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Amino Acid Content of Rabbit Urine and Plasma[1]

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The content of amino acids in plasma and urine from male albino rabbits was determined by ion-exchange chromatography and compared to values for other species. In general, the amino acids present in the plasma and urine of rabbits are those commonly found in other species. In contrast to human urine in which the content of histidine is usually greater than the two methylated derivatives, the content of histidine in rabbit urine is lower than either 1-methylhistidine or 3-methylhistidine. The plasma amino acid pattern is unusual in that glycine content is higher than alanine content; this is the reverse of the pattern in man and the cat.

Fasting the rabbits for 88 hr. caused approximately a 66% decrease in the total amount of urinary amino acids. A 12- or 88-hr. fast caused the plasma total amino acid content to decrease approximately 30%. Valine, isoleucine, and leucine contents of the plasma, however, were increased.

INTRODUCTION

Other than the work of Duchateau and Florkin (1) who used microbiological methods to determine the content of 15 amino acids in plasma from normal rabbits, and of Engel and Pollak (2) who used paper chromatography to determine the pattern of amino acids in rabbit serum, information on the quantitative distribution of amino acids in biological fluids of normal rabbits does not appear to be available. The purpose of this investigation was to determine the content of amino acids in the urine and plasma of normal rabbits by means of ion-exchange chromatography, and to compare the values to those found in other species.

EXPERIMENTAL

Animals and Ration

Albino male rabbits weighing at least 3 kg. were housed individually and fed whole oats and fresh cabbage. With this ration, unlike many of the commercial rations, the rabbit produces an acid urine, free of undesirable sediment. The animals had access to water at all times.

Collection of Urine and Blood

Eight rabbits who had received the ration for a minimum of 4 days were placed into individual metabolism cages. Urine collections were made daily on six of the animals (phenol preservative), and refrigerated. Three or four consecutive 24-hr. samples for each animal were pooled, and aliquots frozen. Three of the above rabbits were then fasted for 88 hr. Urine collections were made as before.

Blood was withdrawn from the marginal vein of the ear of three of the above rabbits while they were receiving the ration, and from five who had been fasted for 12 hr. Three of these latter animals were fasted an additional 76 hr. (total hours of fasting, 88) and blood was again withdrawn. Plasma was separated by centrifugation.

Chemical Methods

Urine was prepared for amino acid analysis by the method of Stein (3). Plasma was prepared for analysis by the method of Stein and Moore (4). Amino acids and other ninhydrin-reacting substances in the samples were determined by the quantitative ion-exchange chromatographic method of Spackman, Stein, and Moore (5). Unknown peaks 1 and 2 in Tables I and III refer to com-

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TABLE I

FREE AMINO ACIDS IN THE URINE OF MALE RABBITS FED AN OATS AND CABBAGE RATION OR FASTED FOR 88 HOURS

Amino acid	Ration[a]	Fasted[b]
	mg./24 hr.	*mg./24 hr.*
Unknown peak 1	1.8 ± 0.8	—[c]
Unknown peak 2	9.3 ± 3.2	3.2 ± 1.1
Taurine	15.6 ± 9.8	7.4 ± 11.1
Aspartic acid	0.6 ± 0.3	<0.3
Threonine	1.0 ± 0.2	0.5 ± 0.2
Serine	2.6 ± 0.7	1.0 ± 0.2
Asparagine plus glutamine	2.4 ± 0.8	1.0 ± 0.4
Sarcosine	2.0 ± 1.4	<0.3
Proline	0.8 ± 0.4	<0.3
Glutamic acid	2.1 ± 0.9	0.6 ± 0.2
Citrulline	0.6 ± 0.3	<0.3
Glycine	7.3 ± 2.4	1.0 ± 0.4
Alanine	2.5 ± 1.4	0.6 ± 0.2
Valine	0.3 ± 0.1	<0.3
Cystine	0.7 ± 0.2	<0.3
Cystathionine	1.1 ± 0.4	—
Methionine	0.8 ± 0.1	<0.3
Isoleucine	0.8 ± 0.4	<0.3
Leucine	0.8 ± 0.4	<0.3
Tyrosine	0.7 ± 0.3	<0.3
Phenylalanine	0.8 ± 0.2	<0.3
β-Alanine	3.2 ± 0.6	—
β-Aminoisobutyric acid	1.0 ± 0.5	—
Ornithine	1.9 ± 0.8	1.2 ± 1.1
Ethanolamine	1.9 ± 0.9	0.4 ± 0.1
Lysine	3.0 ± 1.2	1.5 ± 1.3
1-Methylhistidine	9.8 ± 2.7	5.9 ± 2.9
Histidine	0.7 ± 0.2	0.4 ± 0.1
3-Methylhistidine	3.7 ± 0.9	1.6 ± 0.9
Arginine	1.2 ± 0.9	0.4 ± 0.1
Total of means[d]	81.0	26.7

[a] Mean ± S.D. for six animals; a total of twenty-one 24-hr. collections were made.

[b] Mean ± S.D. for three animals; a total of nine 24-hr. collections were made.

[c] Value not obtained for technical reason.

[d] Values of <0.3 are not included in the total.

pounds eluted in the same chromatographic positions as phosphoserine plus cysteic acid, and phosphoethanolamine from standard amino acid solutions by Spackman *et al.* (5) and this laboratory.

RESULTS

URINARY AMINO ACIDS

Table I lists the amounts of 30 amino acids and other ninhydrin-reacting substances in the urine of male albino rabbits. Amounts of amino acids excreted ranged from 15.6 ± 9.8 mg. taurine to 0.3 ± 0.1 mg. valine per 24 hr. by the animals fed the oats and cabbage ration. In decreasing order, taurine, 1-methylhistidine, unknown peak 2, glycine, and 3-methylhistidine accounted for approximately 56% of the amino acids in the urine.

If the animals were fasted for 88 hr., the amount of amino acids excreted decreased to approximately one-third of the nonfasting value. Taurine was still the highest in amount (7.4 ± 11.1 mg.), while a number of amino acids were excreted in quantities of less than 0.3 mg./24 hr. (Table I). Taurine, 1-methylhistidine, unknown peak 2, 3-methylhistidine, and lysine, in decreasing order, accounted for approximately 73% of the amino acids in the fasting urine.

PLASMA AMINO ACIDS

Twenty-six amino acids and other ninhydrin-reacting substances were determined in the plasma of the rabbits (Table II). Amounts of amino acids ranged from 14.95 ± 5.08 mg. asparagine plus glutamine to 0.16 ± 0.06 mg. aspartic acid per 100 ml. plasma from animals fed the oats and cabbage ration. In decreasing order, asparagine plus glutamine, glycine, proline, lysine, alanine, and arginine accounted for approximately 66% of the amino acids in the plasma.

If the animals were fasted for 12 hr., the amount of amino acids in the plasma decreased to approximately 69% of the nonfasting value. Prolongation of the fasting to 88 hr. caused less than a 5% further decrease. The asparagine plus glutamine moiety was still the most abundant in the plasma, 9.58 ± 2.32 mg. and 10.51 ± 1.08 mg. per 100 ml., respectively, for animals fasted 12 and 88 hr. These two amino acid derivatives along with glycine, alanine, lysine, arginine, and proline accounted for nearly 60% of the amino acids in the plasma of rabbits fasted for 12 and 88 hr.

TABLE II

FREE AMINO ACIDS IN THE PLASMA OF MALE RABBITS FED AN OATS AND CABBAGE RATION, FASTED FOR 12 HOURS AND FOR 88 HOURS

Amino acid	Ration[a]	Fasted	
		12 hr.[b]	88 hr.[c]
	mg./100 ml.	*mg./100 ml.*	*mg./100 hr.*
Taurine	1.04 ± 0.32	0.84 ± 0.45	0.87 ± 0.37
Hydroxyproline	0.48 ± 0.02	0.22 ± 0.24	0.27 ± 0.05
Aspartic acid	0.16 ± 0.06	0.16 ± 0.18	0.09 ± 0.01
Threonine	1.43 ± 0.32	1.18 ± 0.37	1.17 ± 0.11
Serine	3.45 ± 0.76	2.12 ± 0.42	1.71 ± 0.10
Asparagine plus glutamine	14.95 ± 5.08	9.58 ± 2.32	10.51 ± 1.08
Proline	4.39 ± 0.87	2.33 ± 0.63	2.42 ± 0.72
Glutamic acid	1.60 ± 0.33	1.43 ± 0.92	0.75 ± 0.10
Citrulline	2.16 ± 0.53	1.95 ± 0.64	0.84 ± 0.04
Glycine	14.70 ± 3.45	6.64 ± 0.38	4.66 ± 0.16
Alanine	3.91 ± 0.68	4.37 ± 1.26	3.13 ± 0.78
Valine	1.59 ± 0.12	1.81 ± 0.39	2.04 ± 0.12
Cystine	0.92 ± 0.37	0.48 ± 0.34	1.34 ± 0.23
Cystathionine	0.23 ± 0.10	0.20 ± 0.15	0.17 ± 0.04
Methionine	0.65 ± 0.07	0.39 ± 0.21	0.54 ± 0.11
Isoleucine	0.74 ± 0.40	0.83 ± 0.14	1.07 ± 0.22
Leucine	1.31 ± 0.42	1.18 ± 0.13	1.50 ± 0.34
Tyrosine	1.56 ± 0.25	1.06 ± 0.25	0.88 ± 0.12
Phenylalanine	1.05 ± 0.70	1.10 ± 0.24	0.83 ± 0.09
Ornithine	2.48 ± 0.85	1.65 ± 0.11	1.59 ± 0.11
Ethanolamine	—[d]	0.08 ± 0.02	0.06 ± 0.05
Lysine	4.04 ± 0.93	3.49 ± 1.03	3.76 ± 0.54
1-Methylhistidine	0.35 ± 0.07	0.29 ± 0.12	0.53 ± 0.23
Histidine	1.54 ± 0.28	1.22 ± 0.22	1.40 ± 0.24
3-Methylhistidine	0.25 ± 0.10	0.34 ± 0.06	0.60 ± 0.19
Arginine	3.83 ± 0.56	2.77 ± 0.51	2.42 ± 0.38
Total of means	68.81	47.71	45.15

[a] Mean ± S.D. for three animals.
[b] Mean ± S.D. for five animals.
[c] Mean ± S.D. for three animals.
[d] Value not obtained for technical reason.

Although the total amount of amino acids in the plasma decreased during fasting, most of this decrease was caused by lower levels of eight of the amino acids: serine, asparagine, and glutamine; glutamic acid; citrulline; glycine; tyrosine; ornithine; and arginine. Glycine showed the greatest decrease. The content of valine, cystine, isoleucine, leucine, 1-methylhistidine, and 3-methylhistidine was increased by the 88-hr. fast.

DISCUSSION

The amino acid content of urine from rabbits fed the oats and cabbage ration, and of plasma from rabbits fasted 12 hr. are presented for comparison with values for those of human subjects (4, 6, 7) and the cat (8) in Table III. To our knowledge, these are the only other mammals in which comparable studies of the urinary and plasma amino acids have been done. In general, the amino acids usually found in the urine and plasma of man and the cat are also present in the urine and plasma of rabbits, although the amounts differ among the species.

Except for β-alanine, considerably lower amounts of each of the amino acids are

TABLE III

CONTENT OF FREE AMINO ACIDS IN URINE AND PLASMA OF RABBITS AND OTHER MAMMALS

Amino acid	Urine		Plasma			
	Rabbit[a]	Human[b]	Rabbit[c]	Cat[d]	Human Male[e]	Human Female[f]
	mg./24 hr.		*mg./100 ml.*			
Unknown peak 1	1.8	5.3				
Unknown peak 2	9.3	29.5				
Taurine	15.6	77.8	0.84	0.7	0.55	1.06
Hydroxyproline			0.22			
Aspartic acid	0.6	6.5	0.16	0.1	<0.03	0.05
Threonine	1.0	18.3	1.18	1.4	1.39	1.36
Serine	2.6	42.8	2.12	2.1	1.12	1.25
Asparagine plus glutamine	2.4	79.0	9.58			
Sarcosine	2.0	2.3				
Proline	0.8		2.33	2.3	2.30	1.42
Glutamic acid	2.1	3.8	1.43	1.8	0.70	0.59
Citrulline	0.6		1.95	<0.1		
Glycine	7.3	126.6	6.64	2.3	1.54	1.70
Alanine	2.5	14.4	4.37	7.0	3.41	2.48
Valine	0.3	3.3	1.81	2.4	2.88	2.11
Cystine	0.7	6.8	0.48	0.4	1.18	0.79
Cystathionine	1.1	20.5	0.20			
Methionine	0.8	4.2	0.39	0.4	0.38	0.24
Isoleucine	0.8	3.0	0.83	0.8	0.89	0.67
Leucine	0.8	6.8	1.18	1.6	1.69	1.17
Tyrosine	0.7	17.1	1.06	0.7	1.03	0.66
Phenylalanine	0.8	10.0	1.10	0.9	0.84	0.73
β-Alanine	3.2	3.8		<0.03		
β-Aminoisobutyric acid	1.0	43.7		<0.04		
Ornithine	1.9	1.9	1.65	0.2	0.72	0.44
Ethanolamine	1.9	11.6	0.08	<0.04		
Lysine	3.0	22.4	3.49	2.8	2.72	1.41
1-Methylhistidine	9.8	39.0	0.29	1.4	<0.11	
Histidine	0.7	110.1	1.22	1.4	1.15	0.82
3-Methylhistidine	3.7	32.5	0.34	0.1	<0.08	
Arginine	1.2	3.0	2.77	1.4	1.51	0.58

[a] Fed oats and cabbage ration.
[b] Hubbard *et al.* (6).
[c] Fasted for 12 hr.
[d] Tallan *et al.* (8).
[e] Stein and Moore (4).
[f] Soupart (7).

excreted in 24 hr. by the rabbit than by human beings. The pattern between the two species also differs. Taurine, 1-methylhistidine, unknown peak 2, and glycine, in decreasing order, are the four most abundant amino acids in rabbit urine; while glycine, histidine, asparagine plus glutamine, and taurine are the four most abundant in human urine.

The rabbit has a mean excretion of histidine of 0.7 mg. which is lower than the amounts of 1-methylhistidine (9.8 mg.) and 3-methylhistidine (3.7 mg.) excreted. This is the reverse of the finding in man who excretes more histidine (110.1 mg.) than either 1-methylhistidine (39.0 mg.) or 3-methylhistidine (32.5 mg.) (Table III). Little is known of the origin of the methylated derivatives of histidine in urine except for the work of Brown *et al.* (9) who

found labeled 1-methylhistidine in urine from monkeys and human subjects given uniformly labeled histidine-C^{14}. No labeled 1-methylhistidine was found in the urine of rats given L-histidine-2-C^{14}. Fink *et al.* (10), and McManus (11) have reported that 1-methylhistidine excretion is increased in vitamin E-deficient rabbits. The consensus is that increased excretion of methylhistidine might be related to abnormal metabolism of anserine or carnosine which may have important roles to play in the proper functioning of the muscle. In this laboratory it has been found that more 1-methylhistidine than histidine is excreted by a high percentage of patients with psoriasis (6). Also, the urinary ratio of 3-methylhistidine to histidine is abnormally high in many of these patients.

Further studies of histidine metabolism in the normal rabbit, who has a urinary pattern of histidine and methylated histidine excretion similar to many of the human subjects with psoriasis, may lead to information of the metabolic pathway by which these methylated derivatives of histidine arise in the urine.

Citrulline, glycine, ornithine, lysine, and arginine occur in appreciably higher amounts in rabbit plasma than in cat plasma; serine, glutamic acid, glycine, alanine, ornithine, lysine, and arginine values are considerably higher than in human plasma (Table III). The most striking difference among these is in glycine; plasma from rabbits contained a mean of 6.64 mg., while cat plasma contained 2.3 mg., and human plasma 1.54–1.70 mg./100 ml. The amounts of the other amino acids determined in rabbit plasma are either lower or approximately the same as those in cat and human plasma.

The pattern of amino acids in the plasma differs among the three species in that glycine content exceeds alanine in the rabbit. This is not true for man or the cat. If one disregards the asparagine plus glutamine moiety of plasma because values for only the rabbit are available, glycine, lysine, alanine, proline, and arginine, in decreasing order, are the five most abundant amino acids in rabbit plasma. Alanine, lysine, valine, proline, and serine are most abundant in cat plasma; and alanine, valine, lysine, glycine, and proline in human plasma.

That fasting may cause an increase in the branched-chain amino acids of the plasma has been reported by Swendseid *et al.* (12) for one human subject fasted for 14 days, and by Charkey *et al.* (13) for six human subjects fasted for 2 days. The present study confirms this finding in another species, the rabbit; valine, isoleucine, and leucine content of the plasma increased when the animals were fasted for 88 hr. Lysine, threonine, methionine, and arginine content decreased in the rabbit plasma; these amino acids are reported to be decreased in plasma from human subjects fasted for 2 days (13).

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