AMINO-ACID CONTENT OF SERUM IN RHEUMATOID ARTHRITIS

BY

ERIK NETTELBLADT AND BRITT-MARIE SANDELL

From the Medical Department III and the Central Clinical Laboratory, Södersjukhuset, Stockholm, Sweden

In an earlier investigation (Hammarsten, Jonsson, Lindgren, Neri, Nettelblatt, Plum, and Sandell, 1960), it was found that an amino-acid mixture had a stimulating effect upon erythropoiesis in the *in vitro* cultivation of bone marrow. This effect was more pronounced on bone marrow from cases of rheumatoid arthritis than on marrow from patients with other diseases. A possible interpretation of this result is that there is a lack of free amino acids in serum from cases of rheumatoid arthritis.

On the basis of this finding, a study was undertaken of the amino-acid content of the serum in cases of rheumatoid arthritis, and to this end methods have been devised which permit the determination of seventeen different amino acids with relatively satisfactory reliability.

Methods

Serum was first deproteinized by passage on a cation column (Zeokarb 225 H⁺, 4.5NC). Inorganic salts and proteins passed the column, and the amino acids were eluted from the column with concentrated ammonia. The ammonia solution was evaporated to dryness and the residue, containing the amino acids, was dissolved again in a known amount of 10 per cent. isopropyl alcohol in water. An aliquot of the isopropylalcohol solution corresponding to 0.5 ml. serum was then separated with the help of two-dimensional paper chromatography. The solvents used in the first direction were methanol, pyridine, and water (80 : 4 : 20), and in the second direction tert. butanol, diethylamine, methyl ethylketone, and water (33 : 4 : 40 : 27) according to the method of Redfield (1953). After drying, the papers were stained with 0.4 per cent. solution of ninhydrin in acetone containing 5 per cent. acetic acid. The spots obtained were then cut out and eluted in 5 ml. 60 per cent. ethyl alcohol, and the colour was read in a spectrophotometer at 570 m μ . The result was evaluated by comparison with standard solutions which were treated as test samples and run in the same chromatographic vessel.

A test of the method is presented in Table I, where the results of five analyses are compared with the weighed amount. Furthermore, the experiments were performed

TABLE I
RECOVERY OF AMINO ACIDS AFTER PASSAGE ON FIVE
DIFFERENT ZEOKARB COLUMNS

Amino Acid	Values Obtained		Percentage Recovery	Weighed Quantity
	5 ml. solution Mean $\pm$ S.E.	2 ml. solution Mean $\pm$ S.E.		
Alanine ..	8.78 0.15	3.24 0.18	99 91	8.9 3.56
Arginine ..	10.00 1.09	2.90 0.20	57 42	17.42 6.89
Aspartic Acid	12.56 0.74	3.90 0.58	94 73	13.31 5.34
Glutamic Acid	14.82 0.60	5.72 0.14	101 98	14.71 5.88
Glutamine ..	12.78 0.55	5.28 0.29	88 90	14.60 5.85
Glycine ..	8.26 0.32	3.95 0.32	110 132	7.51 3.00
Histidine ..	9.54 0.37	4.00 0.10	62 65	15.50 6.20
Leucine ..	13.30 0.19	4.46 0.53	101 85	13.17 5.27
Lysine.. ..	11.44 0.48	4.14 0.22	78 71	14.62 5.86
Methionine ..	14.54 0.55	4.74 0.26	97 96	14.92 4.92
Phenylalanine	15.62 0.28	5.96 0.42	95 90	16.50 6.60
Serine	10.30 0.17	4.00 0.14	98 95	10.51 4.21
Threonine ..	11.06 0.20	3.82 0.14	93 80	11.90 4.77
Tryptophan ..	17.84 0.89	6.32 0.10	87 77	20.40 8.17
Tyrosine ..	15.50 0.59	6.93 0.17	86 96	18.12 7.25
Valine	12.76 0.20	4.66 0.14	109 99	11.72 4.70

with two concentrations of amino acid standards, and the loss with reference to the concentration was then studied. The loss on the columns was very marked for the following amino acids: arginine, histidine, and lysine. The amount recovered, on the other hand, seemed to be proportional to the concentration, with the possible exception of arginine and aspartic acid, which at a lower concentration gave a lower percentage recovery.

As regards methionine, it was observed that after passage on the column this amino acid divided on subsequent paper chromatography into two spots. The irregular spot came in this connexion to coincide with the glycine spot. As the concentration of methionine is normally very small, the value for the glycine concentration in serum will not be essentially changed thereby. No correction of the glycine values for methionine was therefore made.

The proline concentration was determined by a special method. When the amino acids had been dissolved in 10 per cent. isopropyl alcohol as described above, one-dimensional paper chromatograms were developed in propanol: water (7:3) for 8 hours according to the method of Boissonnas (1950). When these strips of paper had dried they were stained with 0.4 per cent. isatin in acetone, washed with 1 N HCl according to the method of Pasieka and Morgan (1956), and then evaluated with a Spinco Analytrol Densitometer.

The variance $\frac{S.D. \times 100}{M}$ was 15 per cent.

Material

All the cases examined were fasting when the blood sample was taken, but there was no standardization of diet before the analyses. Serum was used for the analyses, which were started on the same day as the samples were taken. Normal sera from subjectively healthy persons belonging to the hospital staff were used as controls. The erythrocyte sedimentation rate, haemoglobin, serum glutamic oxalacetic transaminase (S-GOT), and protein electrophoresis were normal and the urine was free from glucose and protein.

All the cases of rheumatoid arthritis used in this study fulfilled the criteria for classical rheumatoid arthritis of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959).

Results

Table II shows the serum concentration of seventeen different free amino acids in normal persons. When the material was divided according to sex, no difference was observed, and total values are therefore given in the Tables.

TABLE II
SERUM CONTENT OF FREE AMINO ACIDS
IN NORMAL SUBJECTS

Amino Acid	No. of Cases	Serum Content (μmoles/litre) Mean ± S.E.	
Alanine	34	307	11.2
Arginine	40	94	6.8
Aspartic Acid	20	79	11.3
Glutamic Acid	53	117	8.2
Glutamine	43	460	24.6
Glycine	41	277	20.0
Histidine	39	111	9.7
Leucine	49	158	9.1
Lysine	39	202	15.4
Methionine	35	16	1.0
Phenylalanine	40	82	5.2
Proline	18	269	23.5
Serine	40	142	14.2
Threonine	40	166	11.8
Tryptophan	37	58	2.9
Tyrosine	39	84	7.7
Valine	41	165	8.5

Table III (opposite) shows the serum concentration of free amino acids in cases of rheumatoid arthritis. For some of the amino acids statistically significant differences from normal could be demonstrated (Table IV). The concentration of glutamic acid is above normal in rheumatoid arthritis, whereas glutamine is lower than normal. The ratio glutamic acid: glutamine in serum was calculated for each individual case and comparisons were made between the mean ratios of the groups, with the following results:

Rheumatoid arthritis = 0.60; Normal = 0.20;
Difference = 0.40; $t = 5.71$; $P < 0.001$.

In order to study the specificity of these findings, investigations were carried out on sixteen cases with different internal medical diseases (including pneu-

TABLE IV
SERUM CONTENT OF THE FIVE FREE AMINO ACIDS WHICH SHOWED A STATISTICALLY SIGNIFICANT
DIFFERENCE BETWEEN RHEUMATOID ARTHRITIS AND NORMAL SERA

Amino Acid	Rheumatoid Arthritis		Normal Subjects		Difference	<i>t</i>	<i>P</i>
	No. of Cases	μmoles/litre Mean ± S.E.	No. of Cases	μmoles/litre Mean ± S.E.			
Arginine	17	67 7.7	40	94 6.8	- 27	2.55	<0.05
Glutamic Acid	16	243 40.9	53	117 8.2	+ 126	3.02	<0.01
Glutamine	17	262 29.1	43	460 24.6	- 198	5.13	<0.001
Histidine	16	56 8.0	39	111 9.7	- 55	4.37	<0.001
Tyrosine	16	53 4.6	39	64 7.7	- 31	3.34	<0.01

TABLE III

SERUM CONTENT OF FREE AMINO ACIDS IN CASES OF RHEUMATOID ARTHRITIS

Amino Acid	No of Cases	Serum Content (μmoles/litre) Mean ± S.E.
Alanine	17	344 19.1
Arginine	17	67 7.7
Aspartic Acid	16	98 18.2
Glutamic Acid	16	243 40.9
Glutamine	17	262 29.1
Glycine	17	278 20.1
Histidine	16	56 8.0
Leucine	16	151 10.1
Lysine	17	227 20.1
Methionine	15	21 4.2
Phenylalanine	15	115 16.6
Proline	57	209 13.9
Serine	16	155 10.8
Threonine	14	148 26.9
Tryptophan	17	46 6.0
Tyrosine	16	53 4.6
Valine	16	171 13.0

monia, cardiosclerosis, hypertonia, nephritis, and anaemia). The results were within normal limits, except for the tyrosine concentration, which showed a subnormal value (55 ± 6.5) similar to that observed in the rheumatoid arthritis group (53 ± 4.6).

Discussion

Several writers, including Trnavská and Sitaj (1960) and more recently Armstrong, Bocking, and Derrick (1962), have shown that the total amount of free amino acids in plasma or serum in cases of rheumatoid arthritis is lower than normal. In the present investigation the total amount of free amino acids was low, but there was no significant difference noted between the arthritic and normal cases. As regards the individual free amino acids, low values were recorded for arginine, glutamine, tyrosine, and histidine, while the concentration of glutamic acid was higher in cases of rheumatoid arthritis than in normal subjects. Using microbiological methods, Brodie, Wallraff, Borden, Holbrook, Stephens, Hill, Kent, and Kemmerer (1950) found low concentrations of arginine, histidine, and threonine in the serum of patients with rheumatoid arthritis. Trnavská and Sitaj (1960) found a significant reduction of proline and histidine, while arginine and threonine were normal. We found low values of proline in the rheumatoid arthritis group, but the difference was not statistically significant:

Rheumatoid arthritis $M = 209 \pm 13.9$;
Normal $M = 269 \pm 23.5$.

To ascertain whether the deviation in the serum

concentration of free amino acids is specific for rheumatoid arthritis, we also analysed a series of cases of other internal medical diseases. The choice of such control material is very difficult, but the cases chosen showed the same general characteristics with respect to chronicity and nutrition, as the cases of rheumatoid arthritis.

Our controls gave the same serum concentrations of individual free amino acids as had been found in the normal subjects, with the exception of tyrosine. Any differences found between the normal group and the rheumatoid arthritis group were not due to medical treatment, as all therapy was discontinued during the taking of the samples, and none of the patients was treated with cortisone or ACTH, which affect the amino-acid metabolism (Derrick and Hanley, 1957). It therefore seems probable that the differences found for arginine, glutamine, histidine and glutamic acid are due to rheumatoid arthritis.

On the other hand, this series showed no correlation between the serum amino-acid levels and pathological activity, chronicity, or duration of disease. Nor was it possible to observe any correlation between the amino-acid changes and the serum concentration of gamma-globulins, alpha globulins, or C-reactive protein. It may well be, however, that the methodological error is too large for such correlations to be detected.

The glutamic acid : glutamine ratio was considerably higher in the cases of rheumatoid arthritis than in the normal subjects. This may be because the formation of amide from glutamic acid is prevented, for according to Elliott (1953) this formation is dependent on adenosine triphosphate (ATP). Györki and Sandell (1959) found that the ATP content of erythrocytes in cases of rheumatoid arthritis was different from normal. The high glutamic acid : glutamine ratio in rheumatoid arthritis might also be explained by enzymatic disturbances.

The tryptophan metabolism has also been shown to be disturbed in cases of rheumatoid arthritis (Bett, 1962), but we obtained a slightly lower serum concentration of free tryptophan in our cases of rheumatoid arthritis, and the difference was not statistically significant.

It seems doubtful whether the low serum levels of certain free amino acids observed by us are related to the clinical symptoms, since large amounts of amino acids have been supplied to patients with rheumatoid arthritis without affecting the pathological activity, anaemia, or reticulocytes. It seems likely that the deviations from normal observed by us demonstrate changes in the amino-acid and protein metabolism in cases of rheumatoid arthritis.

Summary

The serum concentration of free amino acids has been investigated by paper chromatography in a series of cases of rheumatoid arthritis. Low concentrations of arginine, glutamine, tyrosine, and histidine were found in rheumatoid arthritis, while the glutamic acid levels were higher than normal. The significance of these findings is briefly discussed.

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La teneur du sérum en amino-acides dans l'arthrite rhumatismale

RÉSUMÉ

On détermina le taux sérique des amino-acides libres au moyen de la chromatographie sur papier dans une série de cas d'arthrite rhumatismale. Ce taux fut bas pour l'arginine, la glutamine, la tyrosine et l'histidine, tandis que celui de l'acide glutamique s'éleva au dessus de la normale. On discute ici brièvement l'importance de ces résultats.

Contenido sérico de amino-ácidos en la artritis reumatoide

SUMARIO

Se determinó la concentración sérica de amino-ácidos libres por la cromatografía sobre papel en una serie de casos de artritis reumatoide. Esta concentración fué baja con respecto a la arginina, glutamina, tirosina e histidina, mientras que la del ácido glutámico fué superior a lo normal. El significado de estos hallazgos se discute brevemente.