LXV. A METHOD FOR OBTAINING UNCONTAMINATED SPECIMENS OF URINE FROM THE BILLY GOAT; WITH SOME NOTES UPON THE NORMAL METABOLISM OF THIS ANIMAL.

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THE method described in this communication¹ was applied during the war to a study of the normal urine of the goat. By its means some data upon the normal metabolism of the goat were collected, and although by no means complete, it has been thought worth while to put them upon record as they are in general agreement with the work of others. Steenbock, Nelson and Hart [1914] showed that the calf used ammonia for the purpose of neutralising acid given in the diet. Winterberg [1898] had previously shown that the rabbit used ammonia under similar circumstances. Some previous observations upon the goat have been made by Baer [1906], who showed that under conditions of starvation, the output of ammonia was increased.

The notes dealt with in this paper are concerned with the urine of the goat upon different diets, during starvation, and after feeding with acid and alkali. For this purpose the titratable alkalinity of the urine and the hydrogen ion concentration have been studied, as well as the ammonia excretion, and in some cases the excretion of chlorides. The reaction of the urine is expressed throughout in the usual Sørensen notation. For the estimation of the titratable alkalinity, $P_H = 7.45$ has been used as the titration end point.

I. METHODS.

For the preservation of the samples of urine, chloroform and toluene were used. No precautions were taken to avoid loss of CO_2 during the collection of the samples.

1. *The Collection of Samples of Urine.* A crate of design and dimensions shown in Fig. 1, made of wood, was used for the goat in all experiments. It has a three-inch zinc lined bottom from which the urine runs out and is collected in a suitable vessel.

¹ Published by permission of the Controller, Chemical Warfare Department. This work was done two years ago. It was hoped that an opportunity might arise to clear up some of the points, but as none is likely to appear at present, I have published these notes in the hope that they may be useful.

GOAT CRATE

Length [without extension for trough]	.....37 $\frac{1}{2}$ "
Breadth.....	13 $\frac{1}{2}$ "
Depth [without trestles].....	26 $\frac{1}{2}$ "
Feeding tray.....	13 $\frac{1}{2}$ x 17"
Battens	3"

Fig. 1.

In order to avoid contamination with faeces billy goats were used. A bag was devised by Lc.-Cpl. Morgan, which is attached to the goat and into which it passes its faeces. This is shown in Fig. 2.

Fig. 2.

A is chest-band made of two-inch webbing, 3 feet in length. *d* in centre buckles to strap *d'* on bag in Fig. 2 *C*.

B is girth-band made of elastic 1 inch in breadth by 2 feet 6 inches in length, *a*, *b*, *c*, in centre fixed on slant, buckled to tapes *a'*, *b'*, *c'* in Fig. 2 *C*.

C is faeces bag of waterproof jaconet; sizes vary with animals. Medium size, flat, length 12 inches, width 8 inches. For a 56 lb. animal width 4 inches is sufficient. A band of elastic (*e*) 12 inches long is fixed round the opening. On this are fixed tapes *a'*, *b'*, *c'*, *d'*, made of elastic to prevent chafing. *a'* and *c'* are 5½ inches, *b'* is 3 inches and *d'* 18 inches in length. *a'*, *b'*, *c'* buckle to *a*, *b*, *c*, in Fig. 2 *B*

To fix.

1. Put on chest-band *A*. If it tends to slip put it between the forelegs.
2. Connect *C* to *B* by the small tape *b'* and buckle *b*. Buckle round the girth-band, and buckle *d'* to *d*; then adjust the other small straps *a'* and *c'*. The back strap *d'* (Fig. 2 *C*) is fastened to girth-band by a large safety pin. The bag is removed easily by undoing the safety pin, and slipping the whole over the beast's legs.

2. *Determination of Hydrogen Ion Concentration.* This has been studied colorimetrically, using a comparometer and indicators similar to those developed by the American workers [see Clark and Lubs, 1917] on the subject. Pheno and cresol red were the indicators used practically throughout. A three-tube comparometer was used similar to that of S. W. Cole.

Standard phosphate, borate and potassium chloride mixtures were used for the comparison.

3. *Titrateable Alkalinity.* 10 cc. samples of urine are titrated with acid ($N/10$ HCl) or alkali ($N/10$ NaOH) according as the urine is more acid or more alkaline than $P_H = 7.45$. The estimations are made in a comparometer. The control urine tubes receive similar dilutions to the sample that is being studied.

The titration end point has been taken as $P_H = 7.45$ which is approximately the reaction of normal blood.

Determinations of titrateable alkalinity using phenolphthalein, instead of phenol red or cresol red and boiling off the CO_2 formed, were done on some goats. The results in the main run hand in hand with those obtained by using the $P_H = 7.45$ standard, as shown in the following table.

Table I.

Determinations of titrateable alkalinity using (1) $P_H = 7.45$ as an end point, and (2) $P_H = 9.0$ (phenolphthalein) as an end point.

+ = cc. $N/10$ alkali (NaOH).

- = cc. $N/10$ acid (HCl).

	$P_H = 7.45$	$P_H = 9.0$ (phenolphthalein)
Goat		
A.	+ 43.5	+ 101.5
24 hours sample	+ 85.1	+ 639.4
	+ 43.5	+ 375
	+ 25.5	+ 196.4
	- 21.8	- 29
	- 227.5	- 214.5
B.	+ 27.0	+ 66.6
24 hours sample	+ 49.5	+ 253.0
	+ 31.8	+ 206.5
	+ 33.7	+ 266.8
C.	- 12.3	- 24.5
	- 5.7	- 37.1
	+ 3.6	+ 244.0
	+ 15.5	+ 49.6
	- 4.6	- 41.4

The results are parallel on the whole, though there are larger increments in the titration value with phenolphthalein. The comparison was made by Lt. T. W. Wilson, U.S.A.

4. *Estimation of ammonia. (amino-acid).* Samples have been tested by Sørensen's formaldehyde method with the following modification by Lieut. Wilson for the analysis of alkaline urines. The alkaline urine is acidified to $P_H = 7.0$, raised to the boiling point and cooled quickly. After this the estimation is proceeded with in the standard way.

5. *Estimation of chlorides.* The chlorides were estimated by Volhard's method.

II. CALCULATION OF RESULTS AND EXPLANATION OF DIAGRAMS.

As a general rule 24 hourly specimens have been collected. The results have been expressed as cc. of $N/10$ alkali per 24 hours, and grams NH_3 per 24 hours.

In a few instances the urine was collected in periods shorter than 24 hours. As the rate of excretion is of most interest here, the results are calculated in times of 24 hour periods, *i.e.* the average hourly rate of excretion during a period over which the specimen is collected is multiplied by 24.

Suppose a 15 hour sample of urine measured 170 cc., the result for the cc. of alkali per 24 hours has been calculated on a volume of 272 cc.

$$(\text{= } 170 \times \frac{24}{15} \text{ cc.}).$$

In this way the comparison of the rate of excretion in irregular periods is possible.

The results have been plotted on diagrams. In the diagrams the results given for a definite day say 6. viii. 18 represent the amounts excreted, the P_H for the same urine etc., for the 24 hours including the night 6. viii. 18 to 7. viii. 18.

III. THE NORMAL URINE AND ITS VARIATION WITH DIFFERENT DIETS.

As in other animals the acidity and the ammonia excretion in the urine of the goat vary considerably with the diet.

Most of the goats used in these experiments, when taken from the goat pen, excreted an acid urine. They were placed on the experimental clover hay diet. The equilibrium reached by the urine tended to run roughly parallel to the H-ion concentration of an infusion of the hay or grass sample. The infusion was made by heating the hay gently in water and allowing it to stand for two hours.

Good clover hay was alkaline, about $P_H = 8.5$. A goat's urine on this diet has also a P_H of about 8.5.

Poorer samples of meadow hay and grass, the latter especially, had a more acid infusion value. Meadow hay infusion had a P_H of 8.0, and fresh grass of between 6.0 and 7.0. The goat's urine on this latter diet always showed a more acid value than on the former. On fresh grass in fact, the urine tended to be actually acid. The time taken to reach this equilibrium was between two and three days. A delay of two or three days would be expected from the fact that fresh food is not received into an empty stomach in the goat, but into a stomach that contains normally a large quantity of food in process of digestion.

The results given in Tables II–IV are typical of those obtained when the normal goat is changed from a diet of grass to one of hay.

Generally speaking, a goat which has been on a grass diet in a pen must have a urine of low hydrogen ion concentration, for at the time of removal from the pen the P_H usually lies between 5.0 and 6.0. In two to three days

on a good clover hay diet the P_H rises and tends to become constant at a value of 8.5. While this P_H remains constant, the titratable alkalinity shows large daily variations.

The excretion of ammonia (and amino-acid) varies from 0.05–0.3 gram ammonia per 24 hours. Amounts larger than 0.6 g. per 24 hours have not so far been found. As would be expected the chlorides show large variations.

Table II. (Goat No. 2403, weight 38 lb.)

Date	Hours	Diet, hay lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	cc. acid 24 hrs
16. iii. 18	22	1½	—	375	1040	8.4	53
17. iii. 18	24	½	—	265	1040	8.1	26.5
18. iii. 18	24	1½	—	195	1035	8.5	41
19. iii. 18	24	1½	—	310	1042	8.5	106
20. iii. 18	24	1½	—	185	1042	8.5	44
21. iii. 18	24	1½	—	260	1030	8.5	68
22. iii. 18	24	1½	500	215	1040	8.5	99
23. iii. 18	24	1½	500	200	1032	8.5	36
24. iii. 18	24	1½	250	200	1033	8.4	38

No ammonia or chloride estimations.

Table III. (Goat No. 3144, weight 41 lb.)

Date	Hours	Diet, hay lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	cc. acid 24 hrs	NH ₃ g. per 24 hrs	NaCl g.	Faeces
16. v. 18	24	1	nil	315	1033	8.5	59.8	0.147	—	F
17. v. 18	24	1½	1000	345	1034	8.5	63.8	0.111	—	F
18. v. 18	24	1½	900	475	1031	8.5	73.6	0.464	—	F
19. v. 18	24	1	500	485	1032	8.5	48.5	0.181	—	F
20. v. 18	24	1½	1500	455	1033	8.5	50.0	0.092	2.13	F
21. v. 18	24	1½	1050	570	1031	8.5	51.3	0.198	2.66	F

Table IV. (Goat No. 3520, weight 52 lb.)

Date	Hours	Diet, hay lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	cc. acid 24 hrs	NH ₃ g. per 24 hrs	NaCl g.
6. vi. 18	24	1½	2000	520	1022	8.7	72.8	0.0265	1.946
7. vi. 18	24	1½	500	395	1036	8.7	98.7	0.026	2.22
8. vi. 18	24	1	1000	230	1040	8.7	37.9	0.027	1.05
9. vi. 18	24	1	500	190	1041	8.7	38.0	0.029	0.111
Goat taken out of the crate with diarrhoea.									
18. vi. 18	24	1	500	205	1042	8.1	8.2	0.070	1.99
19. vi. 18	24	1	1000	235	1043	8.7	44.6	0.05	1.69
20. vi. 18	24	1	1000	230	1040	7.45	nil	0.18	1.99
21. vi. 18	24	1½	1000	300	1037	8.1	15.0	0.107	1.20
22. vi. 18	24	1½	1000	375	1032	8.5	77.9	0.26	1.50
23. vi. 18	24	1½	1000	325	1040	8.5	52	0.064	2.54
24. vi. 18	24	1½	1000	300	1042	8.5	66	0.051	3.28

The effect on the urine of a change from rich to poor hay is shown in the case of goat No. 3128 (Table V). From 11. v. 18–17. v. 18 it was fed on a good clover hay diet. The P_H value started low, when it came out of the grass pen, and then rose. It was taken out of the crate from 17–28th. Beginning on 28th it was fed for three days on a poor sample of meadow hay (infusion value $P_H = 6.2$), on 31. v. 18 the normal clover hay diet was given to it again, when it reached a proper equilibrium $P_H = 8.5$.

Table V. (Goat No. 3128, weight 66 lb.)

Date	Hours	Diet lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	cc. acid 24 hrs	NH_3 g. per 24 hrs	NaCl g.	
11. v. 18	24	1½	nil	210	1027	7.2	-4.2	0.117	—	
12. v. 18	24	1	500	160	1030	6.4	-8.0	0.129	—	
13. v. 18	24	1½	1350	195	1030	8.1	17.5	0.061	—	
14. v. 18	24	1½	nil	385	1042	8.5	113.5	0.065	—	Clover hay diet. Infusion value $P_H = 8.5$
15. v. 18	24	1½	875	175	1035	8.1	20.1	0.035	—	
16. v. 18	24	1½	1000	330	1041	8.1	21.4	0.072	—	
17. v. 18	24	1½	1500	280	1040	8.3	49	0.053	—	
28. v. 18	24	2	1000	275	1034	5.0	-50.9	0.225	3.410	Poor meadow hay diet. Infusion value $P_H = 6.2$
29. v. 18	24	2½	445	445	1017	5.2	-46.7	0.287	3.09	
30. v. 18	24	1	750	—	—	6.0	(not free from faeces)			
31. v. 18	24	2½	1000	480	1035	8.3	38.4	0.497	6.25	Clover hay diet. Infusion value $P_H = 8.5$
1. vi. 18	24	1	1000	950	1023	8.5	195	0.274	3.44	
2. vi. 18	24	1½	500	655	1028	8.5	81.9	0.155	2.60	
3. vi. 18	24	1½	1000	500	1037	8.5	107.5	0.042	1.81	

Fig. 3. Showing titratable alkalinity, P_H and ammonia content of urine of Goat No. 3520, normal, fed on clover hay diet.

The case is not quite so simple as it was thought at first, as can be seen from goat No. 4360 (Table VI). This goat was fed throughout on a diet of freshly-cut grass giving an infusion value of $P_H = 5.5-5.7$. For the first three days the P_H dropped, and then rose again, showing some form of compensation. Owing to the urgency of other work further experiments could not be done to settle this point.

Table VI. (Goat No. 4360, weight 58 lb.)

Date	Hours	Diet	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	cc. acid 24 hrs	Ammonia g. per 24 hrs	
5. viii. 18	24	not weighed	nil	875	1016	8.1	+175.5	—	Grass diet. Infusion value $P_H = 5.5-5.7$
6. viii. 18	24	—	500	750	1024	6.7	- 11.2	—	
7. viii. 18	24	—	500	920	1022	6.3	- 18.4	—	
8. viii. 18	24	—	500	575	1036	7.3	- 2.8	—	
9. viii. 18	24	—	nil	690	1036	8.1	+ 27.6	0.22	
10. viii. 18	No specimen								
11. viii. 18	24	—	500	885	1028	8.1	44.2	0.27	
12. viii. 18	24	—	300	650	1036	8.1	26	0.14	
13. viii. 18	24	—	200	565	1044	8.1	22.6	0.11	

Table VII. (Goat No. 2697, weight 39 lb.)

(The urine was free from protein and sugar.)

Date	Vol. of urine cc.	Sp. Gr.	P_H	cc. N/10 acid	Protein	Sugar	Auton
9. iv. 18	245	1028	6.3	+12.3	—	—	—
10. iv. 18	285	1030	7.3	+ 5.7	—	—	—
11. iv. 18*	400	1026	8.4	-36.0	—	—	—
12. iv. 18	310	1026	8.1	-15.5	—	—	—
13. iv. 18	230	1030	7.1	+ 4.6	—	—	—

* Starved 24 hours.

Table VIII. (Goat No. 2720, weight 57 lb.)

Date	Hours	Diet, hay lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P_H	To P_H 7.45		
							cc. acid 24 hrs	NH ₃ g. per 24 hrs	Faeces
9. iv. 18	24	1	—	240	1040	8.4	25.2	—	F
10. iv. 18	24	1½	500	260	1050	8.5	65	0.022	F
11. iv. 18	24	1½	500	190	1060	8.1	12.3	0.0014	F
12. iv. 18*	24	nil	500	320	1025	8.3	17	0.032	F
13. iv. 18	24	2	350	270	1034	6.7	-7.81	0.1	F
14. iv. 18	24	1½	500	300	1048	8.5	75.5	0.038	C
15. iv. 18	24	1½	500	210	1050	8.3	21	0.032	F
16. iv. 18	24	1½	1250	1975	1048	8.1	13.8	0.028	F
17. iv. 18	24	2½	1500	150	1048	7.5	7.5	0.04	F

16th and 17th, traces of albumin.

* Starved 24 hours. F=Urine free of faeces. C=Urine contaminated with faeces.

The data for the urine of goat No. 2720 are shown graphically in Fig. 4.

Effect of Starvation.

Tables VII, VIII and IX show the effect of starvation on the urine of the normal goat. When this period of starvation was 24 hours, the maximum acidity was reached between the 24th and 48th hour after the commencement of starvation; when the period of starvation was 48 hours, the maximal acidity occurred between the 48th and 72nd hour after the last food was taken. In the four cases observed the period of maximal acidity was not during the period of starvation, but after it had ceased in each case.

Table IX. (Goat No. 2879, weight 52 lb.)

Date	Hours	Diet, hay lb.	Water cc.	Vol. of urine cc.	Sp. Gr.	P _H	To P _H 7.45		NH ₃ g. per 24 hrs	Faeces	Sugar	Albu- min
							cc. acid 24 hrs	cc. acid 24 hrs				
25. iv. 18	24	$\frac{3}{4}$	—	300	1036	6.7	-13.5	.232	—	—	—	—
26. iv. 18	24	$1\frac{1}{4}$	800	210	1045	6.9	-6.3	.132	F	—	—	—
27. iv. 18	24	1	500	195	1048	8.3	+11.7	.071	F	slight trace	—	—
28. iv. 18	24	1	800	155	1048	8.5	+41.07	.022	F	—	—	—
29. iv. 18	24	2	1250	225	1043	8.3	+16.87	.049	F	—	—	—
30. iv. 18	24	$1\frac{1}{2}$	1250	255	1047	7.8	3.82	.142	F	—	—	—
1. v. 18	24	$1\frac{1}{4}$	1000	225	1039	8.3	14.62	.070	F	slight trace	—	—
2. v. 18	24	nil	1250	530	1017	8.3	18.55	.072	—	—	—	—
3. v. 18	24	nil	125	517	1015	7.6	2.58	.105	F	—	—	—
4. v. 18	24	$1\frac{1}{4}$	1000	255	1020	6.9	-6.37	.166	F	slight trace	—	—
5. v. 18	24	$1\frac{1}{4}$	1250	230	1040	8.3	+13.8	.168	F	—	—	—
6. v. 18	24	1	1100	305	1040	8.3	+3.81	.067	F	—	—	—

The Effect of feeding with alkali.

Table X shows the effect of feeding two goats on alkali. In each case 10 g. of sodium bicarbonate was given in a single dose. As in other cases the animal had been kept in the house on a hay diet sufficiently long for the titratable alkalinity of the urine to be constant for a couple of days before the dose. In each case the urine showed a considerable rise in alkalinity on the day of the meal, which had entirely passed off on the day subsequent to it.

Table X. (Goats Nos. 2441, 2453.)

Alkali. Goat No.	Days	Titratable alk.	Remarks
2441	1st	62	10 g. NaHCO ₃ fed
	2nd	110	
	3rd	97	
	4th	169	
	5th	107	
	6th	27	
2453	1st	5	10 g. NaHCO ₃ fed
	2nd	69	
	3rd	75	
	4th	154	
	5th	27	
	6th	49	

The course of this experiment on goat No. 2453 is shown in Fig. 5.

Fig. 4. Showing the effect of starvation on the titratable alkalinity, the hydrogen ion concentration and the ammonia content of the urine of a normal goat (No. 2720, see text).

Fig. 5. Effect of feeding with $NaHCO_3$ on titratable alkalinity of goat urine.

IV. EFFECT OF FEEDING WITH ACID.

Table XI shows the effect of the administration of a dose of hydrochloric acid to a goat. The urine became acid to litmus in the first 24 hour period, the maximum acidity was reached in the period 24-48 hours after administration of the acid. As the urine became acid the excretion of ammonia rose, reaching a maximum in the same period as the maximal acidity and fell as the alkalinity became re-established.

The data which were obtained for the urine of goat No. 2904 are represented in Fig. 6.

Fig. 6. Effect of administration of HCl on the titratable alkalinity, hydrogen ion concentration and ammonia content of goat's urine.

Table XI. (Goat No. 2904.)

Date	Hours	Diet, hay	Water	Vol. of urine	Sp. Gr.	P _H	cc. N/10 acid per 24 hrs	NH ₃ g. per 24 hrs	Faeces
		lb.	cc.	cc.					
26. iv. 18	24	1½	500	210	1036	6.7	-15.7	0.287	F
27. iv. 18	24	1	250	235	1042	8.1	+11.7	0.125	F
28. iv. 18	24	1	520	220	1038	8.1	14.3	0.057	F
29. iv. 18	24	1½	1000	285	1035	8.1	9.97	0.046	F
30. iv. 18*	24	1	1250	340	1033	6.5	-11.9	0.407	F
1. v. 18	24	1½	1000	375	1033	6.0	-24.3	0.784	F
2. v. 18	24	1½	1500	370	1035	6.7	-16.6	0.600	F
3. v. 18	24	1½	500	305	1033	7.8	+6.1	0.194	F
4. v. 18	24	1	1000	330	1040	8.1	24.7	0.072	F
5. v. 18	24	1	1250	335	1037	8.5	68.7	0.039	F

* At 11 a.m. on 30th 30 cc. of strong HCl in 200 cc. water were given by stomach tube.

DISCUSSION.

The use of ammonia by the goat for neutralisation of acid ingested is another case in which the original distinction between the herbivorous and carnivorous animal has broken down. It is in agreement with the work of Steenbock, Nelson and Hart [1914] upon the production of ammonia for neutralisation purposes by the calf. Fitz, Alsberg and Henderson [1907] showed that rabbits after the ingestion of acid excreted an increased amount of phosphates in the urine. Their paper gives no recorded analyses of the ammonia output, and as Winterberg has shown that rabbits fed on oats utilise ammonia for neutralisation, the rabbit would not seem to be really exceptional.

SUMMARY OF RESULTS.

1. A method is described of obtaining urine specimens uncontaminated by faeces from the billy goat for the study of its metabolism.

2. The results which have been obtained show that the goat's urine responds to alterations in the acidity of the diet. With the addition of acids to the diet, the P_H of the urine gets less, and the titratable alkalinity falls. As the urine passes the neutral point and becomes actually acid there is a tendency for ammonia to be excreted.

3. The goat though herbivorous uses ammonia for the neutralisation of ingested acid, and in this respect confirms the conclusions of others that there is no real distinction between the herbivorous and carnivorous animal in this respect.

4. The goat readily excretes alkali when this is administered by the mouth.

5. The goat's urine reacts to a period of starvation by becoming more acid. In one or two days' starvation the urine reaches its most acid value after the starvation is stopped.

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