Free amino acid patterns of plasma, erythrocytes and leucocytes in hypoproteinaemia

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1. In pregnancy, severe hypoproteinaemia, cirrhosis and anaemia, plasma free α-amino nitrogen (FαAN) was found to be lowered. The non-essential:essential amino acid ratios, determined by paper chromatography, were increased in hypoproteinaemia and anaemia.

2. The erythrocytic FαAN content increased in early hypoproteinaemia and values lower than normal were found in severe hypoproteinaemia. There was also a rise in anaemia, which was due to increased cellular non-essential amino acids.

3. The leucocytic FαAN was reduced in all hypoproteinaemic states. The increased FαAN values in patients with thyrotoxicosis demonstrated that, in catabolism, leucocytic uptake of amino acids is increased. The non-essential:essential amino acid ratios were decreased in cirrhosis, possibly due to poor biosynthesis of non-essential amino acids by the liver.

In nutritional protein deficiency, a fall in plasma free amino acid concentration, and alterations in several essential and non-essential amino acids have been reported by various workers (Cravioto, 1958; Westall, Roitman, De la Pena, Rasmussen, Cravioto, Gomez & Holt, 1958; Arroyave, Wilson, de Funes & Béhar 1962; Holt, Snyderman, Norton, Roitman & Finch, 1963). Whitehead (1964) derived ratios from four non-essential and four essential amino acids in diagnosing the severity of protein deficiency. Arroyave (1970) demonstrated that valine:glycine ratios are more discriminating for early protein-energy malnutrition than ratios obtained from groups of amino acids. The applicability of such ratios has been doubted in diagnosing marasmic kwashiorkor (Anasuya & Narasinga Rao, 1968; Committee Report, 1970). Björnesjö, Mellander & Jagenburg (1968) demonstrated that the free amino acid content of erythrocytes was increased in protein deprivation and anaemia. So far, no information is available regarding free α-amino acid content of plasma, erythrocytes and leucocytes in various hypoproteinaemic disorders. It would be of interest if leucocytes, with their shorter life-span, were the cells to show earliest amino acid changes in hypoproteinaemia.

In this study an attempt has been made to investigate the amino acid patterns quantitatively and qualitatively in plasma, erythrocytes and leucocytes in some hypoproteinaemic states and in the hypermetabolic state, thyrotoxicosis.

EXPERIMENTAL

Subjects

The adult subjects investigated were suffering from hypoproteinaemia – twenty-five pregnant women, ten cases of cirrhosis, sixteen cases of nephrosis; nutritional hypoproteinaemia – eleven cases; iron deficiency – twenty-eight cases; thyrotoxicosis

† For reprints.

– eight cases. Seventy-six children suffering from marasmus and from undernutrition were also studied. In addition, investigations were made on the following control subjects: thirty normal adults, forty-five children (aged 1–5) and fifteen non-pregnant women.

Methods

Venous blood (5 ml) was collected in fasting state in a siliconized tube using heparin as anticoagulant and allowed to sediment at 4° for 30 min. The unsedimented cells in plasma were decanted into another tube and centrifuged at 1000 rev/min for 5 min. The plasma was decanted separately and the bottom plug in the tube was treated with 2·5 ml of chilled, distilled water, mixed well with a Pasteur pipette and immediately treated with 2·5 ml of cold hypertonic saline (18 g NaCl/l) and centrifuged. The supernatant was drained off, the leucocytes were suspended in the desired quantity of isotonic saline and the leucocyte counts per ml were made. The details of methodology are described in a separate communication (Gupta & Agarwal, 1972).

Chemical analyses

The plasma, erythrocyte and leucocyte free α-amino acid nitrogens (FαAN) were determined according to the method of Goodwin (1968) except that the protein-free filtrate from leucocytes was obtained by treatment with trichloroacetic acid (100 g/l).

Free amino acid patterns were determined by a combination of electrophoresis at 320 V and paper chromatography with butanol–acetic acid–water mixture as solvent. The determination of groups of amino acids was done spectrophotometrically as reported earlier (Gupta & Agarwal, 1972). The amino acid groups were: I, lysine, arginine and histidine; II, leucine, isoleucine, phenylalanine and methionine; III, valine, tyrosine and tryptophan; IV, glutamic acid, threonine, serine, aspartic acid, taurine, cystine and cysteine; and V, alanine and glycine. The ratios of the total 'non-essential':'essential' amino acids, groups (IV+V): groups (I+II+III), were calculated (see footnote to Table 4).

RESULTS

FαAN in pregnancy

Plasma, erythrocyte and leucocyte FαAN concentrations decreased with increase in gestational age. However, a statistically significant fall was noted only for plasma ($P < 0{\cdot}10$). The value for erythrocytes during 10–20 weeks of gestation (11·95, SD ± 0·30) was higher than for non-pregnant women but statistically not significant (Table 1). The relationships between gestational age and plasma, erythrocyte and leucocyte FαAN were studied by calculating the correlation coefficients and since these coefficients were significant (0·81, 0·62 and 0·54 respectively) regression coefficients were calculated and also found to be significant (Table 1).

Table 1. *Concentrations of free α-amino acid nitrogen in plasma, erythrocytes and leucocytes in pregnant women suffering from hypoproteinaemia and their correlation with gestational age*

(Mean values and standard deviations; number of subjects – all different – in parentheses)

Gestational age	Plasma (P) (mg/l)	Erythrocytes (E) (mg/l)	Ratio E: P*	Leucocytes (μg/10⁶ cells)
Non-pregnant women	52·7 ± 1·8 (15)	89·2 ± 21·6 (15)	2·30	1·76 ± 0·31 (15)
10–20 weeks	38·9 ± 4·3 (6)	108·9 ± 21·3 (7)	3·96	1·39 ± 0·31 (4)
21–30 weeks	35·3 ± 6·7 (8)	81·9 ± 31·2 (9)	3·28	1·29 ± 0·38 (10)
31 weeks–full term	34·2 ± 6·2 (12)	78·1 ± 27·1 (12)	3·23	1·16 ± 0·18 (17)
Correlation coefficient	0·81	0·62		0·54
Regression coefficient†	−0·048	−0·208		−15·16
Goodness of fit of regression lines (*P*)	< 0·001	< 0·001		< 0·01

* Calculated on a water-basis assuming the water contents of erythrocytes and plasma to be 65% and 92% respectively (Björnesjö, 1963).
† With gestational age as independent variable.

Table 2. *Free α-amino acid nitrogen in normal and abnormal states of adults*

(Mean values and standard deviations; number of subjects in parentheses)

Subjects	Plasma (P) (mg/l)		Erythrocytes (E) (mg/l)		Ratio E:P*	Leucocytes (μg/10⁶ cells)	
	Mean	SD	Mean	SD		Mean	SD
Normal	55·7	10·9 (30)	90	26 (16)	2·30	1·70	0·44 (10)
Anaemia	50·8	4·1 (28)	197	47 (20)	5·51	1·02	0·26 (7)
Nephrosis	54·2	21·0 (16)	124	54 (16)	3·24	1·16	0·20 (4)
Cirrhosis	44·9	7·3 (10)	119	38 (10)	3·76	1·13	0·34 (6)
Thyrotoxicosis	52·3	6·4 (8)	108	27 (8)	2·93	1·83	0·49 (7)

* Calculated on a water-basis assuming the water contents of erythrocytes and plasma to be 65% and 92% respectively (Björnesjö, 1963).

Table 3. *Relationship between plasma albumin values and concentrations of free α-amino acid nitrogen in erythrocytes and leucocytes*

(Mean values and standard deviations; number of subjects in parentheses)

Plasma albumin (g/l)	α-Amino acid N: Plasma (mg/l)	Erythrocytes (mg/l)	Leucocytes (μg/10⁶ cells)
< 20	44·0 ± 10·8 (12)	102·3 ± 35 (12)	1·31 ± 0·04 (3)
20–30	42·6 ± 11·2 (21)	156·0 ± 59 (21)	1·04 ± 0·52 (5)
31–40*	50·0 ± 12·0 (39)	154·0 ± 55 (39)	1·03 ± 0·48 (5)
> 41	60·0 ± 14·0 (12)	108·0 ± 53 (12)	1·81 ± 0·38 (12)
Correlation coefficient	0·678	0·060	0·886
Regression coefficient	+1·061	—	+293·8
Goodness of fit of regression lines (*P*)	< 0·001	—	< 0·001

* Including ten normal subjects.

Table 4. *Pattern of amino acids in plasma, erythrocytes and leucocytes, as determined by spectrophotometry (extinction × 1000: approximate values, see p. 152)*

(Mean values and standard deviations; number of subjects in parentheses)

Subjects	Amino acid group*									
	'Essential'†						'Non-essential'‡			
	I		II		III		IV		V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Normal (22)										
Plasma	62	11	37	15	36	17	112	43	76	30
Erythrocytes	39	23	19	14	22	18	272	61	71	33
Leucocytes	108	63	81	59	71	44	193	95	117	75
Anaemia (20)										
Plasma	39	21	21	15	18	27	83	45	78	39
Erythrocytes	35	12	15	8	13	9	311	55	125	69
Leucocytes	102	37	78	37	52	29	201	75	097	47
Hypoproteinaemia (10)										
Plasma	34	9	25	10	26	12	85	35	56	19
Erythrocytes	28	7	13	6	22	12	211	52	51	13
Leucocytes	63	28	43	22	30	13	126	48	56	25
Nephrosis (5)										
Plasma	37	9	25	6	42	21	74	12	57	25
Erythrocytes	27	9	18	8	12	4	212	11	34	5
Leucocytes	46	3	60	35	31	27	168	48	61	31
Cirrhosis (5)										
Plasma	53	9	28	9	34	10	93	5	46	9
Erythrocytes	35	8	12	2	18	10	218	30	57	23
Leucocytes	86	25	70	27	39	13	180	56	56	16
Full-term pregnancy (12)										
Plasma	39	10	21	7	19	6	76	24	53	29
Erythrocytes	35	15	25	17	17	9	300	1	54	35
Leucocytes	74	42	45	37	32	16	132	49	74	31
Thyrotoxicosis (3)										
Plasma	37	3	28	12	38	3	97	20	59	15
Erythrocytes	25	3	13	10	19	19	194	22	46	4
Leucocytes	60	11	131	93	67	15	476	195	133	26

* I, lysine, arginine and histidine; II, leucine, isoleucine, phenylanine and methionine; III, valine, tyrosine and tryptophan; IV, glutamic acid, threonine, serine, aspartic acid, taurine, cystine and cysteine; and V, alanine and glycine.

† Apart from tyrosine in group III.

‡ Apart from threonine in group IV.

FαAN in normal subjects and patients

The plasma FαAN values were decreased significantly only in cirrhosis ($P < 0.001$) and anaemia ($P < 0.05$). In nephrosis with hypoalbuminaemia, the plasma FαAN remained unaltered. The leucocyte FαAN was significantly reduced in anaemia ($P < 0.005$), cirrhosis ($P < 0.02$) and nephrosis ($P < 0.01$) (Table 2).

Relationship between plasma albumin and FαAN values

This relationship was studied in eighty-four out of ninety-two subjects listed in Table 2, including twenty-two normal subjects with serum albumin > 36 g/l and

Table 5. *Non-essential:essential* free amino acid ratios in plasma, erythrocytes and leucocytes of normal and abnormal states of adults*

(Mean values and standard deviations; number of subjects in parentheses)

Subjects	Plasma Mean	Plasma SD	Erythrocytes Mean	Erythrocytes SD	Leucocytes Mean	Leucocytes SD
Normal	1·40	0·27 (22)	5·51	2·73 (18)	1·47	0·52 (17)
Anaemia	2·18	1·07 (20)	8·54	4·79 (17)	1·44	0·52 (19)
Nutritional hypoproteinaemia	1·73	0·53 (11)	4·29	2·07 (10)	1·54	0·59 (11)
Nephrosis	1·25	0·24 (5)	3·55	1·09 (3)	1·70	0·95 (4)
Cirrhosis	1·24	0·23 (5)	4·48	1·20 (5)	1·30	0·54 (5)
Pregnancy, full term	1·57	0·86 (8)	4·36	2·06 (12)	1·52	0·45 (13)
Thyrotoxicosis	2·01	0·78 (3)	5·65	2·50 (3)	2·54	0·45 (3)

* See footnote to Table 4.

Table 6. *Non-essential:essential free amino acid ratios in plasma, erythrocytes and leucocytes of children* (1–5 *years*)

(Mean values and standard deviations; number of subjects in parentheses)

Subjects	Plasma Mean	Plasma SD	Erythrocytes Mean	Erythrocytes SD	Leucocytes Mean	Leucocytes SD
Normal	1·52	0·39 (45)	5·12	2·02 (44)	1·56	0·45 (31)
Undernourished	1·61	0·36 (21)	6·42	3·80 (21)	1·54	0·35 (13)
Marasmus	1·6	0·35 (6)	5·92	2·89 (6)	1·61	0·51 (5)

leucocyte FαAN > 1·48 μg/10^6 cells. The plasma FαAN was decreased in low plasma albumin groups. The erythrocyte FαAN in mild to moderate hypoproteinaemic groups was significantly increased ($P <$ 0·01). In the plasma albumin group < 20 g/l the observed erythrocyte FαAN was normal. The leucocyte FαAN was found to be significantly lowered in all types of hypoproteinaemia (Table 3). The relationship between plasma albumin and plasma leucocytic FαAN was found to be significant. There was no correlation between plasma albumin and erythrocyte FαAN concentrations.

Amino acid patterns (*Table* 4) *and non-essential:essential ratios* (*Table* 5) *in normal adults and patients*

Plasma. A tendency for a decrease in amino acids in all groups of amino acids was observed in anaemia (excepting group V), hypoproteinaemia, nephrosis (except group III) and pregnancy. The mean values for non-essential amino acids (groups IV and V) were significantly reduced in pregnancy ($P <$ 0·005). The plasma non-essential:essential amino acid ratios were significantly increased only in hypoproteinaemia ($P <$ 0·1) and anaemia ($P <$ 0·005). The extinction values given in Table 4 are from spectrophotometric determinations. As different amino acids gave variable intensities of colour, the values are not precise (see Gupta & Agarwal, 1972).

Erythrocytes. A significant decrease was noted in the amino acids of groups IV and V in hypoproteinaemia ($P <$ 0·01), whereas in anaemia there was a significant increase in the non-essential amino acids of group V ($P <$ 0·01). The erythrocyte non-essential: essential amino acid ratio was significantly increased in anaemia ($P < 0.001$) mainly due to increases in the non-essential acids. The fall in the ratio for cirrhosis, pregnancy and nephrosis was not significant ($P > 0.2$).

Leucocytes. In hypoproteinaemia and pregnancy, amino acids of groups I, II, III and V were significantly decreased ($P < 0.005$). Significant reductions in amino acids were also observed for groups I, III and V in nephrosis, and groups III and V in cirrhosis ($P < 0.005$). In thyrotoxicosis, the non-essential amino acids were markedly increased in the leucocytes whereas the concentration of these amino acids decreased in the plasma and erythrocytes. The non-essential: essential amino acid ratios were not significantly altered in any of the conditions.

Amino acid ratios in children

The results obtained in children (1–5 years) suffering from malnutrition in the urban and rural population demonstrated that the plasma FαAN was unaltered, the mean from seventy-six observations being 55, SD $\pm$ 12.5 mg/l. The leucocyte FαAN was 1.6 μg/10^6 cells in six children; twelve had values of < 0.9 μg/10^6 cells. The remaining children had values between 0.9 and 1.6 μg/10^6 cells. The plasma and erythrocyte non-essential: essential amino acid ratios were increased in marasmus ($P < 0.01$) and undernourished ($P < 0.001$) children. The non-essential: essential amino acid ratios of leucocytes showed no significant change (Table 6).

Erythrocyte: plasma FαAN ratios (calculated on a water-basis) in adults

The ratios of FαAN in the water of erythrocytes and plasma were calculated for adults assuming that the water contents were 65% and 92% respectively (Björnesjö, 1963). Increased erythrocyte: plasma ratios were observed in pregnancy, anaemia, cirrhosis and nephrosis (Tables 1 and 2).

DISCUSSION

The results of the study reported here demonstrated that plasma FαAN decreased during pregnancy in hypoproteinaemic women: the observations are in accordance with those of Bonsnes (1947) and Björnesjö (1963). Plasma valine, tryptophan and tyrosine (group III) did not alter in nephrosis and cirrhosis, whereas amino acids in other groups were reduced. The increase in erythrocyte non-essential amino acids observed in anaemia may have been induced by hypoxia (Bange & Bange-Barnoud, Rashidi, Dion & Peres, 1968; Björnesjö, Jarnulf & Lausing, 1968). During treatment of anaemic patients with iron, erythrocyte FαAN was further increased (unpublished observations) thus confirming the observations of Björnesjö, Mellander *et al.* (1968).

So far, alterations in free amino acid patterns in leucocytes have been reported only in various types of infections and leukaemias (Hill, 1960; McMenamy, Lund & Wallach, 1960; Cline, 1965; Lee, Bolger & Bridges, 1969). We found that the leucocyte FαAN was markedly reduced in all the hypoproteinaemic disorders irrespective of their mechanism(s), and that essential amino acids were reduced in all hypoproteinaemic disorders excepting cirrhosis, in which a reduction of non-essential amino acids was the main change observed. In thyrotoxicosis, non-essential amino acids were markedly increased; this is difficult to explain.

Studies on leucocyte amino acids in various metabolic states should offer a fruitful field for further work.

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