Plasma Levels of Free Amino Acids in Normal Subjects Compared with Patients with Rheumatoid Arthritis.* (18087)

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Evidence has been presented recently to show that abnormal metabolism of amino acids may occur in rheumatoid arthritis. DeVries and Alexander(1) have reported low plasma glycine in some cases of rheumatoid arthritis. We have reported increased plasma levels as well as increased urinary excretion of histidine in patients with rheumatoid arthritis treated with adrenocorticotrophic hormone and with 17-hydroxy-11-dehydrocorticosterone(2). Urinary excretions of other amino acids in patients with this disease have also been reported(3-5).

The present investigation was undertaken to determine whether a difference exists between plasma levels of "free" amino acids of normal individuals and patients with rheumatoid arthritis. The plasma levels of "free" arginine, glycine, histidine, lysine, phenylalanine, serine and threonine will be reported.

Experimental. The control subjects were research associates who were apparently normal and in good health. The patients with rheumatoid arthritis had been under observation of this group from 6 months to 15 years. The average age of the control males was 34.2 years in contrast to 40.6 years for males with rheumatoid arthritis. The average age for the control females was 34.7 years compared to 50.3 years for females with rheumatoid arthritis—a difference of 15.6 years. Ackermann, Hoffstatter, and Kountz(6) have reported that no difference exists in the plasma histidine values in groups with as great a variation in age as 46.7 years. With few exceptions patients with rheumatoid arthritis were hospitalized for observation and treatment at the time this study was begun. All forms of medication were discontinued for 3 days prior to the time the blood samples were taken. The patients were on a rigidly controlled diet containing 70 g of protein per day. The diet of the control group was adequate but no attempt was made to keep an accurate account of protein intake. Kirsner, Sheffner and Palmer(7) have reported that a variation of as much as 35 g of protein per day had no significant effect on the plasma levels of the "free" amino acids we will report in this paper. After a 12-14 hour fasting period, 30 ml of blood were obtained by venipuncture and prevented from clotting by the use of heparin. Plasma tungstic acid filtrates were prepared according to the method of Hier and Bergeim(8). The filtrates were stored at −15°C. At the time of assay they were thawed, adjusted to a pH of 6.9 and diluted so that the final volume represented a 1:5 or 1:10 dilution of the original

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TABLE I. Plasma Levels of 7 "Free" Amino Acids.

	Normals		Rheumatoid arthritics		
Amino acid	No. of cases	Mean ± S.E.	No. of cases	Mean ± S.E.	Value of "t"
		Men			
Arginine	8	22.1 ± 1.58 (16.4-29.3)	21	15.6 ± 1.23 (6.7-24.3)	3.25*
Glycine	8	26.0 ± 2.05 (15.7-34.0)	9	24.0 ± 2.71 (17.1-40.4)	0.59
Histidine	12	15.2 ± 0.54 (12.2-18.2)	23	11.1 ± 0.51 (6.8-16.9)	5.50*
Lysine	8	32.3 ± 1.63 (25.9-39.2)	21	29.5 ± 1.60 (14.6-41.6)	1.23
Phenylalanine	10	12.9 ± 1.43 (7.5-23.9)	17	12.6 ± 1.00 (6.1-23.8)	0.17
Serine	6	11.4 ± 2.07 (7.4-20.3)	13	11.8 ± 1.23 (5.4-20.5)	0.17
Threonine	8	20.7 ± 2.61 (13.2-36.9)	21	13.2 ± 0.80 (8.8-19.5)	2.75*
		Women			
Arginine	15	22.1 ± 0.95 (17.2-29.1)	28	14.8 ± 1.04 (5.3-22.8)	5.19*
Glycine	22	27.1 ± 1.71 (14.3-49.5)	16	28.5 ± 3.00 (14.8-60.2)	0.41
Histidine	28	13.9 ± 0.39 (9.5-17.8)	38	10.8 ± 0.54 (3.2-18.1)	4.61*
Lysine	18	28.5 ± 1.68 (17.1-38.6)	26	26.3 ± 1.57 (12.2-38.5)	0.96
Phenylalanine	21	10.9 ± 0.46 (8.1-15.6)	29	10.9 ± 0.39 (6.9-15.1)	0.00
Serine	15	13.3 ± 0.95 (6.0-18.1)	21	12.9 ± 1.04 (4.6-23.4)	0.29
Threonine	18	17.9 ± 1.21 (7.9-27.4)	28	13.0 ± 0.40 (9.4-17.9)	3.58*

* Highly significant P less than 0.01.

plasma. It was found that by diluting more than the original 1:3 volume as recommended by Hier and Bergeim(8) the microbiological response fell on the steeper and more sensitive part of our standard curve. Furthermore, the higher dilutions enabled us to run more assays on each individual. The assay procedure and media, with slight modifications, were those of Henderson and Snell(9). Glycine, histidine, lysine, phenylalanine and serine were measured with *Leuconostoc mesenteroides P-60*; threonine, and arginine with *Streptococcus fecalis R*. The filtrates were assayed in duplicate and at three levels. In all other details the procedures as previously described(8,9) were followed. As a check on the precision of the method in our laboratory, assays in quadruplicate were made on pooled tungstic acid filtrates and yielded us the same "(±) 10% of the mean" as reported by Hier and Bergeim(10). In order to determine the effect of freezing and thawing on plasma filtrates duplicate assays were made on 5 pooled filtrate aliquots. One of the aliquots was stored without freezing in a refrigerator at 10°C while the other 4 were frozen and thawed from one to 4 times. The differences from the means fell well within the (±) 10% accuracy of the method.

Results. Table I compares the amount of 7 "free" amino acids found in the plasma of normal individuals with values found for patients with rheumatoid arthritis. It will be seen that no significant differences exist between the values for males and females in either group. The amounts of arginine, histidine, and threonine are significantly lower in the group with rheumatoid arthritis. The

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values for glycine, lysine, phenylalanine and serine do not show a significant difference.

Discussion. It is generally conceded that plasma values for various amino acids are relatively constant(7). It seems, therefore, that any significant differences are worthy of consideration. Although the changes we have demonstrated may be small in gamma per ml, they are nevertheless valid as shown by statistical analysis. The normal values established in this study compare favorably with those found in the literature(6,7,10).

Summary. Microbiological determinations of plasma values for arginine, glycine, histidine, lysine, phenylalanine, serine, and threonine have been determined in a group of normal individuals and compared with the values obtained from patients with rheumatoid arthritis.

1. The values obtained for arginine, histidine, and threonine in patients with rheumatoid arthritis were lower than those obtained from the normal group. These differences were highly significant(11). 2. The values for glycine, lysine, phenylalanine and serine were not significantly different in the group with rheumatoid arthritis from those found in the group of normal individuals.

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Free Amino Acids in Sea-Urchin Eggs and Embryos.* (18088)

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Free amino nitrogen has been determined in embryos of various animals; however, as yet there has been little attempt to analyze the sources of this qualitatively. Li and Roberts(1), using the paper chromatographic method, found no free amino acids in eggs and embryos of the frog. Drilhon and Busnel (2), also using chromatographic methods, found free glutamic acid, serine, alanine, and valine in the eggs of *Bombyx mori.* In later stages of development other amino acids appeared.

The present work was undertaken to investigate the occurrence of free amino acids in sea-urchin eggs, and to determine whether qualitative changes take place during development. Unfertilized sea-urchin *(Strongylocentrotus purpuratus)* eggs were extracted with 75% ethanol at 3-5°C for 2 days; 1.5 cc of ethanol were used per 50 mm^3 of eggs packed by centrifugation. The supernatant was analyzed for amino acids and peptides by means of 2-dimensional paper chromatography as described by Consden *et al.*(3). Whatman filter paper No. 1 was used with water saturated phenol as the first solvent and a n-butanol-acetic acid-water mixture (approximately 11:3:4) as the second. Amino acids and peptides were revealed by spraying the papers with 0.2% ninhydrin and heating to approximately 80°C for 10-20 minutes. Amino acid spots were tentatively identified by position and checked by adding pure amino acids to the unknown.

Free amino acids identified in the unfertilized sea-urchin eggs were glycine, glutamic acid, lysine, threonine, alanine, valine, leucine (or isoleucine). A ninhydrin-positive spot with R_f values slightly less than alanine was found to be glutamine. Addition of pure glutamine to the extract caused intensification of this spot. Acid hydrolysis of the material

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