THE NORMAL PROTEIN METABOLISM OF THE RAT.

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The highly interesting results obtained by Mendel and Osborne[1] on feeding single specific proteins to rats lend added interest to any detailed information that may be obtained concerning the nature of the metabolism of the rat as compared with that of any other animal or of man. The small size of the animal has heretofore prevented the accumulation of much information of a quantitative nature. The total urinary nitrogen determinations of Mendel and Osborne and those of Hatai[2] represent so far as we know all that has been done in this line. By means of the new microchemical method,[3] recently published from this laboratory it is possible to obtain more detailed figures. Because of the limited quantity of urine comprising the twenty-four-hour amounts, some changes in these methods were found necessary. In every case the twenty-four-hour urine of each animal (5–50 cc. in amount) was made up to 50 cc. From this stock solution portions were taken directly for ammonia, uric acid, creatinine and creatine determinations. For total nitrogen and urea determinations a dilution of a part of this solution to twice its volume brought the nitrogen content to the desired amount (1 ± mgm. per 1 cc.). In the smallest rats this second dilution was unnecessary, the quantity being sufficiently reduced in the first dilution.

The uric acid analysis followed the general scheme of its determinations in human urine as published by Folin and Denis.[4] Ten cubic centimeters of rat urine were found to contain a sufficient quantity of uric acid for the determination. A drop of acetic acid

[1] Carnegie Institution of Washington, publication 156, Pts. I and II.

[2] *Amer. Journ. of Physiol.*, xiv, p. 102, 1905.

[3] This *Journal*, xi, 1912.

[4] *Ibid.*, xiv, p. 95, 1913.

(instead of hydrochloric) was used to acidify when driving off the hydrogen sulphide. After the reaction with the uric acid reagent and sodium carbonate the blue solution was filtered into a 25 cc. volumetric flask and made up to the mark with washings. It was then read against a standard of 1 mgm. of uric acid in 100 cc.

The creatinine determinations offered at the beginning considerable difficulty owing to the small amount of creatinine and its great dilution in rat urine. The original standard, potassium bichromate solution, could not be used and had to be replaced by creatinine solutions of known concentration. Other factors had to be modified—the amounts of picric acid and alkali, and the time necessary for the development of the maximum color had to be worked out. Our standard creatinine solutions were made of approximately the same concentration as the urine. These solutions and the urine were then treated exactly alike and, by developing the color simultaneously in both, constant and seemingly reliable results were obtained. The modified procedure is as follows:

To 5 cc. of urine and to 5 cc. of the standard creatinine solution, each in a 25 cc. volumetric flask, add 2.5 cc. of saturated picric acid solution from a burette, and then 1 cc. of 10 per cent sodium hydroxide solution. Let stand for ten minutes, fill up to the mark with water and then determine the color as in the original method.

The creatine determination was made, in terms of creatinine, by the same method and appeared as an addition to the previously obtained creatinine figure. It was converted into creatinine by adding 0.5 cc. of 2N HCl to 5 cc. of urine in a 100 cc. Erlenmeyer flask, evaporating to rather less than 2 cc. volume on a water bath, and continued heating at this volume for two hours. To maintain the volume a small top-shaped glass bulb was inserted in the mouth of the flask for condensation. At the end of the time indicated the volume was again made up to approximately 5 cc. by adding water to the flask on a balance until the weight was the sum of 5 grams and the previously-taken weight of the flask. Beyond this the treatment was exactly the same as with creatinine except that an additional 0.5 cc. of NaOH was added to neutralize the HCl used.

Each rat was kept in a specially constructed small cage resting on top of a funnel. Each day's urine was collected in 2 cc. of normal hydrochloric acid. The twenty-four-hour aspect is only approximate since no attempt was made to have the bladder emptied at the end of each day. The duration of the experiment is long enough, however, so that the average of all the figures should come close to the true twenty-four-hour results. The urines were analyzed in the case of rats M, X and A over a period of nine days, and in the case of rat G, fifteen days.

In tables 1 and 2 are given the analyses of a series of approximately twenty-four-hour urines obtained from two large mature rats, a female (M) weighing 290 grams and a male (X) weighing 197 grams. To save space only the last six days are recorded.

Table 3 represents a summary of the results recorded in tables 1 and 2, together with summaries of the results obtained from two young growing rats, A and G, weighing 40 to 50 grams. We have made several other series of similar studies but these deal chiefly with tumor-bearing rats and will be described elsewhere by Ordway and Morris.

The rats were kept on a purine-free diet consisting of powdered crackers and water.

It will be seen, from examination of the average results, that the percentage composition of rat urine differs but little from that of human urine. Being small animals but voracious feeders the total nitrogen per kilo of body weight is much larger than in the case of man. The percentage relationship between the amounts of total nitrogen, urea nitrogen and ammonia nitrogen is almost the same as in man. Creatinine nitrogen shows a somewhat larger value for the rats, reaching 15 mgm. per kilo in the mature rats, while man usually eliminates 7–11 mgm. Small amounts of creatine were always found in the urine. This is rather interesting in view of the excessive feeding of rats and the suggestion of Folin and Denis[5] that the creatine in the urine of children might be due to an "excessively high level of protein consumption."

The most striking and interesting feature of the analyses is the fact that the urine of rats contains quite as much uric acid in proportion to body weight as does human urine. Through the

[5] This *Journal*, xi, p. 253, 1912.

TABLE 1.

Female rat weighing 290 grams (M).

DATE (DEC.)	16		18		19		20		21		AV. 24 HOURS	
	mgm.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total N	172.5	100.0	184.5	100.0	165.0	100.0	190.5	100.0	155.0	100.0	173.5	100.0
Urea N	147.9	86.0	139.1	75.4	130.0	78.8	150.0	78.7	103.8	67.0	143.2	77.3
Ammonia N	9.1	5.3	9.4	5.1	9.5	5.8	9.4	4.9	8.2	5.3	9.1	5.2
Uric Acid N	0.52	0.3	0.73	0.4	0.71	0.43	0.83	0.43	0.65	0.42	0.69	0.40
Creatinine N	4.8	2.8	4.7	2.6	3.9	2.4	4.3	2.2	4.7	3.0	4.5	2.65
Creatinine + Creatine N	5.0	2.9	4.6	2.6	3.6	2.3	4.7	2.5	4.7	3.0	4.7	2.71

TABLE 2.

Male rat weighing 197 grams (X).

DATE (DEC.)	16		17		18		19		20		21		AV. 24 HOURS	
	mgm.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
Total N	144.0	100.0	127.0	100.0	119.0	100.0	118.0	100.0	116.0	100.0	132.0	100.0	126.0	100.0
Urea N	115.3	80.1	109.8	86.5	102.7	86.3	88.7	75.2	101.0	87.1	118.1	89.5	105.9	84.0
Ammonia N	8.2	5.7	7.1	5.6	6.6	5.5	6.3	5.4	5.4	4.7	6.9	5.2	6.7	5.3
Uric Acid N	0.43	0.3	0.75	0.59	0.62	0.52	0.43	0.36	0.62	0.54			0.52	0.41
Creatinine N	3.1	2.1	3.3	2.6	2.6	2.2	3.0	2.5	2.4	2.1	3.2	2.4	2.9	2.30
Creatinine + Creatine N	3.2	2.2	3.7	2.9	2.9	2.5	2.7	2.2	2.4	2.1	3.3	2.5	3.0	2.38

TABLE 3.

Summary of percentages.

	RAT											
	M			X			A			G		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Weight	292.0	289.0	290.5	197.0	197.0	197.0	42.7	41.0	41.9	51.5	46.5	49.0
Urea N	86.0	67.0	77.3	89.5	75.2	84.0	78.2	64.1	71.3	87.7	61.1	76.3
Ammonia N	8.6	4.9	5.2	8.5	5.2	5.3	11.7	6.1	9.0	10.5	4.8	7.0
Uric Acid N	0.43	0.29	0.40	0.59	0.30	0.41	0.47	0.21	0.35	0.67	0.15	0.48
Creatinine N	3.0	2.2	2.65	2.6	2.1	2.30	1.3	1.0	1.08	1.7	1.1	1.37
Creatinine N + Creatine N	3.1	2.3	2.71	2.9	2.1	2.38	1.5	1.1	1.16	1.8	1.0	1.44

*Summary in grams per kilo body weight.**

	RAT			
	M	X	A	G
Total N	0.598	0.639	1.068	0.594
Urea N	0.463	0.537	0.761	0.453
Ammonia N	0.031	0.034	0.096	0.041
Uric Acid N	0.0024	0.0026	0.0037	0.0029
Creatinine N	0.0155	0.0147	0.0115	0.0082
Creatinine N + Creatine N	0.0162	0.0148	0.0124	0.0086

*(Figures based on average weights, both of animals and metabolism products.)

investigations of Wiechowski[6] and more recently of Hunter and Givens[7] we have learned that mammals other than man convert the greater part of the uric acid into allantoin, and the urines of such animals therefore contain very little if any uric acid. The metabolism of rats is, however, in this respect like that of man.

In view of this highly curious similarity it seemed necessary also to determine whether the blood of rats is as rich in uric acid as is human blood. Folin and Denis[8] have recently shown that domestic animals, whose urine contains allantoin instead of uric acid, uniformly show mere traces of uric acid in the blood. The blood from six full-grown rats was collected over a little powdered potassium oxalate, and the uric acid, total non-protein nitrogen and urea were determined by the methods of Folin and Denis. The figures obtained for 100 grams of blood were as follows: Uric acid, 2 mgm.; non-protein nitrogen, 38; urea, 22. The experiment was repeated twice, each time using for the analysis the mixed blood of six normal white rats, and 2.4 mgm. and 2.5 mgm. respectively of uric acid per 100 grams of blood were found. These are substantially the same figures as Folin and Denis found for normal human blood. The purine metabolism of rats is, therefore, like that of man and unlike that of other mammals hitherto investigated. Jones and Rohdé[9] published some experiments on purine ferments of the rat a few years ago, and one of the conclusions reached is interesting in connection with our results: "The results of this work show that the organ extracts of the rat jointly and severally are incapable of exhibiting either adenase or xanthooxidase. There is, therefore, no way for uric acid to be formed by the purine ferments in extracts of the organs of the animal. Nevertheless rats' urine contains uric acid. From 50 cc. of urine we were able to isolate enough uric acid for complete identification. Presumably the organs also contain uric acid which might be detected by methods of sufficient refinement but the substance cannot be produced by the action of organ extracts on purine bases."

[6] *Biochem. Zeitschr.*, xxv, p. 433, 1910.
[7] This *Journal*, xiii, p. 372, 1912.
[8] *Ibid.*, xiv, p. 31, 1913.
[9] *Ibid.*, vii, p. 237, 1909.

It seems rather remarkable that the one animal which (excepting man) produces the most uric acid in the course of normal metabolism should lack the ferments capable of producing it. In this connection we can state that investigations conducted by J. B. Sumner in this laboratory have shown that aqueous extracts from rat livers are as capable of destroying uric acid as similar extracts obtained from the livers of cats and sheep. The significance of "purine ferments" as obtained from organ extracts in relation to the formation and elimination of uric acid in the course of normal metabolism is, therefore, far from clear.