J. Physiol. (1963), **169**, *pp.* 39–61
With 7 text-figures
Printed in Great Britain

THE EFFECT OF ABSORPTION ON THE ACIDITY OF RUMEN CONTENTS

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(*Received* 7 *January* 1963)

The role of the saliva in neutralizing the large quantities of fatty acid which are produced by the fermentations in the rumen of the sheep is well known. However, there is evidence that the absorptive properties of the reticulo-rumen epithelium also play a part in maintaining a relatively stable acidity of the rumen contents. Danielli, Hitchcock, Marshall & Phillipson (1945) observed that when fatty acid was absorbed from solutions introduced into the isolated reticulo-rumen, continual additions of acid were necessary in order to keep the pH below 6. It was suggested by Danielli *et al.* that at a low pH, un-ionized fatty acid was leaving the rumen more rapidly than the corresponding anion.

Another mechanism involved in the neutralization of fatty acid was discovered by Masson & Phillipson (1951). These workers observed that when alkaline solutions of fatty acid were introduced into the isolated reticulo-rumen the pH drifted towards 7·3–7·8 and sometimes passed through a minimal value. Further, the appearance of total CO_2, mainly as bicarbonate, was closely related to the disappearance of fatty acid.

The way in which the apparent interaction between fatty acid uptake and bicarbonate appearance occurs is difficult to investigate, since there are changes in seven known species of particles which may affect the pH. These are hydrogen and hydroxyl ions, carbon dioxide, bicarbonate and carbonate, ionized and un-ionized fatty acid; in addition there are unknown contributions of metabolites by the epithelium.

The aim of the present work was to determine the forms in which fatty acid and bicarbonate enter and leave the solutions in the rumen. Observations were made by means of a comparatively simple system in which fatty acid was omitted from the solutions in the rumen so that information might be obtained concerning the movements of CO_2 and bicarbonate and thus the changes as a result of including fatty acid could be assessed. Subsequently the observations were extended to the plasma side of the epithelium. Throughout this paper the term CO_2 is used to describe dissolved CO_2 + undissociated carbonic acid; total CO_2 refers to CO_2 + bicarbonate.

METHODS

Preparation of animals. Small sheep were fitted with two permanent ebonite rumen cannulae in the dorsal sac of the rumen several months before an absorption experiment. Food was withheld after the morning feed on the day previous to the experiment, so that by the morning of the experiment the contents of the reticulo-rumen were sufficiently fluid to facilitate their removal and replacement with 150 mM-NaCl. The sheep was anaesthetized with sodium pentobarbital, the trachea cannulated, a carotid artery and a jugular vein exposed and the oesophagus ligated in the cervical region. Laparotomy was performed through a right paracostal incision and the junction of the reticulum and omasum was exposed and ligated, care being taken to avoid including the epiploic vessels in the ligature (Masson & Phillipson, 1951); then the abdominal wound was sutured. The 'isolated' reticulo-rumen sac was rinsed with tap water until the drainings appeared clean. During an absorption experiment the animal was placed on its right side with the rumen cannulae uppermost.

TABLE 1. Composition of solutions introduced into the rumen. All solutions contained creatinine 200 mg/l. Most solutions were gassed with mixtures of N_2 and CO_2. The initial CO_2 concn. is given in Table 4

		Concentration (mM)				
Expt.	Period	Na^+	K^+	Cl^-	HCO_3^-	CH_3COO^-
1	1	156	—	46	110	—
	2	180	—	180	—	—
2	1	165	15	180	—	—
	2	165	15	30	—	150
3, 4	1	168	—	91	77	—
	2	168	—	—	77	91
5	1	180	—	156	24	—
	2	180	—	—	24	156
6	1	180	—	—	24	156
	2	180	—	156	24	—
7	1	180	—	—	30	150
	2	180	—	150	30	—
8	1	180	—	—	15	165
	2	180	—	165	15	—
9	1	180	—	165	15	—
	2	180	—	—	15	165
10	1	180	—	—	24	156
	2	180	—	156	24	—
11	1	180	—	—	15	165
	2	180	—	165	15	—

Samples of blood were taken from the right ruminal vein through the abdominal incision, and in order to accomplish this without undue disturbance these absorption experiments were performed with the sheep on its left side, with its single rumen cannula projecting through a hole in the table. Solutions were introduced into the rumen through an oesophageal tube.

Absorption experiment. The reticulo-rumen was filled with 1·5–2 l. of solution of composition given in Table 1. Gas above the liquid was expelled either through the cannula, or a large bore hypodermic needle inserted through a purse-string suture in the rumen wall. Samples were removed by gentle suction through a glass tube in a bung in the cannula. Part of the sample was run directly under liquid paraffin and cooled to await total CO_2 and pH measurements. A further 25 ml. was preserved by adding 0·5 ml. 10 N-H_2SO_4. Throughout the absorption period the rumen contents were mixed by pressing gently on the sheep's

abdomen. The rumen was rinsed three times with 1 l. of tap water after draining each test solution and before introducing the next solution.

An attempt was made to keep the temperature of the animal constant by covering the animal and adjusting the ambient temperature. The temperature of the solution in the rumen or the rectal temperature was recorded and the values given in Table 2 were usually maintained within $\pm 1°$ C.

Blood samples were removed anaerobically, solid heparin being used as anticoagulant. When samples were taken from both the carotid artery and a rumen vein, they were removed millilitre for millilitre simultaneously from both sites.

The electrical potential between jugular blood and rumen contents was measured by a procedure described previously (Dobson & Phillipson, 1958).

TABLE 2. The experimental temperature and sheep parameters for each experiment. The epithelium of the reticulum and rumen was stripped and weighed after drying at 105° C. The pK_1' for plasma was taken from a nomogram relating pK_1' to pH and temperature (Severinghaus, Stupfel & Bradley, 1956). The pK_1' for a rumen solution was taken as 6·10 for an ionic strength of 180 mM-NaCl at 38° C (Hastings & Sendroy, 1925). The variation with temperature has been assumed to be similar to plasma at pH 7·4

Expt.	Sheep wt. (kg)	Dry wt. of epithelium (g)	Temp. (°C)	pK_1' of CO_2	
				Rumen soln.	Plasma
1	36	43	36	6·10	6·11
2	43	43	36	6·10	6·11
3	33	31	37	6·10	6·11
4	26	40	36	6·10	6·11
5	27	31	38	6·09	6·10
6	20	23	36	6·10	6·11
7	20	31	33	6·12	6·13
8	28	39	38	6·09	6·10
9	28	33	37	6·10	6·11
10	39	45	39	6·09	6·10
11	32	46	37	6·10	6·11

The amount of a constituent of the rumen solution at the time of taking a sample was calculated from its concentration and the concentration of creatinine, initially 200 mg/l., which proved suitable as a volume marker. In ten absorption periods of 1–2 hr 99·4 % $\pm$ 0·1 (S.E.) of the creatinine introduced into the rumen was recovered. A negligible amount of Jaffé-positive material entered a rumen solution free of creatinine and adsorption of creatinine on to the rumen epithelium did not occur.

pH measurement. In Expts. 1–5 the rumen pH was recorded at 2–4 min intervals with a glass electrode introduced through an airtight seal in one rumen cannula. A 3·5 M-KCl/agar bridge in a 2 mm bore polyvinyl chloride tube made a connexion from the rumen contents to a calomel electrode kept at constant temperature. The potential of the electrode system was read on a battery-operated Cambridge pH meter on the mV scale and at the end of each experiment the electrode system was calibrated with borate and phthalate buffers at the experimental temperature. The glass electrode used was selected for its small drift under the operating conditions. The errors of this method are not easy to assess, as they are largely systematic, but it is unlikely that the error in any reading exceeded $\pm$ 0·03 pH units, whereas the error in pH differences within a period was probably less.

In Expts. 4–11 the pH was measured on the samples of rumen solutions and whole blood under anaerobic conditions and at the experimental temperature (Table 2) in an apparatus similar to that described by Astrup & Schrøder (1956); the measurements were made within a few hours of sampling. The electrode system was calibrated with borate and phthalate buffers made up as described in British Standard 1647:1950. The S.E. of the mean of

duplicate estimations was $\pm 0{\cdot}0034$ pH units. In two experiments where the two methods of pH measurement were used together, in twenty-one observations, the mean difference was $0{\cdot}002 \pm 0{\cdot}0003$ (S.E.) pH units.

CO_2 and bicarbonate. Total CO_2 was measured manometrically on plasma and rumen samples with the apparatus of Van Slyke & Neill (1924). The S.E. of the mean of duplicate determinations was $\pm 0{\cdot}10$ mM except in Expts. 2–4, when the S.E. was $\pm 0{\cdot}2$ mM. The concentrations of CO_2 and bicarbonate were calculated from the total CO_2, pH and pK_1' (Table 2) by means of the Henderson–Hasselbalch equation. CO_2 in gases was measured with a Haldane apparatus.

Creatinine was measured by the Jaffé reaction after traces of protein were removed by shaking 1 ml. of acidified sample with 5 ml. saturated picric acid solution, diluting to 25 ml. and filtering through Whatman No. 42 paper. 1 ml. of 1·5 N-NaOH was added to 10 ml. of filtrate and the optical density at 520 mμ was read after 60 min. The accuracy of the determination was improved by minimizing the exposure of the colour to light, and by allowing the solutions to stand at least 10 min in the dark in the Beckman spectrophotometer before reading. Blank and standard solutions were measured concurrently to compensate for differences in colour intensity due to temperature difference, and small exposures to light. The S.E. of the mean of duplicate determinations was $\pm 0{\cdot}7$ μg/ml.

Acetate was estimated as steam-volatile acid in Markham stills and the distillate was titrated with 0·05 N-$Ba(OH)_2$, phenol red being used as the indicator. The S.E. of the mean of duplicate estimations was $\pm 0{\cdot}2$ mM. Fatty acid appearing in solutions in the rumen was estimated in large volumes of solution removed at the end of an absorption period. The solutions were made alkaline, concentrated by boiling and then steam-distilled on a large scale to reduce contamination with lactic acid. The distillate was then concentrated, redistilled in a Markham still and the proportion of each of the lower fatty acids determined by gas chromatography (James & Martin, 1952).

Other chemical methods. Phosphate was estimated by the method of Berenblum & Chain (1938); lactate by the method of Barker & Summerson (1941); pyruvate by the method of Freidemann & Haugen (1943); total ketone bodies by the method of Greenberg & Lester (1944); ammonia and urea by the method of Conway (1957). Haemoglobin on whole blood samples was determined spectrophotometrically (Dobson, 1959).

Conventions. All numerical values of the passage or rate of passage of a substance from the rumen solution are given a positive sign.

RESULTS

Gaseous CO_2 exchange

The changes in partial pressure of CO_2 within a gas-filled rumen give a convenient and direct measure of CO_2 exchange uncomplicated by the movements of bicarbonate. The washed isolated reticulo-rumen sac of an anaesthetized sheep was drained, moistened with about 200 ml. of 167 mM-NaCl and flushed out with the gas mixture to be investigated. The rumen was then inflated to a pressure of 5 cm of water with the gas mixture and samples were withdrawn under mercury into gas pipettes through a two-way tap. The pressure did not change during the course of the two experiments depicted in Fig. 1.

These experiments establish that there is a two-way exchange of CO_2 across the rumen epithelium with the equilibrium lying in the region of the blood partial pressure. This is considerably lower than the partial

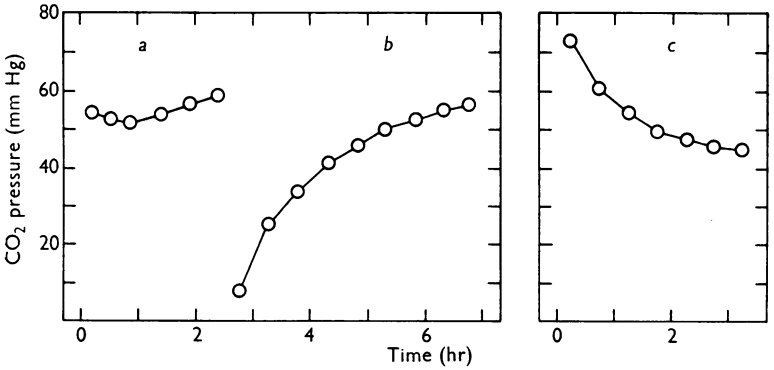

Fig. 1. Changes in the partial pressure of CO_2 with gases in the rumen. The gas mixtures used were approximately (*a*) 10 % CO_2–90 % N_2; (*b*) 100 % N_2; and (*c*) 15 % CO_2–85 % N_2.

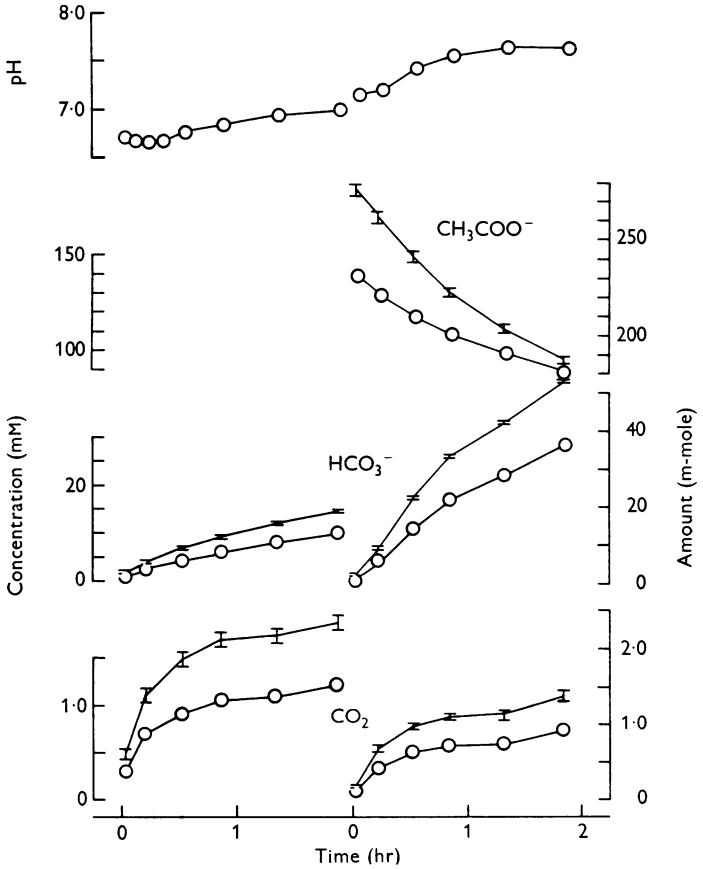

Fig. 2. Expt. 2. The appearance of CO_2 and HCO_3^- in solutions in the rumen when their initial concentrations are low. The first period is in the absence of acetate, the second with acetate present. Concn. ○; vertical bars show amount ±2 S.E.

pressure found with normal rumen contents—about 50 % CO_2 (Turner & Hodgetts, 1955). Since the volume changes are unknown it is not possible to calculate the amounts of CO_2 exchanged.

CO_2 and bicarbonate exchanges

The first group of experiments was undertaken to establish the course of changes in CO_2 and bicarbonate when these occurred fairly quickly. The entry of CO_2 and bicarbonate into the rumen solution is illustrated in Fig. 2; acetate was absent from the first period and present in the second.

In the absence of acetate both CO_2 and bicarbonate appeared rapidly in the initial stages, but the rate of CO_2 appearance decreased after the first half hour; the rate of bicarbonate entry decreased slightly as the concentration in the rumen increased. The net over-all effect of these changes appeared to be responsible for the minimal value in the pH

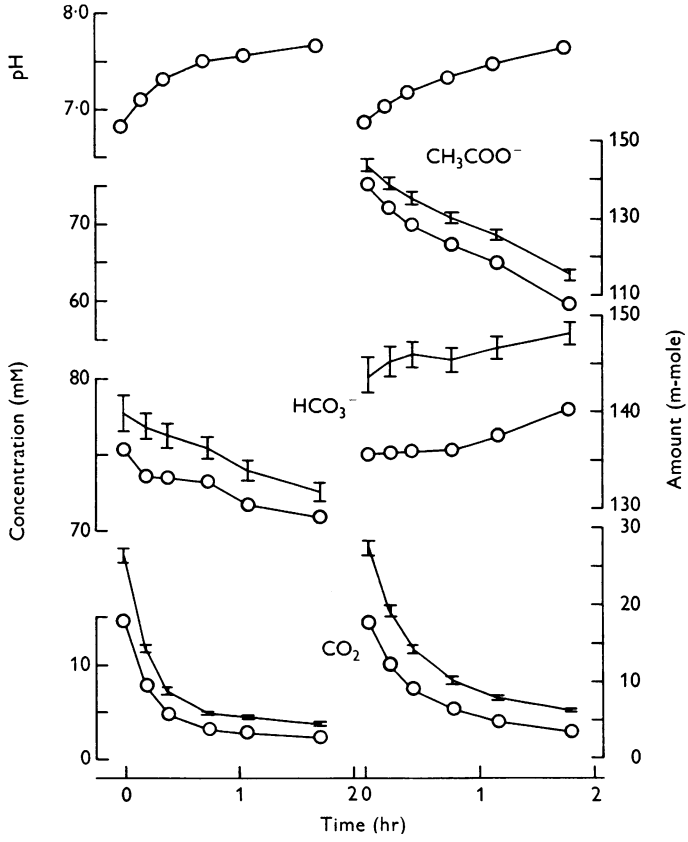

Fig. 3. Expt. 4. The changes in CO_2 and HCO_3^- in solutions in the rumen when the initial concentrations are high. The first period is in the absence of acetate, the second with acetate present. Concn. ○; vertical bars show amount ±2 S.E.

reading; the minimal pH was less marked in this period than in two other periods on different sheep with a similar solution in the rumen. In these latter experiments the initial pH values were 6·75 and 6·91 and the minimal values observed were 6·57 and 6·39 after 20 and 17 min respectively.

In the presence of acetate the most striking difference was the rapid rate of bicarbonate accumulation in the rumen. This rate decreased a little with time, as did the rate of acetate disappearance. The appearance of CO_2 was similar to that in the previous period although it tended to accumulate more slowly. The pH increased throughout the period but the initial increase was slow when CO_2 was entering rapidly; thereafter the increase was faster as the bicarbonate increased alone. As the concentration of bicarbonate increased, the pH change occurred more slowly, owing to the increase in buffering capacity of the rumen solution.

When the initial concentrations of CO_2 and bicarbonate were high in the test solutions, CO_2 disappeared very rapidly at first, with the result that the pH increased (Fig. 3). The disappearance of CO_2 was slower in the presence of acetate.

In the absence of acetate, the bicarbonate disappeared steadily from the rumen at 0·08 m-mole/min, but when acetate was present bicarbonate accumulated at a rate of $-0{\cdot}04$ m-mole/min with acetate being absorbed at a rate of 0·27 m-mole/min. In another experiment with similar solutions, bicarbonate uptake in the absence of acetate absorption was 0·16 m-mole/min. When 0·35 m-mole/min acetate was absorbed the bicarbonate uptake was reduced to 0·04 m-mole/min. The bicarbonate appearance per mole of acetate lost was $+0{\cdot}11$ and $-0{\cdot}14$ in these two experiments. These ratios are much higher than those found at lower concentrations of bicarbonate in the rumen (Fig. 6). Hence it appears that any relation between acetate uptake and bicarbonate appearance is superimposed on an absorption of bicarbonate at the high concentration used.

In a second group of absorption experiments an attempt was made to compare the steady-state concentration of CO_2 in the rumen solution with that of the blood. The CO_2 concentration of the test solutions introduced into the rumen was near that expected (approximately 1·2 mM) and in six periods on three sheep the changes in CO_2 and bicarbonate were followed, taking four or five samples from the rumen during each period; carotid blood samples were taken at the middle and end of each period. An example of the maintenance of a steady state in the rumen over $1\frac{1}{2}$ hr is shown in Fig. 4.

It is important that the steady-state concentration should be observed when the volume changes due to absorption are minimal, as this concentration is dependent on rate of volume change. The changes in these experiments were very small, as can be seen from the changes in creatinine concentration given for each period in Table 5.

In the attainment of this steady state the concentration changes of CO_2 were reasonably exponential with time in all but one of twelve periods where it could be tested. Five periods in the absence of acetate had an exponential half-life of 9–25 min and in six periods with acetate present the half-life was 25–35 min. Both these groups include a period where the concentration was decreasing. Thus, providing the initial concentration

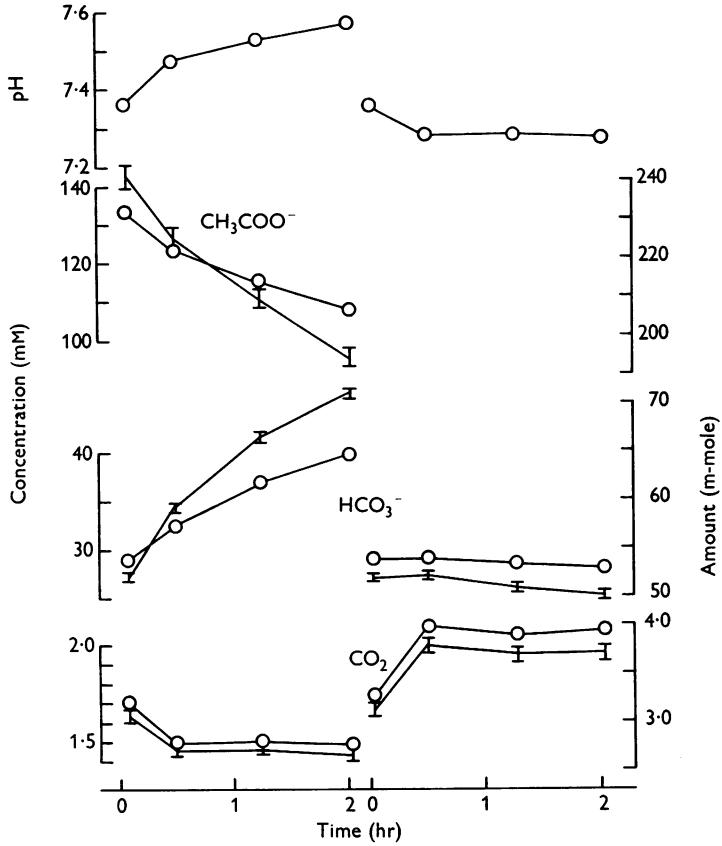

Fig. 4. Expt. 7. The effect of acetate on the steady-state concentration of CO_2 in the rumen. In the first period acetate is present, in the second it is absent. Concn. ○; vertical bars show amount ±2 S.E.

was within 50% of the steady value, the difference between the rumen concentration of CO_2 and its final steady value became less than the errors of its estimation after three half-lives. Working within these limitations the number of samples from the rumen was reduced to allow more plasma samples to be analysed. In two further experiments the rumen solutions were left in for 90 min, and a rumen sample taken then was assumed to have reached the steady state.

The rumen steady-state concentrations of CO_2 with corresponding plasma concentrations are given in Table 3. The corresponding bicarbonate concentration and pH are given in Tables 4 and 5. The acetate uptakes are given for each solution in which this ion was included in Tables 3 and 4. The differences in CO_2 concentration between the steady rumen level and the arterial blood are summarized in Table 6. The two last experiments in Tables 3–6 were somewhat different from the others, and are described separately. In the absence of acetate, the steady concentration of CO_2 in the rumen was higher than the arterial or venous levels. When acetate was absorbed, the steady-state rumen concentration was near or even below the arterial concentration, and in one experiment it was also appreciably below the venous concentration. The steady-state rumen concentration was about 0·5 mM higher in the absence of acetate than in its presence, an increase of about 30 %. This effect is seen most clearly in Fig. 4 where the initial CO_2 concentration in both periods was 1·7 mM. In the first period, when acetate was present, the rumen concentration dropped to 1·5 mM; in the second period, when acetate was absent, the rumen concentration rose to 2·1 mM. Both final values were held reasonably steady.

The corresponding bicarbonate concentrations are shown in Table 4. It was hoped to be able to define the steady-state concentration of bicarbonate

TABLE 3. The concentration of CO_2 in rumen solutions and plasma in the presence and absence of acetate. The plasma samples were taken at the following approximate times:

(*a*) 45 min after putting the solution in the rumen;
(*b*) 5 min before the final rumen samples were taken;
(*c*) 5 min after the final rumen sample was taken.

The corresponding bicarbonate concentrations and measured pH are given in Tables 4 and 5. The uptake of acetate is expressed on the basis of the dry weight of the stripped epithelium

		CO_2 concentration (mM)								
		Rumen solution			Plasma					
			Final		Carotid artery			Rumen vein		Acetate uptake (μ-mole $min^{-1}g^{-1}$)
Expt.	Period	Initial	90 min	120 min	(*a*)	(*b*)	(*c*)	(*b*)	(*c*)	
5	1	0·91	—	1·77	1·32	1·32	—	—	—	0
	2	0·80	1·29	—	1·24	1·20	—	—	—	13·5
6	1	0·81	—	1·62	—	1·53	—	—	—	6·3
	2	0·88	—	2·02	1·48	1·43	—	—	—	0
7	1	1·70	—	1·48	1·43	1·64	—	—	—	13·0
	2	1·73	—	2·07	1·72	1·64	—	—	—	0
8	1	1·28	1·29	—	—	1·61	1·63	1·42	1·38	20·9
	2	0·95	1·83	—	—	1·59	1·56	1·61	1·53	0
9	1	1·03	1·72	—	—	1·60	1·64	1·64	1·70	0
	2	0·85	1·16	—	—	1·55	1·48	1·49	1·40	16·8
10	1	1·08	1·51	—	—	—	1·31	—	1·53	5·3
	2	0·89	1·85	—	—	1·25	1·25	1·53	1·55	0
11	1	1·07	1·34	—	—	1·60	1·52	1·67	1·56	1·8
	2	0·97	1·94	—	—	1·45	1·40	1·68	1·60	0

TABLE 4. The concentration of HCO_3^- in rumen solutions and plasma in the presence and absence of acetate, together with the changes in amount of bicarbonate (± S.E.), acetate and ammonia in the rumen solution. The plasma samples were taken at the following approximate times:

(*a*) 45 min after putting the solution in the rumen;
(*b*) 5 min before the final rumen sample was taken;
(*c*) 5 min after the final rumen sample was taken.

The corresponding CO_2 concentrations and measured pH are given in Tables 3 and 5. The mean potential is measured between the blood and rumen contents

		HCO_3^- concentration (mM)											
		Rumen solution			Plasma					Loss from rumen solution (m-mole)			
			Final		Carotid artery			Rumen vein					Mean potential
Expt.	Period	Initial	90 min	120 min	(*a*)	(*b*)	(*c*)	(*b*)	(*c*)	HCO_3^-	CH_3COO^-	NH_4^+	(mV)
5	1	22·9	—	23·8	24·1	23·9	—	—	—	−1·8 ± 0·4	0	−1·4	27·2
	2	23·0	33·8	—	24·7	24·5	—	—	—	−21·0 ± 0·4	38	−0·2	25·0
6	1	23·3	—	30·1	—	29·8	—	—	—	−8·6 ± 0·2	17	−0·3	36·7
	2	23·6	—	23·2	30·2	29·2	—	—	—	−0·42 ± 0·24	0	−0·2	28·1
7	1	28·9	—	39·8	25·5	27·3	—	—	—	−18·8 ± 0·4	47	−0·4	30·2
	2	28·8	—	27·9	28·0	27·8	—	—	—	1·9 ± 0·4	0	−0·6	22·9
8	1	16·5	31·3	—	—	30·6	30·3	28·4	28·2	−28·2 ± 0·4	70	−0·4	—
	2	15·1	15·9	—	—	29·6	29·0	29·3	29·0	−1·6 ± 0·3	0	−0·5	—
9	1	16·4	16·8	—	—	27·8	27·9	27·9	27·5	−0·81 ± 0·32	0	−0·8	—
	2	15·7	31·5	—	—	30·2	30·2	26·4	26·0	−31·3 ± 0·4	53	−0·5	—
10	1	24·6	24·5	—	—	—	20·8	—	22·1	0·4 ± 0·7	21	—	—
	2	23·9	22·3	—	—	21·6	21·6	24·2	24·6	5·1 ± 0·6	0	—	—
11	1	16·5	16·5	—	—	22·1	21·4	22·5	22·6	0·3 ± 0·3	8	−0·2	—
	2	15·7	14·0	—	—	22·0	21·7	23·8	23·2	2·8 ± 0·3	0	−0·2	—

TABLE 5. The pH in rumen solutions and plasma in the presence and absence of acetate, together with changes of creatinine concentration. The plasma samples were taken at the following approximate times:

(*a*) 45 min after putting the solution in the rumen;
(*b*) 5 min before the final rumen sample was taken;
(*c*) 5 min after the final rumen sample was taken.

The corresponding CO_2 and HCO_3^- concentrations are given in Tables 3 and 4

		pH								
		Rumen solution			Plasma					
			Final		Carotid artery			Rumen vein		Increase in creatinine concn.
Expt.	Period	Initial	90 min	120 min	(*a*)	(*b*)	(*c*)	(*b*)	(*c*)	(%)
5	1	7·50	—	7·23	7·35	7·35	—	—	—	0·1
	2	7·56	7·52	—	7·39	7·40	—	—	—	−0·9
6	1	7·57	—	7·38	—	7·39	—	—	—	−1·0
	2	7·54	—	7·17	7·41	7·41	—	—	—	−3·2
7	1	7·36	—	7·56	7·37	7·34	—	—	—	0·4
	2	7·35	—	7·26	7·33	7·35	—	—	—	0·7
8	1	7·21	7·48	—	—	7·37	7·36	7·39	7·40	2·0
	2	7·30	7·04	—	—	7·36	7·36	7·35	7·37	0·1
9	1	7·31	7·10	—	—	7·34	7·33	7·33	7·31	−0·1
	2	7·38	7·54	—	—	7·39	7·41	7·35	7·37	−1·7
10	1	7·46	7·31	—	—	—	7·29	—	7·25	0·0
	2	7·53	7·18	—	—	7·33	7·33	7·29	7·29	−0·6
11	1	7·30	7·20	—	—	7·24	7·25	7·23	7·26	−1·2
	2	7·32	6·97	—	—	7·28	7·29	7·25	7·26	−1·8

in the absence of acetate, but the changes in these experiments were rather small. Only two experiments, 7 and 8, gave reasonably reliable changes. These suggest a value for the steady-state concentration lying between plasma concentration and about half this level. The change in Expt. 6 is not large enough to be significant, neither is that in Expt. 9 when allowance is made for imperfections in the volume marker. The change in Expt. 5, though definite, may be associated with the relatively large increase in rumen ammonia in this period. This reservation is discussed in detail below.

The venous–arterial concentration differences for CO_2 are given in Table 6. The difference was small when no acetate was present in the rumen in Expt. 8, and tended to be positive in Expt. 9. However, in the presence of acetate, the venous–arterial difference became negative, especially in the first experiment. Thus the lowering of the rumen steady concentration is associated with a lowering of the venous level. The relative lowering was less on the blood side of the epithelium, but the changes appear to operate in the same sense. It is clear from these observations that the rumen steady concentration of CO_2 does not arise by an equilibrium of the rumen contents with venous or arterial blood.

In the absence of acetate the bicarbonate concentrations in the venous plasma are very close to the arterial levels (Table 4), as might be expected

from the small rumen changes. By contrast, in the presence of acetate the bicarbonate level in the plasma leaving the rumen is lower than that entering; furthermore, bicarbonate is appearing in the rumen. In this instance the changes on both sides of the epithelium are in an opposite sense, as would be expected from a transfer of bicarbonate across the epithelium. These changes in concentration are too large to be due to changes in haemo-concentration. In Expt. 9, in which this effect was checked, the arteriovenous difference in haemoglobin concentration was 2% or less. The final levels of CO_2 concentration both with and without acetate are similar to the previous experiments, but the venous–arterial concentration of CO_2 appears to be greater than the others.

TABLE 6. Summary of CO_2 concentration differences between the final rumen concentration and the plasma, with and without acetate absorption. The final (rumen without acetate)–(with acetate) is corrected for the difference in arterial concentration of CO_2 between the two periods in an experiment

	CO_2 concentration difference (mM)					
	Final rumen–arterial		Final rumen (without acetate)–(with acetate)		Plasma Venous–arterial	
Expt.	Without acetate	With acetate	Uncorrected	Corrected	Without acetate	With acetate
5	0·45	0·09	0·48	0·36	—	—
6	0·59	0·09	0·40	0·50	—	—
7	0·43	−0·16	0·59	0·59	—	—
8	0·25	−0·33	0·54	0·58	−0·01	−0·22
9	0·10	−0·36	0·52	0·46	0·05	−0·08
Mean	0·36	−0·13	0·51	0·50	—	—
10	0·60	0·20	0·34	0·40	0·29	0·22
11	0·52	−0·22	0·60	0·74	0·22	0·06

Depressed acetate uptake

The sheep were starved after the morning feed on the day preceding the experiment, and usually the rumen contents were removed on the morning of the experiment. In Expts. 10 and 11, the last two in Tables 3–6, the treatment was different. The sheep were starved the whole of the day previous to the experiment, and the rumen contents replaced by saline that evening to allow an early start to the experiments the following day.

This treatment appeared to affect the uptake of fatty acid from the rumen. The relation between acetate and bicarbonate movements was also affected, as the uptake of acetate was not accompanied by the appearance of bicarbonate. There does appear, however, to be a much larger uptake of bicarbonate from the chloride solutions than was found in the previous experiments with similar bicarbonate concentrations. Similar results were obtained in two other experiments. One of these received the usual

pre-treatment except that excessive washing of the rumen was necessary in order to remove the wool this sheep had eaten.

Other weak electrolytes

It is possible for any weak electrolyte entering the rumen to act as a hydrogen-ion carrier and it is important to know the possible magnitude of this transport before interpreting pH changes. The amount of lactate, phosphate, and ammonia appearing in the rumen solution in Expts. 1 and 2 increased linearly with time. The results are summarized in Table 7 by giving their mean rate of entry, and further observations on ammonia entry are given in Table 4.

TABLE 7. The appearance of lactate, pyruvate, phosphate and ammonia in the rumen solution

		Rate of loss (m-mole/hr)			
Expt.	Period	Lactate	Pyruvate	Phosphate	Ammonia
1	1	−0·36	−0·02	−0·09	−0·24
	2	−0·30	−0·01	−0·10	−0·90
2	1	−0·45	−0·02	−0·50	−1·15
	2	−0·55	−0·04	−0·20	−0·50

Urea, a possible precursor of the ammonia, was present in all the samples examined from the rumen. In Expts. 5 and 6 samples taken under liquid paraffin were stored overnight at room temperature, and since the urea initially present did not hydrolyse it appeared that urease activity was absent from the samples. However, in the first period of Expt. 7 the urea which appeared in the early part of the absorption period had partially disappeared towards the end, owing possibly to a low urease activity. By contrast to the linear appearance of ammonia, the entry of urea decreased as the concentrations in the rumen increased (Fig. 5). The initial entry of nitrogen in these two forms was −4·8 m-mole/hr in the first period and −2·0 m-mole/hr in the second, while in six other periods it lay between −0·5 and −4·0 m-mole/hr.

Pyruvate was estimated in single samples taken towards the end of each period in Expts. 1 and 2. In calculating the results given in Table 7 it has been assumed that the traces found in the rumen have appeared in proportion to the time.

Pennington (1952) found that ketone bodies did not enter the rumen solutions when acetate was absorbed. In Expt. 1, where there was no fatty acid in the rumen, less than −0·05 m-mole of ketone bodies entered. Thus the effect on the rumen pH of the appearance of acetoacetic and β-hydroxybutyric acids is negligible.

The appearance of steam-volatile fatty acids in solutions with no

acetate present was not followed during an absorption period, as the method used for their estimation was not sufficiently sensitive. Instead, large samples were taken from the solutions remaining at the end of an absorption period; the results are given in Table 8.

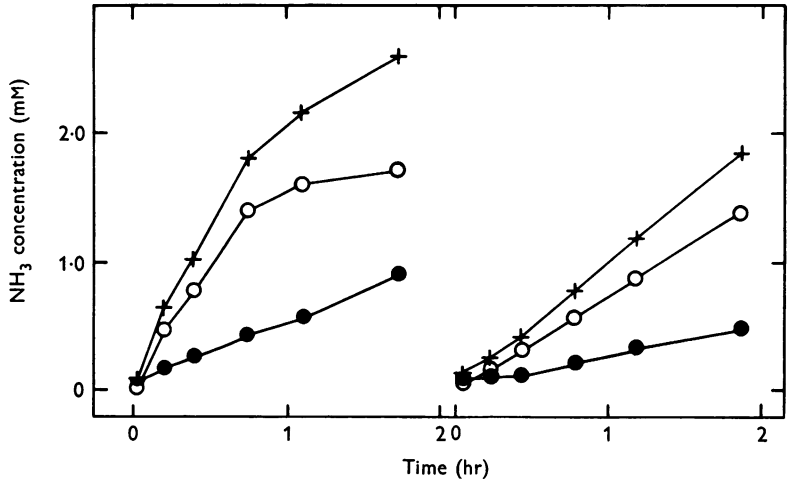

Fig. 5. Expt. 4. The appearance of ammonia and urea in solutions in the rumen. NH_3, ●; urea, as NH_3, ○; urea $+NH_3$, +.

TABLE 8. The appearance of steam-volatile fatty acid in the rumen solution

Expt.	Period	Final concn. (mM)	Final amount (m-mole)	Time (min)	Acetate (%)
2	1	0·31	0·56	115	> 95
4	1	0·44	0·74	116	98
5	1	0·23	0·42	127	99

It is concluded that these acidic weak electrolyte movements will give rise to hydrogen-ion movements less than $-1{\cdot}0$ m-mole/hr, and in most cases the possible contribution of ammonia to hydrogen-ion movements is less than 1·0 m-mole/hr.

The interaction between bicarbonate and acetate movements

The linear relation between the appearance of total CO_2 in the rumen and the absorption of short-chain fatty acids (Masson & Phillipson, 1951) was confirmed for acetate during the course of experiments published elsewhere (Dobson & Phillipson, 1958; Dobson, 1959). Eighteen observations of acetate uptake and bicarbonate appearance in the rumen of six sheep are summarized in Fig. 6. The correlation found between acetate disappearance and bicarbonate appearance is not due to differences in

rumen size in the different sheep. Approximately 0·5 mole of bicarbonate enters the rumen for each mole of acetate leaving it, and there is a suggestion that more bicarbonate enters per mole of acetate uptake when the mean bicarbonate concentration is low.

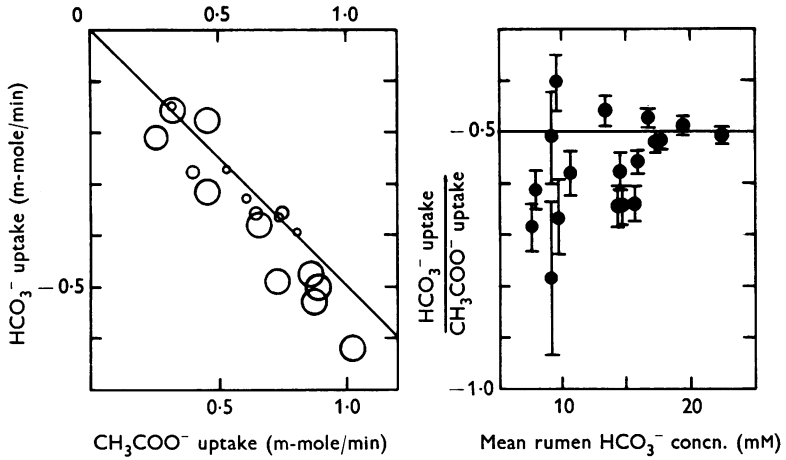

Fig. 6. The relation between acetate loss and bicarbonate gain of solutions in the rumen. The circles in the left-hand diagram and vertical lines on the right-hand diagram represent approximately ± 1 S.E. The lines are drawn to indicate the appearance of 1 mole of bicarbonate for each 2 moles of acetate disappearance.

DISCUSSION

CO_2 and bicarbonate penetration in the absence of acetate

The changes of pH of a solution are determined by the movements of the differently ionized forms of weak electrolytes into and out of the solution, if we assume that the ionization products of water may be regarded as weak electrolytes. In these experiments the major weak electrolytes present are CO_2 and bicarbonate, with small concentrations of metabolic products, and traces of hydrogen, hydroxyl and carbonate ions. It is also assumed that carbonate plays no part in pH changes in the rumen.

The contribution of metabolic products to the system can, for convenience, be reduced to their ability to carry hydrogen ions in or out of the system. Further, as the movement of hydroxyl ions in one direction is indistinguishable from a movement of hydrogen ions in the opposite direction, it is unnecessary to consider movements of this ion separately.

With these simplifying assumptions the system can be considered in terms of movements of bicarbonate, CO_2 and hydrogen ions. The CO_2 and bicarbonate can be considered to be independently variable quantities at the pH and total CO_2 concentration encountered (Warburg, 1922). For

instance, when CO_2 enters the solution the small amount of bicarbonate formed when it ionizes can be neglected. The movements of CO_2 and bicarbonate were down their concentration gradients when the gradients across the rumen epithelium were large. These movements can take place in either direction starting from the same pH. Both CO_2 and bicarbonate movements will therefore participate in the pH changes in the rumen solutions.

Rumen steady-state concentrations

Before these investigations were started it was assumed that CO_2 would equilibrate in the rumen at its plasma level, whereas bicarbonate would equilibrate so that its electrochemical gradient across the epithelium vanished. With the electrical potential making the plasma 30 mV positive to rumen contents the bicarbonate concentration in the rumen would, therefore, tend towards about one-third of its plasma concentration.

Neither of these assumptions proved correct, since the rumen steady-state concentrations of both CO_2 and bicarbonate were higher than had been expected. The steady-state concentration of CO_2 was about 0·4 mM higher than plasma and the bicarbonate steady-state concentration lay between the plasma concentration and half this level, although it could not be defined with any precision owing to its slow rates of entry and leaving near the equilibrium concentration.

Tissue respiration. A simple explanation of the raised steady concentration of CO_2 can be given by considering the effect of CO_2 production within the epithelium of the rumen. When no CO_2 passes into or out of the rumen solution, all the metabolic CO_2 produced by the epithelium must pass into the plasma. If this is diffusing down its concentration gradient, the concentration of CO_2 will rise as the epithelium is traversed from plasma to rumen contents and the concentration within the rumen solution must be the same as that just within the epithelium adjacent to the rumen contents, because there is no passage into the rumen. Hence the steady rumen concentration must be greater than the plasma. A similar situation has been observed with the epidermis which equilibrates with 8·5% CO_2 (Shaw & Messer, 1931).

Hydrogen ion movements. A raised steady-state concentration of CO_2 would result from the addition of hydrogen ions to the rumen solution. However, this process would lower the bicarbonate steady-state concentration, whereas it is observed to be higher than expected. In addition, a suitable mechanism for the transfer of hydrogen ions is not apparent. Simple diffusion would not account for the addition of hydrogen ions as, at times, the movement would be against its electrochemical gradient. The dissociation of metabolic acids would account for only about half the

necessary rate of addition of hydrogen ions, even if they appeared in the rumen solution solely in the un-ionized form. A third possibility, namely the forced exchange of sodium with hydrogen ions, would give rise to the appearance of ten times too much hydrogen ion. It may be mentioned that similar quantities of sodium are taken up from sodium chloride as from sodium acetate (A. Dobson, unpublished observations).

Transfer of hydrogen ions from plasma to rumen contents thus seems unlikely to play any major part in the equilibrium under discussion. This conclusion is in keeping with some observations on the pH of blood draining the rumen. When the rumen pH is low, no depression of the blood pH is noticed unless a readily penetrating anion is present (Dobson & Phillipson, 1956).

Ammonia and urea. Both ammonia and urea appear in the solution in the rumen, but urease activity in the solution was very low. Nevertheless, as normal rumen contents have a high urease activity (Pearson & Smith, 1943) as little as 20 ml. of contents would account for the observed ammonia production. This volume could easily remain within the outermost desquamating layer of epithelium. Sufficient activity could thereby survive the washing treatment to account for the ammonia found.

The ammonia production in the rumen may account for the raised steady-state concentration of bicarbonate. If the ammonia arises from urea, the over-all reaction in the presence of bicarbonate buffers would be:

$$(NH_2)_2CO + CO_2 + 3H_2O \rightarrow 2NH_4^+ + 2HCO_3^-.$$

For every mole of ammonia produced, a mole of bicarbonate is formed. A similar production of bicarbonate would occur if the ammonia diffused into the rumen solution in the un-ionized form. It may be noted that the CO_2 consumption which would accompany either of these mechanisms would depress the steady-state concentration of CO_2 only a small amount, about 0·1 mM. A similar estimation of the effect of the bicarbonate production on the bicarbonate steady-state concentration cannot be made, as the equilibration of bicarbonate shows no sign of being exponential.

There is a similarity between the initial rate of entry of ammonia + urea in the present work and the rate at which ammonia entered the rumen in the experiments of Houpt (1959). Since Houpt could not detect urea in the rumen solutions it is possible that he encountered a high urease activity. The decrease in rate of entry of urea in the present experiments with time is possibly due to the increase in urea concentration in the rumen.

It is concluded that the experimental results are concordant with the hypothesis that the epithelium of the rumen is permeable to CO_2 and bicarbonate and that exchanges of hydrogen ion are not important. The production of CO_2 within the membrane raises the steady-state concen-

tration of CO_2 above that of the plasma. Small amounts of ammonia appearing in the rumen solution may explain the equilibration of bicarbonate at a higher concentration than its electrochemical gradient would allow.

The effect of acetate absorption

About half the uptake of acetate from the rumen is accompanied by strong electrolyte absorption (Dobson, 1959). This part of the acetate is therefore leaving the rumen solution as the anion. The other part of the acetate uptake exchanges for bicarbonate in the over-all reaction, but

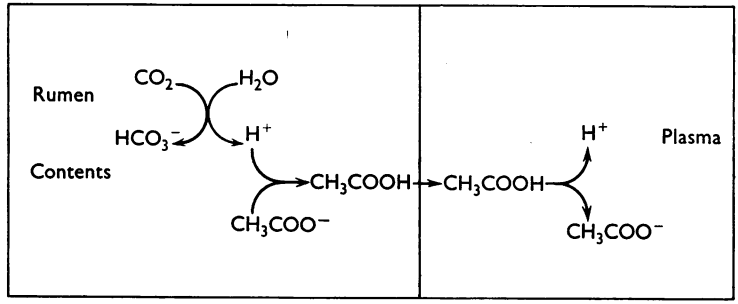

Fig. 7. The effect of the selective absorption of un-ionized acetate upon CO_2 and HCO_3^- in the rumen. The position of the membrane within the layers between plasma and rumen contents is not defined. Un-ionized acetate diffuses through the barrier down its concentration gradient. It ionizes continuously on the plasma side of the membrane, producing hydrogen ions and acetate. On the rumen contents side of the membrane the reverse process takes place. The supply of hydrogen ion is maintained by the conversion of CO_2 to HCO_3^-.

a direct exchange of the two anions seems unlikely. Such a mechanism does not account for the alteration in the steady-state concentration of CO_2 when acetate is absorbed. In addition, although there is evidence that both anions penetrate by a non-specific mechanism, a close coupling between the two anion fluxes must be postulated in order to allow the movement of acetate to raise the steady-state concentration of bicarbonate.

Differences in the steady-state levels of bicarbonate and CO_2 cannot be due to differences in the rate of appearance of metabolic acids, phosphate or ammonia, or in the potential across the rumen epithelium. Although the steady-state concentration of CO_2 would be lowered by an inhibition of CO_2 production within the epithelium, it could not be depressed below the plasma level. It is necessary that there should be a consumption of CO_2 for this to happen.

The simultaneous lowering of the steady-state concentration of CO_2 and raising of that of bicarbonate when acetate is present suggests a removal of hydrogen ions at some place within the epithelium or rumen

solution. If at least one membrane between the rumen solution and plasma were very much more permeable to the un-ionized fatty acid than its anion, this would give rise to a consumption of hydrogen ion as the un-ionized fatty acid passed across the membrane down its concentration gradient (Fig. 7). The bicarbonate produced would then be equivalent to the free fatty acid passing through this barrier. The observed bicarbonate appearance associated with fatty acid uptake in any absorption period must be corrected for bicarbonate diffusion across the epithelium in order to give the bicarbonate produced by the movement of free acid. It is not possible to give an estimate free of criticism for the correction for diffusion. The data relevant to this are given in Table 9 and in Fig. 6.

TABLE 9. The mean amount of bicarbonate appearing for the disappearance of 1 mole of acetate for different concentrations of bicarbonate

	Rumen HCO_3^- concn.		
Source	No. of observations	Range of mean (mM)	$\frac{\Sigma\Delta HCO_3^-}{\Sigma\Delta Ac^-}$
Fig. 1	7	7·5–10·5	−0·61
Fig. 1, Expt. 2	7	13·2–15·6	−0·59
Fig. 1	5	16·6–22·4	−0·50
Expts. 5–9	5	23·5–34·4	−0·48
Expts. 3, 4	2	74·6–77·7	0·00

The amount of bicarbonate appearing per mole of acetate loss is calculated for different experiments grouped according to the mean bicarbonate concentration during the period. The mean of several observations is taken to reduce the scatter with appropriate weighting for the precision of each observation. In Expts. 5–9 the bicarbonate movement in the absence of acetate was small. When acetate was present the diffusion correction for bicarbonate will thus be small, and tend to reduce the bicarbonate appearance, because the concentration of bicarbonate increased during the absorption period when acetate was present. Experiments 5–9 give a value of −0·48. The correction would be in the opposite direction for the group of observations with a mean rumen bicarbonate concentration of 7·5–10·5 mM, as this is below the equilibrium level for bicarbonate in the absence of acetate. This group gives a value of −0·61. The corrected value cannot, therefore, be far different from −0·5.

It is concluded that approximately half the acetic acid absorbed from the rumen is leaving in the un-ionized form and half as the anion, even though only about 0·5 % of free acid is un-ionized at this pH. Because the concentration of free acid is low, below 1 mM, a boundary within the epithelium must be much more permeable to the free acid than to the anion. In the past it has been argued that the proportion of free acid is

reduced to negligible proportions at neutral pH, and the permeability of the rumen wall to the anion alone can be studied (Danielli *et al.* 1945). This assumption now appears unjustified.

When a solution free of fatty acid is placed in the rumen, acetate passes into it; the concentration and composition of the steam-volatile fatty acid in the rumen is similar to that observed in the plasma under these conditions in the rumen (Kiddle, Marshall & Phillipson, 1951; Masson & Phillipson, 1951). This observation supports the idea that fatty acid passes passively across the rumen epithelium.

The results presented cannot be used to infer that the equilibration of CO_2 in the presence of acetate is slower than in its absence as appears in Expt. 4 (Fig. 3). It is well known that the rate of absorption of fatty acids decreases in successive absorption periods in acute experiments (Masson & Phillipson, 1951). This makes a comparison of rate of absorption between periods of dubious value.

The neutralization of rumen contents

The acids which are formed by the fermentations in the rumen are neutralized by the plasma in two ways. First, by the production of an alkaline saliva which is poured into the rumen contents down the oesophagus; secondly, by the selective absorption of acid through the rumen epithelium, where it is neutralized by the plasma through the mediation of the tissue buffers.

Since a large part of the fatty acid produced in the rumen is absorbed in the rumen and omasum, and providing the epithelium of the omasum behaves in a manner similar to that of the rumen, a little under half the total fatty acid produced will thus be absorbed in the un-ionized form at neutral rumen pH. At a lower pH, when the proportion of un-ionized fatty acid in the contents is increased, it seems reasonable to suppose that a greater proportion of fatty acid would be absorbed in the un-ionized form.

A rough estimate of the ability of the saliva to neutralize fatty acid can be made from the data of Kay (1960). The total volume of the parotid, inferior molar, palatine, buccal and pharyngeal salivas secreted each day is between 5·7 and 16 l. The secretions of these glands are similar in their bicarbonate and phosphate content, and other salivary secretions will have negligible neutralizing power. Kay found the mean concentration of bicarbonate was 112 mM and phosphate 24 mM in parotid saliva collected over 24 hr, and the following calculation is therefore based on this composition.

The pK'_1 of carbonic acid was taken as 6·1, the pK'_2 of phosphoric acid as 6·85 and the pK′ of fatty acid as 4·65. As the partial pressure of CO_2

within the rumen is normally about 50 %, it was further assumed that the CO_2 concentration was held constant at 12 mM by CO_2 production and absorption. This last assumption, which is necessary to represent conditions in the rumen, makes the neutralization ability of the saliva dependent on its concentration, especially at pH of 6·5 or above. This complication has been ignored. It can then be calculated that 10 l. of saliva, a reasonable daily output, will bring 1 mole of fatty acid to pH 6·5; 1·3 mole to pH 6; 1·5 mole to pH 5·5, 1·9 mole to pH 5·0 and 3·3 mole to pH 4·5.

The daily production of fatty acid in the rumen of a sheep on a maintenance level of nutrition is estimated to be 3·2 mole/day (Blaxter, 1962). Since the pH of the normal rumen contents is about 6·5, there must exist ways for the neutralization and removal of acid other than by saliva. On this basis about 2 mole/day fatty acid would be absorbed in the un-ionized form.

Depressed fatty-acid uptake

Treatments which can depress the uptake of fatty acid from the rumen include cannulation during the course of an experiment (Masson & Phillipson, 1951) and starvation (Armstrong, Blaxter & Graham, 1957) in addition to those described here, namely leaving the rumen filled overnight with NaCl, and excessive washing. The decreased absorption encountered in successive periods in anaesthetized sheep (Masson & Phillipson, 1951) may also be a related phenomenon. The previous treatment of the epithelium appears to affect its absorption properties.

An understanding of the mechanism of this depressed uptake is desirable, because it may form a basis for investigating the control of absorption. In addition, the lack of information concerning such phenomena must be remembered whenever the results from experimental conditions are extrapolated to the normal sheep.

The loss of the relation between fatty-acid uptake and bicarbonate appearance may be more apparent than real. There is a greater uptake of bicarbonate with chloride solutions in those experiments in which depressed fatty-acid uptake was observed than in those with more normal uptake. Thus when acetate is present and is taken up at the reduced rate we may be seeing the net result of an appearance of bicarbonate due to fatty-acid uptake, and its loss due to absorption. This accords with the observation that steady-state concentration of CO_2 is similar between the experiments with normal and depressed fatty-acid uptake.

Physiological phenomena

It is well known that in many biological systems the stimulatory properties of a solution at low pH depend on the anions present at a given hydrogen ion concentration and buffer molarity. To cite examples en-

countered in the rumen, there are the effects on gastric motility (Ash, 1956, 1959), and the stimulation of the saliva secretion (Ash & Kay, 1959) and rumen venous blood flow (Dobson & Phillipson, 1956).

Since the penetration of free fatty acid at neutral pH has been demonstrated, it is possible that the effects of different buffers depend on the ability of the un-ionized acid to penetrate. When the penetration of the un-ionized acid is possible, the buffer would be able to reduce the pH beyond the selective membrane even though the membrane is relatively impermeable to hydrogen ions.

SUMMARY

1. The epithelium of the reticulo-rumen sac is permeable to both carbon dioxide and bicarbonate ion.

2. The steady-state concentration of carbon dioxide in the rumen in the absence of fatty acid is above the concentration in the plasma. The steady-state concentration is lowered when acetate is present, and can be depressed by acetate below the plasma concentration.

3. The steady-state concentration of bicarbonate in the rumen in the absence of fatty acid is difficult to define, but is probably below the plasma concentration. The steady-state concentration is raised to several times the plasma concentration when acetate is present.

4. The uptake of fatty acid from the rumen is accompanied by a consumption of CO_2 and production of bicarbonate within the rumen solution, due to the penetration of un-ionized fatty acid. Near neutrality about half the fatty acid leaves in the un-ionized form. This allows the plasma to neutralize the fatty acids produced in the rumen through the mediation of tissue buffers. The amount of fatty acid neutralized directly in this way appears to be similar to the amount neutralized by the saliva.

5. The passage of small quantities of ammonia, lactate, phosphate and acetate into the rumen contents has been observed. Pyruvate and ketone body appearance is negligible.

We wish to thank Dr A. T. Phillipson for his advice and encouragement throughout this work. Mrs Madeleine Duncan, Mr J. Dunnett, Mr G. Main and Mr L. E. Vowles are thanked for their skilled technical assistance, Mr R. S. Reid for the gas chromatography estimation, and Mr J. Appleton for the ketone body determination.

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