E meal (22.8±5.5%) and from Su_2-O_2 E meal (24.8±8.0%). For WK meals differences were less between varieties: normal 26.8±4.6, O_2-O_2 30.1±8.3, and Su_2-O_2 31.6±9.7% of

intake.

With ordinary maize, Scrimshaw, Bressani et al demonstrated that both lysine and tryptophan had to be supplemented to attain consistently improved nitrogen retentions in children (3, 4). Despite the increased lysine and tryptophan of opaque-2 containing maize, lysine is still first-limiting for animals (5) and for adult humans (6).

In the course of the studies summarized above, plasma samples were obtained at selected times for free amino acid determinations. This paper reports the results of these, as they help explain the differences in protein utilization.

MATERIALS AND METHODS

In the evaluation of the three endosperm meals (Normal, O_2-O_2 and Su_2-O_2), each of six children received consecutive diets based on each of the three meals in one of six different sequences. Maize provided all the 6.4% protein calories in isoenergetic, isonitrogenous diets of 100 to 125 Kcal/kg/day. Each diet period lasted nine days and the 27 days of maize diets were preceded and followed by a casein control diet. In this report the diet sequences are identified by the maize meal fed during the last of the three nine-day periods, as this was likely to be the main determinant of the amino acid patterns found in plasma. For WK meals the same protocol was followed. Four children participated in both studies, two in the E meal studies only, and two in the WK meal studies only.

The children were 10 to 25 months of age, were free of apparent infection, had no evidence of malabsorption, and were well advanced in their convalescence.

A sample of fasting (8 hours) plasma was obtained before the first meal of the last day of the third maize diet period in each sequence. This was expected to reflect the effect of 26 days of maize (E or WK) consumption and eight days of the particular meal by which the sequence was identified. Three and four hours after the first meal, additional plasma samples were obtained. There should have been twelve sets of three samples, each of two sets corresponding to one of the six meals as last in sequence: Normal E, O_2-O_2 E, Su_2-O_2 E, Normal WK, O_2-O_2 WK, and Su_2-O_2 WK. One of the Normal E meal set of samples was not available for analysis.

Plasma free amino acid levels were determined by liquid column chromatography and photofluorometry (7). They were expressed as actual molar concentrations (μmole/liter) and as molar ratios: the ratio of the concentrations of total essential (and the semi-essentials cystine and tyrosine) amino acids to total amino acids (TEAA/TAA), and the ratio of the concentration of each essential amino acid to that of the total essentials (EAA/TEAA).

RESULTS

Table I summarizes the pertinent fasting plasma free amino acid levels, and contrasts them with those previously found in infants and children consuming modified cow milk or casein as the sole source of protein, at 6.4% to 7.0% protein calories, and at less than 6.4% protein calories (7). Subsequent comparisons have been made with the

lower level of milk protein intake because the significantly poorer absorptions of nitrogen from any of the maize meals than from milk protein made the "effective" intakes from maize more like those of the lower level.

Total free amino acid concentrations were all within two standard deviations (S.D.) of the milk protein values and 7 of 11 were within one S.D.

Four of five TEAA/TAA ratios from the E meals were more than two S.D.'s below the mean for milk protein. The lowest ratio (0.160) corresponded to the diet period with the poorest nitrogen absorption (45% of intake) of the entire study. The next lowest (0.170) corresponded to the poorest absorption in all the O_2-O_2 E studies. The two other low values (0.196 and 0.177) corresponded to the two poorest absorptions of nitrogen from the Su_2-O_2 E meal. The fact that so many of the poorest absorptions were seen in the last of three consecutive periods of E meal consumption suggests that protein digestion tends to deteriorate during prolonged maize E meal consumption.

Only one of the six TEAA/TAA ratios from the WK studies was more than two S.D.'s below the milk protein mean, and this corresponded to the poorest nitrogen absorption of all the WK studies. The mean ratio for all the WK studies, 0.229, was higher, but not quite significantly so, than that for all the E studies, 0.192.

When TEAA/TAA ratios are low, with normal TAA levels, the levels of the non-essential amino acids must obviously be higher. This was explained by higher alanine levels in all cases, and by elevations of

glycine, proline, or asparagine-glutamine in some cases but not others. In all 11 studies, plasma free ornithine was more than one S.D. below the milk protein mean, and in 6 of 11 it was more than two S.D.'s below that mean.

In 6 of 11 studies, plasma tyrosine was more than one S.D. below the milk protein mean and in 4 of those 6, it was more than two S.D.'s below the mean. There were no other consistent changes in any of the other essentials not listed in the table.

Table I also lists the concentrations of lysine, tryptophan, leucine and isoleucine as their molar fractions of TEAA. In only one E study was there a significantly low fasting lysine/TEAA ratio of 0.067, corresponding to one of the O_2-O_2 studies. In the WK studies there was only one suggestively low ratio of 0.120, corresponding to one of the Normal maize studies. The very low lysine/TEAA ratio corresponded to the study with the poorest nitrogen absorption from O_2-O_2 E meal, and to a low TEAA/TAA ratio.

In only one E study, with Normal maize, was the tryptophan/TEAA ratio more than one S.D. below the mean for milk protein. In all the WK studies this ratio was well within one S.D. of that mean. The mean for the WK studies was significantly ($P<0.05$) higher than for the E studies.

In all five E studies the leucine/TEAA ratio was more than one S.D. above the mean for milk protein; in four of five it was more than two S.D.'s above that mean, and in two studies it was more than three S.D.'s above. In two WK studies the leucine/TEAA ratio was within one S.D. of the mean for milk protein; in two it was two S.D.'s above

that mean. In the remaining two studies, with Normal WK meal, the leucine/TEAA ratio was three S.D.'s above the mean for milk protein.

The isoleucine/TEAA ratio was below the mean for milk protein in all five E and six WK studies but in only one of each set was it more than two S.D.'s below, and just barely so.

In 5 of 11 studies the valine/TEAA ratio was lower at 3 or 4 hours postprandially than fasting, but the fall was minimal. Also in five studies, not the same, there was a similar slight fall in threonine ratios. Both the ½ cystine/TEAA and methionine/TEAA ratios were normal to begin with and showed little or no change postprandially. The low tyrosine/TEAA ratios seen in the fasting state tended to correct themselves postprandially. It is most likely that the valine and threonine ratios, being high to begin with, fell only as a result of the increase in leucine/TEAA ratios.

Figure 1 illustrates the postprandial changes in the EAA/TEAA ratios for lysine, tryptophan, leucine and isoleucine after the E meals. It is quite likely that the values at 1 and 2 hours, not determined, might have been well above the lines drawn to join the fasting and 3-hour ratios.

The lysine/TEAA ratio for the one subject (#568) consuming the Normal E meal increased at 3 hours to more than 2 S.D.'s above the mean for milk protein but fell at 4 hours to well below the fasting level. This same child, who during this diet period had the poorest absorptions and retentions of nitrogen of the entire study, also had the lowest fasting TEAA/TAA ratio and tryptophan/TEAA ratio. Postprandially, this last ratio fell to a level which we see only in

kwashiorkor or in pellagra. He had the highest fasting leucine ratio and the lowest isoleucine ratio, neither one of which changed significantly at 3 and 4 hours. It seems likely that tryptophan and not lysine was first-limiting.

In one child consuming O_2-O_2 E meal (#567), the very low fasting Lys/TEAA ratio fell even further postprandially, and his normal Trp/TEAA ratio also fell, but not to the same degree. Fasting leucine, already high, rose to the highest level in the study but without an adverse effect on isoleucine. In the other child consuming O_2-O_2 E meal (#560), Lys/TEAA dropped from a normal fasting level to 2 S.D.'s below the mean for milk protein at 4 hours, while tryptophan remained unchanged at a normal ratio. The Leu/TEAA ratio rose steeply at 3 hours and fell slightly at 4 hours, while Ile/TEAA fell more than one S.D. at 3 hours and recovered at 4 hours. For both of these children consuming O_2-O_2 E meal, lysine seems to have been clearly first-limiting.

After Su_2-O_2 E meals, the Lys/TEAA ratio fell very slightly in one child, quite steeply in the other, while changes in Trp/TEAA were very modest. Their Leu/TEAA ratios were not as high to begin with, but did rise steeply, particularly at 4 hours, when Ile/TEAA ratios fell only moderately. For one child, lysine may have been first-limiting, but for the other, lysine and tryptophan may have been equally limiting.

Figure 2 illustrates the postprandial changes in EAA/TEAA ratios for the same four essential amino acids after the WK meals.

For one child consuming Normal WK meal (#567), the relatively low fasting Lys/TEAA ratio fell very steeply at 3 hours, with some recovery at 4 hours. This is the same child with a very low fasting ratio after O_2-O_2 E meal and a further fall postprandially (Figure 1). He had a moderate postprandial fall in Trp/TEAA, a very steep rise in Leu/TEAA at 3 hours, and only a modest drop in Ile/TEAA. The other child consuming Normal WK meal had a modest postprandial fall in lysine, tryptophan, and isoleucine ratios, and a more moderate rise in the already high Leu/TEAA ratio. For the first of these two children lysine seemed clearly first-limiting; for the second, lysine and tryptophan, and possibly isoleucine, seemed equally limiting.

The two children consuming O_2-O_2 WK meal had modest postprandial falls in their normal Lys/TEAA ratios, no fall in Trp/TEAA ratios, and very modest increases in Leu/TEAA ratios. One had a moderate fall in Ile/TEAA at 3 hours and a recovery at 4 hours; the other had a very steep fall in Ile/TEAA at 4 hours, after a moderate increase at 3 hours. This may represent laboratory error or a true limitation of protein synthesis by isoleucine deficiency. There were minimal indications of lysine limitation and none at all for tryptophan.

One child (#553) had no postprandial fall of his normal Lys/TEAA ratio after Su_2-O_2 WK meal, the other (#561) had a distinct drop to 2 S.D.'s below the milk protein mean. The first, conversely, had a sharp drop in Trp/TEAA, and the second, an insignificant fall from a high normal fasting ratio. This would suggest that tryptophan was first-limiting for #553 and lysine for #561. Child #553 also had a sharp 3-hour fall in Ile/TEAA which was not seen in #561. Neither one had a very impressive postprandial increase in Leu/TEAA ratios.

DISCUSSION

Although the fasting TEAA/TAA ratio is a generally reliable indicator of protein intake or absorption, and to a limited degree of protein quality (8), neither the fasting methionine/TEAA ratio (9), nor the fasting lysine/TEAA ratio (10) has proven to be an effective or consistent indicator of inadequacy. On the other hand, the postprandial changes in these two individual ratios have been shown to identify a deficiency of one of these two essential amino acids in the diet (9, 10).

Normal maize has been found to be almost equally limiting in lysine and tryptophan for small children: both amino acids had to be supplemented to obtain a consistent increase in nitrogen retention, with one producing no effect in some children if the other was not added (3, 4). In the same studies, the further addition of methionine had an adverse effect on nitrogen retention, but the further addition of isoleucine had a further supplementary effect. To our knowledge, similar studies have not been carried out in children consuming O_2-O_2 maize, and certainly not in children consuming Su_2-O_2 maize. Studies with opaque-2 in adults do suggest that lysine is still first-limiting (6).

In view of the above, it would not be surprising to find disparate results in the fasting and postprandial plasma amino acids of children consuming maize, and this seems indeed to be the case, quite different from the experiences with soy and wheat proteins, both clearly limiting in a single amino acid. Because six different maize meals were studied and there were only two sets of plasma samples for each

meal (only one for the Normal E meal), the results found cannot clearly be attributed to each meal, as they might have been due to individual variations in amino acid requirements or absorption.

The fasting TEAA/TAA ratios quite consistently identified poor nitrogen absorption, particularly from the E meals. Individual fasting EAA/TEAA ratios were only consistent in identifying the high leucine content of all the meals, particularly those of the normal maize, whether as the endosperm meal or as the whole kernel meal. In one case definitely, and possibly in another, the inadequacy of dietary lysine was evident from the fasting Lys/TEAA ratio. Interestingly, these two sets of samples were from the same child, once after consuming the O_2-O_2 E meal for eight days (which followed nine days of Normal E and nine days of Su_2-O_2 E meals), and the other after consuming Normal WK meal for eight days (after nine days of O_2-O_2 WK and nine days of Su_2-O_2 WK). This may be an example of a higher individual requirement for lysine. Although the mean fasting Trp/EAA ratio was significantly higher after the WK meals than after the E meals, both were within the normal range. Fasting isoleucine/TEAA ratios were moderately low, but never strikingly so. Thus, with only occasional exceptions, the fasting EAA/TEAA ratios were again ineffective in singling out essential amino acid inadequacies.

Postprandial changes, measured only at 3 and 4 hours after the first meal of the day, were much more helpful. They identified lysine as clearly first-limiting in both children consuming O_2-O_2 E meal, one of two children consuming Su_2-O_2 E meal, one of two consuming Normal WK meal, and one of two consuming Su_2-O_2 WK meal. In one child

consuming O_2-O_2 WK meal, lysine may also have been first-limiting. Tryptophan was clearly first-limiting in the one child consuming Normal E meal and probably first-limiting for one of two consuming Su_2-O_2 WK meal. In one child consuming O_2-O_2 WK meal, if not a laboratory error, a steep fall in Ile/TEAA ratio at four hours may have identified isoleucine as first-limiting. In the remaining two studies, one with Su_2-O_2 E and one with Normal WK meal, lysine and tryptophan may have been equally limiting.

These plasma free amino acid studies suggest that lysine is probably the most common first-limiting factor in Normal maize or in O_2-O_2 and Su_2-O_2, but that tryptophan, and perhaps occasionally isoleucine, can also be first-limiting. The excess of leucine, particularly in Normal maize, was invariably evident, even in fasting plasma. The persistently normal methionine/TEAA ratios confirm the fact that this amino acid is not limiting in maize.

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FOOTNOTES

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2. Graham, G.G., Glover, D.V., Lopez de Romaña, G., Morales, E. & MacLean, W.C., Jr. Nutritional value of normal, opaque-2, and sugary-2 opaque-2 maize hybrids for infants and children. I. Digestibility and utilization. Submitted to J. Nutr.

Table I

Fasting plasma free amino acids of infants and small children consuming milk or casein (ref. 7), maize endosperm, or maize whole kernel meals as the sole source of dietary protein. (Subject #'s in parenthesis after type of meal)

Protein Source % Protein Kcal	TAA µmole/ liter	TEAA/TAA	EAA/TEAA			
			Lys	Trp	Leu	Ile
Modified Milk or Casein						
6.4-7.0% (n=81)	2633	0.287	0.147	0.065	0.120	0.070
$\bar{X}$ ± S.D.	±439	±0.030	±0.042	±0.017	±0.017	±0.011
Modified Milk or Casein						
<6.4% (n=8)	2428	0.250	0.146	0.063	0.112	0.070
$\bar{X}$ ± S.D.	±295	±0.023	±0.022	±0.019	±0.012	±0.011
Endosperm Meals - 6.4%						
Normal (#568)	2842	0.160	0.167	0.040	0.207	0.046
O_2-O_2 (#560)	2602	0.256	0.150	0.058	0.147	0.058
O_2-O_2 (#567)	2460	0.170	0.067	0.060	0.170	0.060
Su_2-O_2 (#549)	2578	0.196	0.139	0.044	0.125	0.055
Su_2-O_2 (#553)	2379	0.177	0.140	0.071	0.140	0.059
Whole Kernel Meals - 6.4%						
Normal (#567)	2857	0.204	0.120	0.051	0.153	0.057
Normal (#549)	2616	0.229	0.150	0.072	0.149	0.062
O_2-O_2 (#565)	2495	0.195	0.158	0.074	0.113	0.047
O_2-O_2 (#560)	2361	0.253	0.132	0.077	0.136	0.060
Su_2-O_2 (#553)	1845	0.255	0.136	0.072	0.136	0.055
Su_2-O_2 (#561)	2793	0.236	0.150	0.092	0.098	0.050

Figure 1

Fasting and postprandial molar ratios (EAA/TEAA) for lysine, tryptophan, leucine and isoleucine in five children consuming Normal (N), opaque-2 opaque-2 (O), or sugary-2 opaque-2 (S) endosperm maize meals. Mean ± one and two standard deviations of milk protein values (reference 7) are indicated by shaded and clear areas. (Subject number in parenthesis after type of meal).

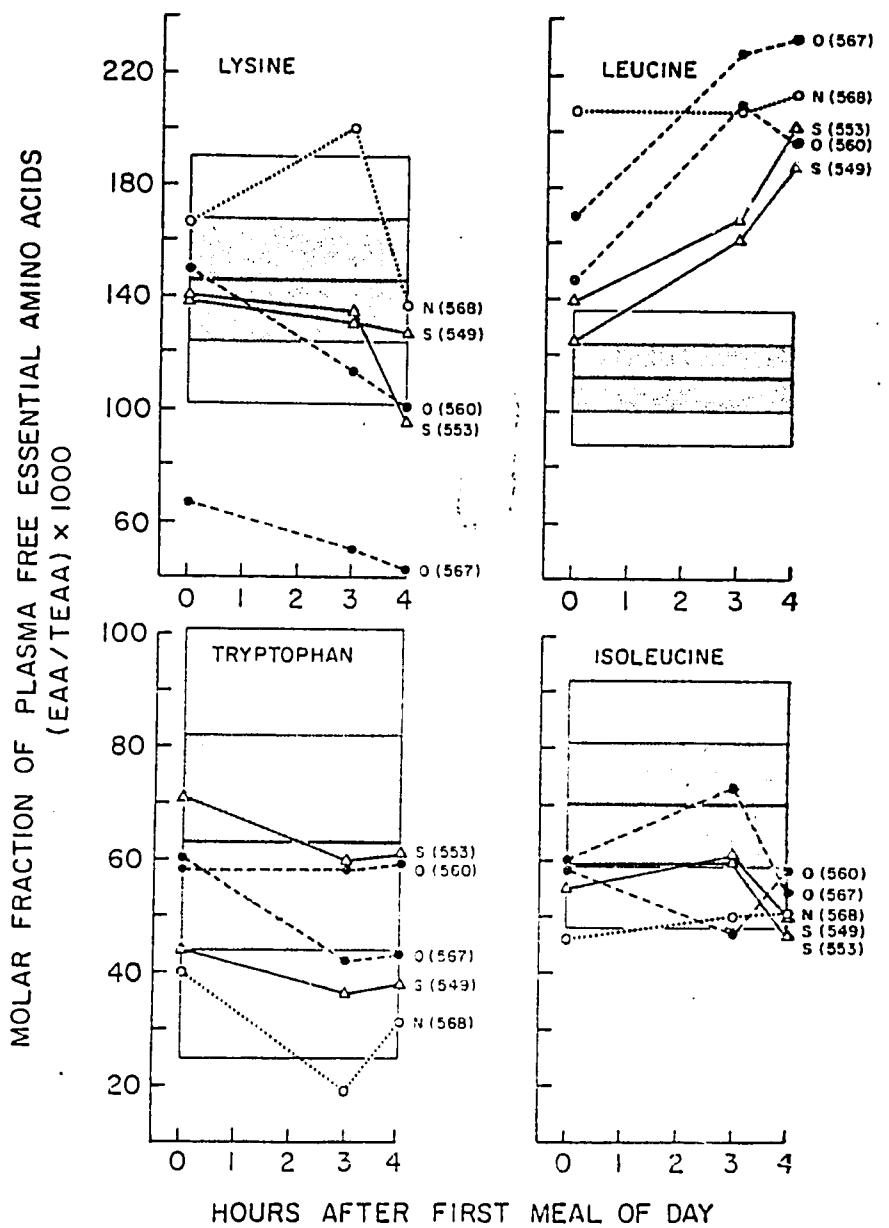

Figure 2

Fasting and postprandial molar ratios (EAA/TEAA) for lysine, tryptophan, leucine and isoleucine in six children consuming Normal (N), opaque-2 opaque-2 (O), or sugary-2 opaque-2 (S) whole kernel maize meals. Mean ± one and two standard deviations of milk protein values (reference 7) are indicated by shaded and clear areas. (Subject number in parenthesis after type of meal).

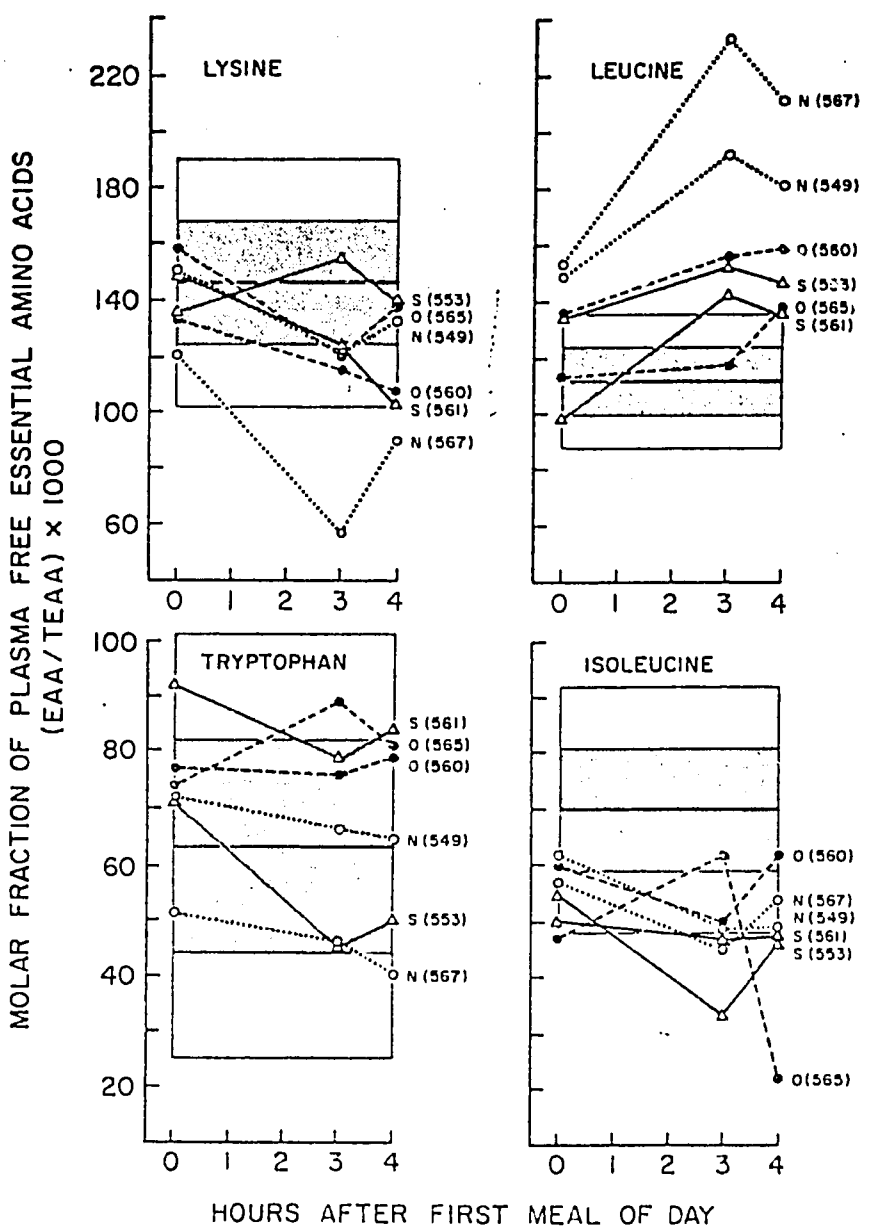

Table 1

Summary of children participating in these studies. All data are those at the beginning of the studies.

Patient No.	Hospital Day	Age Mos	Height Cm	Height Age[1] Mos	Weight Kg	Weight Age[1] Mos	[Albumin] g/dl
525	167	22	73.0	10.5	9.02	9.0	3.87
549	320	26	81.1	17.5	9.68	10.8	4.81
561	214	21	72.6	10.3	7.96	6.8	4.38
568	78	26	75.6	12.5	8.85	8.5	3.72
569	97	16	70.4	8.5	9.20	9.5	3.71
570	20	27	71.7	9.5	7.16	5.4	3.96
571	20	12	68.5	7.3	6.58	4.5	3.74
572	112	6	61.7	3.5	5.87	3.3	3.63
575	81	10	59.5	2.7	4.74	1.3	3.76

[1] Age to which child's height or weight corresponds to the 50th percentile value of the Boston reference data.

Table 2

Proximate analysis of four samples of sorghum evaluated in these studies. All values per 100 g except lysine.[1]

	954114	P721-0P9	954063	IS11758
Nitrogen-mg	1916.78	1983.97	1638.31	2326.40
Protein (N x 6.25)-g	11.98	12.40	10.24	14.54
Lysine-g/100 g protein	2.2	3.0	2.1	2.9
Fat-g	3.35	3.73	3.15	5.02
Ash-g	1.68	2.06	1.62	2.48
Fiber-g	3.28	4.22	1.30	5.62
Carbohydrate-by difference-g	71.11	69.24	74.54	63.84
Moisture-g	8.60	8.35	9.15	8.50
Energy-Kcal	362.51	360.13	367.47	358.70

[1]Analysis of nitrogen was carried out in the Instituto de Investigacion Nutricional. Lysine content is that reported by Purdue University. All other analyses were done by the Food Analysis Laboratory of the Department of Nutrition, Universidad Nacional Agraria, La Molina (Lima) Peru.

Table 3

Composition of the 4 sorghum and casein control diets per 100 Kcal[1,2]

	954114	P721-0P9	954063	IS11758	Casein Control
Sorghum - g	13.36	12.90	15.63	11.00	——
Soybean-Cottonseed (80:20) oil - ml	3.28	3.24	3.23	3.16	3.74
Sucrose - g	6.40	6.97	4.25	8.88	5.63
Dextrimaltose - g	——	——	——	——	6.00
Corn Starch - g	——	——	——	——	5.00
Casec R[3] - g	——	——	——	——	1.86
Calorie Distribution					
Protein - %	6.4	6.4	6.4	6.4	6.4
Fat - %	30.0	30.0	30.0	30.0	30.0
Carbohydrate - %	63.6	63.6	63.6	63.6	63.6

[1]Diets as shown provided 6.4% of energy as protein. After the initial six patients, protein calories in the sorghum diets were increased to 8.0%. Fat continued to provide 30% of calories.

[2]All diets were supplemented with a major and trace mineral mixture to meet or exceed Recommended Dietary Allowances. Na and K intakes were constant at 2.1 and 4.7 mEq/100 Kcal. Polyvisol and folic acid provided necessary vitamins.

[3]Casec (Mead Johnson): 1 g provides 0.86 g protein, 0.02 g fat, 3.70 Kcal.

Table 4

Sequence of dietary periods

Period No.	1	2	3	4	5	6	7
No. Days	9	9	7	9	9	7	9
Patient No.							
1	C	S_1	S_2	C	S_3	S_4	C
2	C	S_1	S_2	C	S_4	S_3	C
3	C	S_2	S_1	C	S_2	S_4	C
4	C	S_2	S_1	C	S_4	S_3	C
5	C	S_1	S_4	C	S_2	S_3	C
6	C	S_1	S_4	C	S_3	S_2	C
7	C	S_4	S_1	C	S_2	S_3	C
8	C	S_4	S_1	C	S_3	S_2	C