THE ABSORPTION OF AMMONIA THROUGH THE RUMEN OF THE SHEEP

By J. P. Hogan*

[*Manuscript received January 9, 1961*]

Summary

In sheep anaesthetized with chloralose, the transport of ammonia across the rumen epithelium increases with the concentration gradient at pH 6·5. The movement of volatile fatty acids across the rumen epithelium at pH 6·5 increases the transport of ammonia. The effects both of ammonia concentration and of the movements of the fatty acids on the transport of ammonia were so reduced at pH 4·5 that they were either absent or within experimental error. The transport of ammonia was not affected within the limits of the measurements either by changes in the concentrations of sodium, potassium, chloride, carbon dioxide, or lactate in the rumen or by the net movement of water into and out of the rumen. The use of the changes in concentration of ammonia in the rumen veins to indicate transport of ammonia from the rumen is discussed.

I. Introduction

The factors that affect the transport of the ions Na^+, K^+, Cl^-, and the steam volatile fatty acids across the rumen epithelium of the sheep have been extensively studied in recent years. In general, it seems that the two main forces controlling the outflux of ions from the rumen to the blood are the concentration gradient and the potential difference which exists between the blood and rumen contents and which is positive for the blood (Phillipson 1955; Dobson and Phillipson 1958); these forces appear to be sufficient to account for the net fluxes of the ions studied except Na^+. Little account has been taken of the transport of ammonia from the rumen, however, despite evidence that the movement of this substance may be both extensive and of considerable importance to the nitrogen status of the animal (McDonald 1948; Chalmers, Cuthbertson, and Synge 1954).

In the range of pH values normally found in the rumen, viz. 4·5–7 (Phillipson 1942; Briggs, Hogan, and Reid 1957), ammonia is present mainly as the NH_4^+ ion. Ammonium ions must leave the rumen against the potential gradient which is positive for the blood (Dobson and Phillipson 1958) but are assisted by the concentration gradient (McDonald 1948; Lewis, Hill, and Annison 1957). The present experiments described here, however, show that other factors also affect the transport of ammonia from the rumen. In this paper, ammonia refers to both dissociated and undissociated forms. In the discussion it is differentiated into free ammonia (NH_3) and ammonium (NH_4^+).

II. Material and Methods

Scottish Blackface and Merino sheep of 28–36 kg body weight, which had previously been cannulated with either ebonite or rubber cannulae in the dorsal rumen sac, were used in these experiments. The surgical techniques for the isolation of

* Division of Animal Physiology, C.S.I.R.O., Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

the rumen by ligating the oesophagus and the reticulo-omasal orifice were identical with those of Masson and Phillipson (1951). The absorption of ammonia and, in some experiments, of other ions were measured by the net changes in the solution in the rumen. The error in the recovery of solutions containing ammonia 2 min after they had been poured into the rumen in six sheep varied between $+0\cdot7$ and $-1\cdot1\%$. In the following experiments, net changes less than 2% of the ammonia added were not regarded as significant.

Samples of blood were drawn by syringe from anaesthetized sheep after exposing the carotid artery and jugular and posterior (right) rumen veins. In several experiments the rate of blood flow was measured in part of the posterior rumen vein by using a simple bubble flow meter (Soskin, Priest, and Schutz 1934). Samples of blood were readily obtained through a side-arm in the apparatus.

Arterial samples were obtained in conscious sheep from exteriorized carotid loops, and venous samples through polyethylene tubing passed into the rumen veins from inside the rumen. Access to these veins was obtained through fistulae 4 in. in diameter which had been prepared in the dorsal rumen sac. These fistulae were fitted with cannulae made from polyvinyl chloride.

In several experiments the rumen was washed out and refilled with $0\cdot9\%$ w/v sodium chloride solution, which was left in the rumen for 60 min. During this time the concentration of ammonia in the right ruminal vein was about $3\cdot5$ μg nitrogen/ml compared with 1 μg nitrogen/ml in the peripheral circulation. Similar results were obtained with conscious sheep. In some experiments the concentration of urea nitrogen in the right ruminal vein was 1–2 mg/100 ml less than in blood from the carotid artery but in other experiments no such difference was observed. At the end of 60 min, less than $0\cdot5$ m-equiv. ammonia nitrogen could be recovered from the solution in the rumen. It was concluded that the net movement of ammonia into the rumen either as ammonia or produced from urea during an absorption experiment would not affect the results.

In two experiments, solutions of identical composition were placed in the rumen during three successive 1-hr periods. Losses of ammonia in the three periods of the first experiment were 14, 16, and 16 m-equiv., and in the second experiment 10, 9, and 9 m-equiv., respectively. These experiments indicated that the rate of transport of solutions did not vary appreciably during 3 hr. Nevertheless, all experiments were repeated several times with the order of addition of solutions randomized to avoid errors caused by changes in the preparation. Two experiments showed that the rate of transport of ammonia was the same whether ammonia was added as the sulphate, chloride, or lactate. In subsequent experiments ammonia was added as the sulphate.

Ammonia in the solutions placed in the rumen was estimated by the method of Conway (1947) or by steam distillation in a microKjeldahl apparatus. Blood ammonia was determined by the microdiffusion techniques of Conway (1947) or Seligson and Seligson (1951), the blood being measured by syringe into the diffusion vessels immediately after sampling. The corrections made by Conway for the estimation of ammonia in human blood were used in analyses performed by the Conway method after tests had shown that the corrections were also valid for the blood of the sheep.

Total steam volatile fatty acids were estimated by the method of McAnally (1944). The pH of solutions was measured with either a Cambridge or a Pye Universal pH meter.

Sodium and potassium were determined with an "E.E.L." flame-photometer and chloride by the electrometric method of Sanderson (1952).

III. Results

(a) *Effect of Concentration of Ammonia and of pH on the Rate of Transport across the Epithelium*

Many preliminary experiments indicated that, at about pH 6·5, the transport of ammonia across the rumen epithelium increased when the concentration of ammonia in the rumen rose. These results were confirmed in greater detail in two experiments (Fig. 1). Ammonium sulphate solutions were prepared in mixed phosphate

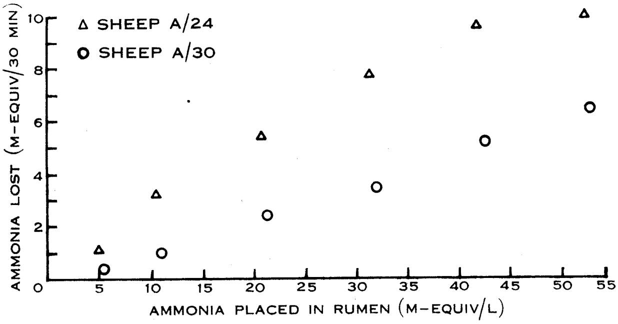

Fig. 1.—Effect of increased concentration of ammonia in the rumen at pH 6·5 on its rate of transport. The solutions placed in the rumen (2 l. of each) contained 0·002–0·026M $(NH_4)_2SO_4$, 0·018M KH_2PO_4, 0·018M Na_2HPO_4, 0·035M sodium acetate, and 0·004–0·077M NaCl.

and acetate buffers at pH 6·5 to give final concentrations of ammonia ranging from 3 to 53 m-equiv/l. The solutions were adjusted to 0·15M with sodium chloride, and the order in which the different solutions were placed in the rumen was randomized in each experiment. In both sheep there was an increase in the rate of transport of ammonia as the concentration of ammonia rose. The differences between the two sheep in the rates of transport of ammonia correspond to the net rates of transport of acetate, which for sheep A/24 were 27–33 m-equiv/30 min (mean 29 m-equiv.) compared with 13–15 m-equiv/30 min (mean 14 m-equiv.) for sheep A/30. There was no indication that a more rapid transport of ammonia affected the passage of the fatty acids.

Losses of ammonia again varied with concentration when solutions of ammonium sulphate ranging in concentration from 15 to 30 mM were prepared in phosphate buffers at pH 6·5 and in sucrose solutions (Table 1). These solutions were all left in the rumen for 60 min.

At pH 4·5, however, the concentration of ammonia in the rumen did not affect its rate of passage across the epithelium. Table 2 shows the results of experiments in which 15 and 30 mM solutions of ammonium sulphate were prepared in acetate and in phosphate buffers at pH 4·5. There were no consistent differences in the rate of transport of ammonia despite appreciable changes in the concentration of ammonia in the rumen.

TABLE 1

EFFECT OF AMMONIA CONCENTRATION ON THE TRANSPORT OF AMMONIA FROM PHOSPHATE BUFFERS AT pH 6·5 AND FROM SUCROSE IN THE RUMEN

Sheep No.	Solution*	pH Range	Rumen Ammonia			
			Concentration (m-equiv/l)	In (m-equiv.)	Out (m-equiv.)	Loss (m-equiv.)
A/15	1	6·2–6·2	60	119	106	13
	2	6·3–6·2	30	60	54	6
	1	6·2–6·2	60	119	109	10
	2	6·2–6·2	30	60	59	1
A/29	1	6·5–6·3	60	119	109	10
	2	6·5–6·5	30	59	54	5
	1	6·5–6·4	60	119	109	10
	2	6·5–6·5	30	59	55	4
A/39	3	7·1–6·7	30	60	54	6
	4	6·9–6·7	60	120	107	13
	5	7·1–6·7	46	90	82	8
A/40	3	7·0–6·6	30	59	54	5
	4	6·8–6·6	60	119	110	9
	5	6·9–6·6	46	89	83	6

* Solutions (2 l. of each placed in the rumen):

1: 0·03M $(NH_4)_2SO_4$+0·038M KH_2PO_4+0·038M Na_2HPO_4+0·045M NaCl.

2: 0·015M $(NH_4)_2SO_4$+0·038M KH_2PO_4+0·038M Na_2HPO_4+0·06M NaCl.

3: 0·015M $(NH_4)_2SO_4$+0·135M sucrose.

4: 0·03M $(NH_4)_2SO_4$+0·12M sucrose.

5: 0·023M $(NH_4)_2SO_4$+0·127M sucrose.

(b) *Effect of Volatile Fatty Acids on the Transport of Ammonia*

The experiments shown in Figure 1 suggested that the movement of the volatile fatty acids from the rumen might affect the movement of ammonia. In investigating this further, use was made of the data of Masson and Phillipson (1951) who showed that the rate of transport of the volatile fatty acids across the rumen epithelium is related to their concentration. Three experiments were performed in which the transport of ammonia from solutions of sodium acetate or butyrate at pH 6·5 were observed. The concentration of ammonia in all solutions was 30 m-equiv/l, while the concentration of volatile fatty acids varied between 0 and 100 m-equiv/l. Figure 2 shows the extent of the transport of the fatty acids and Figure 3 the relationship between the transport of fatty acids and ammonia. It may be noted that far more

TABLE 2

EFFECT OF CONCENTRATION OF AMMONIA AT pH 4·5 ON ITS RATE OF TRANSPORT FROM THE RUMEN

Sheep No.	Solution*	pH Range	Rumen Ammonia			
			Concentration (m-equiv/l)	In (m-equiv.)	Out (m-equiv.)	Loss (m-equiv.)
A/2	1	4·5–4·9	30	58	57	1
	2	4·3–5·2	60	116	110	6
	2	4·5–5·0	60	116	112	4
	1	4·6–5·0	30	59	54	5
A/3	1	4·5–4·8	30	61	54	7
	2	4·5–5·0	60	116	112	4
	2	4·5–4·9	60	121	115	6
	1	4·5–5·0	30	59	54	5
A/34	3	4·5–5·0	60	120	119	1
	4	4·5–5·0	30	60	59	1
	3	4·5–4·9	60	120	118	2
	4	4·6–4·9	30	60	58	2

* Solutions (2 l. of each placed in the rumen):

1: 0·015M $(NH_4)_2SO_4$+0·03M CH_3COONa+0·045M CH_3COOH+0·06M NaCl.

2: 0·03M $(NH_4)_2SO_4$+0·03M CH_3COONa+0·045M CH_3COOH+0·045M NaCl.

3: 0·03M $(NH_4)_2SO_4$+0·056M KH_2PO_4+0·056M NaH_2PO_4+0·008M NaCl.

4: 0·015M $(NH_4)_2SO_4$+0·056M KH_2PO_4+0·056M NaH_2PO_4+0·023M NaCl.

fatty acid than ammonia left the rumen. When only small quantities of fatty acids were present, increases in the transport of the fatty acids did not affect the movement of ammonia (Table 3).

At pH 4·5, the rate of transport of volatile fatty acids was greatly increased as the concentration rose. However, the rate of transport of ammonia did not seem to be related to the movement of the fatty acid (Fig. 4).

(c) *Effect of other Molecules*

The rate of transport of ammonia at a concentration of 30 m-equiv/l at pH 6·5 was not affected by changes in the concentration of lactate (0–80 m-equiv/l), potassium (0–135 m-equiv/l), sodium (0–135 m-equiv/l), or chloride (0–150 m-equiv/l).

When carbon dioxide was bubbled through solutions in the rumen, there was an increase in the net rate of transport of chloride from the rumen from 8 to 15 m-equiv/hr, but no change in the rate of transport of ammonia. The net movements of water into and out of the rumen were varied by altering the tonicity of the solution in the rumen. Water movements which varied between a gain of 40 ml and a loss of 170 ml showed no appreciable effect on the movement of ammonia.

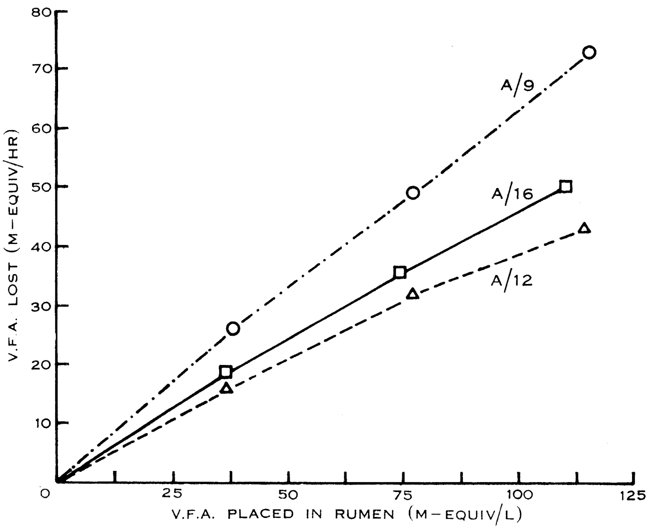

Fig. 2.—Effect of concentration of volatile fatty acids in the rumen on their rate of transport at pH 6·5. The solutions placed in the rumen (2 l. of each) contained for sheep A/9 and A/16, 0·015M $(NH_4)_2SO_4$; 0·09M KH_2PO_4; 0·09M Na_2HPO_4, 0–0·110M sodium acetate and 0·007–0·117M NaCl. For sheep A/12 sodium butyrate replaced sodium acetate.

(d) *Rate of Transport of Ammonia in the Blood Draining the Rumen*

In a number of experiments an estimate of the quantity of ammonia carried in part of the posterior rumen vein was made while net losses of ammonia from the rumen were being measured. In Table 4 the results of two experiments are shown, in which 15 and 30 mM solutions of ammonium sulphate in Krebs–Ringer–bicarbonate buffers containing acetate were added to the rumen. The higher concentration of ammonia in the rumen was accompanied by increased concentrations in the rumen vein; the rate of blood flow was reduced but this would not be sufficient to account for the great increase in the ammonia concentration. In both experiments the concentration of ammonia in the rumen vein indicated qualitatively the rate of transport of ammonia across the rumen epithelium. The third section of Table 4 presents results of analyses performed on blood from the posterior rumen vein of a conscious sheep. The concentration of ammonia nitrogen increased from 5 μg/ml blood when phosphate buffers alone were in the rumen to 18 μg/ml when the concentration of ammonia in the rumen was brought to 20 m-equiv/l; when the concentration of ammonia in the rumen was further increased to 40 m-equiv/l, the concentration of blood ammonia nitrogen rose to 29 μg/ml. These results suggest that the observations made on the anaesthetized sheep above are qualitatively similar to those which occur in a standing conscious sheep.

When the rumen pH was altered with acetate and phosphate buffers, however, there did not seem to be such a clear relationship between net losses of ammonia from the rumen and the concentration of ammonia in the rumen vein. Table 5 shows the results of two experiments. With sheep S/32 the addition of the more acid solution to the rumen was accompanied by an appreciable fall in the concentration of

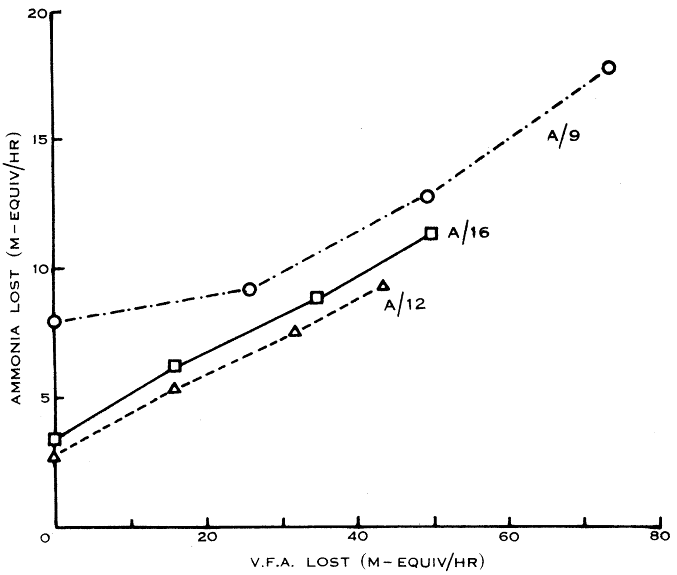

Fig. 3.—Effect of volatile fatty acid transport on ammonia transport from the rumen at pH 6·5. The solutions used were as described for Figure 2.

ammonia in the blood; however, the rate of blood flow was so greatly increased that the movement of ammonia in this part of the rumen vein was much the same in both absorption periods. This result did not reflect the net losses from the rumen. In sheep S/34 the replacement of a solution in the rumen at pH 4·5 with another at pH 6·5 produced a slight increase in the rate of flow of blood in the rumen vein. This was not expected in view of previous results (Dobson and Phillipson 1956). It may be seen, however, that the concentration of ammonia in the vein was simultaneously increased and that the ammonia carried in this part of the vein gave some indication of the net losses of ammonia from the rumen. It appears from these two experiments that the use of the concentration of ammonia in the rumen vein to indicate qualitatively the transport of ammonia from the rumen could be misleading under conditions where the rate of blood flow in the vein might alter appreciably.

IV. Discussion

Most investigations of the transport of ions across the rumen epithelium have referred to the net flux—the balance between the outflux of the ions from the rumen to the blood and the influx from the blood to the rumen. This is true for sodium, potassium, and chloride, the concentration of which in arterial blood may be similar to that in rumen contents; however, it should not apply to ammonia, the concentration of which in arterial blood is not normally more than 0·1 mM (McDonald 1948; Lewis, Hill, and Annison 1957). Ammonia from the blood would have to enter the rumen against an appreciable concentration gradient, for the concentration of ammonia in the rumen usually lies between 7 and 60 m-equiv/l (McDonald 1948; Annison *et al.* 1954).

Table 3

Effect of small quantities of volatile fatty acids (v.f.a.) on the transport of ammonia

Sheep No. A/21

Solution*	pH	Ammonia Nitrogen (m-equiv.)			V.F.A. (m-equiv.)		
		In	Out	Loss	In	Out	Loss
1	6·3–6·0	181	169	12	0	0	0
2	6·4–6·5	181	169	12	3·4	2·6	0·8
3	6·5–6·4	180	169	11	10·4	8·5	1·9
4	6·5–6·4	180	171	9	7·0	5·4	1·6

* Solutions (2 l. added to the rumen):

1: 0·045M $(NH_4)_2SO_4$+0·038M Na_2HPO_4+0·038M KH_2PO_4+0·029M NaCl.

2: 0·045M $(NH_4)_2SO_4$+0·038M Na_2HPO_4+0·038M KH_2PO_4+0·027M NaCl+0·002M CH_3COONa.

3: 0·045M $(NH_4)_2SO_4$+0·038M Na_2HPO_4+0·038M KH_2PO_4+0·023M NaCl+0·006M CH_3COONa.

4: 0·045M $(NH_4)_2SO_4$+0·038M Na_2HPO_4+0·038M KH_2PO_4+0·025M NaCl+0·004M CH_3COONa.

Some ammonia may be formed in the rumen during an experiment, however, for it appears from arteriovenous differences that urea is at times removed from the blood as it passes through the rumen. That some of this urea is converted to ammonia is suggested by the fivefold concentration of ammonia in the right ruminal vein above the arterial level in both conscious and anaesthetized sheep whose rumens have been emptied, washed out, and filled with 0·9% sodium chloride solution. Nevertheless, as the concentration of ammonia in the rumens of these sheep, even after an hour, did not exceed 0·2 m-equiv/l, it is assumed that rumen ammonia derived either directly from ammonia or indirectly from urea in the blood would not affect the interpretation of the present experiments. The losses of ammonia from the rumen recorded in these experiments, while in reality a net flux, may therefore be regarded as an outflux from the rumen to the blood. Since these experiments were completed, Houpt (1959) has described experiments in which ammonia accumulated at the rate of 2 m-mole/hr in the washed-out, isolated rumen. These figures are about four times as high as those found here. In the absence of information on the diets of Houpt's sheep or their feeding routine before the rumen was emptied, it is not possible to discuss the differences in the two sets of results. The important point, however, is that Houpt

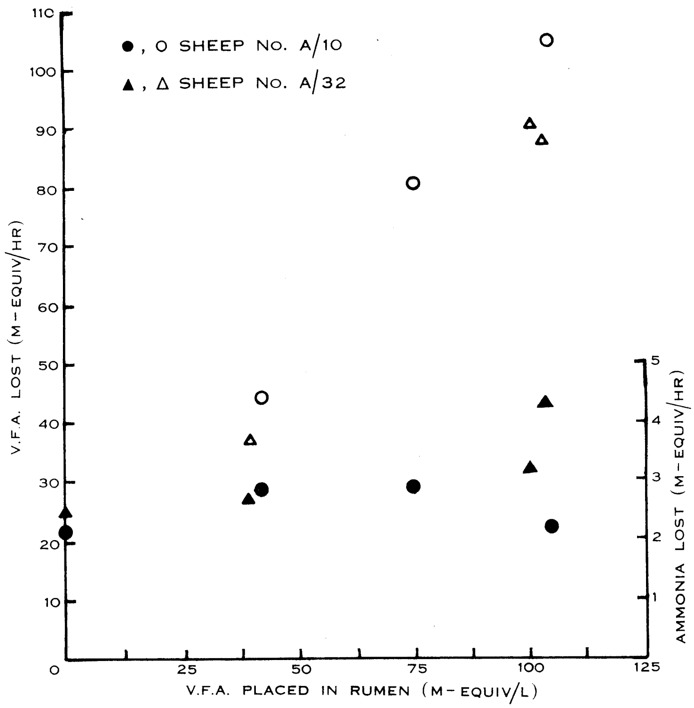

Fig. 4.—Effect of concentration of volatile fatty acids at pH 4·5 on the rate of transport of fatty acids (○, △) and of ammonia (●, ▲) from the rumen. The solutions placed in the rumen (2 l. of each) contained 0·015M $(NH_4)_2SO_4$, 0·019M KH_2PO_4, 0·019M NaH_2PO_4, 0–0·054M acetic acid, 0–0·044M sodium acetate, and 0–0·098M NaCl.

observed that ammonia accumulated at a relatively steady rate during several hours. This confirms our idea that ammonia would enter the rumen during each of the four periods of an absorption experiment to about the same extent, and thus the "endogenous" production of ammonia during an absorption experiment would not affect the comparisons of the transport of ammonia during the individual absorption periods.

The rate of transport of ammonia across the rumen epithelium was increased by raising the concentration of ammonia in the rumen from 30 to 60 m-equiv/l at pH 6·5. This occurred when the other constituents in the rumen were either acetate which is readily transported across the epithelium, phosphate which is usually transported only slowly (Scarisbrick and Ewer 1951), or sucrose. However, at pH 4·5, the rate of transport of ammonia from the rumen was not altered appreciably with changes in concentration. It thus appears that the change in pH from 6·5 to 4·5 affected the mechanism of transport of ammonia from the rumen. This could have occurred either by altering the rumen epithelium in some way or by alterations in the ionic composition of the solutions.

TABLE 4

RATE OF FLOW OF BLOOD AND THE CONCENTRATION OF AMMONIA IN PART OF THE RIGHT RUMINAL VEIN OF THE SHEEP

Sheep No.	Solution*	Rumen Ammonia (m-equiv/l)	Rumen pH	Rate of Blood Flow (ml/min)	Concn. of Ammonia Nitrogen in Rumen Vein (μg/ml)	Total Ammonia Nitrogen in Blood (mg/min)	Net Loss of Ammonia Nitrogen from Rumen (m-equiv/hr)
S/27	1	30	6·2–6·9	19	29	0·55	10
	2	60	6·6–7·0	14	53	0·74	24
	1	30	6·6–6·9	19	29	0·55	7
S/30	1	30	6·3–7·0	20	14	0·28	9
	2	60	6·3–6·9	12	35	0·42	15
	1	30	6·5–6·9	16	24	0·38	2
G/77†	3	0	6·5	—	5		
	4	20	6·5	—	18		
	5	40	6·5	—	29		

* Solutions (4 l. for S/27 and S/30, 2 l. for G/77):

1: 0·015M $(NH_4)_2SO_4$+0·021M CH_3COONa+0·114M Krebs–Ringer–bicarbonate.

2: 0·03M $(NH_4)_2SO_4$+0·021M CH_3COONa+0·099M Krebs–Ringer—bicarbonate.

3: 0·075M KH_2PO_4+0·075M Na_2HPO_4.

4: Solution 3+0·01M $(NH_4)_2SO_4$.

5: Solution 4+0·01M $(NH_4)_2SO_4$.

† Conscious sheep.

At the pH of the present experiments, almost all the ammonia would be present as NH_4^+. However, some free ammonia is always present in equilibrium with NH_4^+ according to the equation $NH_4^+ \rightleftharpoons H^+ + NH_3$. The proportions of NH_3 and NH_4^+ may be calculated from the Henderson–Hasselbach equation which Bromberg, Robin, and Forkner (1960) have shown approximates to

$$pK_a = pH + \log\,(\text{total ammonium}/NH_3).$$

These authors estimated the pK_a of this system, in anaesthetized dogs, to be 9·15.

From their figures it may be calculated that the ratio total ammonium: NH_3 in plasma at pH 7·4 is 56 : 1 and in rumen contents at pH 6·5 and 4·5, 450 : 1 and 45000 : 1 respectively. Plasma, which has a total ammonium concentration of 100 μg nitrogen/100 ml or 71 μ-equiv/l would thus have 1·25 μ-equiv/l NH_3. Rumen fluids containing 30 m-equiv/l ammonium at pH 6·5 and 4·5 would have respectively 67 μ-equiv/l and 0·67 μ-equiv/l NH_3. Thus while there is a much greater concentration of NH_3 in the rumen at pH 6·5 than in the blood, at a rumen pH of 4·5 the reverse obtains.

TABLE 5

EFFECT OF LOWERING THE pH OF RUMEN CONTENTS ON THE RATE OF FLOW OF BLOOD AND THE CONCENTRATION OF AMMONIA IN THE RIGHT RUMEN VEIN

Sheep No.	Solution*	Rumen pH	Rate of Blood Flow (ml/min)	Ammonia Nitrogen in Venous Blood Leaving Rumen (μg/ml)	Total Ammonia Nitrogen in Blood (mg/min)	Net Loss of Ammonia Nitrogen from Rumen (m-equiv/hr)
S/32	1	6·4–6·6	26	31	0·81	14
	2	4·4–4·8	55	11	0·60	6
	1	6·4–6·5	22	29	0·64	11
S/34	2	4·9–5·8	49	14	0·69	1
	1	6·5–6·8	56	28	1·57	17
	2	4·4–5·6	32	16	0·51	4

* Solutions (4 l. of each):

1: 0·015M $(NH_4)_2SO_4$+0·075M CH_3COONa+0·008M HCl+0·021M KH_2PO_4+0·031M Na_2HPO_4.

2: 0·015M $(NH_4)_2SO_4$+0·038M CH_3COONa+0·038M CH_3COOH+0·03M HCl+0·01M KH_2PO_4+0·019M Na_2HPO_4.

These calculations suggest that one mechanism for the transport of ammonia across the rumen epithelium is by diffusion as NH_3. They also indicate why ammonia is transported from the rumen in accordance with its concentration gradient at pH 6·5 but not at pH 4·5.

The effect of the fatty acids at pH 6·5 on the transport of ammonia appeared only when large quantities of acid were being absorbed. The absence of any "mole for mole" relationship indicated that there was only an indirect effect on ammonia transport. At pH 6·5, most of the fatty acid would be present as the anion and it has been suggested that it is absorbed as such (Danielli *et al.* 1945). It is known (Masson and Phillipson 1951) that when a volatile fatty acid anion leaves the rumen it is replaced by an influx of bicarbonate. The quantity entering is about half the fatty acid disappearing. The remainder of the fatty acid presumably passes from the rumen accompanied by a corresponding number of positively charged ions such as the ammonium ion.

The rate of transport of ammonia from the rumen at pH 4·5 was not appreciably affected by the movement of the volatile fatty acids. At this pH, most of the fatty acid crosses the rumen as free fatty acid (Danielli *et al.* 1945) which would require neither the movement of cations nor exchange with anions.

In the absence of volatile fatty acids the transport of ammonia from solutions containing 30 m-equiv/l ammonia in the rumen was usually small. However, ammonia was lost from the rumen in every experiment. That this was a real transport across the epithelium and not merely adsorption onto it is shown by the 10- to 40-fold increase in the concentration of ammonia in the rumen veins as soon as solutions containing ammonia were added to the rumen. The rise in blood ammonia was so extensive that it could not be accounted for merely by a decrease in the rate of flow of blood through the vein, and indeed, a reduction in the rate of blood flow was observed only when acetate was also present in the rumen at about pH 6·5.

Attempts were made to use the change in concentration of ammonia in the rumen vein as a semiquantitative indication of changes in the rate of transport of ammonia across the rumen epithelium. This method, if successful, would have been a more sensitive indication of transport from the rumen than net losses measured with large volumes of fluid. However, the observation that the addition of ammonia to the rumen was at times accompanied by a drop in the rate of flow of blood in the rumen vein, suggested that measurements of the concentration of ammonia without an accurate estimate of the rate of flow of the blood could be meaningless.

The movement of ammonia across the rumen epithelium was observed to be independent within experimental error of variations in the concentration in the rumen of sodium, potassium, lactate, chloride, and carbon dioxide, and of the net movement of water into and out of the rumen.

While it appears from the present experiments that, of all the substances crossing the rumen epithelium, the volatile fatty acids alone affect the transport of ammonia, it must be remembered that these results record virtually an outflux of volatile fatty acids and ammonia but a net flux of other ions. The present conclusions on the factors affecting the transport of ammonia must therefore be regarded as tentative until the estimation of the true fluxes of other ions may be made.

V. Acknowledgments

This work was commenced at the Rowett Research Institute, Aberdeen, Scotland. I wish to thank Dr. D. P. Cuthbertson for providing facilities, Dr. A. T. Phillipson for his advice and guidance, and Messrs. L. E. Vowles and J. Dunnett for their assistance. The help of Messrs. D. R. Williams and D. J. Bogsanyi of this Laboratory is also gratefully acknowledged.

VI. References

Annison, E. F., Chalmers, M. I., Marshall, S. B. M., and Synge, R. L. M. (1954).—*J. Agric. Sci.* **44**: 270.

Briggs, P. K., Hogan, J. P., and Reid, R. L. (1957).—*Aust. J. Agric. Res.* **8**: 674.

Bromberg, P. A., Robin, E. D., and Forkner, C. E., Jr. (1960).—*J. Clin. Invest.* **39**: 332.

Chalmers, M. I., Cuthbertson, D. P., and Synge, R. L. M. (1954).—*J. Agric. Sci.* **44**: 254–62.

Conway, E. J. (1947).—"Microdiffusion Analysis and Volumetric Error." (Crosby, Lockwood and Son, Ltd.: London.)

Danielli, J. F., Hitchcock, M. S. W., Marshall, R. A., and Phillipson, A. T. (1945).—*J. Exp. Biol.* **22**: 75–84.

Dobson, A., and Phillipson, A. T. (1956).—*J. Physiol.* **133**: 76P.

Dobson, A., and Phillipson, A. T. (1958).—*J. Physiol.* **140**: 94.

Houpt, T. R. (1959).—*Amer. J. Physiol.* **197**: 115.

Lewis, D., Hill, K. J., and Annison, E. F. (1957).—*Biochem. J.* **66**: 587.

Masson, M. J., and Phillipson, A. T. (1951).—*J. Physiol.* **113**: 189.

McAnally, R. A. (1944).—*J. Exp. Biol.* **20**: 130.

McDonald, I. W. (1948).—*Biochem. J.* **42**: 584.

Phillipson, A. T. (1942).—*J. Exp. Biol.* **19**: 186.

Phillipson, A. T. (1955).—*Vet. Rec.* **67**: 1048.

Sanderson, P. H. (1952).—*Biochem. J.* **52**: 502.

Scarisbrick, R., and Ewer, T. K. (1951).—*Biochem. J.* **49**: lxxix.

Seligson, D., and Seligson, H. (1951).—*J. Lab. Clin. Med.* **38**: 324.

Soskin, S., Priest, W. S., and Schutz, W. J. (1934).—*Amer. J. Physiol.* **108**: 107–17.