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National Research Centre, Dokki, Cairo, and the Paediatric Department, Faculty of Medicine, University of Cairo, Egypt

Red blood cells amino-acid pattern in protein energy malnutrition and the effect of oral dosing with single amino acids

F. A. El-Shobaki, N. El-Shawarby, M. F. S. El-Hawary, and *R. Sakr*

With 5 tables

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Studies performed by different authors on plasma amino acid pattern in protein energy malnutrition (PEM) revealed definite state of hypo-aminoacidemia (1, 2, 3, 4, 5) with relative increase of amino acids in moderate kwashiorkor, attributable to tissue destruction (6). The R.B.C.'s amino acids received little attention and studies performed on R.B.C.s revealed contradicting findings (7, 8, 9). *Sakr* et al. (9), reported almost normal red blood cell amino acids in protein calorie malnutrition with some elevation in certain non-essential amino acids. *Arhammer* et al. (7) reported elevation of R.B.C.'s amino acids only in kwashiorkor patients who died during the first week of treatment, while *Michail* (8) found increased level of amino acids in R.B.C.s of all malnourished cases without differentiation into kwashiorkor or marasmus.

The present work aims to investigate the R.B.C.'s amino acid pattern in protein energy malnourished cases representing different forms and grades of the disease. It is also planned to study the effect of oral dosing with individual amino acids on the total amino acid content of R.B.C.s in an attempt to investigate the effect of the disease on the mode of amino acid transport from plasma to R.B.C.s.

Material and methods

The material of this study were 139 PEM cases 3–30 months old. On clinical grounds as was described earlier (10), cases were divided into kwashiorkor (15 moderate and 21 severe) marasmic (18 2nd and 42 3rd grade) and 43 cases suffering from marasmic kwashiorkor. 17 healthy normals of similar age range and socio-economic standard were included to serve as controls. All patients and controls were free from any associate urinary tract infection or any other ailment that might derange the pattern investigated.

Fasting venous heparinized blood samples were obtained from patients and controls. Plasma was separated and R.B.C.s were washed three times with saline, then diluted with double its volume with distilled water and freezed at -20 °C overnight to ensure complete haemolysis (11). Individual amino acids in the haemolysate were determined by the method described by *Levy* and *Chang* (12) and amino acids were calculated as mg/100 ml R.B.C.s.

Five cases of each of the groups studied were given an aqueous oral dose, equivalent to 200 mg/1 kg body weight of cysteine, tryptophan and lysine. Blood samples were then collected 1, 2, 3, 4 and 6 hours after the dose and the total amino acid nitrogen of R.B.C.s were determined by the method of *Ya Pin Lea* (13).

Table 1. Essential amino acids in R.B.C.s of control and malnourished cases (mg/100 ml).

	Control	Moderate Kwashiorkor	Severe Kwashiorkor	2nd Marasmus	3rd Marasmus	Marasmic KWO	Nutritional Oedema
Trypt.	0.1	–	–	–	0.15	0.52*)	–
± S.E.	0.0				0.00	0.1	
Lysine	1.94	0.8*)	0.8*)	1.72	0.34*)	0.9*)	1.5
	0.3	0.2	0.1	0.1	0.1	0.3	0.2
Penylalanine	0.74	0.1*)	–	0.4*)	–	0.34*)	0.86
	0.1	0.0		0.1		0.1	0.1
Threonine	1.0	0.45*)	0.94	0.91	0.8	0.72	0.55*)
	0.1	0.1	0.3	0.2	0.1	0.2	0.1
Valine	2.95	1.15*)	1.39*)	2.43	1.47*)	2.35*)	0.63*)
	0.5	0.2	0.4	0.4	0.1	0.4	0.1
Methionine	0.88	0.45*)	0.9	3.31*)	0.73	0.61	0.6
	0.1	0.1	0.4	0.5	0.2	0.1	0.1
Leucine + Isoleucine	2.08	0.93*)	0.97*)	1.63*)	1.80*)	1.13*)	0.52*)
Total	9.69	3.88	5.00	10.4	5.29	6.57	4.66

*) Difference from controls is statistically significant ($P < 0.05$)

Table 1. Continued. Non-essential amino acids in R.B.C.s of control and malnourished cases (mg/100 ml).

	Control	Moderate Kwashiorkor	Severe Kwashiorkor	2nd Marasmus	3rd Marasmus	Marasmic KWO	Nutritional Oedema
Alanine	1.9	1,37	1.84	1.45	1.46	1.67	1.49
	0.1	0.2	0.2	0.1	0.2	0.3	0.2
Glycine	2.99	1.57*)	2.02	2.43	1.43*)	2.62	1.41*)
	0.3	0.2	0.1	0.3	0.3	0.6	0.2

Table 1. Continued. Non-essential amino acids in R.B.C.s of control and malnourished cases (mg/100 ml).

	Control	Moderate Kwashiorkor	Severe	2nd Marasmus	3rd	Marasmic KWO	Nutritional Oedema
Glutamic acid	0.76	2.26*)	2.40*)	1.78*)	1.80*)	2.80*)	2.07*)
0.1	0.3	0.3	0.3	0.3	0.2	0.3	
Aspartic acid	3.05	2.58	3.19	2.99	1.64*)	5.12*)	2.54*)
0.8	0.4	0.3	0.2	0.2	0.8	0.3	
Arginine	0.67	0.22*)	–	2.86*)	–	0.33*)	–
	0.1	0.00		0.2		0.1	
Histidine	0.38	–	–	6.15*)	–	0.09*)	–
	0.00			1.5		0.00	
Serine	1.85	2.01*)	0.86*)	1.96	1.15	2.71	1.26
	0.2	0.3	0.3	0.3	0.1	0.4	0.2
Tyrosine	2.27	0.68*)	0.86*)	2.64	0.52*)	1.27*)	1.40*)
	0.3	0.2	0.3	0.3	0.1	0.2	0.2
Glutamine	1.39	1.99	2.19*)	2.01	1.15	2.11*)	1.38
	0.2	0.2	0.4	0.1	0.1	0.3	0.2
Asparagine	3.78	1.86*)	1.56*)	4.08	3.91	4.04	1.35*)
	0.4	0.2	0.2	0.3	0.5	0.8	0.2
Cysteine	2.28	1.54*)	1.89	2.26	1.23*)	2.23	1.99
	0.4	0.1	0.3	0.6	0.2	0.5	0.2
Total	21.32	16.08	16.81	30.61	14.29	24.99	14.89

Table 2. R.B.C.'s total amino nitrogen (mg amino nitrogen as leucine/100 ml R.B.C.s) before and after dosing with cysteine.

Time of sampling	F.	1	2	3	4	6 hrs
Controls						
Mean	58.0	84.0	92.4	91.4	77.4	54.8
± S.E.	15.2	13.6	10.4	14.1	6.3	9.6
Kwashiorkor						
Moderate	70.0*)	78.7	122.3*)	85.8*)	82.5	91.5*)
	7.6	5.2	12.8	9.2	8.9	7.1
Severe	78.6*)	70.2	82.8	78.0	48.4*)	65.4
	7.3	6.6	7.4	4.6	8.9	10.3
Marasmus						
2nd grade	95.0*)	85.8	100.6	106.7)*	109.2)*	91.6)*
	6.6	6.7	9.3	13.5	19.6	21.3
3rd grade	117.8*)	148.0*)	115.8*)	112.4*)	115.8%6	96.0*)
	8.3	6.5	10.9	6.5	7.1	6.9
Marasmic Kwashiorkor	80.6*)	88.0	94.2	102.0	90.2*)	73.8*)
	16.9	17.3	24.2	24.2	32.1	20.4

*) The difference from controls is statistically significant ($P < 0.05$)

Table 3. R.B.C.s total amino nitrogen (mg amino nitrogen as leucine/100 ml R.B.C.s) before and after dosing with tryptophan.

Time of sampling	F.	1	2	3	4	6 hrs
Controls						
Mean	75.7	87.0	124.0	103.0	97.3	119.2
± S.E.	8.0	6.7	15.8	11.2	7.1	16.8
Kwashiorkor						
Moderate	82.0*)	100.2*)	110.6	87.6*)	86.4	78.3*)
	5.3	9.1	11.4	7.3	16.1	14.0
Severe	51.0*)	70.6	69.0*)	61.0*)	69.6*)	59.2*)
	4.2	6.2	6.8	10.4	12.5	13.8
Marasmus						
2nd grade	100.2*)	135.0*)	107.0	95.6	106.2*)	88.2*)
	17.2	14.5	16.2	8.6	8.2	11.8
3rd grade	78.0	68.0	85.2*)	75.6*)	91.5	92.3*)
	2.7	7.2	8.8	9.8	8.3	13.0
Marasmic						
Kwashiorkor	100.8*)	91.8	95.3*)	96.0*)	108.7*)	–
	5.9	7.1	7.8	8.6	8.9	–

*) The difference from controls is statistically significant ($P < 0.05$)
– Not determined

Table 4. R.B.C.'s total amino nitrogen (mg amino nitrogen as leucine/100 ml R.B.C.s) before and after dosing with lysine.

Time of sampling	F.	1	2	3	4	6 hrs
Controls						
Mean	81.0	114.2	87.0	112.4	87.8	73.8
± S.E.	7.9	7.4	10.5	9.4	18.3	13.3
Kwashiorkor						
Moderate	83.0	123.3	140.4*)	115.4	114.8*)	109.2*)
	15.1	21.5	35.3	21.8	20.1	16.2
Severe	78.6	77.4*)	90.8	66.4*)	91.8*)	81.0
	8.9	20.5	11.2	8.2	10.2	13.1
Marasmus						
2nd grade	66.0*)	75.0*)	91.5	89.5*)	97.0	107.8*)
	12.1	13.5	15.8	20.8	9.9	10.9
3rd grade	80.8*)	101.4	123.0*)	98.2*)	97.8	83.5
	5.8	10.7	12.1	14.4	8.9	7.5
Marasmic						
Kwashiorkor	75.6	71.4*)	75.2*)	65.8*)	87.6	82.0
	9.2	8.9	4.8	12.8	11.4	10.6

*) The difference from controls is statistically significant ($P < 0.05$)

Results

The level of fasting individual amino acids in R.B.C.s of the different groups is shown in table 1. The level of fasting total amino nitrogen and their values after oral dosing is tabulated in tables 2, 3 and 4. The transport index is given in table 5.

Table 5. Transport index of studied amino acids from plasma to R.B.C.s.

	Tryptophan	Lysine	Cysteine
Controls	1.66	1.41	1.59
Moderate KWO	1.42	1.35	1.37
Severe KWO	1.30	1.30	1.05
2nd grade Marasmus	1.68	1.66	1.15
3rd grade Marasmus	1.16	1.49	1.26
Marasmic KWO	1.08	1.09	1.27

Discussion

The fasting level of R.B.C.'s individual amino acids in our healthy controls are not in fair agreement with comparable values reported by other authors (14). Lysine, leucine, alanine, arginine and histidine were lower, while methionine, cystine, glysine, serine and tyrosine were higher. Such variation in amino acid values are accepted since it is dependent on the dietetic and environmental conditions of the subjects.

In moderate and severe kwashiorkor patients a drop in total essential and non-essential R.B.C.'s amino acids occurred. Certain aminco acids showed elevated levels. These were glutamine, serine and glutamic and aspartic acids in severe kwashiorkor.

In 2nd grade marasmus the total R.B.C.'s essential and non-essential amino acids were higher than normal. Most of the R.B.C.'s individual amino acids showed elevated values, particularly the non-essential ones (table 1). In 3rd grade marasmus the R.B.C.'s amino acids in general showed lower values than normal.

In marasmic kwashiorkor the essential amino acids were less than its normal level while the non-essential ones were somewhat higher and the total amino acids were within normal range.

The reported drop in total amino acids in R.B.C.'s of kwashiorkor patients is comparable with data previously reported in plasma of similar cases (15) which was attributed to dietary protein insufficiency. Such similarity of general pattern in serum and R.B.C.'s amino acids may indicate a sort of equilibrium between extra and intracellular components.

Our finding of decreased R.B.C.'s amino acids in kwashiorkor cases are contradicting with that reported by *Michail* (8). However, this author considered malnutrition cases as a whole group without categorization into kwashiorkor and marasmus. It is suggested that the elevated values reported by *Michail* (8) are due to inclusion of marasmic cases among her P.C.M. group. The elevated R.B.C.'s amino acids in 2nd grade marasmic cases is in corcordance to that reported by *Arhammaar* et al. (7) for kwashiorkor patients. The later author mentioned that elevation of erythrocyte amino acids occurred only in kwashiorkor patients who died during treatment, and attributed this elevation to the associating infection.

Estimation of the fasting level of the total amino nitrogen in R.B.C.s given in terms of mg leucine/100 ml R.B.C.s are in harmony with those determined by chromatography. Moderate kwashiorkor patients showed more or less normal level, severe cases had lower value, while marasmic patients showed higher levels.

Following oral administration of each of the tested amino acids, a rise in R.B.C.'s total amino nitrogen occurred similar to that previously reported in plasma (15). In this study the transport index was calculated as the ratio between maximum level of R.B.C.'s amino acids attained as compared to the fasting level. This index for normal infants amounted to 1.4, 1.59 and 1.66 for cysteine, lysine, and tryptophan, respectively. The transport index of the diseases groups is lower than that of normals (table 6), particularly in marasmic kwashiorkor given tryptophan or lysine and in severe kwashiorkor given cysteine. This indicates retarded rate of transport of amino acids to red blood cells, which may be either due to lower concentration than normal of amino acids in plasma of malnourished cases as has been found earlier (15) or due to abnormalities in red cell membrane as described by *Coward* (16). *Oxender* and *Christensen* (17) suggested two mediators for the transport of neutral amino acids across cell membrane, one is capable of active transport against concentration gradient and the other serves for exchange. *Schwartzman* et al. (18) stated that the extracellular sodium and potassium deprivation caused amino acid exchange diffusion to be quantitatively more sensible than active transport. Owing to deficiency of sodium in protein calorie malnutrition (19), it might be expected that active transport of amino acids is retarded under such condition.

It may be concluded that the R.B.C.'s amino acids are also affected by protein energy malnutrition, in a similar way to plasma, in spite of being to a large extent dependable on plasma.

Summary

R.B.C.'s amino acid pattern was investigated in control and PEM subjects. The effect of oral dosing with cysteine, tryptophan and lysine on total amino acid nitrogen level of R.B.C.s was also studied. Results revealed that in moderate and severe kwashiorkor the total essential and non-essential R.B.C.'s amino acids were decreased. In 2nd grade marasmus the total R.B.C.'s amino acids in general showed lower values than normal. After oral administration of the tested amino acids, the transport index calculated as the level of maximum value of the total R.B.C.'s amino acids reached to the fasting level was lower in malnourished cases relative to controls except in 2nd grade marasmic cases given tryptophan or lysine. This criteria was considered to indicate lower rate of amino acid transport to R.B.C.s due to lower concentration in plasma or shift of transport mechanism from active transport to exchange diffusion.

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Authors' address:

Dr. *F. A. El-Shobaki,* National Research Centre, Dokki, Cairo (Egypt)