16077

Urinary Excretion of Amino Acids by the Rat.*

B. S. SCHWEIGERT. (Introduced by P. B. Pearson.)

From the Department of Biochemistry and Nutrition, Agricultural and Mechanical College of Texas, College Station, Texas.

The adaptation of microbiological methods for the determination of amino acids in urine[1-8] has afforded considerable information on the levels of various amino acids excreted by several species as influenced by the amounts ingested. A paucity of information is available, however, on the influence of dietary deficiencies, such as specific amino acid or vitamin deficiencies, on the excretion of amino acids in the urine. In earlier work[2,9] it was shown that vitamin B_6 deficient rats metabolized tryptophane to microbiologically inactive products at a rate comparable to that observed for vitamin B_6 supplemented animals. In the present study the effect of the ingestion of tryptophane deficient diets and the ingestion of diets deficient in riboflavin or nicotinic acid on the amounts of certain amino acids excreted in the urine was determined.

Experimental. Care of animals and composition of diets. Weanling rats were housed in single cages and were fed the experimental diets for varying periods of time. Variations in the composition of the diets used were either in the level or kind of protein or in the B vitamin supplement added. Corn oil was added to all rations at a level of 4.7%, vitamin A and D concentrate 0.3%, salt mixture 4.0% and sucrose to 100%. Unless indicated otherwise, each diet contained 250 μg each of thiamin and pyridoxine, 300 μg of riboflavin, 2 mg of calcium pantothenate, 100 mg each of choline and inositol and one mg of nicotinic acid per 100 g. The diets were fed *ad libitum*. A brief designation of the diets is as follows:

No. 7—24% casein.
8—Same as No. 7 except no riboflavin was added.
9—12% casein.
10—12% casein and 12% gelatin.
11—Same as No. 10 except no nicotinic acid was added.
12—12% oxidized casein† plus adequate quantities of cystine and methionine. This ration was designed to be deficient in tryptophane.
13—Same as No. 12 plus 0.2% *dl*.tryptophane.

† The oxidized casein used in this investigation was prepared by the method of Toennies, *J. Biol. Chem.*, 1942, **145**, 667.

Urine collections were made by the use of metabolism cages fitted with outside feeders. The urine was collected during a 2- or 3-day period and food consumption records were also kept for these periods. Collections were made at least twice during the experimental period and data on at least two animals were obtained for each series of collections. Thus data on the amino acids excreted were obtained at different times throughout the experimental period. The urine samples from each group were combined prior to analysis so that data were obtained on 4 or more rats in each group. The collections were made at the same time for each series of deficient and sup-

* Frances Panzer and Helen Keene assisted with the amino acid analyses.

1 Schweigert, B. S., Sauberlich, H. E., and Elvehjem, C. A., *Science*, 1945, **102**, 275.

2 Schweigert, B. S., Sauberlich, H. E., Elvehjem, C. A., and Baumann, C. A., *J. Biol. Chem.*, 1946, **164**, 213.

3 Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, 1946, **166**, 417.

4 Steele, B., Sauberlich, H. E., Reynolds, M., and Baumann, C. A., *J. Nutrition*, 1947, **33**, 209.

5 Harvey, C. C., and Horwitt, M. K., *Fed. Proc.*, 1947, **6**, 259.

6 Hier, S. W., and Bergeim, O., *Fed. Proc.*, 1947, **6**, 261.

7 Frankl, W., and Dunn, M. S., *Arch. Biochem.*, 1947, **13**, 93.

8 Dunn, M. S., Camien, M. N., Shankman, S., and Block, H., *Arch. Biochem.*, 1947, **13**, 207.

9 Schweigert, B. S., and Pearson, P. B., *J. Biol. Chem.*, 1947, **168**, 555.

TABLE I.
Effect of Feeding Diets Low in Tryptophane on Urinary Excretion of Certain Amino Acids.*

Diet No. and description	Time on experiment, days	Wt change per day, g	Histidine: Amt excreted daily, mg	Histidine: Ingested amino acid excreted, %	Arginine: Amt excreted daily, mg	Arginine: Ingested amino acid excreted, %	Threonine: Amt excreted daily, mg	Threonine: Ingested amino acid excreted, %	Phenylalanine: Amt excreted daily, mg	Phenylalanine: Ingested amino acid excreted, %	Tryptophane: Amt excreted daily, mg	Tryptophane: Ingested amino acid excreted, %
No. 12 (12% oxid. casein basal)	7-14	—0.9	0.15	2.1	0.28	2.3	0.17	1.4				
No. 13 (12% oxid. casein + tryptophane)	7-14	+2.2	0.13	0.95	0.16	0.73	0.14	0.64				
No. 12 (Group 2)†	4-10	—1.5	0.22	1.4	0.24	1.0	0.36	1.5	0.14	0.50	0.037	—
No. 13 (Group 2)†	4-10	+1.0	0.36	0.98	0.56	1.0	0.43	0.76	0.59	0.89	—	—
No. 7 (24% casein)	14-26	+4.4	0.25	0.34	0.64	0.57	0.54	0.48	0.36	0.27	0.15	0.41
No. 9 (12% casein)	14-26	+3.5	0.22	0.59	0.58	1.0	0.30	0.52	0.36	0.45	0.07	0.37

* Since the amounts excreted per day were very similar for urine collections made at different times during the experimental period, the values indicated are the averages for all collections made during the experimental period. The weight change was computed for only that period during which urine collections were made.

† Rats with an initial weight of 150 g were used in these experiments.

TABLE II.
Excretion of Amino Acids by Riboflavin and Nicotinic Acid Deficient Rats.*

Diet No. and description	Time on experiment, days	Wt change per day, g	Histidine: Amt excreted daily, mg	Histidine: Ingested amino acid excreted, %	Arginine: Amt excreted daily, mg	Arginine: Ingested amino acid excreted, %	Threonine: Amt excreted daily, mg	Threonine: Ingested amino acid excreted, %	Phenylalanine: Amt excreted daily, mg	Phenylalanine: Ingested amino acid excreted, %	Tryptophane: Amt excreted daily, mg	Tryptophane: Ingested amino acid excreted, %
No. 7 (riboflavin supp.)	14-54	+3.1	0.17	0.25	0.48	0.46	0.68	0.65	0.40	0.32	0.18	0.53
No. 8 (riboflavin def.)	14-54	+0.3	0.22	0.67	0.64	1.3	0.59	1.17	0.45	0.76	0.14	0.85
No. 10 (12% casein, 12% gelatin + N.A)	14-45	+2.8	0.37	0.77	1.41	0.80	0.81	0.92	0.46	0.47	0.13	0.66
No. 11 (12% casein, 12% gelatin — N.A.)	14-45	+0.4	0.18	0.75	0.61	0.68	0.32	0.72	0.28	0.55	0.058	0.58

* Since the amounts excreted per day were very similar for urine collections made at different times during the experimental period, the values indicated are the averages for all collections made during the experimental period. The weight change was computed for only that period during which urine collections were made.

plemented groups. The urine samples were stored in a refrigerator prior to analysis.

Amino acid analyses. Histidine, arginine, threonine, phenylalanine and tryptophane determinations were made after neutralization and proper dilution of the urine samples. These amino acids were chosen for analysis since it seemed likely that amino acids of this type may be more difficult to metabolize than the simpler amino acids. In addition, the methods for determining these amino acids in urine are quite accurate while the reliability for others is somewhat uncertain. *S. faecalis* R was used as the test organism for the determinations of threonine, arginine and histidine. The medium used has been described previously.[10] Tryptophane and phenylalanine were determined with *L. arabinosus* as the test organism.[1,11]

In some cases the occurrence of bound forms of the amino acids was investigated. Aliquots of the urine samples were hydrolyzed with one N HCl at 15 pounds pressure for 5 hours, neutralized, diluted to an appropriate volume and assayed for amino acids. By this procedure, data were obtained on the amounts of certain amino acids that were excreted in combined form, since the same samples were analyzed for amino acids prior to hydrolysis.

Results and Discussion. The amounts of histidine, arginine, threonine, phenylalanine and tryptophane excreted per day and the percentage of each amino acid ingested that was excreted in the urine are shown in Tables I and II. The latter figures were computed on the basis of the amount of each amino acid consumed and of the amount of each amino acid excreted per day. The composition accepted for casein was: histidine 2.6%, arginine 3.6%, threonine 4.0%, phenylalanine 4.7% and tryptophane 1.2%. For gelatin the values used were 0.6% histidine, 7.8% arginine, 1.8% threonine, 2.0% phenylalanine and 0.2% tryptophane. The amounts in casein and gelatin were determined by microbiological methods. From these data and from the food consumption data the amounts of each amino acid ingested per day were calculated.

With the methods employed, the values for urine shown in Tables I and II represent the amount of each amino acid present in the urine that is microbiologically available. These values have also been termed the amount of apparent free amino acid present. Undoubtedly, the free amino acids comprise the largest portion measured, but it is recognized that certain amino acids present as peptides are utilized to some extent by the test microorganisms.[12] In order to determine the total amount present, it is necessary to hydrolyze the urine samples to liberate the bound forms of the amino acids.

It will be noted that even when rations deficient in an amino acid or a vitamin are fed for considerable periods of time, the amounts of the amino acids excreted per day are not materially different than those excreted by the control animals. Similarly, no difference was observed in the amounts excreted when determinations were made at different times in the experimental period. The percentage of the ingested amino acids excreted in the urine was somewhat higher, however, for the tryptophane or riboflavin deficient groups than for the respective control groups. The differences in the percentages of the ingested amino acids excreted were not as consistent, however, for the older rats fed the tryptophane deficient and supplemented diets (Table I). Rats fed a nicotinic acid deficient ration excreted approximately the same percentage of these amino acids as did the controls. In general, the amounts of each amino acid excreted when the control diets were fed (No. 7, 9, 10 and 13) were roughly proportional to the amino acid intake. The amount of arginine excreted, for example, was much higher when ration 10 (12% casein + 12% gelatin) was fed than when ration 9 (12% casein) or ration 7 (24% casein) was fed. This is undoubtedly due to the higher amounts of arginine ingested when ration 10

[10] Greenhut, I. T., Schweigert, B. S., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, **162**, 69.

[11] Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Strong, F. M., *J. Biol. Chem.*, 1944, **155**, 183.

[12] Schweigert, B. S., and Snell, E. E., *Nutr. Abs. and Rev.*, 1947, **16**, 497.

was fed since this ration contains gelatin which is very high in arginine.

Thus it appears that the deficiencies studied do not markedly affect the ability of the rat to metabolize the ingested amino acids to microbiologically inactive products even when the animals are losing weight at a rapid rate. It is quite possible, of course, that prolonged or chronic deficiencies may cause the rat to excrete much larger quantities of these amino acids. It is also possible that the metabolism of other amino acids not studied was materially affected. Some tests conducted with lysine and methionine indicate that they also were excreted in small amounts when diets deficient in tryptophane or riboflavin were fed. Preliminary studies indicate that vitamin B_6 deficient rats do not show large changes in the amounts of these amino acids excreted. These results are quite different from those observed with the mouse[13,14] in that this species excretes large amounts of certain amino acids when deficient diets are fed. Thus an average of 37.3% of the ingested amino acids was excreted by mice when fed a tryptophane or methionine deficient diet while an average of only 2.6% was excreted when adequate diets were fed. Differences in the percentage of the ingested tryptophane that was excreted when normal diets were fed to rats as compared to mice have been discussed in earlier work.[2]

Data obtained on the amounts of histidine, arginine, threonine and phenylalanine determined after acid hydrolysis of the urine samples support the same conclusions as those on the amounts of microbiologically available amino acids (Table III); namely, that the amounts of these amino acids excreted are not appreciably affected by the ingestion of deficient diets by the rat. The results show that about ¼ to ½ of the total amount excreted is measured as the free amino acid. These results are in agreement with those reported by Sauberlich and Baumann[3] and Albanese *et al.*[15]

TABLE III.

Effect of Acid Hydrolysis on Amount of Certain Amino Acids Found in Urine.* (All values expressed as mg excreted per day.)

Diet No.	Dietary regimen	Histidine		Arginine		Threonine		Phenylalanine	
		before	after	before	after	before	after	before	after
7	(24% casein)	0.25	1.4	0.64	1.6	0.54	1.5	0.36	1.6
9	(12% casein)	0.22	0.72	0.58	1.3	0.30	1.0	0.36	1.1
10	(12% casein, 12% gelatin)	0.37	1.2	1.41	3.0	0.81	1.7	0.46	1.8
11	(12% casein, 12% gelatin — N.A.)	0.18	0.78	0.61	1.8	0.32	0.83	0.28	1.3
12	(12% oxid. casein — tryptophane)	0.15	0.50	0.28	0.44	0.17	0.60	—	—
13	(12% oxid. casein + tryptophane)	0.13	0.36	0.16	0.42	0.14	0.35	—	—

* The values were obtained on the same samples before and after acid hydrolysis.

[13] Sauberlich, H. E., Pearce, E. L., and Baumann, C. A., *Fed. Proc.*, 1947, **6**, 420.

[14] Pearce, E. L., Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, 1947, **168**, 271.

Although measurements of the amino acids in the urine and balance studies to ascertain the amount of any amino acid that is retained by the body are of great value, it should be recognized that alterations in the degree of metabolism of any amino acid are not necessarily determined by the microbiological methods used. Thus, when diets deficient in a specific amino acid or a vitamin are fed and no large differences in the excretion of the unchanged amino acid is observed, it may be quite possible that the amino acid is only partially metabolized and these partial metabolites excreted in the urine are microbiologically inactive. That marked changes in the amounts of the amino acids excreted in the urine do occur with mice fed various deficient diets has already been observed. Further studies on the specificity of these amino acid methods and their reliability with respect to blood and urine analysis will aid in obtaining more information on the metabolism of specific amino acids by various species.

Summary. Rats have been fed diets deficient in tryptophane, riboflavin or nicotinic acid and the amounts of microbiologically available histidine, arginine, threonine, phenylalanine and tryptophane excreted in the urine were determined. No large changes were noted in the amounts of these amino acids excreted in the urine when rations deficient in nicotinic acid were fed. Rats fed a riboflavin or a tryptophane deficient diet excreted approximately twice as much of the ingested amino acids as animals fed adequately supplemented diets. In all cases, however, the amounts excreted were small, less than 2.5 per cent of those ingested. After acid hydrolysis of the urine samples, a 2-4 fold increase was observed in the values for histidine, arginine, threonine and phenylalanine.

[15] Albanese, A. A., Holt, L. E., Frankston, J. E., and Irby, V., *Fed. Proc.*, 1946, **5**, 118.

16078

Vasoconstrction Elicited by Addition of Plasma to Vasoinactive Tissue Extracts.*

E. MYLON AND J. H. HELLER. (Introduced by M. C. Winternitz.)

From the Laboratory of Pathology, Yale University School of Medicine.

Perfusion experiments on the rabbit's ear have shown that several vasoinactive, renin-containing protein fractions of hog kidney became vasoconstrictive when the perfusion fluid (Ringer-Locke) was supplemented by minute amounts of epinephrine.[1]

Identical effects were observed when the kidney fractions were replaced by similarly prepared fractions of other organs—especially liver.[2]

A change from vasoinactivity to vasoconstriction in the rabbit's ear was previously reported[3] when plasma instead of Ringer-Locke was used for perfusion with kidney fractions (renin). This constriction was not attributed to the direct combined effect of the kidney fraction and plasma, but to the formation of a special vasoconstrictive substance, angiotonin (hypertensin).

Since plasma is known to contain traces of epinephrine,[4] it seemed desirable to investigate whether this epinephrine content might have contributed to the constrictive effect elicited by kidney- or other tissue-fractions.

* Aided by a grant from the Commonwealth Fund.

[1] Mylon, E., Horton, F. H., and Levy, R. P., PROC. SOC. EXP. BIOL. AND MED. 1947, **66**, 375.

[2] Mylon, E., Horton, F. H., and Levy, R. P., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 378.

[3] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[4] Shaw, E. H., *Biochem. J.*, 1938, **32**, 19.