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THE RATES OF ABSORPTION OF ACETIC, PROPIONIC AND *n*-BUTYRIC ACIDS

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Numerous experiments on the absorption of acetic, propionic and *n*-butyric acids have provided considerable information on the rates at which individual acids disappear from the rumen under the conditions of the experiment and of the concentrations appearing in the blood stream, but none of these experiments simulates normal conditions sufficiently to allow deductions to be made of the quantities of these acids normally absorbed.

Recently, Johnson (1951) studied the concentrations of these acids in the rumen of fasting goats and sheep after the administration of large quantities of propionic and *n*-butyric acids, and came to the conclusion that butyric acid was absorbed in greater quantity than propionic acid. It is known that more butyrate can be absorbed from the rumen than can be accounted for by the butyrate entering the blood stream (Masson & Phillipson, 1951), and this is due to metabolism of butyrate by the rumen epithelium (Pennington, 1952) which *in vitro* is more pronounced than metabolism of propionate or acetate. The experiments described here were designed to study (*a*) the absorption of these three acids quantitatively by introducing a steady inflow of a solution of these acids at a suitable pH and in amounts that would maintain the concentrations in the rumen at physiological levels, and (*b*) to see whether the information so gained could be used to determine the rates of absorption in unanaesthetized sheep that were fasting or feeding.

METHODS

Experimental

One-year-old Scottish Blackface sheep, with the rumen previously cannulated, were used. The sheep were starved for 18 hr prior to the experiment to facilitate the removal of food from the rumen. The rumen was emptied and washed clean with warm saline. The sheep were anaesthetized by the intravenous injection of pentobarbitone, and the rumen was isolated in the manner described by Danielli, Hitchcock, Marshall & Phillipson (1945).

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The solution introduced into the rumen was Krebs's bicarbonate Ringer in which NaCl was substituted by sodium acetate, propionate and *n*-butyrate to give concentrations of 63 ± 9, 21 ± 3, and 15 ± 3 m.equiv/l. These concentrations are within the range usually found in the rumen. A similar solution, except that it contained different proportions of the three acids, was introduced into the rumen from a burette at the rate of 1 ml./min. The concentrations of acids in this solution were adjusted as the experimental series progressed until the concentrations in the rumen were steady throughout the experimental period. In this way the rate of introduction of acid served as an index to the rate of absorption. The solution in the rumen was gassed with carbon dioxide at 15 min intervals throughout the experimental period.

In later experiments comparisons were made between solutions at neutral and acid pH values, and between the presence or absence of inorganic ions and carbon dioxide.

Experiments on two unanaesthetized sheep were conducted in a metabolism cage. Both were accustomed to standing in the cage. The solution, containing fatty acids in proportions similar to their rate of absorption in the acute experiment, was introduced into the rumen by connecting a reservoir held 6 ft. above the cage to the rumen cannula by means of polythene tubing. The rate of flow was regulated by Mohr clamps and a drop counter to 60 ± 2 ml./hr.

Chemical

Fatty acids were separated from rumen contents or from saline solutions by steam distillation in the presence of an equal volume of saturated magnesium sulphate solution containing 2·5% (v/v) concentrated sulphuric acid. Individual acids were separated by the gas-liquid chromatographic technique of James & Martin (1952). Recovery of individual acids was 95% or over.

RESULTS

The course of one experiment is given in Table 1. This experiment was the fourth of the series, and the rate at which acids were introduced was very close to the total quantity found to be absorbed. The concentrations of the three

TABLE 1. The absorption of acetic, propionic and butyric acids from the washed, isolated rumen of a 20 kg sheep

Time	Volume (ml.)	pH	m.equiv acid in rumen: Acetic	Propionic	Butyric	Total
0	3000	5·7	183	62	55	300
2 min	3000*	5·8	183	60	53	296
1 hr	3044†	5·7	185	65	56	306
2 hr	3098†	5·6	186	68	59	313
3 hr	3144†	5·6	187	73	64	324
4 hr	3200	5·8	188	76	69	333
4 hr	1000 (washings)	—	—	—	—	5
Rate of introduction of fatty acids (m.equiv/hr)			33	20	25	78
Mean absorption (m.equiv/hr)			32	16	21	69

* Assumed.

† Volume calculated assuming a uniform increase throughout the experimental period.

acids in the rumen showed very little variation throughout the 4 hr. The total left at the end of the experiment was 338 m.equiv while 312 m.equiv were added during the 4 hr to the 300 m.equiv introduced at the start of the experiments so that 274 m.equiv were absorbed. The rate of introduction—78 m.equiv/hr—was close to the rate of absorption, namely 68·5 m.equiv/hr.

The actual losses from the rumen per hour in order of magnitude were found to be acetic > butyric > propionic. It is interesting to compare the ratio of the acids in the rumen at the start of the experiment with ratios in which the acids disappeared from the rumen; thus the propionic/acetic ratios were 1 : 2·9 and 1 : 2·0 for the rumen and for the rate of disappearance respectively; the ratios butyric/acetic were 1 : 3·3 and 1 : 1·5 respectively. This is a clear indication that the higher acids disappear at rates that are disproportionate to their concentrations when compared to acetic acid. It also shows that the low concentration of butyric acid in the rumen is no indication of its relative importance.

TABLE 2. The absorption of acetic, propionic and butyric acids from the washed, isolated rumen of sheep in the presence of Krebs's bicarbonate saline and carbon dioxide when these acids were introduced at different rates

Expt. no.	Wt. sheep (kg)	Rate of introduction of acids (m.equiv/hr) Acetic	Propionic	Butyric	Duration of expt. (hr)	pH of rumen contents	Mean absorption (m.equiv/hr) Acetic	Propionic	Butyric	Total
1	29	9	27	62	3	5·6–5·8	22	18	42	82
2	26	16	16	31	3	5·6–5·8	31	17	27	75
3	24	18	12	28	4	5·6–5·8	34	14	22	70
4	20	33	20	25	4	5·6–5·8	32	16	21	69

TABLE 3. The absorption of acetic, propionic and butyric acids from the washed, isolated rumen of sheep at different pH or in the presence or absence of Krebs's bicarbonate saline and carbon dioxide

Expt. no.	Wt. sheep (kg)	Composition of the solution in rumen apart from fatty acids	Rate of introduction of acids (m.equiv/hr) Acetic	Propionic	Butyric	Duration of expt. (hr)	pH of rumen contents	Mean absorption (m.equiv/hr) Acetic	Propionic	Butyric	Total
5	26	Saline + CO_2	33	16	26	2	6·5	34	9	13	56
6		Saline + CO_2, acidified with HCl				2	5·8	31	16	23	70
7	34	No saline or CO_2 but acidified with HCl	31	16	25	2	5·0	40	24	33	98
8		Saline + CO_2	32	17	24	2	6·4	0	13	8	21
9	29	No saline or CO_2	36	18	19	1½	6·0	50	35	25	110
10		Saline + CO_2, acidified				1½	6·0	48	17	18	83

The results of a series of four similar experiments, including the one presented in detail, are given in Table 2. The high rate of absorption of butyric acid found in the first experiment was due to the high proportion of butyric acid in the solution introduced into the rumen so that the concentration rose to 2·5 times its original value. Otherwise approximately the same rates of absorption occurred in spite of the differences in the rates of introduction of the acids. In a second series of experiments given in Table 3 a comparison of the rates of absorption at two levels of pH showed an increased rate of absorption at an acid pH. The rate increased more with butyric acid than with propionic acid, which is in accordance with the earlier work of Danielli *et al.* (1945).

In addition, the presence of Krebs's bicarbonate Ringer and carbon dioxide appeared to have a depressing effect on absorption, particularly of propionic and butyric acids. In both experiments in which the inorganic ions were omitted the rate of absorption of propionic acid was greater than in any of the other experiments. An alteration of pH in the first of these two experiments could have produced this result, but in the second the pH of the solution was the same and the same depression was found. The complete cessation of absorption of acetic acid that occurred in the second of these experiments is interesting but cannot be explained. The fact that it can occur, however, while absorption of the remaining two acids continues even at a reduced rate is worth recording.

Two experiments were done on unanaesthetized sheep in which a solution of tbe fatty acids was introduced over a 24 hr period while the sheep was feeding normally and later over a shorter period following a 24 hr fast. The results are shown in Fig. 1. During a control period before the experiment, water was introduced into the rumen at the same rate and quantity as the fatty acid solution, in order to study the normal feeding variation of fatty acid. The sheep were fed 1 lb. hay at 7.30 a.m. and 4 p.m., and 0·5 lb. concentrates at 11 a.m. Zero time in Fig. 1 is immediately before the feeding of hay at 7.30 a.m. In Expt. 1 the solution introduced was a mixture of redistilled fatty acids. In Expt. 2 a mixture of fatty acids and their Na salts (pH 5·8) was introduced.

Both experiments show that the continual introduction of either free fatty acids or a buffered solution of fatty acids and their Na salts into the rumen, although causing a considerable increase in the fatty acid concentrations when compared to the control period, did not increase it outside the range that can occur in the rumen under normal feeding conditions (Phillipson, 1942; Schambye, 1951; El-Shazly, 1952). In both experiments a rise in the concentration of fatty acids occurred towards the end of the period of introducing the acids. The rate of introduction in the first experiment was 45 m.equiv/hr and in the second 55 m.equiv/hr. These quantities superimposed upon the fatty acid produced as a result of the fermentative processes within the rumen were too great for the sheep to tolerate indefinitely, and if continued would clearly have given rise to abnormally high concentrations in the rumen. An abrupt rise in total fatty acid occurred in the first experiment on introducing the fatty acid solution into the rumen during fasting. The rumen became very acid and the sheep showed signs of discomfort. A similar, but smaller rise, was observed in the second sheep which received a solution of fatty acids and then Na salts buffered to pH 5·8 during the fasting period.

The most interesting features of these experiments were the proportions of acid in the rumen. The data for both experiments are given in Table 4. The composition of the solution of acids introduced in the first experiment was

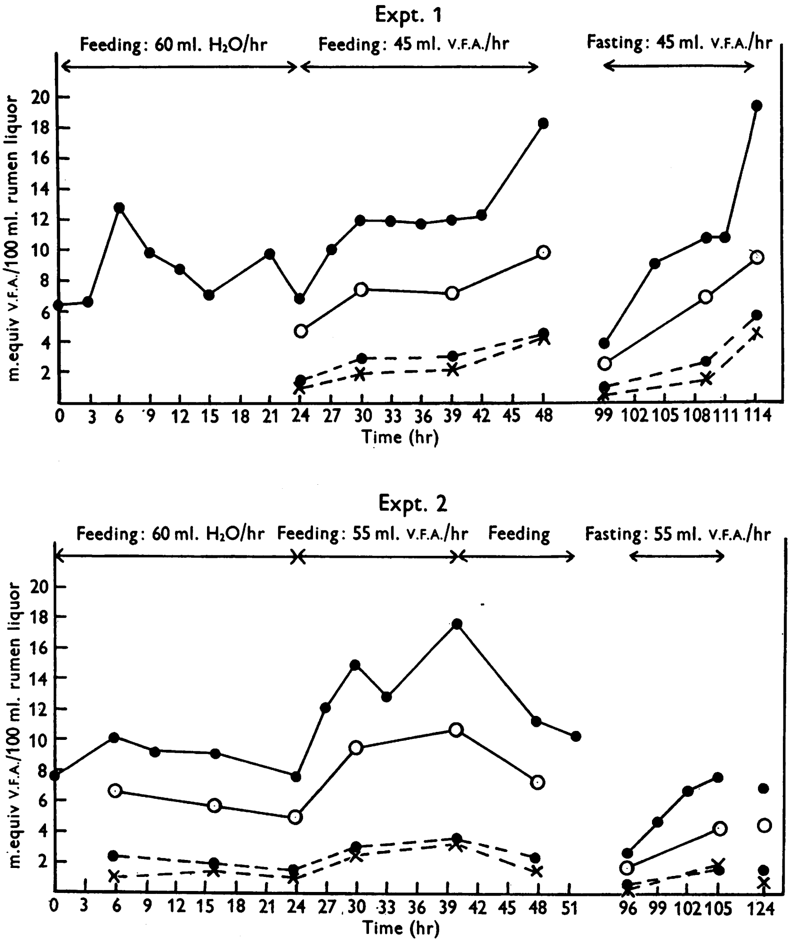

Fig. 1. The concentrations of total volatile fatty acids (V.F.A.) and individual acids in the rumen liquor of sheep during feeding and fasting when water or a solution of the fatty acids (V.F.A.) containing 1 m.equiv in 1 ml. is introduced into the rumen. ●—●, V.F.A.; ○—○, acetic acid; ●- - -●, propionic acid; × - - - ×, butyric acid. Expt. 1: The fatty acid solution contained acetic 38%, propionic 27% and butyric 35%. Expt. 2: The fatty acid solution contained acetic 43%, propionic 21% and butyric 36%.

38 : 27 : 35 expressed as a percentage of the total molecules for acetic, propionic and butyric acids respectively. The proportion of butyric and propionic acids increased during the feeding period but butyric acid remained the smallest fraction in the rumen. It rose, however, to 29% of the total during fasting.

During feeding and fasting the proportions of acids tended to move towards the composition of the mixture introduced, showing that it contained rather too much of the higher acids.

TABLE 4. The changes in total volatile acid, pH and individual acids during periods in which a fatty acid solution or water was introduced into the rumen continuously during feeding or fasting conditions

Treatment of sheep	Hours	Total volatile acid (m.equiv/ 100 ml.)	pH of rumen contents	% of total: Acetic	Propionic	Butyric	Higher acids
Expt. 1							
Feeding with introduction of 60 ml. water/hr, 0–24 hr	0	6·4	6·4	68	19	13	—
	24	6·6	6·4	68	19	13	—
Feeding with introduction of 45 m.equiv fatty acid/hr, 24–48 hr	30	11·8	5·8	62	23	15	—
	39	11·9	5·4	59	24	17	—
	48	18·3	4·8	53	24	23	—
Fasting* with introduction of 45 m.equiv fatty acid/hr, 99–114 hr	99	3·7	6·9	65	23	12	—
	109	10·7	—	63	24	13	—
	114	19·3	4·0	48	23	29	—
Expt. 2							
Feeding with introduction of 60 ml. water/hr, 0–24 hr	6	10·1	6·1	65	23	10	
	16	9·1	6·2	61	21	16	2
Feeding with introduction of 55 m.equiv fatty acid/hr, 24–40 hr	24	7·6	6·5	65	20	12	2
	30	15·1	6·4	63	20	16	1
	40	17·8	5·9	60	20	18	2
Introduction of fatty acids, food available	48	11·3	6·4	65	21	12	2
Fasting† with introduction of 55 m.equiv fatty acid/hr, 96–105 hr	96	2·6	7·1	67	20	13	0
	105	7·8	6·5	55	21	24	0
Feeding	124	7·0	6·1	65	23	12	2

* The sheep was fed between 48–72 hr and food was withheld from 72 hr until the end of the experiment.
† Food was withheld from the 52nd to the 105th hour. The sheep was then fed.

In the second experiment the proportion of acetic acid in the solution introduced was increased and the proportions were 43 : 21 : 36 for the three acids. During the 16 hr feeding period the proportion of the acids in the rumen remained remarkably constant, but during the shorter fasting period the proportions again shifted towards those of the solution introduced, although the changes were not so pronounced as in the previous experiment.

DISCUSSION

These experiments do not contradict the earlier work of Danielli *et al.* (1945) and Gray (1947, 1948) that the rate of disappearance of free fatty acid from the rumen increases with the length of the hydrocarbon chain, and they provide useful information on the quantitative significance of this fact when physiological concentrations are maintained in the rumen at a pH of 5·6–6·5. Considerable emphasis has in the past been placed upon the influence of pH on the rates of absorption of these acids from the rumen: these experiments show that concentration has an even greater influence on the quantities of individual acids disappearing from the rumen. Acetic acid was absorbed in greatest quantity owing to its concentration and although butyric and, to a less extent, propionic acids were absorbed at rates that were relatively more rapid than acetic they were not absorbed in greater quantity. Our general conclusion is that the quantitative order of absorption from the concentrations usually present in the rumen of both anaesthetized and unanaesthetized cannulated sheep at a pH of 5·6–6·5 is as follows: acetic > butyric > propionic, a result predicted by Danielli *et al.* (1945) from calculations based on data available for hay-fed sheep.

Recently Gray & Pilgrim (1951), from a comparison of the ratios of these three acids in the rumen of sheep fed wheaten and lucerne hay, concluded that propionic acid had the greatest relative rate of absorption and that the relation of butyric and acetic acids differed for the two different rations they fed. The ratio of any two acids to each other depends upon their respective rates of production and of absorption. Considering the diverse nature of the substrates present in fodders such as these, it is probable that short chain fatty acids are produced at differing rates in the rumen; in addition, as the concentrations of the acids in the rumen changed so the rates of absorption almost certainly varied, for there is evidence to show that this is so when acetate is present in the rumen (Masson & Phillipson, 1951; Parthasarathy & Phillipson, 1953). The interpretation of a ratio based on two variables can only be tentative.

The quantities of acids absorbed in these experiments appeared to be influenced by the presence or absence of Krebs's bicarbonate saline gassed with carbon dioxide while the apparent absorption of these acids from the rumen of fasting sheep was less than from feeding sheep. The studies of Gray, Pilgrim, Rodda & Weller (1952) showed that rumen bacteria can cause the transmutation of ^{14}C in the carboxyl groups of acetic and propionic acids to butyric and valeric acids respectively. Even so, the changes in activity which they found do not suggest that this could not account quantitatively for the results found in these experiments, in which 45 and 55 m.equiv acid/hr were introduced into the rumen of sheep. If, in the light of Pennington's work (1952), one makes the assumption that absorption of these acids is related to their metabolism by the rumen epithelium, it is possible to suppose that the absorption can be altered or inhibited by the flow of other substrates from the rumen that are not fatty acids, and the possible role of inhibitors cannot be overlooked. The only sure way at present available of determining quantitatively the rate of absorption from the rumen is the use of the isolated rumen of the anaesthetized sheep. The method devised by one of us (W.H.P.) of comparing the results of acute experiments with those obtained in unanaesthetized sheep is a useful check in relating the one to the other, and demonstrates that the use of pentobarbitone as an anaesthetic does not radically alter the normal course of events.

The experiments emphasize the importance of butyric acid. Taking the mean absorption rate for the acute experiments—acetic 32, propionic 18, and butyric 23 m.equiv/hr—and converting these values to kilogram calories we find that of the total energy of 25·3 kcal/hr, 6·7 are represented by acetic acid, 6·6 by propionic acid, and 12·0 by butyric acid. The proportion of butyrate reaching the venous blood stream is small in comparison to the proportion in the rumen (Kiddle, Marshall & Phillipson, 1951). Metabolism of butyric acid by the rumen epithelium (Pennington, 1952) and the production of ketone bodies by these tissues therefore is of considerable physiological significance.

SUMMARY

1. The quantities of acetic, propionic and *n*-butyric acids absorbed from physiological concentrations of these acids in the rumen of anaesthetized sheep at pH 5·6–6·5 and in the presence of Krebs's bicarbonate saline solution and carbon dioxide were in the order of acetic > butyric > propionic acids.

2. The rate of absorption of these acids was influenced by the presence of Krebs's bicarbonate saline and carbon dioxide.

3. The quantities in which these acids disappeared from the rumen of feeding sheep, as far as could be judged by the constancy of their relative proportions, were in the same order.

4. The rate of disappearance of acetic, propionic and butyric acids from the rumen of fasting sheep was slower than from feeding sheep.

5. Butyric acid forms the largest source of energy in the form of fatty acids absorbed from the rumen.

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