

Ammonia in the large intestine of herbivores

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1. The object was to investigate the importance of urea as a source of ammonia in the large intestine of herbivores. Urea was present in small intestinal contents of slaughtered horses in concentrations similar to those in blood but, in the small intestine of slaughtered sheep, the urea was less than in blood.
2. There was little ammonia in small intestinal contents of slaughtered horses but considerable ammonia was present in small intestinal contents of slaughtered sheep. The ammonia in small intestinal contents of the slaughtered sheep was probably formed from urea, as ileal contents taken from a sheep with an ileal cannula contained considerable urea and little ammonia.
3. The ammonia concentration in caecal contents of sheep was related to the concentration of urea in blood except when ileal contents were prevented from entering the caecum.
4. Ileal digesta of sheep contained more free amino nitrogen than did caecal digesta.
5. Ammonia was absorbed more rapidly than water from the caecum of sheep. The rate of absorption was related to the concentration of ammonia in the caecum.

Ammonia is an important metabolite in the rumen, where it is formed mainly from the deamination of amino acids released by proteolysis of ingested protein and by the hydrolysis of blood urea which enters the rumen in saliva or by passage across the rumen wall (Kay & Hobson, 1963). Rumen ammonia which is not utilized by the rumen microflora for synthesis of microbial protein is absorbed and transported to the liver for conversion into urea. This urea may be excreted in the urine or recycled to the rumen (McDonald, 1948).

The presence of ammonia in the contents of the large intestine has been reported (McDonald, 1948) but little is known about its origin or fate. It has been shown that some ammonia is produced in caecal contents by deamination of amino acids (Hecker, 1971). This paper presents experimental results which indicate that blood urea is also a source of ammonia in the large intestine and that ammonia is absorbed rapidly from the caecum.

EXPERIMENTAL

Animals and feeding

Eleven Clun Forest ewes or wethers of at least 1 year of age were used. One (L49) had a caecal and a re-entrant ileal cannula approximately 50 cm from the ileocaecal junction while two (K19 and K97) each had rumen and caecal cannulas. The other eight were used in acute experiments. They were kept in pens and allowed free access to water, salt licks and meadow hay in hay nets. Sufficient hay was placed in the nets to ensure that there was always some remaining. Each morning, this was removed and

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replaced by fresh hay. During one period of 10–12 d, hay was replaced for sheep K 19 and L 49 by barley straw (0.25% nitrogen). During a further period of 16 d, a concentrate supplement consisting mainly of crushed oats, barley and maize meal (2.8% N) was given to sheep K 19. This diet together with 20 g of urea was given to sheep K 19 for a further 10 d.

The five horses were slaughtered at a local slaughter-house. Their previous management was unknown.

Sampling

Caecal contents were obtained from the sheep by suction into tubes of internal diameter 6–10 mm. Caecal liquor was obtained from caecal contents by squeezing through one layer of surgical gauze. Blood was drawn into heparinized syringes from jugular veins. Ileal digesta were collected by disconnecting the cannula and allowing the digesta to flow into a container. When the ileal cannula was disconnected for periods of several hours, the proximal end was plugged except for a period of about 3 min each hour when digesta were allowed to flow into a container.

Samples of digesta were taken from the following parts of the digestive tract of the horses: the stomach; the proximal, the middle and the distal thirds of the small intestine; the caecum; the right and the left ventral and dorsal colon; the small colon and the rectum. These samples were frozen soon after collection by placing them in contact with solid carbon dioxide. When thawed, they were strained or squeezed through one layer of surgical gauze and the liquor was kept for analysis. Blood from the horses was collected from severed jugular veins.

Samples were taken from the following sites in the digestive tract of eight sheep killed by an overdose of pentobarbitone sodium: the rumen; the abomasum; four parts of the small intestine; the caecum; the colon between the ileocaecocolic junction and the beginning of the spiral colon (colon 1); the centripetal colon (colon 2); the centrifugal colon (colon 3) and the terminal colon and rectum (rectum). Blood was collected also. These samples were treated in the same way as samples from the horses except that digesta from the lower large intestine were macerated with three parts by weight of water before liquor was obtained.

Analytical methods

Dry-matter content of caecal digesta and liquor was measured by heating at 105° in tared vessels until the weight was constant (normally 24 h).

The nitrous oxide method of Peters & van Slyke (1932) was used to measure free α -amino-N after removal of protein, cellular material and ammonia (Hecker, 1971).

Ammonia and urea were measured by micro-diffusion analysis (Conway, 1957). Samples were not deproteinized or diluted before analysis. The analysis was done usually on 0.25 or 0.5 ml as measured with a 1 ml tuberculin syringe. Ammonia was liberated by addition of 1 ml saturated potassium carbonate to the outer chamber. In other dishes, 0.5 ml of urease solution made from Urease Tablets (British Drug Houses Ltd, Poole, Dorset; one tablet per 10 ml of 30% (w/v) ethanol in water) was added; the reaction was allowed to proceed for 20 min; potassium carbonate was then added as before. The ammonia liberated was measured by titration with 0.05 N-HCl

after diffusion for 4 h. Ammonia and urea concentrations were calculated from results obtained from duplicate dishes with and without urease.

Urease activity was measured by incubating liquor from gastro-intestinal contents with 1 vol of 6% (w/v) urea for 10 min under liquid paraffin at room temperature. The ammonia concentrations at the end of this period were corrected for the initial ammonia concentration and for changes in controls diluted with 1 vol of water.

RESULTS

Ammonia and urea in digesta from slaughtered horses and sheep

Concentrations of ammonia and urea and urease activity in samples taken from the gastro-intestinal tracts of slaughtered horses are given in Table 1. There was variation in the amount of ammonia in the stomach, with two horses having considerable amounts (16.4 and 29.0 mg N/100 g) and the other three having comparatively little (less than

Table 1. *Ammonia, urea and urease activity in digesta and plasma from five horses and eight sheep*

(Mean values with their standard errors)

Site	Horses			Sheep	
	Ammonia-N (mg/100 g)	Urea-N (mg/100 g)	Urease activity (mg NH ₃ -N released in 10 min per kg)	Ammonia-N (mg/100 g)	Urea-N (mg/100 g)
Rumen	—	—	—	11.0 ± 3.3	0
Stomach or abomasum	11.3 ± 7.5	4.2 ± 1.1	0	8.6 ± 1.9	0
Small intestine 1	3.8 ± 1.3	11.7 ± 1.1	0	13.7 ± 2.8	2.3 ± 0.7
Small intestine 2	0.9 ± 0.4	12.8 ± 1.4	0	9.5 ± 2.5	4.3 ± 0.8
Small intestine 3	1.3 ± 0.4	13.2 ± 0.9	0	8.1 ± 2.2	3.6 ± 0.9
Small intestine 4	—	—	—	7.0 ± 2.0	2.7 ± 0.9
Caecum	4.8 ± 1.1	0	5.8 ± 2.2	14.6 ± 2.5	0
Right ventral colon or colon 1	4.0 ± 1.4	0	5.7 ± 1.5	11.9 ± 1.3	0
Left ventral colon or colon 2	4.2 ± 1.6	0	5.0 ± 1.3	9.7 ± 1.8	0
Left dorsal colon or colon 3	11.1 ± 2.6	0	4.2 ± 0.5	7.2 ± 1.7	0
Right dorsal colon	14.6 ± 3.7	0	2.6 ± 0.4	—	—
Small colon	10.0 ± 2.6	0	2.9 ± 0.4	—	—
Rectum	14.0 ± 3.3	0	5.2 ± 0.9	7.8 ± 2.3	0
Plasma	0	13.8 ± 0.04	0	0	11.7 ± 1.8

5 mg N/100 g). There was much less ammonia in the first part of the small intestine and the other parts of the small intestine contained little ammonia. More ammonia was present in the large intestine, the greatest concentrations being towards the rectum. Urea was found in small amounts in the stomach and none was found in the large intestine. It was present in appreciable amounts only in the small intestine and here the mean concentration was approximately equal to that of plasma urea. Urease activity was present in all parts of the large intestine but absent from the small intestine and stomach.

Concentrations of ammonia and urea in samples from slaughtered sheep also are given in Table 1. There was no urea in samples of rumen, abomasal or large intestinal

contents. There was more ammonia than urea in small intestinal contents, and the urea concentration in these contents was less than the plasma urea concentration. The ammonia concentration in the large intestine decreased between the caecum and the rectum.

Relation between blood urea and caecal ammonia in sheep

Effect of intravenous injections of urea. In control experiments, samples taken over periods of 6–8 h indicated that concentrations of blood urea and of caecal ammonia were relatively constant, caecal ammonia-N being slightly greater than blood urea-N in most samples. The results of a typical experiment are shown in Fig. 1*a*. Feeding sheep with 450 g of a concentrate mixture consisting mainly of crushed oats, barley and maize meal caused no detectable change in concentrations, but feeding with the same weight of concentrates plus 20 g urea resulted in small increases in the succeeding 6 h.

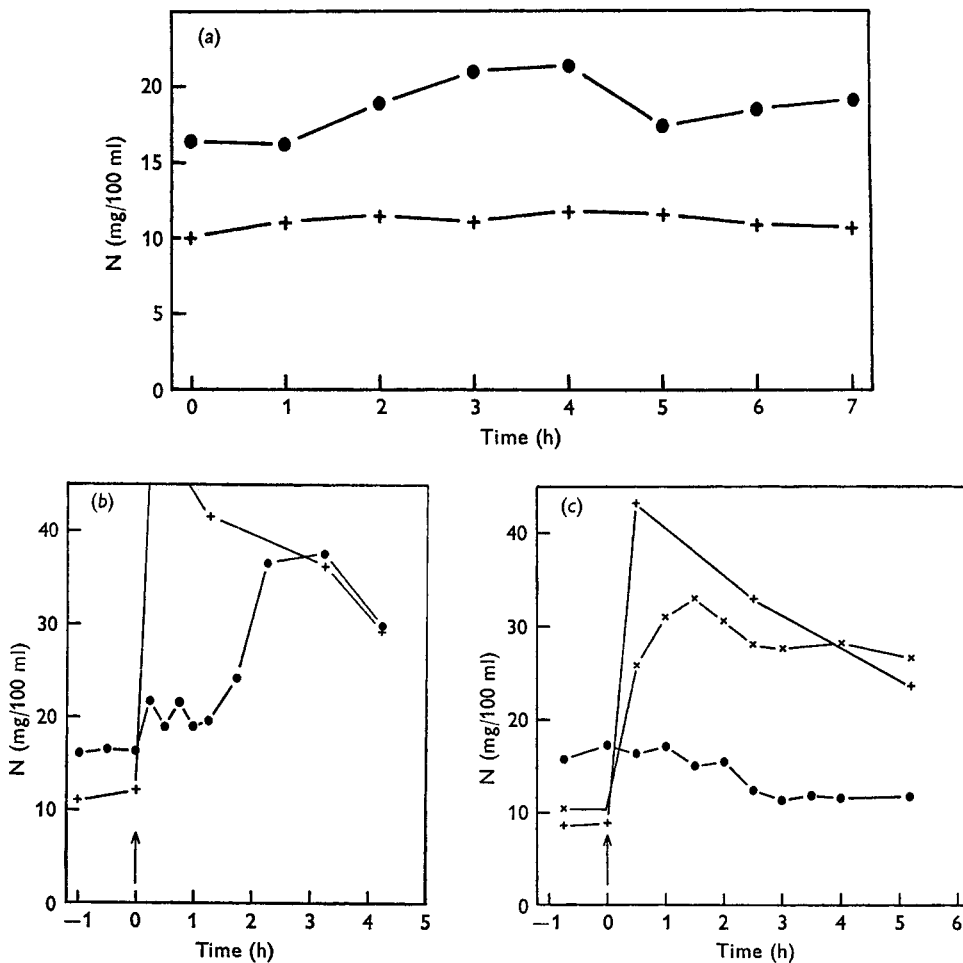


Fig. 1. Relation between concentrations of blood urea and caecal ammonia in sheep L49, (a) under control conditions, (b) after an intravenous injection of urea at 0 h and (c) after disconnecting an ileal re-entrant cannula and then giving an intravenous injection of urea at 0 h. +, blood urea; ●, caecal ammonia; ×, ileal ammonia + urea.

In three experiments blood urea was increased by intravenous injections of between 16 and 24 g urea as a 10% (w/v) solution in 0.5% (w/v) NaCl. In each experiment there was an increase in caecal ammonia-N to a concentration similar to the increased blood urea-N concentration. This increase did not occur immediately, but after intervals of 0.75, 1.75 and 3 h (Fig. 1*b*).

To determine if this increase was due to filling of the caecum with ileal digesta containing increased concentrations of urea or ammonia, the experiment was repeated twice with sheep L49. Before injecting the urea, the ileal re-entrant cannula was disconnected so that none of the digesta in the small intestine could enter the caecum. Samples of ileal digesta were taken for estimation of ammonia and urea concentrations. In response to the urea injection the sum of ileal ammonia-N plus urea-N increased to concentrations similar to those of blood urea-N (Fig. 1*c*). In contrast to the previous experiment no increase in caecal ammonia was seen in the 5 h following the injection.

Table 2. *Urea concentrations in blood of sheep given different diets*

Sheep	Roughage diet (offered <i>ad lib.</i>)	Supplement		No. of samples	Blood urea-N (mg/100 ml)
		Concentrate (g)	Urea (g)		
K19	Barley straw	—	—	10	8.2 ± 0.6
K19	Meadow hay	—	—	15	11.8 ± 0.9
K19	Meadow hay	450	—	11	12.9 ± 1.0
K19	Meadow hay	450	20	7	15.9 ± 0.8
L49	Meadow hay	—	—	10	12.2 ± 0.9
L49	Barley straw	—	—	9	4.8 ± 0.8

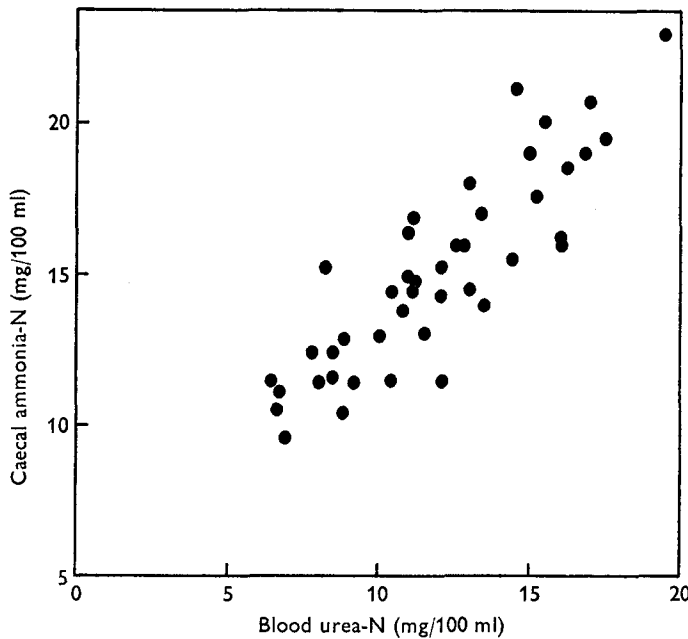


Fig. 2. Relation between concentrations of blood urea and caecal ammonia in sheep K19.

Instead the concentration of ammonia in the caecum decreased, with the regression of caecal ammonia concentration on time being significant on both occasions ($t = 2.24$ and 6.0 ; $P < 0.01$).

Effect of diet. The blood urea concentration of sheep K 19 and L 49 was varied by giving them diets containing different quantities of N. Samples of blood and caecal

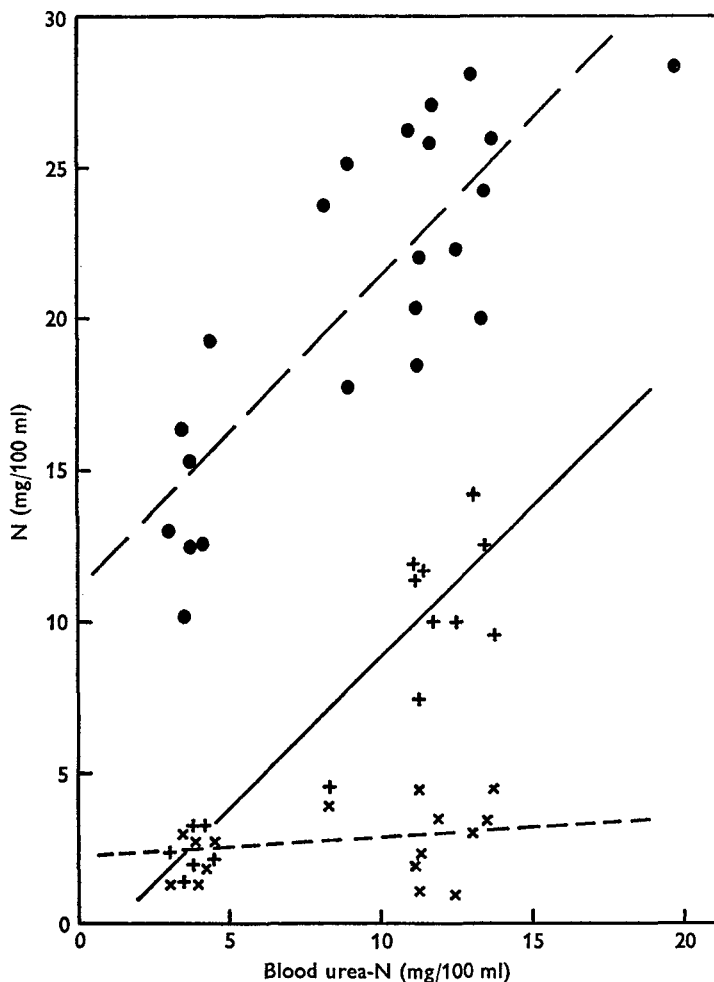


Fig. 3. Relation between concentrations of ileal ammonia (\times), ileal urea ($+$), caecal ammonia (\bullet) and blood urea in sheep L49.

liquor from both sheep and ileal liquor from sheep L49 were taken in the morning before any supplements were given and the concentrations of urea in blood and ileal liquor and of ammonia in caecal and ileal liquors were measured. Details of the diets and the blood urea concentrations are shown in Table 2.

There was a significant correlation ($r = 0.87$) between caecal ammonia and blood urea in sheep K 19 (Fig. 2), with the mean caecal ammonia-N (15.0 mg/100 ml) being greater than the mean blood urea-N (11.9 mg/100 ml). Significant correlations were

obtained in sheep L49 between caecal ammonia and blood urea ($r = 0.82$), between ileal urea and blood urea ($r = 0.93$), but not between ileal ammonia and blood urea ($r = 0.31$) (Fig. 3). In this sheep the mean caecal ammonia-N (20.0 mg/100 ml) was much greater than the mean blood urea-N (8.7 mg/100 ml). There were also highly significant correlations between blood urea and the sum of ileal ammonia-N and urea-N ($r = 0.96$) and between caecal ammonia and the sum of ileal ammonia-N and urea-N ($r = 0.79$) in this sheep.

α -Amino-N in sheep digesta

The mean concentration of free α -amino-N in five samples of caecal liquor (2.00 ± 0.17 (SE) mg/100 ml) was greater than that in five samples of rumen liquor (0.71 ± 0.27 mg/100 ml) but less than that in five samples of ileal liquor (7.2 ± 2.6 mg/100 ml). The differences between the means were significant ($P < 0.05$).

Absorption of ammonia from the caecum

Although in previous experiments the concentration of ammonia in samples of caecal digesta incubated *in vitro* increased (Hecker, 1971), no increase was seen when ileal digesta of sheep L49 were prevented from entering the caecum. An explanation is

Table 3. *Dry-matter percentages, ammonia concentrations and rates of ammonia production in samples of caecal contents of sheep L49 taken at hourly intervals after an ileal re-entrant cannula had been disconnected*

(Ammonia production was measured by incubating contents at 37°. Ammonia absorption was calculated by adding the ammonia produced in 1 h to the change in concentration of ammonia in the caecal contents *in situ* in that hour)

Time (h)	Dry matter (%)			Initial NH ₃ concentration (mg/100 g liquor)			NH ₃ production <i>in vitro</i> (mg N/100 g liquor)			NH ₃ absorption (mg N/100 g liquor)		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0	12.6	10.6	11.2	29.3	22.1	31.6	7.3	4.2	2.2	7.2	5.7	5.5
1	13.1	10.8	11.2	29.4	20.6	28.3	2.9	2.0	2.1	8.4	5.3	5.8
2	14.5	11.3	12.0	23.9	17.3	24.6	2.9	1.9	3.8	5.0	4.3	3.2
3	14.8	12.3	12.9	21.8	14.9	25.2	4.0	2.7	2.1	6.8	3.3	3.5
4	15.9	12.9	13.3	19.0	14.3	23.8	3.8	2.6	3.0	4.3	2.8	3.4
5	16.3	13.0	14.3	18.5	14.1	23.4	3.8	3.1	2.9	4.0	5.9	3.9
6	16.6	15.4	15.4	18.3	11.3	22.4	5.1	3.4	—	5.4	2.9	—
7	17.1	14.8	—	18.0	11.8	—	—	5.0	—	—	4.3	—
8	—	15.4	—	—	12.5	—	—	—	—	—	—	—

that ammonia was absorbed from caecal digesta *in vivo*. The rate of absorption was estimated by measuring the rate of production and the concentrations of ammonia in samples of caecal digesta taken hourly after the ileal re-entrant cannula of sheep L49

had been disconnected. The results are given in Table 3. The dry-matter percentage of the digesta increased with time, indicating that absorption of water occurred. The concentration of ammonia decreased whereas the rate of production, estimated by incubating samples *in vitro* and measuring the change in concentration, was relatively constant except for the seventh sample in Expt 2. The rate of ammonia absorption, calculated by adding the decrease in ammonia concentration in each hour to the amount of ammonia produced in that hour, decreased with time.

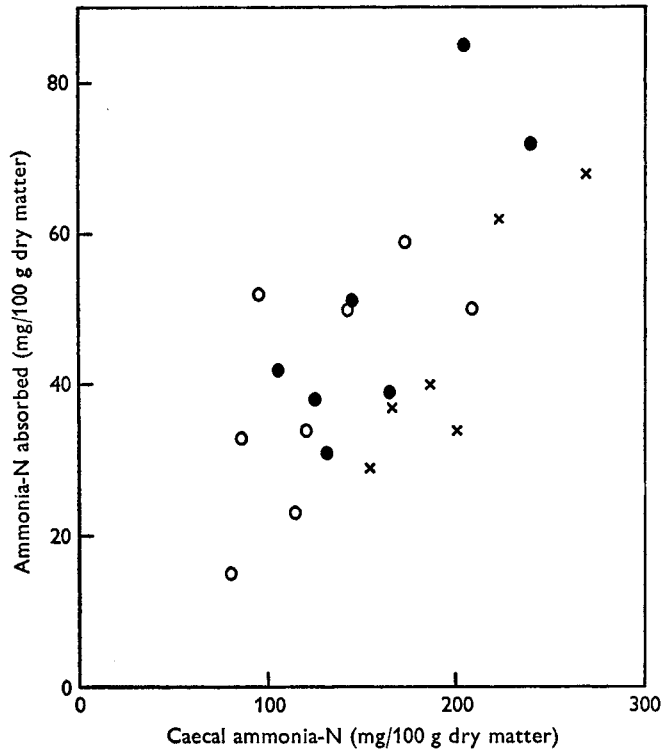


Fig. 4. Relation between ammonia absorbed from caecal contents and caecal ammonia concentration in three experiments (O, ●, x) with sheep L49.

Changes in ammonia concentration were calculated with reference to the amount of dry matter present. This involved an error as some dry matter is digested in the large intestine (Goodall & Kay, 1965; Bruce, Goodall, Kay, Phillipson & Vowles, 1966). However, the error is likely to be small in relation to the changes in water and ammonia that occurred. The amount of ammonia absorbed per 100 g dry matter was related to the mean concentration of ammonia per 100 g dry matter during that hour (Fig. 4). This relation was significant ($r = 0.75$; $P < 0.01$) and the regression of the amount absorbed on the concentration passed almost through the origin indicating that the rate of absorption depended on the concentration.

The total amounts of water and ammonia absorbed during the experiments are given in Table 4. In the three experiments, the mean concentration of ammonia in the

absorbed water was 130 mg/100 g water. As the concentration of ammonia in caecal liquor ranged from 9 to 30 mg/100 g, ammonia was absorbed at a greater rate than water.

Table 4. *Absorption of water and ammonia from caecal contents of sheep L49*

	Expt 1	Expt 2	Expt 3	Mean
Time of duration (h)	7	8	6	7
Water absorbed as g/100 g DM during expt	209	294	244	249
Ammonia-N absorbed as mg/100 g DM during expt	358	316	272	315
Ammonia-N absorbed as mg/100 g water absorbed during expt	171	108	112	130
Mean $\text{NH}_3\text{-N}$ concentration in caecal liquor (mg/100 g)	22.1	15.2	25.2	20.8

DM, dry matter.

DISCUSSION

The results for horses showed a relation between the presence of urease, the presence of ammonia and the absence of urea in gastro-intestinal contents. An exception was the stomach. Urease is likely to be inactive in stomach contents owing to the low pH, but the ammonia present may have resulted from the action of intracellular gastric urease (Kornberg & Davies, 1955). Results for the sheep were similar to those for the horse, the main difference being the presence in the sheep of more ammonia and less urea in the digesta of the small intestine. Other authors have found ammonia in the digesta entering the duodenum and passing from the lower ileum (Clarke, Ellinger & Phillipson, 1966) but it is probable that in that work urea was converted into ammonia before the samples were analysed owing to the prolonged period of collection. When samples were analysed within 5 min of sampling in the present experiments, little ammonia was present and the urea concentration was similar to that of blood urea. The presence of urease activity in sheep ileal digesta, although slight (unpublished observation), would be sufficient to explain the hydrolysis of urea.

The results did not confirm the presence of urea in the large intestine of the horse as reported by Alexander & Davies (1963). The presence of urea would be surprising in view of the fact that urease was found there (Table 1). The method used by Alexander & Davies (1963) to estimate urea is one normally used for blood urea (King & Wootton, 1959). This measures the sum of ammonia and urea and it is possible that the values of Alexander & Davies for urea referred to ammonia in the large intestine.

The results for ileal samples from a live sheep indicated that urea was present in the small intestine at a concentration approaching that of blood urea. When ileal contents entered the caecum, this urea was hydrolysed to ammonia to produce an ammonia-N concentration related to but exceeding the concentration of urea-N in blood. Ammonia would have resulted also from the deamination of amino acids present in ileal contents.

There was comparatively little variation in either blood urea or caecal ammonia concentrations in sheep on the hay diet. This is in contrast to the results of Faichney

(1968), who ascribed marked fluctuations in caecal N fractions, including ammonia, to the filling of the caecum with contents of the small intestine. It is likely that the fluctuations observed by Faichney reflected changes in blood urea concentrations resulting from feeding once daily.

Ammonia is absorbed from rumen contents at neutral pH mainly as the ammonium ion (Hogan, 1961). The mechanism of this absorption is passive diffusion (Hogan, 1961; Gärtner, 1962). The rate of absorption of ammonia from caecal contents varied with the concentration of ammonia and was more rapid than the rate of absorption of water. This indicates that diffusion was the mechanism for ammonia absorption from the large intestine. A similar finding was made by Marty & Raynaud (1964) for the rabbit caecum.

Ammonia absorbed from the large intestine consisted of that produced by the hydrolysis of urea in ileal contents and that produced by the deamination of amino acids. Values from the present experiment and from an earlier one (Hecker, 1971) have been used in the following calculations. If 2 l digesta passed into the large intestine each 12 h (Hogan & Phillipson, 1960) and the blood urea-N concentration was 15 mg/100 ml, approximately 0.6 g urea-N would be 'recycled' to the large intestine each day. Most of this would be absorbed since the sheep of Hogan & Phillipson (1960) passed 290 g faeces/12 h, which might have contained 10 mg ammonia-N/100 g faeces. A further 0.65 g of ammonia-N would be absorbed if there were 700 g digesta in the caecum and proximal colon and 350 g in the remainder of the large intestine (unpublished observation) and if the ammonia production rates in these parts were 4.9 and 1.9 mg N/100 g per 90 min respectively. These amounts of ammonia-N plus a small amount formed by deamination of free amino acids in ileal digesta would suggest that approximately 1.3 g were absorbed daily. This is 14% of the urea entry rate calculated from the formula of Cocimano & Leng (1967) for sheep with a blood urea-N concentration of 15 mg/100 ml. Half of this would depend on the blood urea concentration which would vary with the N intake in food (Lewis, 1957; Preston, Schnakenberg & Pfander, 1965).

It has been assumed by some workers that because the volume of contents in the large intestine is less than that in the rumen and because concentrations of certain metabolites in both organs are similar, the large intestine is of less importance in supplying these metabolites to the body (Hungate, Phillips, McGregor, Hungate & Buechner, 1959). It is not always realized however, that rates of absorption from a hollow viscus depend on the surface area of the wall of the viscus. As the large intestine is a long organ with a narrow diameter, it is likely to have a greater ratio of surface area to volume than the rumen which is a more spherical organ. Thus the rate of absorption per unit volume may be greater for the caecum than for the rumen and this may explain how more than 14% of the urea entry rate could be due to ammonia in blood from the large intestine.

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