The Free Amino Acids in Blood Plasma of Children with Kwashiorkor and Marasmus

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Few studies have been published on plasma amino acid concentrations in patients with kwashiorkor. Qualitative and semi-quantitative information obtained by paper chromatography suggest marked deviations from the normal pattern.[1] Using the quantitative technics of Moore and Stein,[2] Westall et al.[3] and Cravioto et al.[4] obtained detailed information about specific amino acid levels of Mexican children suffering from this severe form of protein malnutrition. Holt et al.[5] refer also to investigations of patients from Dakar and Nigeria, but they give neither clinical descriptions nor numbers of children investigated. An abstract by Norton[6] summarized data from Haiti, Mexico, Dakar and Nigeria. These reports indicate that in kwashiorkor the indispensable amino acids plus tyrosine and cystine tend to be reduced much below normal values.

The postprandial concentrations of individual plasma amino acids may, of course, reflect effects due to the absorption of amino acids from the recently ingested proteins.[7] After overnight fasting, however, the effect of a meal would be eliminated and, therefore, any alteration of the fasting pattern of plasma amino acids should be principally of endogenous origin.

In the present report, concentrations of free plasma amino acids in six patients with kwashiorkor and two with marasmus are compared with those of five children who recovered completely from kwashiorkor. Preliminary observations on the effect of a milk test meal on the plasma amino acid pattern in two patients with kwashiorkor and two patients who re-recovered are also included.

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MATERIALS AND METHODS

Patients with Kwashiorkor or Marasmus

The subjects of the study were six patients with kwashiorkor and two with typical marasmus, selected according to clinical criteria published elsewhere.[8] They were admitted to the INCAP ward for metabolism studies at the hospital of the Sociedad Protectora del Niño in Guatemala City. Some of the characteristics of the cases are given in Table I. No child with severe anemia was included.

Each child received only fluid and electrolyte therapy during the first sixteen to twenty hours of hospitalization. Before dietary therapy was initiated, a 15 to 20 ml. sample of venous blood was taken from the internal jugular vein at about 8 A.M. and placed in a heparinized tube. This sample was used for the determination of the fasting plasma amino acid levels. In two children (A. I. and B. R.) the effect of a milk feeding on the plasma amino acid levels was also investigated. Immediately after the blood sample was obtained, these two children were given a single feeding of a liquid mixture of milk, starch, fat and sugar which furnished 1 gm. of protein per kilogram of body weight. Two and a half hours later, a second blood sample was collected in the same manner as the first.

Children Who Recovered

The five children in the study who recovered were selected from those in INCAP's metabolism ward who were considered completely cured of kwashior-

TABLE I
Some Characteristics of the Children Suffering from Kwashiorkor and Marasmus

Patient	Age	Diagnosis	Weight (kg.)	Height (cm.)	Edema*	Serum Proteins (gm./100 ml.)					
						Total	Albumin	α_1	α_2	β	γ
A. T.	3 yr., 10 mo.	Kwashiorkor	9.89	90	++	4.15	1.60	0.33	0.47	0.40	1.36
B. R.	5 yr., 5 mo.	Kwashiorkor	12.95	90	++	4.20	1.77	0.32	0.68	0.46	0.97
M. V.	1 yr., 6 mo.	Kwashiorkor	7.88	69	+++	3.60	1.43	0.60		0.32	1.25
A. C.	3 yr., 3 mo.	Kwashiorkor	10.06	79	++	4.26	1.44	0.26	0.62	0.44	1.50
T. A.	5 yr., 0 mo.	Kwashiorkor	9.52	95	++	4.07	1.02	0.30	0.65	0.53	1.56
S. A.	6 yr., 0 mo.	Kwashiorkor	13.38	98	+++	3.24	0.65	0.26	0.62	0.47	1.24
E. Ch.	1 yr., 1 mo.	Marasmus	5.47	69	–	5.83	2.89	0.35	0.85	0.75	0.99
R. L.	1 yr., 2 mo.	Marasmus	4.59	68	–	4.91	2.05	0.35	0.67	0.43	1.45

* ++ = moderate; +++ = severe; – = absent.

TABLE II
Some Characteristics of the Children Who Had Recovered from Kwashiorkor and Were Used as Controls in the Study

Patient	Age	Time from Admission (mo.)	Weight* (kg.)	Height (cm.)	Serum Proteins (gm./100 ml.)					
					Total	Albumin	α_1	α_2	β	γ
J. G.	3 yr., 3 mo.	7	11.34	84	7.06	3.77	0.31	0.87	0.89	1.23
T. I.	6 yr., 2 mo.	12	19.50	98	7.33	4.06	0.30	0.86	0.88	1.21
J. A.	5 yr., 11 mo.	15	20.41	106	6.95	4.31	0.86		0.45	1.33
S. A.	6 yr., 4 mo.	4	13.60	92	6.70	3.70	0.20	1.01	0.58	1.21
G. A.	5 yr., 7 mo.	7	13.15	89	6.75	4.31	0.26	0.44	0.59	1.15

* Although the weights of these children are still below the expected figure for their chronological age, their weight:height ratio is normal.

kor. Table II gives some characteristics of the illness in these children. Fasting plasma amino acid levels were determined in three separate samples from one child (G. A.), two from another (J. A.) and one from each of the other three. Samples were collected in the morning, at least fifteen hours after ingestion of the last meal, in the same manner as from the sick children. The effect of a test meal of milk on the plasma amino acid levels was also studied in two of these children (T. I. and J. A.) following the same procedure as for the sick children.

Blood samples were placed under refrigeration within one hour of collection. The plasma was separated from the cells by centrifugation and prepared for ion-exchange resin chromatography following the technic described by Stein and Moore.[2] The resulting protein-free filtrate was then analyzed in a 1.50 m. × 0.9 cm. (i.d.) column of Dowex 50 – × 5 resin, and the separated amino acids were measured colorimetrically with ninhydrin.[9]

The figures for total plasma amino acids are the sums of the twenty amino acids determined individually by the chromatographic method. Tryptophan is not included since it was not determined by this method. Urea was measured in 20 μl. of serum, after incubation with urease at pH 6, protein precipitation with tungstic acid and nesslerization.

RESULTS

Table III gives the individual values for serum urea and total plasma amino acids. The values for amino acids are in agreement with those of previous investigations in other regions of the world.[1,4,5] A relationship between the amino acid levels and the degree of severity of the disease was not apparent. The amino acid levels of the two children with marasmus were intermediate between those of the children with kwashiorkor and those who recovered.

TABLE III
Plasma Urea and Total Amino Acids in Children with Kwashiorkor and Marasmus

Patient	Urea Nitrogen (mg./100 cc. serum)	Total Amino Acids	
		mg./100 cc. Plasma	% of x Recovered
Children with Kwashiorkor			
A. T.	7.8	10.0	47
B. R.	4.6	10.6	50
M. V.	9.2	9.1	43
S. A.	10.7	7.4	35
T. P.	8.3	10.5	50
A. C.	6.3	9.4	44
Average	7.8	9.5	45
Children with Marasmus			
E. Ch.	5.5	12.1	57
R. L.	12.9	14.1	66
Children Recovered from Kwashiorkor			
T. I.	17.9	22.2	...
T. G.	7.9	16.7	...
S. A.	13.8	20.9	...
J. A.	13.9	24.7	...
G. A.	20.9	21.7	...
Average	14.9	21.2	...

The low concentration of urea nitrogen in the children with kwashiorkor confirms findings by other workers.[4,10,11] The average of 7.8 mg. per 100 ml. for kwashiorkor is 52 per cent of the average for the five children who recovered. In one of the children with marasmus, the mean nitrogen level was markedly reduced; in the other, it approached the values for the children who recovered.

Table IV gives the concentrations of the individual plasma amino acids found in the six children with kwashiorkor and in the two with marasmus, as well as the averages for the five children who recovered. The values for the sick children are also expressed in per cent of the average concentrations found in the recovered children.

In general, the concentrations of the essential amino acids were severely depressed in children with kwashiorkor, with phenylalanine and lysine being the least affected and valine and leucine the most affected. Among the non-essential amino acids, tyrosine averaged lowest, followed by cystine, arginine and alanine. The average value of 39 per cent for alanine includes the very low value for one child (S. A.). Excluding this value, the average would be 44 per cent.

The phenylalanine: tyrosine ratio in kwashiorkor (Table V) is consistently elevated due to the much larger decrease in tyrosine than in phenylalanine. This finding agrees completely with results of previous workers.[3,4,6] In two children the arginine: ornithine ratio was definitely lower; in three others, it was about the same, and in one child it was much higher than in the children who recovered. The two children with kwashiorkor who had the lowest arginine: ornithine ratio also had the highest phenylalanine: tyrosine ratio. One (S. A.) had the highest arginine and the lowest ornithine level among all the patients with kwashiorkor who were studied.

In the two children with marasmus, levels of most of the amino acids were intermediate between those found in the children with kwashiorkor and those in the children who recovered except for the low threonine value in one child (F. Ch.) and the low alanine level in another (R. L.). In general, the pattern in this child differed more from that of the patients with kwashiorkor than did that of F. Ch. Lysine and phenylalanine were the amino acids least affected in marasmus.

The nature of these interrelationships is revealed in Figure 1. To construct the "normal" plasma amino acid profile, the amino acids have been arbitrarily arranged according to decreasing concentration (mg. per 100 ml. of plasma) as was done by Holt et al.[5] The values for the malnourished children are compared in each case with this "normal" amino acid profile.

Figure 2 records the changes in the individual plasma amino acid patterns of two patients with kwashiorkor and two children who recovered given the test meal. The post-absorptive profiles of the children who re-

TABLE
Plasma Amino Acids in

Amino Acid	Recovered N = 5 x̄ mg./100 ml. ± S.D.	Children with					
		A. T.		B. R.		M. V.	
		mg./100 ml.	%	mg./100 ml.	%	mg./100 ml.	%
Alanine	2.103 ± 0.40	1.058	50	0.700	33	0.768	36
Valine	1.679 ± 0.48	0.308	18	0.240	14	0.211	13
Glycine	1.607 ± 0.30	1.292	80	1.892	117	1.452	90
Lysine	1.431 ± 0.23	0.723	50	0.739	52	0.517	36
Histidine	1.367 ± 0.14	0.882	64	0.888	65	0.932	68
Glutamic	1.364 ± 0.40	1.113	82	0.723	53	0.733	54
Proline	1.312 ± 0.20	0.748	57	1.113	84	0.467	36
Serine	1.105 ± 0.22	0.765	69	0.891	81	0.977	83
Leucine	1.088 ± 0.34	0.241	22	0.172	16	0.206	19
Tyrosine	0.922 ± 0.28	0.078	8	0.099	11	0.132	14
Arginine	0.810 ± 0.07	0.142	18	0.251	31	0.229	28
Threonine	0.790 ± 0.22	0.262	33	0.369	47	0.185	23
Cystine	0.736 ± 0.14	0.089	12	0.101	14	0.078	11
Phenylalanine	0.729 ± 0.19	0.375	51	0.233	32	0.311	43
Isoleucine	0.629 ± 0.18	0.117	19	0.159	25	0.164	26
Methionine	0.241 ± 0.08	0.092	38	0.058	24	0.088	36
Aspartic	0.070 ± 0.02	0.050	71	...	...	0.046	66
Taurina	0.432 ± 0.10	0.418	97	0.299	53	0.522	120
Ornithine	0.668 ± 0.17	0.252	38	0.241	36	0.251	38
Glutamic glutamine + asparagine	2.151 ± 0.64	1.044	48	1.400	65	0.849	40

NOTE: % = per cent of value in recovered children.

covered were qualitatively similar to their "basal fasting profile." Closer examination revealed differences which, in a previous publication,[12] were tentatively attributed to the quality of the children's protein intake during the two months before the test.

The general tendency of parallelism between the pre- and postabsorptive profiles is seen also for the two children with kwashiorkor. The postabsorptive changes in one child (A. T.) are almost negligible. The increase in amino acid nitrogen was greater in one child (B. R.) than in another (A. T.), but the disproportions in the fasting plasma pattern were not improved by the milk protein feeding. The responses in one child (B. R.) and those of one of the children who recovered (J. A.) are almost the same, both in total plasma amino acids and in most of the individual ones. The difference, therefore, in the postprandial patterns of these two children is mainly due to their widely differing fasting profiles.

COMMENTS

In malnourished children the plasma amino acid patterns do not seem to reflect the composition of the dietary proteins consumed during the development of kwashiorkor or marasmus.* Recent work at INCAP[13] has shown that the plasma amino nitrogen level in children consuming diets completely lacking in protein for periods up to fifteen days did not change significantly, although there was a drop in most of the essential amino acids and a compensating increase in the nonessential amino acids. This clearly differs from the findings in the children with kwashiorkor and marasmus. In these the changes are, for the most part, probably due to a serious failure in various enzymatic pathways associated with the metabolism of the individual amino acids.

As discussed by Cravioto et al.[4] such failure

* Estimated from dietary history data.

IV
Kwashiorkor and Marasmus

Kwashiorkor								Children with Marasmus			
A. C.		T. P.		S. A.		Average		E. Ch.		R. L.	
mg./100 ml.	%	mg./100 ml.	%	mg./100 ml.	%	mg./100 ml.	%	mg./100 ml.	%	mg./100 ml.	%
1.082	51	1.021	48	0.300	14	0.821	39	1.900	90	0.678	32
0.291	17	0.352	21	0.246	15	0.275	16	0.456	27	0.584	35
2.096	130	1.773	110	0.957	60	1.577	98	1.266	79	1.596	99
0.459	32	0.643	45	0.512	36	0.599	42	0.830	58	1.225	86
1.041	76	0.993	73	0.966	71	0.950	70	0.633	46	1.081	79
0.556	41	0.904	66	0.469	34	0.750	55	1.080	79	0.994	73
0.658	50	0.608	46	0.323	25	0.653	50	1.109	84	0.918	70
0.882	75	0.830	71	0.581	49	0.821	74	0.680	62	0.928	84
0.151	14	0.211	19	0.175	16	0.193	18	0.447	41	0.488	45
0.069	8	0.083	9	0.075	8	0.089	10	0.300	32	0.238	26
0.051	6	0.118	15	0.339	42	0.188	23	0.243	30	0.187	23
0.218	28	0.321	41	0.180	23	0.256	32	0.199	25	0.601	76
0.131	18	0.241	33	0.038	5	0.113	15	0.163	22	0.269	36
0.404	55	0.324	44	0.269	37	0.319	44	0.587	80	0.766	105
0.057	9	0.134	21	0.139	22	0.128	20	0.209	33	0.234	37
0.058	24	0.076	32	0.067	28	0.073	30	0.122	51	0.183	76
0.024	34	0.166	237	0.071	101	0.060	86	...	...	0.223	319
0.392	91	0.840	194	0.701	162	0.529	122	0.604	140	1.325	307
0.154	23	0.130	20	0.096	14	0.187	28	0.273	41	0.297	44
0.653	30	0.777	36	0.920	43	0.940	44	1.018	47	1.284	60

in the phenylalanine hydroxylase enzymatic system might explain the greatly reduced concentration of tyrosine. The widely variable arginine:ornithine ratios in kwashiorkor could also be an indication of enzymatic impairment. It may not be a coincidence that the two children with the highest phenylalanine:tyrosine ratios had the lowest arginine:ornithine ratios.

The relatively high lysine level is possibly due to its unique catabolic pathway. Oxidative deamination of lysine by L- or D-amino acid oxidases of mammalian kidney, for example, is extremely low compared with that of other amino acids.[14,15] It is also reasonable to think that the blockage on one of the catabolic paths of phenylalanine, its hydroxylation, could be at least partially responsible for its relatively high value.

As suggested by Holt[16] the extremely low tyrosine concentration may well be a cause of the lack of melanin in the hair of severely malnourished children. The same possibility exists for the effect of insufficient cystine on the alterations in hair texture. Close[17] has reported that the cystine content is low in the hair of patients with kwashiorkor in the Congo.

The remarkable consistency in the amino acid pattern among the children with kwashiorkor was probably a result of the standardized conditions of the sampling, including the strictness of the clinical criteria in selecting the cases. Blood samples were taken at approximately the same time of day after similar overnight fasting periods. Under the fasting conditions, the pattern of the plasma amino acids would be more nearly determined by endogenous factors alone.

The abnormal plasma amino acid pattern in the two children suffering from acute kwashiorkor did not approach normal after the single feeding of milk protein. Although these results must be considered tentative because only two children were tested, it is possible that an

TABLE V
Arginine:Ornithine and Phenylalanine:Tyrosine Ratios in Children with Kwashiorkor and Marasmus

Patients	Phenylalanine: Tyrosine	Arginine: Ornithine
Children with Kwashiorkor		
A. T.	4.81	0.56
B. R.	2.35	1.04
M. V.	2.35	0.91
S. A.	3.59	3.53*
T. P.	3.90	0.91
A. C.	5.85	0.33
Children with Marasmus		
E. Ch.	1.96	0.89
R. L.	3.22	0.63
Children Recovered from Kwashiorkor		
T. I.	0.60	1.25
J. G.	0.90	1.88
S. A.	0.80	1.06
J. A.	0.84	0.95
G. A.	0.92	1.23

* Patient S. A. had the lowest ornithine and the highest arginine value among the children with kwashiorkor.

amino acid mixture with proportionally higher concentrations of those amino acids which are in deficit in the plasma would be more efficiently utilized initially by severely malnourished children.

The findings should also be examined for their possible significance in protein and amino acid metabolism at the cellular level in malnourished children. To maintain an adequate intracellular amino acid environment, a certain concentration of extracellular amino acids must exist. Enriched amino acid environments are associated with accelerated growth.[18,19] Furthermore, efficient protein-anabolism requires the simultaneous presence of each amino acid in correct proportion to the others.

There is already evidence that the various amino acids compete with one another for the mechanisms that concentrate them intracellularly.[20,21] The extreme disproportions found in the plasma amino acids of these malnourished children may impair the active

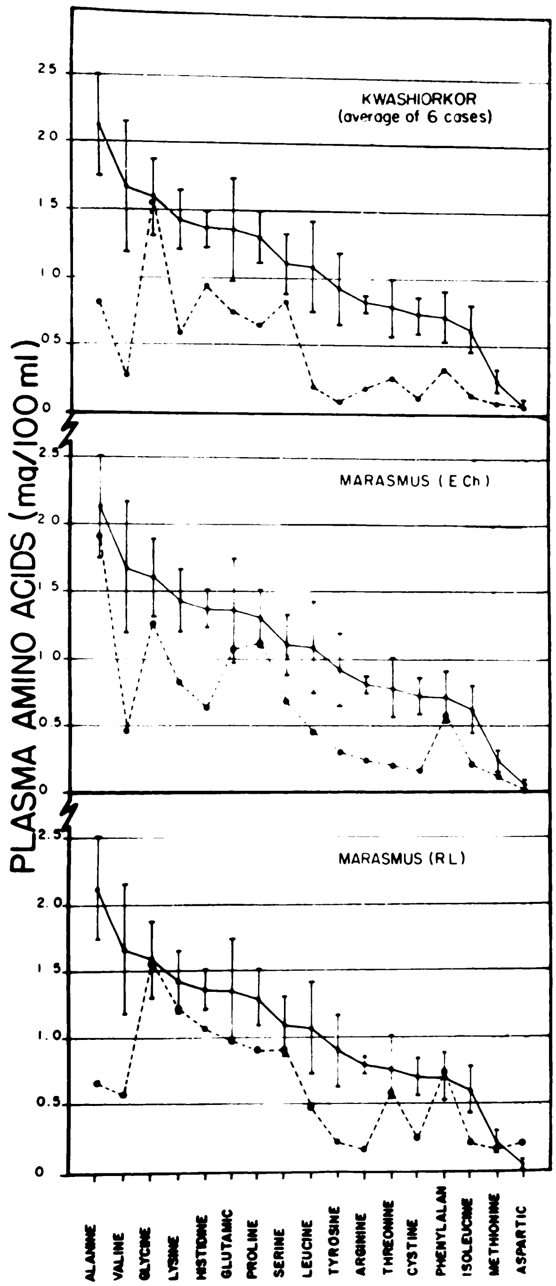

FIG. 1. Fasting plasma amino acids in six children with kwashiorkor and two with marasmus (broken line). The solid line represents the fasting plasma amino "profile" of five healthy children (see text). The vertical lines indicate the standard deviations of the individual amino acid values.

transfer of amino acids into the cells. Such profoundly altered plasma amino acid patterns must result in a greatly altered intracellular free amino acid pool. The mechanisms for protein synthesis would then be presented

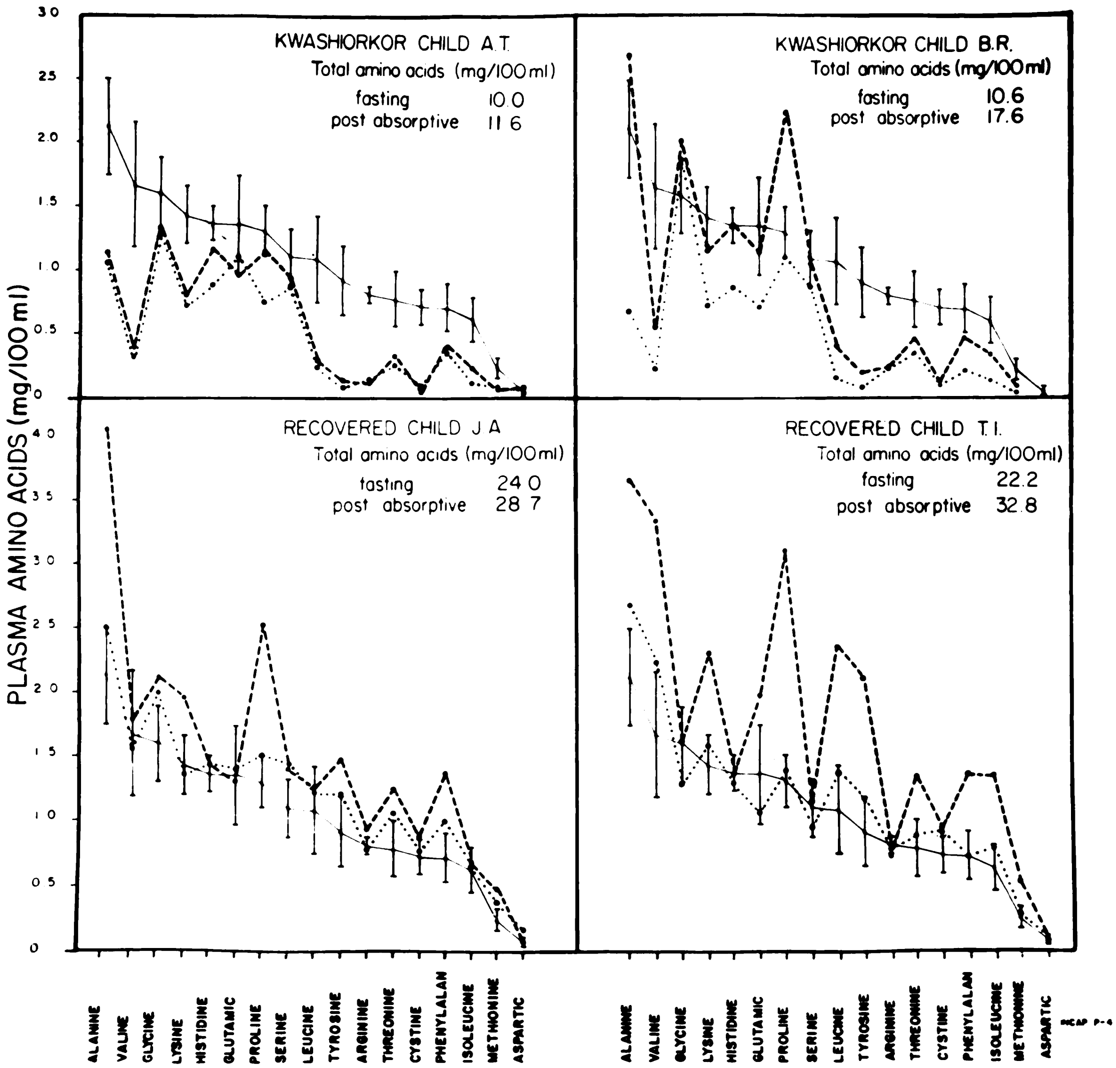

FIG. 2. Effect of single milk feeding on the plasma amino acids of children with kwashiorkor and healthy children. The solid line and the vertical lines are the average plasma amino acid "profile" of five healthy children and the standard deviations for the individual amino acid values as explained in the text. The dotted line and the broken line are, respectively, the fasting and postabsorptive profiles for the children tested.

with an amino acid substrate complex, not only low in concentration, but also of unsuitable composition. Therefore, regardless of its initial cause, the imbalanced combination of amino acids in the severely malnourished child may very well be one of the fundamental inadequacies in cell nutrition adversely affecting normal protein synthesis.

SUMMARY

The plasma-free amino acids of five children with kwashiorkor and two with marasmus have been determined by ion-exchange column chromatography. In agreement with previous workers, the total α-amino acids in children with kwashiorkor were found to be approximately half of those in healthy children. In general, the amino acids most affected were the essential ones with the exception of lysine and phenylalanine. Of the nonessential amino acids, tyrosine, cystine and arginine were the lowest in concentration. Similar changes were observed in the two children with marasmus. In two children with kwashiorkor, the increases in the individual plasma amino acids two and a half hours after a milk feeding did not tend to correct the disproportions found in their fasting amino acid pattern.

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REFERENCES

1. Edozien, J. C., Phillips, E. J. and Collis, W. R. F. The free amino acids of plasma and urine in kwashiorkor. *Lancet*, 1: 615, 1960.
2. Moore, S. and Stein, W. H. Procedures for the chromatographic determination of amino acids on four per cent cross-linked sulfonated polystyrene resins. *J. Biol. Chem.*, 211: 893, 1954.
3. Westall, R. G., Roitman, E., de la Peña, C., Rasmussen, H., Cravioto, J., Gómez, F. and Holt, L. E., Jr. The plasma amino acids in malnutrition. Preliminary observations. *Arch. Dis. Child.*, 33: 499, 1958.
4. Cravioto, J., Gómez, F., Ramos-Galván, R., Frenk, S., Montano, E. L. and García, N. Metabolismo proteico en la desnutrición avanzada: concentración de aminoácidos libres en el plasma sanguíneo. *Bol. Ofic. san. panam.*, 48: 383, 1960.
5. Holt, E. L., Jr., György, P., Pratt, E. L., Snyderman, S. E. and Wallace, W. M. Protein and Amino Acid Requirements in Early Life, p. 63. New York, 1960. University Press.
6. Norton, P. M. Plasma amino acid levels in kwashiorkor. (Abstract.) In: Proceedings of the Fifth International Congress on Nutrition. p. 7. Washington, D. C., September 1–7, 1960.
7. Longenecker, J. B. and Hause, N. L. Relationship between plasma amino acids and composition of the ingested protein. *Arch. Biochem.* 84: 46, 1959.
8. Scrimshaw, N. S. and Béhar, M. Protein malnutrition in young children. *Science*, 133: 2039, 1961.
9. Moore, S. and Stein, W. H. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.*, 211: 907, 1954.
10. Trowell, H. C., Davies, J. N. P. and Dean, R. F. A. Kwashiorkor, p. 167. London, 1954. Edward Arnold, Ltd.
11. Dean, R. F. A. and Schwartz, R. The serum chemistry of uncomplicated kwashiorkor. *Brit. J. Nutrition*, 7: 131, 1953.
12. Arroyave, G., Wilson, D., Béhar, M. and Viteri, F. The development of INCAP vegetable mixtures. II. Biochemical testing. In: National Research Council. Progress in Meeting Protein Needs of Infants and Preschool Children; Proceedings of an International Conference held in Washington, D. C., August 21–24, 1960, p. 49. Washington, D. C., 1961. National Academy of Sciences-National Research Council.
13. Arroyave, G. and Wilson, D. Plasma free-amino acids of children after periods on a nitrogen-free diet. In preparation.
14. Bender, A. E. and Krebs, H. A. The oxidation of various synthetic α-amino-acids by mammalian D-amino acid oxidase, L-amino acid oxidase of cobra venom and the L- and D-amino acid oxidase of *Neurospora crassa*. *Biochem. J.*, 46: 210, 1950.
15. Work, E. Some comparative aspects of lysine metabolism. In: A Symposium on Amino Acid Metabolism, p. 462. Edited by McElroy, W. D. and Glass, H. B. Baltimore, 1955. The Johns Hopkins Press.
16. Holt, E. L., Jr. Oral presentation at "Seminario sobre Desnutrición Infantil," Marzo 17–22, 1958, organizado por la Facultad de Medicina, Universidad del Valle, Cali, Colombia.
17. Close, J. Les modifications chimiques et morphologiques de cheveux, accompagnant le kwashiorkor. *Am. Soc. Belge Méd. Trop.*, 38: 95, 1958.
18. Christensen, H. N. and Streicher, J. A. Association between rapid growth and elevated cell concentrations of amino acids. I. In fetal tissues. *J. Biol. Chem.*, 175: 95, 1948.
19. Christensen, H. N. and Streicher, J. A. Association between rapid growth and elevated cell concentrations of amino acids. II. In regenerating liver after partial hepatectomy in the rat. *J. Biol. Chem.*, 175: 101, 1948.
20. Christensen, H. N. Mode of transport of amino acids into cells. In: A Symposium on Amino Acid Metabolism, p. 63. Edited by McElroy, W. D. and Glass, H. B. Baltimore, 1955. The Johns Hopkins Press.
21. Finch, L. R. and Hird, F. J. R. The uptake of amino acids by isolated segments of rat intestine. II. A survey of affinity for uptake from rates of uptake and competition for uptake. *Biochem. et biophys. acta*, 43: 278, 1960.