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Serum Proteins and Plasma Free Amino Acids in Severe Malnutrition

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Critical Evaluation of New (or Enriched) Protein Sources (For the Prevention of Malnutrition)

The ready availability of relatively simple laboratory determinations for the quantification of the protein "status" of individuals would be very useful in clinical practice as well as in nutritional surveys involving groups of people. The diagnosis of the extreme forms of protein-calorie malnutrition in infants and children, marasmus and kwashiorkor, does not require laboratory assistance. There are other situations in which some estimate of the degree of protein "deficiency" is of great value: recognition of protein malnutrition in individuals without obvious stigmata; assessment of the adequacy and effectiveness of dietary management in the sick and malnourished, or in those in whom dietary restriction of protein or individual amino acids is necessary; and research studies aimed at determining the adequacy of a protein or amino acid source and its level of intake.

Much of the literature on the subject recognizes the serum albumin level as such an indicator, either explicitly or by implication, as when it is used to validate other laboratory determinations. Probably the most convincing documentation of its reliability is in the work of Thomas and Combs, who in a carefully planned study varied the protein and calorie intakes of chicks and demonstrated an excellent correlation between serum albumin levels and body composition (1); an almost equally high correlation was found with total serum proteins. In our studies of convalescent malnourished infants receiving different levels of protein and calories we found serum albumin to be a reliable indicator of protein "adequacy"

(2). In some situations, however, it must be interpreted with caution. In marasmus or starvation, calorie deficiency may be so severe that serum albumin does not give any indication of the severity of a concommitant protein deficiency: its level may be high, normal, or nearly normal, but the signs of calorie deprivation are obvious. Contraction of the extracellular fluid due to losses of sodium and water may result in falsely elevated serum total protein and albumin levels: the values after hydration will be more reliable. Excess sodium and water retention may cause falsely low values. There is no general agreement on the lower limit of normal serum albumin levels, below which protein malnutrition can be confidently stated to exist (3).

Because of the above considerations, and because there is no simple method yet available for the determination of serum albumin in the field, a number of alternate procedures have been suggested as indicators of protein "status", most of them relying on serum or plasma amino acid concentrations. Notable among these are those proposed by Arroyave et al (4, 5) and by Whitehead (6, 7), both of whom felt that these reflected the protein status rather than the composition of the diet associated with the development of the deficiency state. Whitehead developed two simplified techniques, usable in the field, to relate the concentration of some non-essential amino acids to that of a group of essentials, the ratio being

directly correlated with the severity of protein deficiency, gauged in part by the serum albumin levels. Arroyave has proposed the ratios of several essential amino acids, individually, to glycine, as positively correlated with nutritional history and protein status (5). Both authors feel that these ratios or indices are useful for the recognition of marginal or pre-clinical cases of protein malnutrition. In marasmus, however, they do not serve as a gauge of severity (8).

The present report analyses the relationship between serum total protein, serum albumin and plasma amino acid concentrations in 41 severely malnourished infants and children, in an effort to assess their usefulness as clinical or field tools.

Materials and Methods

The patients studied represent 41 nearly consecutive unselected cases of severe malnutrition admitted under our care to the British American Hospital in Lima, Peru. In most of them chronologic age was verified by examination of birth or baptismal certificates. Along with anthropometric measurements, physical examination, routine hematologic studies, serum electrolytes, and appropriate cultures, the initial evaluation included serum total protein, serum albumin, and plasma free amino acid determinations. In all but one case (when it was drawn 15 hours later, after partial rehydration), venous blood samples were obtained within the first hour after admission. Although we have no reliable data on the

length of time elapsed since the last feeding prior to admission, the precarious clinical condition of the great majority fairly well precluded any significant feeding other than some clear liquids in the preceding 24 hours. In only one of them (#12) was there a reliable history of cow's milk ingestion very shortly before admission. Three of the patients (#'s 30, 34 and 37) died within 48 hours of admission.

Serum total proteins were determined by the biuret method, serum albumin by paper electrophoresis (Spinco procedure B). Plasma was sealed in glass ampules, frozen promptly and stored at -20°C, shipped in dry ice within one month to Baltimore, and kept frozen until analyzed (within one month) for free amino acids by liquid column chromatography (9). Tryptophan was determined separately by fluorescence assay (10).

"Length ages" and "weight ages" are those to which the actual measurements of the children correspond on the 50th percentile of a well-known United States standard (11) and, when related to chronologic age, are a crude and arbitrary measure of stunting and weight deficit. The relation of the second to the first of these two "ages" (WA/LA) is an indicator of the degree of wasting (Table 1). The amount of edema present was subjectively quantified from questionable (±) to severe and generalized (4+), as were the skin and hair changes usually associated with kwashiorkor. The same has been done for signs and symptoms of dehydration and acidosis, although the clinical impressions were generally supported by

changes in body weight, hematocrit, serum solids, serum bicarbonate and in some, blood pH. The clinical diagnosis of significant infection, generally confirmed by blood or urine cultures, is indicated by a + sign.

The concentrations of the individual amino acids are expressed in micromoles (μM) per ml, as are the sums of the essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (TAA). Tyrosine and ½ cystine, both considered "semi-essential" because they can spare phenylalanine and methionine, respectively, are included as essential along with the usual eight.

The valine to glycine ratio, Val/Gly, one of those proposed by Arroyave (5), is the ratio of their molar concentrations. The Whitehead "abbreviated" ratio is the ratio of the concentrations of glycine + serine + glutamine + taurine to those of leucine + isoleucine + valine + methionine, determined by paper chromatography (7). In our results the concentration of asparagine is included in the numerator, as it does not separate well from glutamine by liquid column chromatography in our laboratory. The ratio of total essential amino acids (EAA) to total amino acids (TAA) is one which we have used in a number of studies: its values in infants and children receiving milk protein diets have been reported (12).

Results

In Table 1 the patients are listed in descending order of their serum albumin levels. Examination of the table reveals that the first nine patients were 8 months of age or younger, stunted, severely wasted, free of apparent edema and skin or hair changes, and rather frequently dehydrated and acidotic, all characteristic of the severe infantile marasmus associated with diarrhea that we commonly see in urban slums. The last seventeen patients were all over 13 months of age, with six of them over 24 months of age. Almost all of them had prominent edema and skin or hair changes; some were dehydrated but none was obviously acidotic, this last the probable result of severe potassium deficits. The degree of wasting, though prominent in some and masked by edema in others, was generally not as severe as in the first group. Some of them, for example #'s 33, 36 and 40, had significant body fat present and might be called "pure" kwashiorkor, but the majority we would call "marasmic" kwashiorkor. The remaining patients, #'s 10 through 24, were more heterogeneous in age and clinical characteristics; some are further examples of marasmus, others what we might call "marasmus with hypoalbuminemia", and still others "marasmic" kwashiorkor, illustrating the difficulties of classification. The frequency of dehydration and infection reflects our bias toward the admission of the sickest children who cannot be handled successfully on an out-patient basis.

Table 2 again lists the patients in descending order of their serum albumin levels. It gives the molar concentrations of the ten essential and semi-essential amino acids in their plasma, their sum (EAA), the sum of the non-essentials (NEAA), the combined total (TAA), the molar ratio of valine to glycine (Arroyave), the Whitehead abbreviated ratio, and the total essentials and semiessentials as the molar fraction of the total (EAA/TAA). It is apparent that as the serum albumin level decreases, so do the concentrations of the individual essential amino acids, EAA, NEAA, TAA, Val/Gly, and EAA/TAA. The Whitehead abbreviated ratio, reflecting the more marked decrease in essential than in non-essential amino acid concentrations, increases as albumin decreases. In most of the cases of marasmus all of the above values tended to be normal, thus failing to recognize the severe protein deficiency which accompanies but is overshadowed by the extreme energy deficit.

Table 3 lists the correlation coefficients, "r", between serum albumin levels and the plasma free concentrations of each individual amino acid, except asparagine and glutamine, which are expressed as their combined molar concentration. The "P" value of each coefficient is listed, as well as the linear regression equation of each plasma free amino acid's molar concentration (y) on the simultaneously determined serum albumin concentration in g/100 ml (X). The high and very significant correlations of tryptophan, the branched chain amino acid and tyrosine concentrations with serum albumin

are apparent. Slightly less but still high and significant, are the correlation coefficients for the non-essentials alanine, glutamic acid, ornithine, proline, and arginine, and the essentials phenylalanine and methionine. Less so, but still significant, are the correlations for the non-essentials asparagine + glutamine, serine and histidine, and the essentials threonine, lysine and ½ cystine. The correlation coefficients for glycine and taurine were not significant.

Although not in the tables, the correlation coefficient of serum albumin with total serum proteins was extremely high, "r" = 0.947, "P" = 1.4×10^{-9}. For serum globulins and total serum proteins the correlation coefficient was much lower, 0.778. The correlation coefficients for the individual amino acids with total serum proteins, though not reported here, were very similar to those for albumin, but slightly higher, probably reflecting the lesser possibility of laboratory error in the determination of total serum proteins.

Table 4 lists the correlation coefficients "r" between the serum albumin levels of the 41 patients and the sum of the concentrations of essential, non-essential and total amino acids, as well as a number of ratios: EAA/TAA, NEAA/EAA, Val/Gly, Tyr/Gly, ½ Cys/Gly, and both Whitehead ratios. Also listed are their "P" values and the regression equations of the various concentrations (μM/ml), or ratios, on serum albumin (g/100 ml). The highest correlation is with the essential amino acids, followed closely by total amino acids. Even the

concentration of non-essentials has a higher correlation coefficient than any of the ratios, with the sole exception of the valine/glycine ratio.

Figure 1 depicts graphically the linear regression equations of the individual non-essential amino acid concentrations on serum albumin level. For glycine and taurine (not in reality an amino acid) the correlations were not significant, for all the others the "P" value is included. Only arginine, considered essential for young animals and possibly so for the very young human infant, projects to a zero value before zero albumin is reached.

As can be seen in Figure 2, most of the essential and semi-essential amino acids reach a projected value of zero before albumin does, or simultaneously. The most notable exception is lysine, with a relatively high value of more than 0.05 μM/ml at zero albumin. Also relatively "resistant" are threonine and phenylalanine. Despite this, the correlations are significant for all three.

Discussion

The serum albumin level can be used, with some reservations, as a reliable indicator of the protein status of individuals. In numerous evaluations of the protein quality of the diet we have almost invariably found that when a child gains weight on a diet that is not supporting adequate nitrogen retention, the serum albumin level falls significantly in

a matter of days, indicating that the protein "concentration" of the body has fallen (2). If, on the other hand, the child stops gaining or loses weight on a diet which is adequate in calories but inadequate in protein, the serum albumin level does not fall and may actually rise, suggesting that the body composition is being maintained by converting energy from the diet into heat. When dietary energy is inadequate for weight gain, but does meet basal requirements, changes in the serum albumin level probably depend to a great extent on dietary protein. When not even basal energy requirements are met, as in the development of marasmus, severe weight loss ensues but serum albumin may remain at normal levels, even when dietary protein is absent or insignificant.

From the above considerations it is apparent that serum albumin is affected by both protein and calorie intakes and by the relation between these. It cannot be used alone, without regard to the clinical condition of the patient, as an indicator of the body protein content or concentration. This is well illustrated by the present series of patients, in which the first 8 marasmic infants had serum albumin levels above the lower limit of normal in our laboratory, 3.8 g/100 ml. That body protein content and concentration are not normal in this situation is obvious from studies of body composition, both by indirect methods (13) and by analysis of muscle biopsies (14). It seems logical to assume that in extreme and prolonged calorie deficiency, amino acids coming from muscle and the internal organs are used for albumin synthesis. The opposite

situation probably exists early on when weight gain and growth continue despite an inadequate protein intake: serum albumin gives up amino acids to internal organs, muscle, and supporting tissues. If the situation is prolonged, the protein concentration of these tissues decreases and eventually the clinical picture of kwashiorkor develops.

Alterations of the body fluid compartments are characteristic of malnutrition and even in extreme marasmus the extracellular fluid volume is expanded relative to body weight (13). Contraction of the plasma volume, when severe diarrhea supervenes, further complicates the interpretation of concentrations of plasma constituents. Severe infection, also present in some of our cases, can have a direct effect on protein synthesis and catabolism.

In the present series of 41 patients serum albumin was consistently lower in those with manifestations which are considered typical of severe protein deficiency. In a recent report Whitehead et al found a falling serum albumin level to be a reliable indicator of developing kwashiorkor (15). In our patients a very close correlation was found between serum albumin and total serum protein. If the first is a good indicator of protein "status", then the second is equally good, and technically much simpler to determine: there are a number of satisfactory field methods for total serum protein determination. Since albumin normally constitutes more than 50% of total serum protein, and varies more readily than the

globulins, which are relatively insensitive to nutritional alterations, such a high degree of correlation is not surprising.

The relatively high correlations between the concentrations of total amino acids, total essentials, total nonessentials, and most of the individual amino acids, with serum albumin or total protein levels must in part be due to their being affected to a similar degree by changes in hydration. Expansion of the extracellular fluid volume, with or without clinically apparent edema, and dehydration, the result of complicating diarrhea, were undoubtedly common in this series of cases

That changes in hydration do not account for all or most of the variation, however, is apparent from the way in which many of the individual amino acids differed in the degree to which they followed serum albumin changes (Table 3, Figures 1 and 2), and by the relatively high correlation coefficients for the various ratios, particularly the valine/glycine ratio.

The close correlation between serum albumin and plasma amino acid concentrations, particularly those of the essentials, suggests that the one has a determining effect on the others, or vice versa. Of particular interest are the very high correlation coefficients for plasma tryptophan and the branched chain amino acids. The first of these has a recently acquired reputation as the "initiator" of protein synthesis in the liver (16). It has also been shown that, uniquely among the plasma amino acids, two-thirds of the "free"

tryptophan is in reality bound to albumin (17), another possible explanation for the close correlation found. A special role of the branched chain amino acids in liver protein synthesis has also been demonstrated (18).

The relative "resistance" to fall of plasma lysine, and to a lesser degree, that of threonine, is in accord with the observations of others and is probably related to their less efficient catabolic pathways. A greater fall in the concentration of most of the essential amino acids than that of the non-essentials is characteristic of kwashiorkor (19) and determines the changes in the various ratios reported here. Our statistical analysis does not correct for the variance due to changes in hydration, and thus does not adequately recognize that these ratios are independent of volume changes.

Examination of Table 2 reveals that among the marasmic infants with normal serum albumins there are a number with low Val/Gly and EAA/TAA ratios and elevated Whitehead ratios, suggesting that they were more protein "deficient" than their serum protein levels indicate. Conversely, there are a number of children with very low serum albumins and some normal amino acid ratios. Of particular interest are #'s 30, 34 and 37, the three patients who died. Despite serum albumins of 1.73, 1.33 and 1.27 g/100 ml, none had the classical signs of kwashiorkor: edema and skin or hair changes (Table 1). Their Val/Gly ratios of 0.302, 0.238 and 0.492 were all low, but not as low as those of most cases of kwashiorkor. The Whitehead

ratios of 3.976, 3.626 and 3.990, though high, were not as high as those of the more typical kwashiorkor. The EAA/TAA ratios of 0.300, 0.335 and 0.255 were normal in the first two cases, moderately low in the third. Our normal mean for healthy children on milk protein diets is approximately 0.300 (12). These results suggest that their very low serum albumins were more the result of their severe infections than of protein deficiency.

It is apparent from these studies that in severely malnourished infants and children, particularly if they are very sick, initial determinations of serum proteins and plasma amino acids will seldom provide any information that is not clinically evident. The close correlations between serum proteins and some of the amino acid concentrations and ratios suggest that in field studies, or in following the course of a particular patient, the choice of laboratory procedure to be used should be dictated by simplicity and cost. On this basis we would recommend total serum proteins, the plasma valine/glycine ratio, or the Whitehead abbreviated ratio.

Summary

Serum albumin, serum total protein and plasma free amino acids were measured on admission in 41 severely malnourished infants and children, many of whom were dehydrated, acidotic or obviously infected. In order to assess their usefulness in the quantification of protein "status", the correlation coefficients of group and individual amino acid concentrations, as well as various essential to non-essential amino acid ratios, with the serum proteins, was determined. Much of the close correlation between the various concentrations was felt to be due to variations in hydration. Correlation between serum total protein and albumin was so close that it was felt that the first, a much simpler determination, could easily replace the latter as a field tool and in many clinical situations. Plasma concentrations of amino acids, particularly the essentials, were closely correlated with serum proteins, in part the result of changes in hydration. Significantly higher correlation coefficients for tryptophan and the branched chain amino acids suggested a more specific role in determining serum albumin levels. Various amino acid "ratios", particularly valine/glycine, were significantly correlated with serum albumin. There were enough exceptions to suggest that these ratios might recognize severe protein deficiency in infants whose dehydration had elevated their serum albumin levels, or in whom adaptations to extreme calorie deficiency had resulted in conservation of albumin

at the expense of other proteins. Conversely, normal amino acid ratios in patients with hypoalbuminemia might be useful in identifying non-nutritional causes.

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Table 1

Clinical and laboratory findings of 41 severely malnourished young children at time of admission to hospital: body length (L), body weight (W), chronological age (C.A.), length age (L.A.), weight age (W.A.), and serum proteins: total and albumin (Album.).

No. & Sex	Body L cm	Body W kg	AGE In Months C.A.	L.A.	W.A.	WA/LA	Edema	Skin &/ or Hair	Dehydration	Acidosis	Infection	Hct	Serum Protein g/100 ml Total	Album.
1F	59.5	3.81	4.2	3.2	0.3	0.09			3+	3+		38	7.84	4.79
2M	59.0	3.39	4.1	2.3	N.B.				+			37	7.66	4.44
3F	57.3	3.50	3.6	2.3	0.1	0.04			±			35	7.33	4.36
4F	57.0	3.90	6.7	2.1	0.4	0.02					+	30	7.30	4.23
5M	63.0	4.00	4.6	4.1	0.6	0.15		±		+	+	38	7.43	4.09
6F	62.0	4.74	8.0	4.4	1.4	0.32					+	38	7.43	4.05
7M	54.5	3.67	2.7	0.7	0.2	0.29			3+	3+		38	7.87	3.94
8M	51.5	2.25	1.9	0.2	<N.B.			+			+	26	6.96	3.83
9M	57.0	3.46	5.1	1.5	<N.B.				2+		+	38	6.21	3.76
10F	65.0	4.85	15.1	6.1	1.4	0.23			2+	+	+	32	7.63	3.71
11M	72.0	6.32	22.4	9.8	4.1	0.42					+	30	6.82	3.66
12M	66.5	5.42	7.7	6.0	2.4	0.40			2+		+	30	6.44	3.54
13M	55.5	3.13	3.5	1.0	<N.B.						+	43	6.20	3.52
14F	68.3	5.17	16.1	8.1	2.0	0.25					+	35	6.66	3.51
15F	65.0	4.45	11.4	6.1	1.0	0.02			3+	2+	+	32	6.54	3.51
16F	66.0	4.91	8.9	6.7	1.5	0.22		+			+	34	7.06	3.13
17M	70.5	6.50	15.8	8.5	4.4	0.52	±	±	+	+	+	36	5.74	3.01
18F	55.0	3.52	8.8	1.4	0.1	0.07					+	30	6.15	3.00
19M	67.5	5.71	18.2	6.5	3.0	0.46		+				29	7.20	2.92
20M	49.5	2.75	2.3	0.1	<N.B.			+	3+	2+	+	32	5.32	2.88
21M	63.5	4.40	8.8	4.4	1.0	0.23			2+		+	27	6.12	2.82
22F	80.5	9.40	20.4	19.8	11.0	0.56	+	+	+			32	4.29	2.68
23F	71.0	5.74	17.0	9.9	3.0	0.30		+	2+		+	35	4.76	2.27
24F	66.0	5.25	7.7	6.7	2.5	0.37	+		+			40	4.70	2.16
25F	72.5	6.26	16.3	11.0	3.9	0.35	+	+			+	35	4.58	2.12

Table 1 (Cont'd)

No. & Sex	Body L cm	Body W kg	AGE In Months C.A.	AGE In Months L.A.	AGE In Months W.A.	WA/LA	Edema	Skin &/ or Hair	Dehydra-tion	Acido-sis	Infec-tion	Hct	Serum Protein g/100 ml Total	Serum Protein g/100 ml Album.
26M	84.0	9.78	36.6	20.1	11.1	0.55	2+	2+				39	5.51	2.11
27F	66.0	5.12	13.5	6.7	2.0	0.30	2+	+	+			36	4.82	1.98
28M	80.0	7.57	16.1	16.3	6.2	0.38	2+	+	+		+	34	4.65	1.91
29F	75.0	8.76	21.6	12.8	9.1	0.71	3+	+	2+			28	4.15	1.85
30M	81.0	9.27	19.3	17.2	9.7	0.56			2+		+	28	4.65	1.73
31M	81.5	9.30	20.7	17.8	9.8	0.55	3+	2+			+	26	4.67	1.64
32F	73.5	7.00	15.6	11.6	5.4	0.47	3+	2+	+			20	3.93	1.63
33M	85.5	13.05	35.4	21.5	28.5	1.33	3+	3+				33	3.71	1.45
34M	70.0	8.52	26.2	8.3	7.8	0.94			2+		+	31	3.69	1.33
35M	75.0	6.72	15.8	12.1	4.8	0.40	+	+				28	4.15	1.30
36M	74.0	10.47	13.4	11.3	13.7	1.21	4+	2+			+	23	3.86	1.29
37F	77.0	6.49	24.1	14.5	5.3	0.37			2+		+	35	3.10	1.27
38M	69.8	6.56	24.0	8.1	4.6	0.57	3+	3+				28	3.82	1.25
39F	75.0	8.28	16.6	12.8	8.0	0.63	3+	+			+	29	4.30	1.22
40M	80.5	13.00	39.7	16.7	28.0	1.68	4+					33	3.29	0.79
41F	71.5	7.65	15.0	10.4	6.7	0.64	4+	3+			+	19	2.43	0.79

Table 2

Serum albumin (S. Alb.) and plasma free amino acids of 41 severely malnourished young children at the time of admission to the hospital: individual essentials, total essentials (EAA)*, total non-essentials (NEAA), total amino acids (TAA), and Valine/Glycine (Val/Gly), the Whitehead abbreviated (Wh.abb.), and the EAA/TAA ratios.

No.	S. Alb. g/100ml	PLASMA FREE AMINO ACIDS IN uM/ml													RATIOS		
		Trp	Leu	Ile	Val	Tyr	Phe	Met	½ Cys	Thr	Lys	EAA*	NEAA	TAA	Val/Gly	Wh.abb.	EAA%TAA
1	4.79	0.075	0.169	0.059	0.172	0.081	0.073	0.015	0.019	0.077	0.219	0.959	1.620	2.579	1.117	1.065	0.372
2	4.44	0.063	0.151	0.086	0.198	0.057	0.089	0.029	0.064	0.101	0.071	0.909	1.745	2.654	0.712	1.726	0.343
3	4.36	0.043	0.061	0.027	0.089	0.037	0.044	0.023	0.021	0.106	0.111	0.562	2.036	2.598	0.276	3.995	0.216
4	4.23	0.089	0.221	0.133	0.348	0.127	0.065	0.045	0.025	0.289	0.278	1.620	2.458	4.078	1.420	1.011	0.397
5	4.09	0.043	0.039	0.026	0.109	0.081	0.114	0.010	0.016	0.139	0.104	0.681	1.747	2.428	0.452	3.027	0.280
6	4.05	0.082	0.158	0.096	0.239	0.102	0.075	0.032	0.025	0.224	0.220	1.253	2.193	3.446	1.170	1.213	0.365
7	3.94	0.065	0.242	0.134	0.261	0.124	0.148	0.058	0.101	0.357	0.403	1.893	3.045	4.938	0.562	1.599	0.383
8	3.83	0.040	0.058	0.038	0.085	0.024	0.026	0.014	0.027	0.149	0.092	0.553	1.888	2.441	0.220	3.497	0.227
9	3.76	0.041	0.112	0.061	0.202	0.032	0.036	0.011	0.014	0.053	0.088	0.650	1.409	2.059	1.496	1.420	0.316
10	3.71	0.039	0.117	0.081	0.194	0.067	0.065	0.041	0.075	0.106	0.173	0.958	2.326	3.284	0.545	2.614	0.292
11	3.66	0.050	0.200	0.085	0.124	0.083	0.113	0.022	0.011	0.201	0.393	1.282	2.749	4.031	0.379	2.190	0.318
12	3.54	0.050	0.129	0.072	0.213	0.069	0.079	0.028	0.036	0.081	0.139	0.896	1.696	2.592	1.760	1.337	0.346
13	3.52	0.023	0.026	0.016	0.081	0.035	0.033	0.010	0.012	0.164	0.123	0.523	2.025	2.548	0.197	6.737	0.205
14	3.51	0.054	0.068	0.041	0.103	0.016	0.053	0.006	0.003	0.103	0.161	0.608	1.565	2.173	0.566	2.674	0.280
15	3.51	0.044	0.064	0.038	0.096	0.047	0.083	0.013	0.015	0.249	0.150	0.799	2.606	3.405	0.196	5.355	0.235
16	3.13	0.031	0.101	0.055	0.172	0.049	0.056	0.025	0.053	0.175	0.137	0.854	2.396	3.250	0.489	2.578	0.263
17	3.01	0.016	0.030	0.016	0.069	0.026	0.020	0.006	0.010	0.084	0.086	0.363	1.080	1.443	0.283	4.934	0.252
18	3.00	0.072	0.128	0.088	0.191	0.096	0.079	0.031	0.022	0.097	0.137	0.941	1.533	2.474	1.553	1.340	0.380
19	2.92	0.046	0.046	0.019	0.049	0.047	0.052	0.010	0.011	0.119	0.131	0.530	1.542	2.072	0.306	4.484	0.256
20	2.88	0.025	0.048	0.024	0.078	0.024	0.045	0.013	0.019	0.061	0.083	0.420	0.696	1.116	0.684	1.742	0.376
21	2.82	0.028	0.074	0.041	0.120	0.028	0.044	0.010	0.026	0.057	0.072	0.500	0.950	1.450	0.741	1.498	0.345
22	2.68	0.019	0.026	0.014	0.038	0.009	0.060	0.009	0.013	0.039	0.054	0.281	1.345	1.626	0.171	6.253	0.173
23	2.27	0.018	0.038	0.021	0.062	0.017	0.048	0.011	0.009	0.062	0.066	0.352	1.440	1.792	0.199	5.553	0.196
24	2.16	0.022	0.062	0.040	0.081	0.024	0.038	0.016	0.033	0.063	0.084	0.463	1.652	2.115	0.220	4.608	0.219
25	2.12	0.014	0.037	0.020	0.060	0.012	0.038	0.009	0.006	0.084	0.068	0.348	1.237	1.585	0.184	5.095	0.220
26	2.11	0.024	0.100	0.065	0.177	0.026	0.048	0.033	0.019	0.293	0.173	0.960	3.264	4.224	0.237	4.299	0.227
27	1.98	0.020	0.030	0.019	0.056	0.012	0.030	0.008	0.011	0.040	0.093	0.319	0.944	1.263	0.236	3.713	0.253
28	1.91	0.013	0.021	0.010	0.041	0.022	0.031	0.007	0.008	0.079	0.071	0.303	0.879	1.182	0.177	6.139	0.256
29	1.85	0.016	0.020	0.012	0.029	0.005	0.028	0.002	0.003	0.018	0.026	0.159	0.777	0.936	0.220	4.619	0.170
30	1.73	0.038	0.030	0.019	0.060	0.062	0.061	0.016	0.018	0.084	0.102	0.490	1.141	1.631	0.302	3.976	0.300

Table 2 (Cont'd)

No.	S. Alb. g/100ml	PLASMA FREE AMINO ACIDS IN µM/ml													RATIOS		
		Trp	Leu	Ile	Val	Tyr	Phe	Met	½ Cys	Thr	Lys	EAA*	NEAA	TAA	Val/Gly	Wh.abb.	EAA/TAA
31	1.64	0.018	0.077	0.024	0.146	0.026	0.060	0.008	0.010	0.104	0.085	0.558	1.635	2.193	0.547	2.475	0.254
32	1.63	0.025	0.030	0.015	0.030	0.008	0.030	0.006	0.006	0.031	0.046	0.227	1.013	1.240	0.157	5.704	0.183
33	1.45	0.007	0.032	0.024	0.062	0.009	0.026	0.012	0.041	0.030	0.055	0.298	1.075	1.373	0.188	5.169	0.217
34	1.33	0.023	0.052	0.021	0.079	0.062	0.075	0.019	0.039	0.180	0.211	0.761	1.510	2.271	0.238	3.626	0.335
35	1.30	0.026	0.023	0.015	0.044	0.007	0.031	0.005	0.004	0.028	0.086	0.269	0.995	1.264	0.177	6.448	0.213
36	1.29	0.024	0.044	0.016	0.072	0.018	0.030	0.009	0.013	0.055	0.143	0.424	1.282	1.706	0.393	3.865	0.249
37	1.27	0.030	0.061	0.013	0.116	0.016	0.039	0.011	0.022	0.108	0.120	0.536	1.562	2.098	0.492	3.990	0.255
38	1.25	0.017	0.022	0.010	0.032	0.019	0.039	0.009	0.017	0.020	0.056	0.241	0.815	1.056	0.145	6.521	0.228
39	1.22	0.021	0.040	0.021	0.058	0.017	0.033	0.012	0.011	0.084	0.330	0.627	1.558	2.185	0.236	3.611	0.287
40	0.79	0.020	0.028	0.016	0.032	0.020	0.039	0.012	0.007	0.014	0.032	0.220	0.751	0.971	0.327	3.591	0.227
41	0.79	0.020	0.016	0.008	0.028	0.009	0.019	0.004	0.007	0.017	0.044	0.172	1.091	1.263	0.058	12.107	0.136

* Tyrosine and ½ Cystine have also been included as essentials.

Table 3

Correlation coefficients (r) between serum albumin and each plasma free amino-acid; and linear regression of each plasma free amino acid (in μM/ml) on serum albumin (in g/100 ml) of 41 severely malnourished children at the time of their admission to the hospital.

Plasma free amino-acids	r	p	Linear regression equations of plasma amino acids on serum albumin
Tryptophan	0.7442	$<1 \times 10^{-7}$	y = - 0.0003 + 0.013207X
Leucine	0.6570	$<5 \times 10^{-6}$	y = - 0.0159 + 0.033655X
Isoleucine	0.6547	$<5 \times 10^{-6}$	y = - 0.0106 + 0.018941X
Valine	0.6498	$<5 \times 10^{-6}$	y = - 0.0035 + 0.042398X
Tyrosine	0.6436	$<6 \times 10^{-6}$	y = - 0.0088 + 0.018420X
Alanine	0.5664	$<2 \times 10^{-4}$	y = 0.0351 + 0.098532X
Glutamic Acid	0.5602	$<2 \times 10^{-4}$	y = 0.0481 + 0.060437X
Phenylalanine	0.5508	$<2 \times 10^{-4}$	y = 0.0178 + 0.013384X
Ornithine	0.5279	$<5 \times 10^{-4}$	y = 0.0106 + 0.108281X
Proline	0.5240	$<5 \times 10^{-4}$	y = 0.0988 + 0.054416X
Arginine	0.5239	$<5 \times 10^{-4}$	y = - 0.0120 + 0.026188X
Methionine	0.5073	$<1 \times 10^{-3}$	y = 0.0019 + 0.0053951X
Asparagine + Glutamine	0.4942	$<2 \times 10^{-3}$	y = 0.0982 + 0.035222X
Serine	0.4907	$<2 \times 10^{-3}$	y = 0.0693 + 0.032622X
Threonine	0.4887	$<2 \times 10^{-3}$	y = 0.0149 + 0.034858X
Lysine	0.3715	$<2 \times 10^{-2}$	y = 0.0522 + 0.029017X
½ Cystine	0.3711	$<2 \times 10^{-2}$	y = 0.0045 + 0.0065632X
Histidine	0.3160	$<5 \times 10^{-2}$	y = 0.0334 + 0.012735X
Glycine	0.0157	n.s.	y = 0.2656 + 0.0017452X
Taurine	- 0.1050	n.s.	y = 0.0731 - 0.0072243X

Table 4

Correlation coefficients (r) between serum albumin and total, essentials*, non-essentials and several ratios of plasma free amino acids; and linear regression equations of same groups and ratios of the plasma free amino acids (in μM/ml) on serum albumin (in g/100 ml) of 41 severely malnourished young children at the time of their admission to the hospital.

Groups and ratios of plasma free amino acids	r	p	Linear regression equation of groups & ratios of plasma amino acids on serum albumin
Total essential amino acids*	0.6414	$<1 \times 10^{-5}$	$y = 0.0522 + 0.215967X$
Total amino acids	0.6146	$<3 \times 10^{-5}$	$y = 0.8214 + 0.523906X$
Valine/Glycine (Arroyave's)	0.5706	$<2 \times 10^{-4}$	$y = 0.0838 + 0.221974X$
Total non-essentials	0.5522	$<2 \times 10^{-4}$	$y = 0.7223 + 0.307256X$
Total essentials/Total amino acids	0.5296	$<5 \times 10^{-4}$	$y = 0.1870 + 0.030851X$
Tyrosine/Glycine (Arroyave's)	0.5257	$<5 \times 10^{-4}$	$y = 0.0387 + 0.084766X$
Non-essentials/Total essentials*	- 0.5027	$<1 \times 10^{-3}$	$y = 4.1682 - 0.455733X$
Whitehead & Dean ratio	- 0.5021	$<2 \times 10^{-3}$	$y = 4.6710 - 0.596704X$
Whitehead & Dean abbreviated ratio	- 0.4861	$<2 \times 10^{-3}$	$y = 5.5637 - 0.734035X$
½ Cystine/Glycine (Arroyave's)	0.3916	$<2 \times 10^{-2}$	$y = 0.0266 + 0.022729X$

Footnote: *Tyrosine and ½ Cystine have also been included as essentials.

Figure 1

Plot of linear regression equations of plasma free non-essential amino acid concentrations of 41 malnourished infants on their serum albumin concentrations. "P" values are given for those that were significant. Broken lines correspond to "projections" beyond the range of actual values.

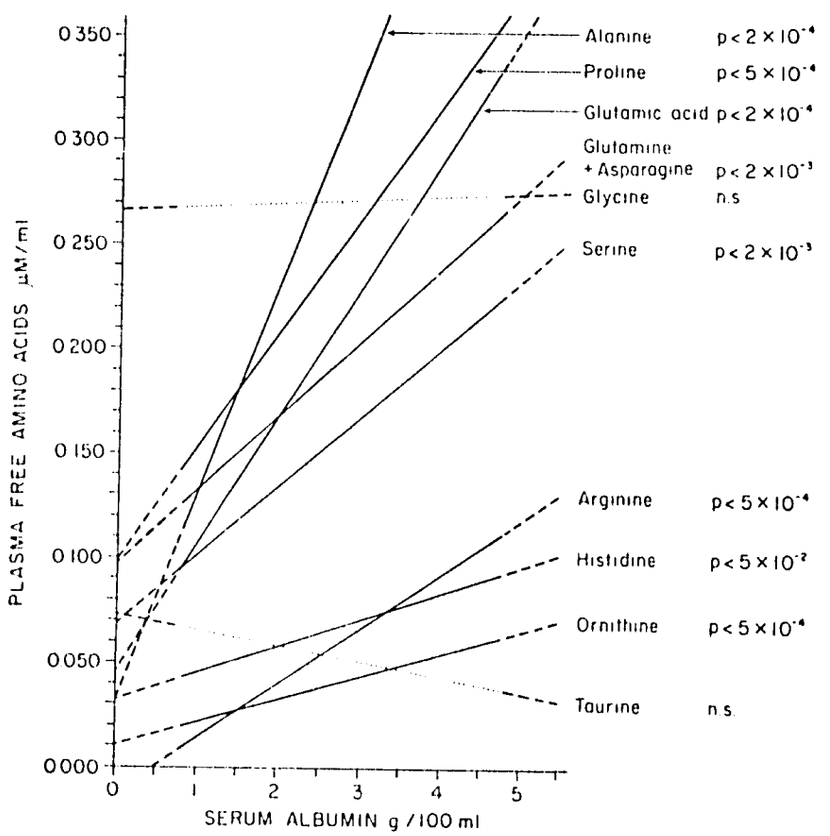
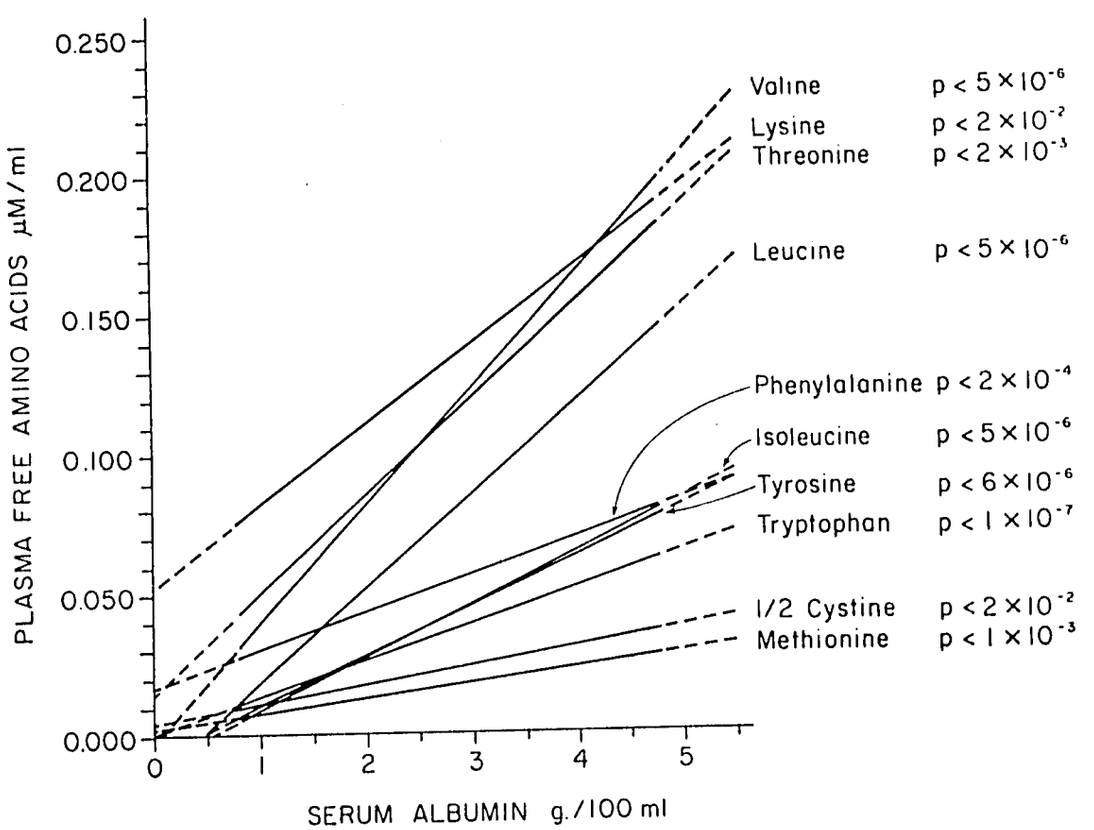


Figure 2

Plot of linear regression equations of plasma free essential amino acid concentrations of 41 malnourished infants on their serum albumin concentrations. The "semi-essential" amino acids, tyrosine and cystine, are included. Broken lines correspond to "projections" beyond the range of actual values.



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Serum Proteins and Plasma Free Amino Acids in Severe Malnutrition

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SUBJECT:

Progress Report - October 5, 1973
Research Contract AID/csd-2946
Critical Evaluation of New (or Enriched) Protein Sources (For the Prevention of Malnutrition)

The ready availability of relatively simple laboratory determinations for the quantification of the protein "status" of individuals would be very useful in clinical practice as well as in nutritional surveys involving groups of people. The diagnosis of the extreme forms of protein-calorie malnutrition in infants and children, marasmus and kwashiorkor, does not require laboratory assistance. There are other situations in which some estimate of the degree of protein "deficiency" is of great value: recognition of protein malnutrition in individuals without obvious stigmata; assessment of the adequacy and effectiveness of dietary management in the sick and malnourished, or in those in whom dietary restriction of protein or individual amino acids is necessary; and research studies aimed at determining the adequacy of a protein or amino acid source and its level of intake.

Much of the literature on the subject recognizes the serum albumin level as such an indicator, either explicitly or by implication, as when it is used to validate other laboratory determinations. Probably the most convincing documentation of its reliability is in the work of Thomas and Combs, who in a carefully planned study varied the protein and calorie intakes of chicks and demonstrated an excellent correlation between serum albumin levels and body composition (1); an almost equally high correlation was found with total serum proteins. In our studies of convalescent malnourished infants receiving different levels of protein and calories we found serum albumin to be a reliable indicator of protein "adequacy"

(2). In some situations, however, it must be interpreted with caution. In marasmus or starvation, calorie deficiency may be so severe that serum albumin does not give any indication of the severity of a concommitant protein deficiency: its level may be high, normal, or nearly normal, but the signs of calorie deprivation are obvious. Contraction of the extracellular fluid due to losses of sodium and water may result in falsely elevated serum total protein and albumin levels: the values after hydration will be more reliable. Excess sodium and water retention may cause falsely low values. There is no general agreement on the lower limit of normal serum albumin levels, below which protein malnutrition can be confidently stated to exist (3).

Because of the above considerations, and because there is no simple method yet available for the determination of serum albumin in the field, a number of alternate procedures have been suggested as indicators of protein "status", most of them relying on serum or plasma amino acid concentrations. Notable among these are those proposed by Arroyave et al (4, 5) and by Whitehead (6, 7), both of whom felt that these reflected the protein status rather than the composition of the diet associated with the development of the deficiency state. Whitehead developed two simplified techniques, usable in the field, to relate the concentration of some non-essential amino acids to that of a group of essentials, the ratio being

directly correlated with the severity of protein deficiency, gauged in part by the serum albumin levels. Arroyave has proposed the ratios of several essential amino acids, individually, to glycine, as positively correlated with nutritional history and protein status (5). Both authors feel that these ratios or indices are useful for the recognition of marginal or pre-clinical cases of protein malnutrition. In marasmus, however, they do not serve as a gauge of severity (8).

The present report analyses the relationship between serum total protein, serum albumin and plasma amino acid concentrations in 41 severely malnourished infants and children, in an effort to assess their usefulness as clinical or field tools.

Materials and Methods

The patients studied represent 41 nearly consecutive unselected cases of severe malnutrition admitted under our care to the British American Hospital in Lima, Peru. In most of them chronologic age was verified by examination of birth or baptismal certificates. Along with anthropometric measurements, physical examination, routine hematologic studies, serum electrolytes, and appropriate cultures, the initial evaluation included serum total protein, serum albumin, and plasma free amino acid determinations. In all but one case (when it was drawn 15 hours later, after partial rehydration), venous blood samples were obtained within the first hour after admission. Although we have no reliable data on the

length of time elapsed since the last feeding prior to admission, the precarious clinical condition of the great majority fairly well precluded any significant feeding other than some clear liquids in the preceding 24 hours. In only one of them (#12) was there a reliable history of cow's milk ingestion very shortly before admission. Three of the patients (#'s 30, 34 and 37) died within 48 hours of admission.

Serum total proteins were determined by the biuret method, serum albumin by paper electrophoresis (Spinco procedure B). Plasma was sealed in glass ampules, frozen promptly and stored at -20°C, shipped in dry ice within one month to Baltimore, and kept frozen until analyzed (within one month) for free amino acids by liquid column chromatography (9). Tryptophan was determined separately by fluorescence assay (10).

"Length ages" and "weight ages" are those to which the actual measurements of the children correspond on the 50th percentile of a well-known United States standard (11) and, when related to chronologic age, are a crude and arbitrary measure of stunting and weight deficit. The relation of the second to the first of these two "ages" (WA/LA) is an indicator of the degree of wasting (Table 1). The amount of edema present was subjectively quantified from questionable (±) to severe and generalized (4+), as were the skin and hair changes usually associated with kwashiorkor. The same has been done for signs and symptoms of dehydration and acidosis, although the clinical impressions were generally supported by

changes in body weight, hematocrit, serum solids, serum bicarbonate and in some, blood pH. The clinical diagnosis of significant infection, generally confirmed by blood or urine cultures, is indicated by a + sign.

The concentrations of the individual amino acids are expressed in micromoles (μM) per ml, as are the sums of the essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (TAA). Tyrosine and ½ cystine, both considered "semi-essential" because they can spare phenylalanine and methionine, respectively, are included as essential along with the usual eight.

The valine to glycine ratio, Val/Gly, one of those proposed by Arroyave (5), is the ratio of their molar concentrations. The Whitehead "abbreviated" ratio is the ratio of the concentrations of glycine + serine + glutamine + taurine to those of leucine + isoleucine + valine + methionine, determined by paper chromatography (7). In our results the concentration of asparagine is included in the numerator, as it does not separate well from glutamine by liquid column chromatography in our laboratory. The ratio of total essential amino acids (EAA) to total amino acids (TAA) is one which we have used in a number of studies: its values in infants and children receiving milk protein diets have been reported (12).

Results

In Table 1 the patients are listed in descending order of their serum albumin levels. Examination of the table reveals that the first nine patients were 8 months of age or younger, stunted, severely wasted, free of apparent edema and skin or hair changes, and rather frequently dehydrated and acidotic, all characteristic of the severe infantile marasmus associated with diarrhea that we commonly see in urban slums. The last seventeen patients were all over 13 months of age, with six of them over 24 months of age. Almost all of them had prominent edema and skin or hair changes; some were dehydrated but none was obviously acidotic, this last the probable result of severe potassium deficits. The degree of wasting, though prominent in some and masked by edema in others, was generally not as severe as in the first group. Some of them, for example #'s 33, 36 and 40, had significant body fat present and might be called "pure" kwashiorkor, but the majority we would call "marasmic" kwashiorkor. The remaining patients, #'s 10 through 24, were more heterogeneous in age and clinical characteristics; some are further examples of marasmus, others what we might call "marasmus with hypoalbuminemia", and still others "marasmic" kwashiorkor, illustrating the difficulties of classification. The frequency of dehydration and infection reflects our bias toward the admission of the sickest children who cannot be handled successfully on an out-patient basis.

Table 2 again lists the patients in descending order of their serum albumin levels. It gives the molar concentrations of the ten essential and semi-essential amino acids in their plasma, their sum (EAA), the sum of the non-essentials (NEAA), the combined total (TAA), the molar ratio of valine to glycine (Arroyave), the Whitehead abbreviated ratio, and the total essentials and semiessentials as the molar fraction of the total (EAA/TAA). It is apparent that as the serum albumin level decreases, so do the concentrations of the individual essential amino acids, EAA, NEAA, TAA, Val/Gly, and EAA/TAA. The Whitehead abbreviated ratio, reflecting the more marked decrease in essential than in non-essential amino acid concentrations, increases as albumin decreases. In most of the cases of marasmus all of the above values tended to be normal, thus failing to recognize the severe protein deficiency which accompanies but is overshadowed by the extreme energy deficit.

Table 3 lists the correlation coefficients, "r", between serum albumin levels and the plasma free concentrations of each individual amino acid, except asparagine and glutamine, which are expressed as their combined molar concentration. The "P" value of each coefficient is listed, as well as the linear regression equation of each plasma free amino acid's molar concentration (y) on the simultaneously determined serum albumin concentration in g/100 ml (X). The high and very significant correlations of tryptophan, the branched chain amino acid and tyrosine concentrations with serum albumin

are apparent. Slightly less but still high and significant, are the correlation coefficients for the non-essentials alanine, glutamic acid, ornithine, proline, and arginine, and the essentials phenylalanine and methionine. Less so, but still significant, are the correlations for the non-essentials asparagine + glutamine, serine and histidine, and the essentials threonine, lysine and ½ cystine. The correlation coefficients for glycine and taurine were not significant.

Although not in the tables, the correlation coefficient of serum albumin with total serum proteins was extremely high, "r" = 0.947, "P" = 1.4×10^{-9}. For serum globulins and total serum proteins the correlation coefficient was much lower, 0.778. The correlation coefficients for the individual amino acids with total serum proteins, though not reported here, were very similar to those for albumin, but slightly higher, probably reflecting the lesser possibility of laboratory error in the determination of total serum proteins.

Table 4 lists the correlation coefficients "r" between the serum albumin levels of the 41 patients and the sum of the concentrations of essential, non-essential and total amino acids, as well as a number of ratios: EAA/TAA, NEAA/EAA, Val/Gly, Tyr/Gly, ½ Cys/Gly, and both Whitehead ratios. Also listed are their "P" values and the regression equations of the various concentrations (μM/ml), or ratios, on serum albumin (g/100 ml). The highest correlation is with the essential amino acids, followed closely by total amino acids. Even the

concentration of non-essentials has a higher correlation coefficient than any of the ratios, with the sole exception of the valine/glycine ratio.

Figure 1 depicts graphically the linear regression equations of the individual non-essential amino acid concentrations on serum albumin level. For glycine and taurine (not in reality an amino acid) the correlations were not significant, for all the others the "P" value is included. Only arginine, considered essential for young animals and possibly so for the very young human infant, projects to a zero value before zero albumin is reached.

As can be seen in Figure 2, most of the essential and semi-essential amino acids reach a projected value of zero before albumin does, or simultaneously. The most notable exception is lysine, with a relatively high value of more than 0.05 μM/ml at zero albumin. Also relatively "resistant" are threonine and phenylalanine. Despite this, the correlations are significant for all three.

Discussion

The serum albumin level can be used, with some reservations, as a reliable indicator of the protein status of individuals. In numerous evaluations of the protein quality of the diet we have almost invariably found that when a child gains weight on a diet that is not supporting adequate nitrogen retention, the serum albumin level falls significantly in

a matter of days, indicating that the protein "concentration" of the body has fallen (2). If, on the other hand, the child stops gaining or loses weight on a diet which is adequate in calories but inadequate in protein, the serum albumin level does not fall and may actually rise, suggesting that the body composition is being maintained by converting energy from the diet into heat. When dietary energy is inadequate for weight gain, but does meet basal requirements, changes in the serum albumin level probably depend to a great extent on dietary protein. When not even basal energy requirements are met, as in the development of marasmus, severe weight loss ensues but serum albumin may remain at normal levels, even when dietary protein is absent or insignificant.

From the above considerations it is apparent that serum albumin is affected by both protein and calorie intakes and by the relation between these. It cannot be used alone, without regard to the clinical condition of the patient, as an indicator of the body protein content or concentration. This is well illustrated by the present series of patients, in which the first 8 marasmic infants had serum albumin levels above the lower limit of normal in our laboratory, 3.8 g/100 ml. That body protein content and concentration are not normal in this situation is obvious from studies of body composition, both by indirect methods (13) and by analysis of muscle biopsies (14). It seems logical to assume that in extreme and prolonged calorie deficiency, amino acids coming from muscle and the internal organs are used for albumin synthesis. The opposite

situation probably exists early on when weight gain and growth continue despite an inadequate protein intake: serum albumin gives up amino acids to internal organs, muscle, and supporting tissues. If the situation is prolonged, the protein concentration of these tissues decreases and eventually the clinical picture of kwashiorkor develops.

Alterations of the body fluid compartments are characteristic of malnutrition and even in extreme marasmus the extracellular fluid volume is expanded relative to body weight (13). Contraction of the plasma volume, when severe diarrhea supervenes, further complicates the interpretation of concentrations of plasma constituents. Severe infection, also present in some of our cases, can have a direct effect on protein synthesis and catabolism.

In the present series of 41 patients serum albumin was consistently lower in those with manifestations which are considered typical of severe protein deficiency. In a recent report Whitehead et al found a falling serum albumin level to be a reliable indicator of developing kwashiorkor (15). In our patients a very close correlation was found between serum albumin and total serum protein. If the first is a good indicator of protein "status", then the second is equally good, and technically much simpler to determine: there are a number of satisfactory field methods for total serum protein determination. Since albumin normally constitutes more than 50% of total serum protein, and varies more readily than the

globulins, which are relatively insensitive to nutritional alterations, such a high degree of correlation is not surprising.

The relatively high correlations between the concentrations of total amino acids, total essentials, total nonessentials, and most of the individual amino acids, with serum albumin or total protein levels must in part be due to their being affected to a similar degree by changes in hydration. Expansion of the extracellular fluid volume, with or without clinically apparent edema, and dehydration, the result of complicating diarrhea, were undoubtedly common in this series of cases

That changes in hydration do not account for all or most of the variation, however, is apparent from the way in which many of the individual amino acids differed in the degree to which they followed serum albumin changes (Table 3, Figures 1 and 2), and by the relatively high correlation coefficients for the various ratios, particularly the valine/glycine ratio.

The close correlation between serum albumin and plasma amino acid concentrations, particularly those of the essentials, suggests that the one has a determining effect on the others, or vice versa. Of particular interest are the very high correlation coefficients for plasma tryptophan and the branched chain amino acids. The first of these has a recently acquired reputation as the "initiator" of protein synthesis in the liver (16). It has also been shown that, uniquely among the plasma amino acids, two-thirds of the "free"

tryptophan is in reality bound to albumin (17), another possible explanation for the close correlation found. A special role of the branched chain amino acids in liver protein synthesis has also been demonstrated (18).

The relative "resistance" to fall of plasma lysine, and to a lesser degree, that of threonine, is in accord with the observations of others and is probably related to their less efficient catabolic pathways. A greater fall in the concentration of most of the essential amino acids than that of the non-essentials is characteristic of kwashiorkor (19) and determines the changes in the various ratios reported here. Our statistical analysis does not correct for the variance due to changes in hydration, and thus does not adequately recognize that these ratios are independent of volume changes.

Examination of Table 2 reveals that among the marasmic infants with normal serum albumins there are a number with low Val/Gly and EAA/TAA ratios and elevated Whitehead ratios, suggesting that they were more protein "deficient" than their serum protein levels indicate. Conversely, there are a number of children with very low serum albumins and some normal amino acid ratios. Of particular interest are #'s 30, 34 and 37, the three patients who died. Despite serum albumins of 1.73, 1.33 and 1.27 g/100 ml, none had the classical signs of kwashiorkor: edema and skin or hair changes (Table 1). Their Val/Gly ratios of 0.302, 0.238 and 0.492 were all low, but not as low as those of most cases of kwashiorkor. The Whitehead

ratios of 3.976, 3.626 and 3.990, though high, were not as high as those of the more typical kwashiorkor. The EAA/TAA ratios of 0.300, 0.335 and 0.255 were normal in the first two cases, moderately low in the third. Our normal mean for healthy children on milk protein diets is approximately 0.300 (12). These results suggest that their very low serum albumins were more the result of their severe infections than of protein deficiency.

It is apparent from these studies that in severely malnourished infants and children, particularly if they are very sick, initial determinations of serum proteins and plasma amino acids will seldom provide any information that is not clinically evident. The close correlations between serum proteins and some of the amino acid concentrations and ratios suggest that in field studies, or in following the course of a particular patient, the choice of laboratory procedure to be used should be dictated by simplicity and cost. On this basis we would recommend total serum proteins, the plasma valine/glycine ratio, or the Whitehead abbreviated ratio.

Summary

Serum albumin, serum total protein and plasma free amino acids were measured on admission in 41 severely malnourished infants and children, many of whom were dehydrated, acidotic or obviously infected. In order to assess their usefulness in the quantification of protein "status", the correlation coefficients of group and individual amino acid concentrations, as well as various essential to non-essential amino acid ratios, with the serum proteins, was determined. Much of the close correlation between the various concentrations was felt to be due to variations in hydration. Correlation between serum total protein and albumin was so close that it was felt that the first, a much simpler determination, could easily replace the latter as a field tool and in many clinical situations. Plasma concentrations of amino acids, particularly the essentials, were closely correlated with serum proteins, in part the result of changes in hydration. Significantly higher correlation coefficients for tryptophan and the branched chain amino acids suggested a more specific role in determining serum albumin levels. Various amino acid "ratios", particularly valine/glycine, were significantly correlated with serum albumin. There were enough exceptions to suggest that these ratios might recognize severe protein deficiency in infants whose dehydration had elevated their serum albumin levels, or in whom adaptations to extreme calorie deficiency had resulted in conservation of albumin

at the expense of other proteins. Conversely, normal amino acid ratios in patients with hypoalbuminemia might be useful in identifying non-nutritional causes.

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Table 1

Clinical and laboratory findings of 41 severely malnourished young children at time of admission to hospital: body length (L), body weight (W), chronological age (C.A.), length age (L.A.), weight age (W.A.), and serum proteins: total and albumin (Album.).

No. & Sex	Body L cm	Body W kg	AGE In Months C.A.	L.A.	W.A.	WA/LA	Edema	Skin &/ or Hair	Dehydration	Acidosis	Infection	Hct	Serum Protein g/100 ml Total	Album.
1F	59.5	3.81	4.2	3.2	0.3	0.09			3+	3+		38	7.84	4.79
2M	59.0	3.39	4.1	2.3	N.B.				+			37	7.66	4.44
3F	57.3	3.50	3.6	2.3	0.1	0.04			±			35	7.33	4.36
4F	57.0	3.90	6.7	2.1	0.4	0.02					+	30	7.30	4.23
5M	63.0	4.00	4.6	4.1	0.6	0.15		±		+	+	38	7.43	4.09
6F	62.0	4.74	8.0	4.4	1.4	0.32					+	38	7.43	4.05
7M	54.5	3.67	2.7	0.7	0.2	0.29			3+	3+		38	7.87	3.94
8M	51.5	2.25	1.9	0.2	<N.B.			+			+	26	6.96	3.83
9M	57.0	3.46	5.1	1.5	<N.B.				2+		+	38	6.21	3.76
10F	65.0	4.85	15.1	6.1	1.4	0.23			2+	+	+	32	7.63	3.71
11M	72.0	6.32	22.4	9.8	4.1	0.42					+	30	6.82	3.66
12M	66.5	5.42	7.7	6.0	2.4	0.40			2+		+	30	6.44	3.54
13M	55.5	3.13	3.5	1.0	<N.B.						+	43	6.20	3.52
14F	68.3	5.17	16.1	8.1	2.0	0.25					+	35	6.66	3.51
15F	65.0	4.45	11.4	6.1	1.0	0.02			3+	2+	+	32	6.54	3.51
16F	66.0	4.91	8.9	6.7	1.5	0.22		+			+	34	7.06	3.13
17M	70.5	6.50	15.8	8.5	4.4	0.52	±	±	+	+	+	36	5.74	3.01
18F	55.0	3.52	8.8	1.4	0.1	0.07					+	30	6.15	3.00
19M	67.5	5.71	18.2	6.5	3.0	0.46		+				29	7.20	2.92
20M	49.5	2.75	2.3	0.1	<N.B.			+	3+	2+	+	32	5.32	2.88
21M	63.5	4.40	8.8	4.4	1.0	0.23			2+		+	27	6.12	2.82
22F	80.5	9.40	20.4	19.8	11.0	0.56	+	+	+			32	4.29	2.68
23F	71.0	5.74	17.0	9.9	3.0	0.30		+	2+		+	35	4.76	2.27
24F	66.0	5.25	7.7	6.7	2.5	0.37	+		+			40	4.70	2.16
25F	72.5	6.26	16.3	11.0	3.9	0.35	+	+			+	35	4.58	2.12

Table 1 (Cont'd)

No. & Sex	Body L cm	Body W kg	AGE In Months C.A.	AGE In Months L.A.	AGE In Months W.A.	WA/LA	Edema	Skin &/ or Hair	Dehydra-tion	Acido-sis	Infec-tion	Hct	Serum Protein g/100 ml Total	Serum Protein g/100 ml Album.
26M	84.0	9.78	36.6	20.1	11.1	0.55	2+	2+				39	5.51	2.11
27F	66.0	5.12	13.5	6.7	2.0	0.30	2+	+	+			36	4.82	1.98
28M	80.0	7.57	16.1	16.3	6.2	0.38	2+	+	+		+	34	4.65	1.91
29F	75.0	8.76	21.6	12.8	9.1	0.71	3+	+	2+			28	4.15	1.85
30M	81.0	9.27	19.3	17.2	9.7	0.56			2+		+	28	4.65	1.73
31M	81.5	9.30	20.7	17.8	9.8	0.55	3+	2+			+	26	4.67	1.64
32F	73.5	7.00	15.6	11.6	5.4	0.47	3+	2+	+			20	3.93	1.63
33M	85.5	13.05	35.4	21.5	28.5	1.33	3+	3+				33	3.71	1.45
34M	70.0	8.52	26.2	8.3	7.8	0.94			2+		+	31	3.69	1.33
35M	75.0	6.72	15.8	12.1	4.8	0.40	+	+				28	4.15	1.30
36M	74.0	10.47	13.4	11.3	13.7	1.21	4+	2+			+	23	3.86	1.29
37F	77.0	6.49	24.1	14.5	5.3	0.37			2+		+	35	3.10	1.27
38M	69.8	6.56	24.0	8.1	4.6	0.57	3+	3+				28	3.82	1.25
39F	75.0	8.28	16.6	12.8	8.0	0.63	3+	+			+	29	4.30	1.22
40M	80.5	13.00	39.7	16.7	28.0	1.68	4+					33	3.29	0.79
41F	71.5	7.65	15.0	10.4	6.7	0.64	4+	3+			+	19	2.43	0.79

Table 2

Serum albumin (S. Alb.) and plasma free amino acids of 41 severely malnourished young children at the time of admission to the hospital: individual essentials, total essentials (EAA)*, total non-essentials (NEAA), total amino acids (TAA), and Valine/Glycine (Val/Gly), the Whitehead abbreviated (Wh.abb.), and the EAA/TAA ratios.

No.	S. Alb. g/100ml	PLASMA FREE AMINO ACIDS IN uM/ml													RATIOS		
		Trp	Leu	Ile	Val	Tyr	Phe	Met	½ Cys	Thr	Lys	EAA*	NEAA	TAA	Val/Gly	Wh.abb.	EAA%TAA
1	4.79	0.075	0.169	0.059	0.172	0.081	0.073	0.015	0.019	0.077	0.219	0.959	1.620	2.579	1.117	1.065	0.372
2	4.44	0.063	0.151	0.086	0.198	0.057	0.089	0.029	0.064	0.101	0.071	0.909	1.745	2.654	0.712	1.726	0.343
3	4.36	0.043	0.061	0.027	0.089	0.037	0.044	0.023	0.021	0.106	0.111	0.562	2.036	2.598	0.276	3.995	0.216
4	4.23	0.089	0.221	0.133	0.348	0.127	0.065	0.045	0.025	0.289	0.278	1.620	2.458	4.078	1.420	1.011	0.397
5	4.09	0.043	0.039	0.026	0.109	0.081	0.114	0.010	0.016	0.139	0.104	0.681	1.747	2.428	0.452	3.027	0.280
6	4.05	0.082	0.158	0.096	0.239	0.102	0.075	0.032	0.025	0.224	0.220	1.253	2.193	3.446	1.170	1.213	0.365
7	3.94	0.065	0.242	0.134	0.261	0.124	0.148	0.058	0.101	0.357	0.403	1.893	3.045	4.938	0.562	1.599	0.383
8	3.83	0.040	0.058	0.038	0.085	0.024	0.026	0.014	0.027	0.149	0.092	0.553	1.888	2.441	0.220	3.497	0.227
9	3.76	0.041	0.112	0.061	0.202	0.032	0.036	0.011	0.014	0.053	0.088	0.650	1.409	2.059	1.496	1.420	0.316
10	3.71	0.039	0.117	0.081	0.194	0.067	0.065	0.041	0.075	0.106	0.173	0.958	2.326	3.284	0.545	2.614	0.292
11	3.66	0.050	0.200	0.085	0.124	0.083	0.113	0.022	0.011	0.201	0.393	1.282	2.749	4.031	0.379	2.190	0.318
12	3.54	0.050	0.129	0.072	0.213	0.069	0.079	0.028	0.036	0.081	0.139	0.896	1.696	2.592	1.760	1.337	0.346
13	3.52	0.023	0.026	0.016	0.081	0.035	0.033	0.010	0.012	0.164	0.123	0.523	2.025	2.548	0.197	6.737	0.205
14	3.51	0.054	0.068	0.041	0.103	0.016	0.053	0.006	0.003	0.103	0.161	0.608	1.565	2.173	0.566	2.674	0.280
15	3.51	0.044	0.064	0.038	0.096	0.047	0.083	0.013	0.015	0.249	0.150	0.799	2.606	3.405	0.196	5.355	0.235
16	3.13	0.031	0.101	0.055	0.172	0.049	0.056	0.025	0.053	0.175	0.137	0.854	2.396	3.250	0.489	2.578	0.263
17	3.01	0.016	0.030	0.016	0.069	0.026	0.020	0.006	0.010	0.084	0.086	0.363	1.080	1.443	0.283	4.934	0.252
18	3.00	0.072	0.128	0.088	0.191	0.096	0.079	0.031	0.022	0.097	0.137	0.941	1.533	2.474	1.553	1.340	0.380
19	2.92	0.046	0.046	0.019	0.049	0.047	0.052	0.010	0.011	0.119	0.131	0.530	1.542	2.072	0.306	4.484	0.256
20	2.88	0.025	0.048	0.024	0.078	0.024	0.045	0.013	0.019	0.061	0.083	0.420	0.696	1.116	0.684	1.742	0.376
21	2.82	0.028	0.074	0.041	0.120	0.028	0.044	0.010	0.026	0.057	0.072	0.500	0.950	1.450	0.741	1.498	0.345
22	2.68	0.019	0.026	0.014	0.038	0.009	0.060	0.009	0.013	0.039	0.054	0.281	1.345	1.626	0.171	6.253	0.173
23	2.27	0.018	0.038	0.021	0.062	0.017	0.048	0.011	0.009	0.062	0.066	0.352	1.440	1.792	0.199	5.553	0.196
24	2.16	0.022	0.062	0.040	0.081	0.024	0.038	0.016	0.033	0.063	0.084	0.463	1.652	2.115	0.220	4.608	0.219
25	2.12	0.014	0.037	0.020	0.060	0.012	0.038	0.009	0.006	0.084	0.068	0.348	1.237	1.585	0.184	5.095	0.220
26	2.11	0.024	0.100	0.065	0.177	0.026	0.048	0.033	0.019	0.293	0.173	0.960	3.264	4.224	0.237	4.299	0.227
27	1.98	0.020	0.030	0.019	0.056	0.012	0.030	0.008	0.011	0.040	0.093	0.319	0.944	1.263	0.236	3.713	0.253
28	1.91	0.013	0.021	0.010	0.041	0.022	0.031	0.007	0.008	0.079	0.071	0.303	0.879	1.182	0.177	6.139	0.256
29	1.85	0.016	0.020	0.012	0.029	0.005	0.028	0.002	0.003	0.018	0.026	0.159	0.777	0.936	0.220	4.619	0.170
30	1.73	0.038	0.030	0.019	0.060	0.062	0.061	0.016	0.018	0.084	0.102	0.490	1.141	1.631	0.302	3.976	0.300

Table 2 (Cont'd)

No.	S. Alb. g/100ml	PLASMA FREE AMINO ACIDS IN µM/ml													RATIOS		
		Trp	Leu	Ile	Val	Tyr	Phe	Met	½ Cys	Thr	Lys	EAA*	NEAA	TAA	Val/Gly	Wh.abb.	EAA/TAA
31	1.64	0.018	0.077	0.024	0.146	0.026	0.060	0.008	0.010	0.104	0.085	0.558	1.635	2.193	0.547	2.475	0.254
32	1.63	0.025	0.030	0.015	0.030	0.008	0.030	0.006	0.006	0.031	0.046	0.227	1.013	1.240	0.157	5.704	0.183
33	1.45	0.007	0.032	0.024	0.062	0.009	0.026	0.012	0.041	0.030	0.055	0.298	1.075	1.373	0.188	5.169	0.217
34	1.33	0.023	0.052	0.021	0.079	0.062	0.075	0.019	0.039	0.180	0.211	0.761	1.510	2.271	0.238	3.626	0.335
35	1.30	0.026	0.023	0.015	0.044	0.007	0.031	0.005	0.004	0.028	0.086	0.269	0.995	1.264	0.177	6.448	0.213
36	1.29	0.024	0.044	0.016	0.072	0.018	0.030	0.009	0.013	0.055	0.143	0.424	1.282	1.706	0.393	3.865	0.249
37	1.27	0.030	0.061	0.013	0.116	0.016	0.039	0.011	0.022	0.108	0.120	0.536	1.562	2.098	0.492	3.990	0.255
38	1.25	0.017	0.022	0.010	0.032	0.019	0.039	0.009	0.017	0.020	0.056	0.241	0.815	1.056	0.145	6.521	0.228
39	1.22	0.021	0.040	0.021	0.058	0.017	0.033	0.012	0.011	0.084	0.330	0.627	1.558	2.185	0.236	3.611	0.287
40	0.79	0.020	0.028	0.016	0.032	0.020	0.039	0.012	0.007	0.014	0.032	0.220	0.751	0.971	0.327	3.591	0.227
41	0.79	0.020	0.016	0.008	0.028	0.009	0.019	0.004	0.007	0.017	0.044	0.172	1.091	1.263	0.058	12.107	0.136

* Tyrosine and ½ Cystine have also been included as essentials.

Table 3

Correlation coefficients (r) between serum albumin and each plasma free amino-acid; and linear regression of each plasma free amino acid (in μM/ml) on serum albumin (in g/100 ml) of 41 severely malnourished children at the time of their admission to the hospital.

Plasma free amino-acids	r	p	Linear regression equations of plasma amino acids on serum albumin
Tryptophan	0.7442	$<1 \times 10^{-7}$	y = - 0.0003 + 0.013207X
Leucine	0.6570	$<5 \times 10^{-6}$	y = - 0.0159 + 0.033655X
Isoleucine	0.6547	$<5 \times 10^{-6}$	y = - 0.0106 + 0.018941X
Valine	0.6498	$<5 \times 10^{-6}$	y = - 0.0035 + 0.042398X
Tyrosine	0.6436	$<6 \times 10^{-6}$	y = - 0.0088 + 0.018420X
Alanine	0.5664	$<2 \times 10^{-4}$	y = 0.0351 + 0.098532X
Glutamic Acid	0.5602	$<2 \times 10^{-4}$	y = 0.0481 + 0.060437X
Phenylalanine	0.5508	$<2 \times 10^{-4}$	y = 0.0178 + 0.013384X
Ornithine	0.5279	$<5 \times 10^{-4}$	y = 0.0106 + 0.108281X
Proline	0.5240	$<5 \times 10^{-4}$	y = 0.0988 + 0.054416X
Arginine	0.5239	$<5 \times 10^{-4}$	y = - 0.0120 + 0.026188X
Methionine	0.5073	$<1 \times 10^{-3}$	y = 0.0019 + 0.0053951X
Asparagine + Glutamine	0.4942	$<2 \times 10^{-3}$	y = 0.0982 + 0.035222X
Serine	0.4907	$<2 \times 10^{-3}$	y = 0.0693 + 0.032622X
Threonine	0.4887	$<2 \times 10^{-3}$	y = 0.0149 + 0.034858X
Lysine	0.3715	$<2 \times 10^{-2}$	y = 0.0522 + 0.029017X
½ Cystine	0.3711	$<2 \times 10^{-2}$	y = 0.0045 + 0.0065632X
Histidine	0.3160	$<5 \times 10^{-2}$	y = 0.0334 + 0.012735X
Glycine	0.0157	n.s.	y = 0.2656 + 0.0017452X
Taurine	- 0.1050	n.s.	y = 0.0731 - 0.0072243X

Table 4

Correlation coefficients (r) between serum albumin and total, essentials*, non-essentials and several ratios of plasma free amino acids; and linear regression equations of same groups and ratios of the plasma free amino acids (in μM/ml) on serum albumin (in g/100 ml) of 41 severely malnourished young children at the time of their admission to the hospital.

Groups and ratios of plasma free amino acids	r	p	Linear regression equation of groups & ratios of plasma amino acids on serum albumin
Total essential amino acids*	0.6414	$<1 \times 10^{-5}$	$y = 0.0522 + 0.215967X$
Total amino acids	0.6146	$<3 \times 10^{-5}$	$y = 0.8214 + 0.523906X$
Valine/Glycine (Arroyave's)	0.5706	$<2 \times 10^{-4}$	$y = 0.0838 + 0.221974X$
Total non-essentials	0.5522	$<2 \times 10^{-4}$	$y = 0.7223 + 0.307256X$
Total essentials/Total amino acids	0.5296	$<5 \times 10^{-4}$	$y = 0.1870 + 0.030851X$
Tyrosine/Glycine (Arroyave's)	0.5257	$<5 \times 10^{-4}$	$y = 0.0387 + 0.084766X$
Non-essentials/Total essentials*	- 0.5027	$<1 \times 10^{-3}$	$y = 4.1682 - 0.455733X$
Whitehead & Dean ratio	- 0.5021	$<2 \times 10^{-3}$	$y = 4.6710 - 0.596704X$
Whitehead & Dean abbreviated ratio	- 0.4861	$<2 \times 10^{-3}$	$y = 5.5637 - 0.734035X$
½ Cystine/Glycine (Arroyave's)	0.3916	$<2 \times 10^{-2}$	$y = 0.0266 + 0.022729X$

Footnote: *Tyrosine and ½ Cystine have also been included as essentials.

Figure 1

Plot of linear regression equations of plasma free non-essential amino acid concentrations of 41 malnourished infants on their serum albumin concentrations. "P" values are given for those that were significant. Broken lines correspond to "projections" beyond the range of actual values.

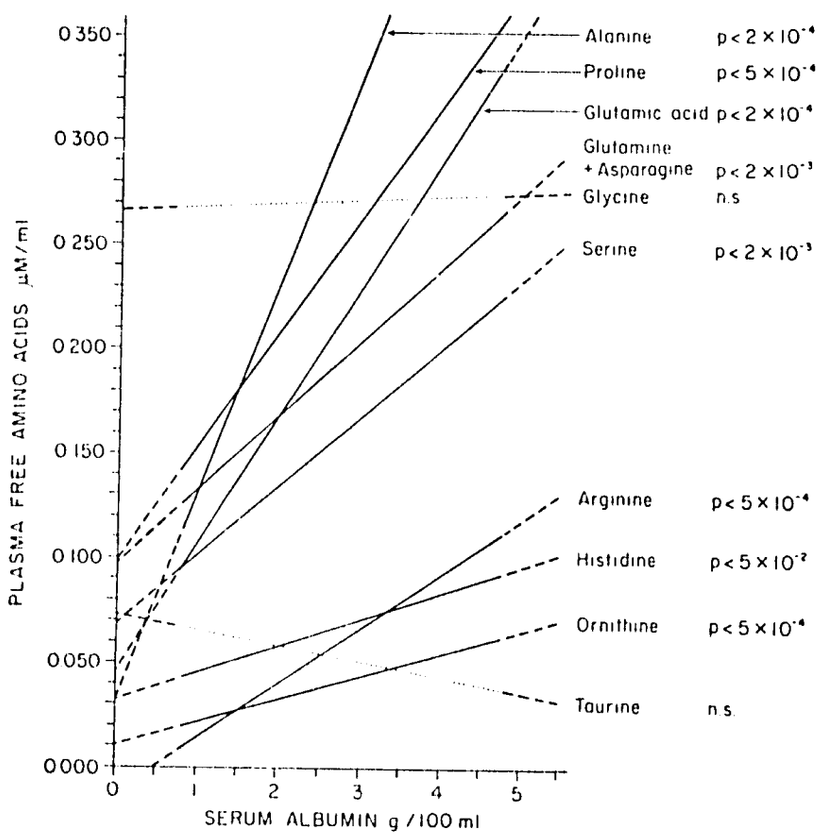
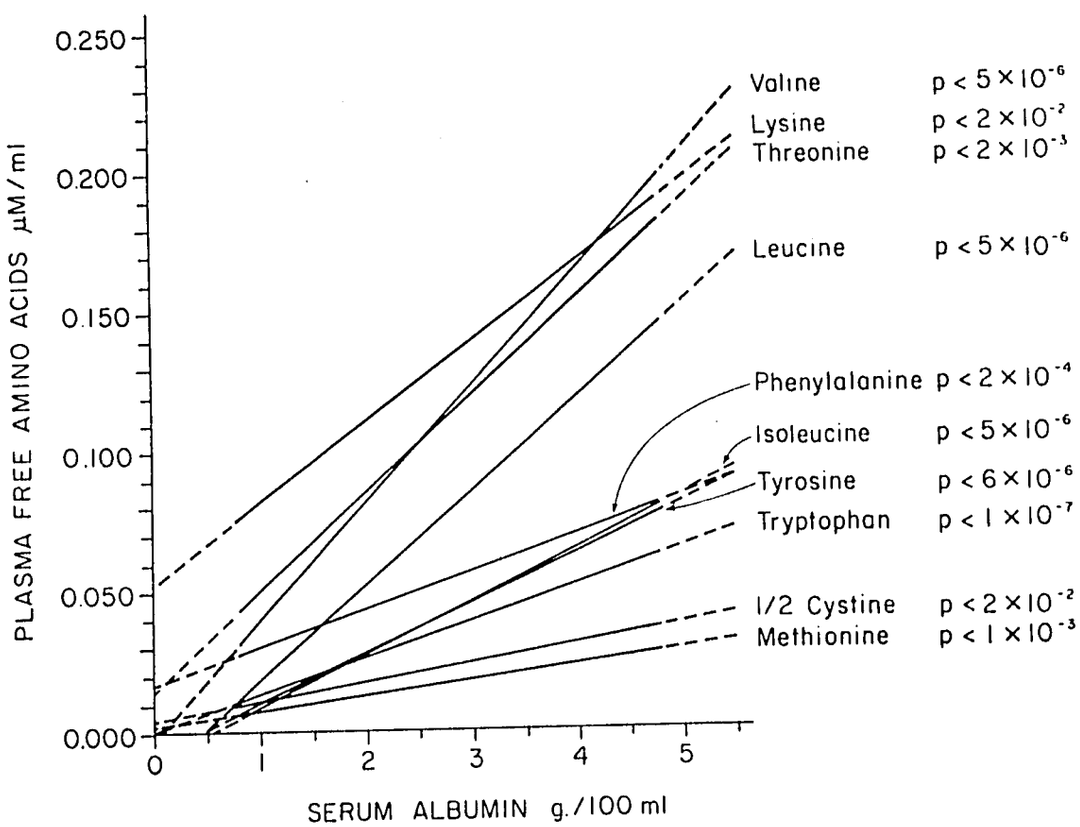


Figure 2

Plot of linear regression equations of plasma free essential amino acid concentrations of 41 malnourished infants on their serum albumin concentrations. The "semi-essential" amino acids, tyrosine and cystine, are included. Broken lines correspond to "projections" beyond the range of actual values.