ARTICLE:
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THE NITROGENOUS CONSTITUENTS OF HEN URINE.

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Analyses of urine from birds have been made by Minkowski (1), Milroy (2), Paton (3), Sharpe (4), and Steel (5); nevertheless in no case has the complete partition of urinary nitrogenous compounds been reported. We have collected such data. It was thought in the beginning that the total urinary nitrogen in bird droppings might be calculated from one constituent, *viz.* uric acid. Such a calculation would be advantageous in the determination of fecal nitrogen in bird droppings, a quantity that is necessary in the determination of digestibility coefficients for protein. Unfortunately our results, taken with those of Steel (5) who analyzed the white portion of bird droppings, indicate that considerable reabsorption of urinary constituents, NaCl, urea, and creatine, occurs in the cloaca of the hen. It appears impossible therefore to calculate the fecal nitrogen with any accuracy from the total and uric acid nitrogen values of bird droppings. The difficulty in obtaining bird urine, uncontaminated with feces, was overcome by using a special technique applicable to unanesthetized, unoperated birds.

Five young hens were used in this experiment. They were kept in cages throughout the entire time, and were fed the same standard ration that is used on the University poultry farm. This ration contains the following per 530 pounds.

	lbs.
Wheat middlings	100
Wheat bran	100
Corn	200
Meat scrap	100
Bone meal	20
Salt	5
Cod liver oil	5

The average weight of the chickens at the beginning of the experiment was 2 kilos. They grew well, gaining an average of 1.2 kilos per bird. They were normal to all appearances.

The first step in the work was to develop a method by which the urine could be collected without contamination with feces. Several investigators have done this by operating upon the birds, separating the urodeum and proctodeum, and forming an artificial anus. The urine and feces were then collected in rubber bags. An objection to this method is that the bowels become inactive and it is necessary to wash out the feces at each collection. The birds cannot be considered normal after such an operation has been performed and there are possibly some effects of trauma.

The procedure used here in the collection of urine was as follows: The hen was given 100 cc. of water by stomach tube and immediately fastened to a specially constructed chicken board. The feces were removed from the cloaca and the cloaca was carefully washed out. A catheter was then inserted into the urodeum near the openings of the ureters. The urine was collected in graduated test-tubes so the rate of flow could be easily determined. At times it was necessary to plug the intestine with cotton to prevent small pieces of feces from coming into the cloaca. Usually, however, this was not necessary unless the period of collection was over 2 hours.

The urine was analyzed for total nitrogen, uric acid, urea, ammonia, creatine, and creatinine. Creatine and creatinine were determined together and reported as creatinine. Total nitrogen was determined by the Kjeldahl method, urea by the Van Slyke and Cullen modification of Marshall's method (6), ammonia by Folin's aeration method (7). Folin's colorimetric methods (8) were used to determine uric acid, creatine, and creatinine.

The first collections of urine were made from hens that were anesthetized with ether. Such urine was dilute and the rate of excretion was very rapid. It was noted that as the effect of the anesthetic wore off the rate of flow decreased. Table I shows the effect of ether on the rate of flow and on the nitrogen content of the urine.

The hens remained quiet during the collection so that it was not necessary to use an anesthetic, but when none was used the urine was so concentrated that the solids precipitated and clogged the catheter. To overcome this difficulty, water was given by mouth immediately before the collection of the urine. Water increased

TABLE I.

Effect of Ether Anesthesia on Urine Flow.

	Ether anesthesia.			No ether.		
Hen No.	Urine.	N per 100 cc.	Total N per hr.	Urine.	N per 100 cc.	Total N per hr.
	cc. per hr.	*mg.*	*mg.*	*cc. per hr.*	*mg.*	*mg.*
1	60	45	27.0	14.3	178	25.5
2	55	48	26.4	12.0	184	22.1

TABLE II.

Nitrogenous Constituents in 100 Cc. of Hen Urine and Their Proportions.

Hen No.	Total N.	Uric acid N.		Urea N.		Ammonia N.		Creatine-creatinine N.		Undetermined N.		Remarks.
	mg.	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	
1 a	101.2	65.0	64.2	11.2	11.0	17.2	16.9	7.5	7.4	0.3	0.29	
b	102.3	63.3	61.7	11.5	11.2	17.0	16.6	8.2	8.0	2.3	2.3	
c	105.0	73.0	69.5	11.6	11.0			8.9	8.8			
d	107.5	65.6	61.0	11.8	10.9	19.4	18.0	9.2	8.6	1.6	1.5	
2 a	69.4	44.8	64.6	7.6	10.2	11.8	17.0	5.0	7.1	0.2	0.3	Ether anesthesia.
b	94.5	61.0	65.5	8.7	9.2	16.1	17.0	7.1	7.5	0.8	0.84	
3 a	158.0	95.0	60.0	16.3	10.2	25.4	16.0					
b	109.0	69.7	64.0	12.0	11.0	16.5	15.1	8.9	8.1	1.9	1.7	
c	85.1	53.1	62.4	8.2	9.6	14.6	17.2	5.7	7.9	2.5	2.9	
4 a	95.4	56.7	59.4	11.0	11.5	17.7	18.5	7.6	8.0	2.4	2.5	
b	101.6	67.0	66.0	9.6	9.4	15.1	14.9	8.2	8.1	0.5	0.49	
5 a	82.5	51.0	62.0	8.6	10.4	14.1	17.1	6.5	7.9	2.3	2.7	
b	126.0	74.9	59.5	13.8	10.9	24.6	19.5	10.2	8.1	2.5	2.0	
c	63.8	38.5	60.0	6.85	10.6	12.1	19.0	5.8	9.1	0.55	0.86	Ether anesthesia.
Average.	100.0	62.9	62.9	10.4	10.4	17.3	17.3	8.0	8.0	1.4	1.4	

the flow of urine but did not affect the relative amounts of nitrogenous constituents.

A number of analyses was made to determine the effects of diuresis produced by ether and water on the relative amounts of the nitrogen compounds in the urine. No effect was found. The diuretics caused merely a dilution of the urine and had no effect on the nitrogenous constituents. Ambard and Wolf (9) report that water diuresis had no effect on the excretion of uric acid.

The volume of urine which is emptied into the cloaca is rather large. In these experiments the amount excreted when no diuretic was used varied greatly, the maximum being about 30 cc. per hour. If this rate is maintained for the entire 24 hours, the daily excretion would be about 700 cc. This value agrees with those obtained by Paton (3) and Sharpe (4) by direct measurement of urine from birds which had been operated upon. When diuretics were used, the flow was increased. Most of the water, therefore, which is excreted with the urine is reabsorbed in the cloaca.

The urine was neutral to litmus in every case tested. The specific gravity was determined on one sample and was found to be 1.004. In most cases it was impossible to determine the specific gravity or the reaction of the urine due to the fact that the urine was run into a solution of lithium carbonate to prevent the precipitation of uric acid.

Table II gives the results of analyses of urine samples which were collected as described above. In nearly every case the collection period was 2 hours. When these data are expressed in percentage of total nitrogen, it is seen that there is not agreat deal of variation in the distribution of nitrogen waste products among the different samples. Of the total nitrogen, an average of 62.9 per cent is in the form of uric acid, 10.4 per cent in the form of urea, 17.3 per cent in the form of ammonia, and 8.0 per cent in the form of creatine-creatinine. Minkowski (1) found from 60 to 70 per cent of the total nitrogen present in the urine of normal geese in the form of uric acid, from 3 to 4 per cent in the form of urea, and from 9 to 18 per cent in the form of ammonia.

The amount of creatinine in the urine was too small to be determined by the method used but the amount of creatine was relatively large. This peculiarity of bird urine has been observed by Paton (3). Creatine was determined by converting it to creatinine by boiling with picric acid and determining the latter.

Chlorides were determined in a few samples. The amount of chlorine found in the urine is much greater than the amount excreted in the combined urine and feces. Chlorides, therefore, are reabsorbed with water in the cloaca.

SUMMARY.

In fourteen trials with five hens, the uric acid nitrogen of hen urine varied from 59.4 to 69.5 per cent of the total urinary nitrogen, with an average of 62.9 per cent. Ammonium nitrogen accounted for 17.3 per cent, with variations between 14.9 and 19.5, urea nitrogen for 10.4 per cent, with variations between 9.2 and 11.2, and creatine plus creatinine nitrogen for 8.0 per cent of the urinary nitrogen with variations between 7.1 and 9.1 per cent. Uric acid nitrogen plus ammonia nitrogen accounted for 80.3 per cent of the urinary nitrogen with variations between 76 and 82.5 per cent. Since an unknown amount of reabsorption of urea, creatine, and creatinine probably occurs in the cloaca of the hen, it does not seem practical, at least without further information, to calculate urinary nitrogen from the amount of uric acid in hen droppings by multiplying uric acid nitrogen by some factor.

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