Moore, J. H. & Tyler, C. (1955*a*). *Brit. J. Nutr.* **9**, 63.
Moore, J. H. & Tyler, C. (1955*b*). *Brit. J. Nutr.* **9**, 81.
Moore, J. H. & Tyler, C. (1955*c*). *Brit. J. Nutr.* **9**, 389.
Mueller, W. J. (1956). *J. Nutr.* **58**, 29.
Pigden, W. J. & Brisson, G. J. (1956). *Canad. J. agric. Sci.* **36**, 146.
Raymond, W. F. & Minson, D. J. (1955). *J. Brit. Grassl. Soc.* **10**, 282.
Schneider, W. (1955). *Z. Tierernähr.* **10**, 269.
Schürch, A. (1952). *Mitt. Lebensm. Hyg., Bern*, **43**, 335.
Schürch, A. F., Crampton, E. W., Haskell, S. R. & Lloyd, L. E. (1952). *J. Anim. Sci.* **11**, 261.
Smith, A. M. & Reid, J. T. (1955). *J. Dairy Sci.* **38**, 515.
Tyler, C. (1946). *J. agric. Sci.* **36**, 275.
Vartiovaara, U. & Roine, P. (1942). *Maataloust. Aikakausk.* **14**, 127.

Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets

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It has been known for many years that organic acids are produced by bacterial fermentation in the rumen. The importance of such fermentation in the nutrition of the ruminant has been adequately appreciated only recently, with the establishment of the relative proportions in which the acids are produced and the discovery of their role in ruminant metabolism.

Elsden (1945) showed that the production of a mixture of acetic and propionic acids, a butyric isomer and traces of a valeric isomer was characteristic of fermentation in the sheep's rumen. Acetic acid was present in greatest amount and the proportion of propionic acid usually exceeded that of butyric. Ample confirmation with a wide variety of diets has since been obtained by Elsden, Hitchcock, Marshall & Phillipson (1946), Schambye & Phillipson (1949), Gray, Pilgrim & Weller (1951), Kiddle, Marshall & Phillipson (1951), Gray, Pilgrim, Rodda & Weller (1952) and El-Shazly (1952). Gray & Pilgrim (1951) reported that as the concentration of total volatile fatty acids in the rumen increased, there was a decrease in the ratio of acetic to propionic acid. A similar difference was shown in the experiments of El-Shazly (1952) between the samples taken before and after feeding, but not in those of Schambye & Phillipson (1949). When diets containing high proportions of flaked maize were fed to lambs by Phillipson (1952), high and sustained concentrations of lactic acid were observed and the ratio of acetic to propionic acid was unusually low. Previously, lactic acid had been found in high concentration only shortly after the feeding of high-sugar diets (Phillipson, 1942; Phillipson & McAnally, 1942), the lactic acid disappearing rapidly.

A few values for steers given by Elsden (1945) and Elsden *et al.* (1946) indicated

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a general similarity to the relative proportions of volatile acids in the rumen liquor of sheep, but, apart from some values published by McClymont (1951), and by Carroll & Hungate (1955) since our work was begun, no values for the cow were available. The present paper describes a study of the volatile fatty acids and lactic acid in the rumen of dairy cows during the feeding of a variety of diets.

EXPERIMENTAL

Animals and their management

Animals. Four Dairy Shorthorn cows were used, each fitted with a large rumen fistula. Apart from cow A, the cows were in milk.

Diet. The diets investigated and the daily ration per cow were as follows:

Exp. 1: cow A, 18 lb. poor meadow hay.

Exp. 2: cow B, 16 lb. poor meadow hay and 15 lb. proprietary dairy cubes balanced for milk production.

Exp. 3: cow A, 18 lb. good meadow hay and 5 lb. concentrates consisting of dredge corn 4, crushed oats 4, bran 2, coconut cake 2, flaked maize 2 and groundnut meal 1, parts by weight.

Exp. 4: cow A, 16 lb. good seeds-hay and 3 lb. decorticated groundnut cake.

Exp. 5: cow B, 22 lb. good seeds-hay and 3 lb. decorticated groundnut cake, together with 10 lb. concentrates consisting of bran 1, groundnut meal $\frac{1}{2}$, crushed oats 1, flaked maize 1 and dredge corn 3 parts by weight.

Exp. 6: cow A, 13 lb. good meadow hay and 50 lb. pulped mangolds.

Exp. 7: cows A and B, free grazing on old permanent pasture.

Exp. 8: cow A, 70 lb. good arable silage.

Exp. 9: cows B and C, (*a*) 16 lb. hay and 20 lb. concentrates, (*b*) 2 lb. hay and 24 lb. concentrates, (*c*) 2 lb. hay, 5 lb. dried straw pulp and 20 lb. concentrates, and (*d*) 18 lb. hay and 10 lb. concentrates. The hay was good-quality lucerne hay, the concentrates consisted of flaked maize 10, fine wheat offal 7 and decorticated groundnut cake 3 parts by weight, and the straw pulp, obtained from the paper-making industry, had been prepared by treatment with sodium hydroxide under pressure.

Exp. 10: cows C and D, (*a*) 16 lb. hay, and concentrates 20 lb. for cow C and 16 lb. for cow D, (*b*) 18 lb. finely ground hay and 18 lb. concentrates, and (*c*) 16 lb. hay and 16 lb. concentrates. The hay was good seeds-hay and the concentrate was similar to that given in Exp. 9.

The diets used for Exps. 1–8 are typical of normal farm practice. Those given in Exps. 9 and 10 were designed as part of an investigation of the effect of variation in the ratio of hay to concentrates and in the physical state of the hay on milk fat content (see Balch, Balch, Bartlett, Bartrum, Johnson, Rowland & Turner, 1955) and included some unusual diets (Exp. 9, *b* and *c*, and Exp. 10, *b*).

Management. Before the beginning of each period of sampling the cows were given the experimental diet for at least 10 days. The daily ration was given in two equal meals, except that the concentrates in Exps. 3–5 and the hay in Exp. 9, *b* and *c*, were given at the evening meal only. Concentrates were given first, followed by the hay, the meal being consumed in about 1 h. Mineral licks and water were available. Except for experiments under grazing conditions, the cows were housed in covered yards or standings.

Sampling

Each investigation lasted for 24 h, samples of rumen liquor being taken just before each feed and then at hourly intervals. In preliminary work the maximum variation observed in the concentration of total volatile acids between samples of rumen liquor taken at the same time from different parts of the rumen was 1·1 m-equiv./100 ml. rumen liquor. The sampling method adopted was to take rumen contents from about 6 in. below the surface of the digesta and to strain the liquor through surgical gauze.

Chemical methods

pH. Rumen liquor was cooled rapidly to room temperature and the pH was measured with a glass electrode. Repeat measurements, carried out during a period of 20 min after the sample was cooled, showed little variation.

Total volatile acids. Rumen liquor was centrifuged to remove suspended solids and diluted with four volumes of water. Equal volumes of diluted liquor and saturated acidified magnesium-sulphate solution (25 ml. conc. H_2SO_4/l.) were mixed and left overnight to precipitate protein. After the protein had been centrifuged off, the total volatile acids were separated by steam distillation and titrated with phenol red as indicator. This determination may include a little lactic acid if any is present in the rumen liquor.

Individual volatile fatty acids. The double-distillation method of Friedemann (1938) was used except that the protein, precipitated by half saturation with magnesium sulphate, was centrifuged off before steam distillation. The mixed fatty acids were then extracted with chloroform containing 5% (v/v) *n*-butanol (Elsden, 1946) and analysed by the method of Moyle, Baldwin & Scarisbrick (1948) with their column I. This procedure gave a separation into acetic, propionic and butyric acids and a mixed fraction containing the higher homologues.

Lactic acid. After removal of soluble carbohydrates and protein by precipitation with copper-lime (Elsden, 1945), lactic acid was determined by the *p*-hydroxydiphenyl colorimetric method of Barker & Summerson (1941). During the mixing of the sample with sulphuric acid, the tubes were cooled in ice to prevent local overheating and oxidation beyond the acetaldehyde stage. Recovery of lactic acid, added as the lithium salt to rumen liquor in concentrations of from 4 to 200 mg/100 ml., ranged from 98 to 103%. Comparison of the values obtained with the colorimetric method and with a standard distillation method (Troy & Sharp, 1935) showed good agreement.

RESULTS

The range and mean values observed for the pH of, and the concentration of total volatile acids in, the rumen liquor during each experiment are given in Table 1.

pH

The pH fluctuated inversely with the concentration of volatile acids. Apart from occasional samples taken just before feeding, the pH did not rise above 7·0, but there was appreciable variation between diets in both the range of pH and the mean pH. The lowest pH and the greatest range (4·30–6·98) were observed with the low-hay diet (Exp. 9, *b*), whereas the lowest sustained pH values were recorded for grazing cows (Exp. 7) when the pH remained between 5 and 6 throughout the experiment. Appreciable variation in the buffering capacity of the rumen contents was observed, the buffering capacity being markedly lower with the low-hay, low-hay–straw-pulp and ground-hay diets given in Exps. 9 and 10 than with the corresponding normal hay diets.

Table 1. *Diurnal variation of pH and concentration of total volatile acids in rumen liquor of cows*

Exp. no.	Cow	pH Range	pH Mean	Total volatile acids (m-equiv./100 ml. rumen liquor) Range	Total volatile acids Mean	pH Range	pH Mean	Total volatile acids (m-equiv./100 ml. rumen liquor) Range	Total volatile acids Mean
		Feeding time 7 a.m., hourly observations during period 7 a.m. to 5 p.m.				Feeding time 5 p.m., hourly observations during period 5 p.m. to 7 a.m.			
1	A	6·31–6·54	6·49	10·5–12·1	11·2	6·20–6·60	6·37	10·7–11·8	11·6
2	B	5·57–6·43	5·90	10·7–13·1	12·0	5·53–6·36	5·93	11·1–13·7	11·9
3	A	6·58–6·96	6·75	8·1–10·4	9·3	6·01–6·58	6·45	9·2–12·1	10·4
4	A	6·62–6·99	6·82	8·8–10·4	9·8	6·51–6·98	6·74	8·9–12·4	10·6
5	B	6·68–6·94	6·85	8·9–9·6	9·0	5·77–6·86	6·26	10·9–15·7	13·4
6	A	6·61–7·00	6·84	8·4–10·4	9·7	6·55–6·92	6·72	8·5–11·7	10·2
		Free grazing, hourly observations during period 7 a.m. to 5 p.m.				Free grazing, hourly observations during period 5 p.m. to 7 a.m.			
7	A	5·26–5·79	5·48	12·4–16·3	14·8	5·07–5·75	5·37	13·4–16·5	15·0
	B	5·41–5·92	5·67	12·2–14·3	13·1	5·09–5·84	5·42	13·2–16·0	14·3
		Feeding time 6 a.m., hourly observations during period 6 a.m. to 6 p.m.				Feeding time 6 p.m., hourly observations during period 6 p.m. to 6 a.m.			
8	A	6·25–6·90	6·49	8·7–13·0	10·8	6·06–6·60	6·30	9·6–12·9	11·2
9, *a*	B	5·51–7·04	6·26	10·6–14·6	12·7	5·72–6·83	6·22	11·3–15·9	13·1
	C	5·45–6·90	6·24	10·6–17·5	13·7	5·40–6·70	6·01	12·5–18·3	14·7
9, *b*	B	4·96–6·80	5·71	7·8–14·7	12·2	4·92–6·60	5·67	9·0–16·6	12·9
	C	4·55–6·98	5·74	5·6–18·5	16·1	4·30–6·90	5·30	6·9–20·0	14·3
9, *c*	B	5·04–6·29	5·55	12·4–18·5	14·9	4·93–6·09	5·47	11·4–18·8	14·8
	C	5·06–6·80	5·91	7·8–20·0	13·5	5·00–6·58	5·84	8·3–19·6	13·1
9, *d*	B	5·90–6·70	6·45	8·4–14·5	11·3	6·00–7·20	6·58	7·7–14·6	11·0
	C	5·90–6·85	6·31	9·4–15·6	12·1	5·90–6·85	6·32	10·6–16·1	13·0
10, *a*	C	5·45–6·55	6·03	10·0–15·2	12·9	5·20–6·30	5·78	11·3–15·7	13·2
	D	5·20–7·00	6·37	7·9–15·3	11·0	5·15–6·80	6·03	8·2–15·0	11·9
10, *b*	C	4·95–6·80	5·96	7·0–21·3	13·3	4·95–7·00	5·90	7·1–20·0	12·4
	D	5·30–6·90	6·11	8·0–15·2	11·9	5·10–6·90	6·01	7·7–12·2	10·8

Concentration of total volatile acids

Only minor fluctuations in concentration of total volatile acids were observed after a feed of hay alone (Exp. 1, and Exps. 3–5 after the 7 a.m. feeds). With different hays the concentration ranged from 8·1 to 12·1 m-equiv./100 ml. rumen liquor. When silage or hay supplemented with mangolds or with concentrates was given the concentration increased to a peak and then declined towards the values before feeding.

Fig. 1, which gives the full results for Exp. 5, illustrates the rapid increase in concentration observed after feeding concentrates. For the different diets given, peak concentrations ranging from 11·4 to 21·3 m-equiv./100 ml. rumen liquor were reached between 2 and 6 h after feeding. It is noteworthy that in Exp. 9 there was a marked increase in the range of concentration of total volatile acids in the rumen when the hay ration was reduced from 16 lb. (Exp. 9, *a*) to 2 lb./day (Exp. 9, *b*), and this increase was maintained when dried straw pulp was added to the low-hay diet in Exp. 9, *c*.

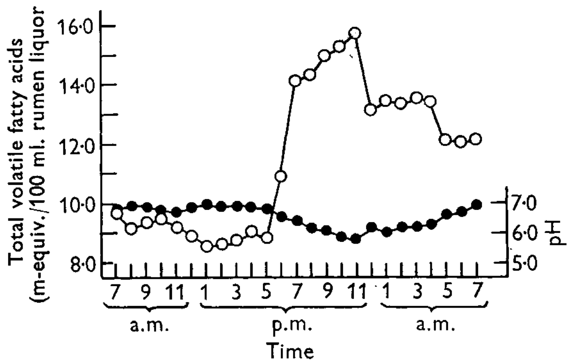

Fig. 1. Exp. 5. pH of, and concentration of total volatile fatty acids in, rumen liquor of cows at hourly intervals over a 24 h period during the feeding of a hay–concentrate diet. Hay fed at 7 a.m., hay and concentrates at 5 p.m. ●—●, pH; ○—○, concentration of volatile fatty acids.

A similar increase in range was observed in Exp. 10 when the diet containing long hay (Exp. 10, *a*) was replaced by a similar diet containing all the hay in a finely ground form (Exp. 10, *b*). Full results for cow C in Exps. 9, *a* and *b*, and 10, *a* and *b*, are given in Figs. 2 and 3 respectively. The lowest concentration measured during all experiments was 5·6 m-equiv./100 ml. rumen liquor, for cow C in Exp. 9, *b*, and the highest 21·3 m-equiv., for cow C in Exp. 10, *b*.

Relative proportions of the individual volatile fatty acids

The mean composition of the mixture of volatile fatty acids in the rumen liquor during the feeding of each diet is given in Table 2. No consistent variations were observed with time after the feeding of a given diet. When the values were placed in descending order of acetic-acid percentage, trends in both the percentages of the other acids and the ratio of hay to concentrates in the diet were revealed. With the exception of the diet containing 18 lb. ground hay (Exp. 10, *b*), the percentage of acetic acid decreased with the decrease in the ratio of hay to concentrates in the diet. Values for propionic acid and the acids higher than butyric increased as the acetic-acid value decreased. With the exception of the diet containing only 2 lb. hay (Exp. 9, *b*), there was a similar increase in butyric-acid content.

Weights of volatile fatty acids

In Exps. 8–10 the rumen was completely emptied at 12 noon after the last sample had been taken, and the weight of water present was determined to calculate the range in the weights of each acid in the rumen liquor during each experiment. The estimated ranges, given in Table 3, were less than those actually present because the weight of digesta in the rumen decreases by some 4 lb./100 min during the time interval between two feeds (Balch, 1952). Hence the values at the peak of fermentation (which usually occurred before 12 noon) were too low and those after 12 noon (when fermentation was declining) were too high.

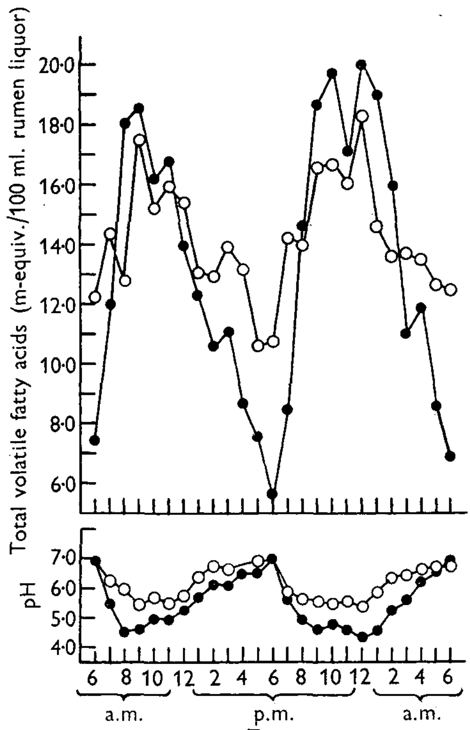

Fig. 2

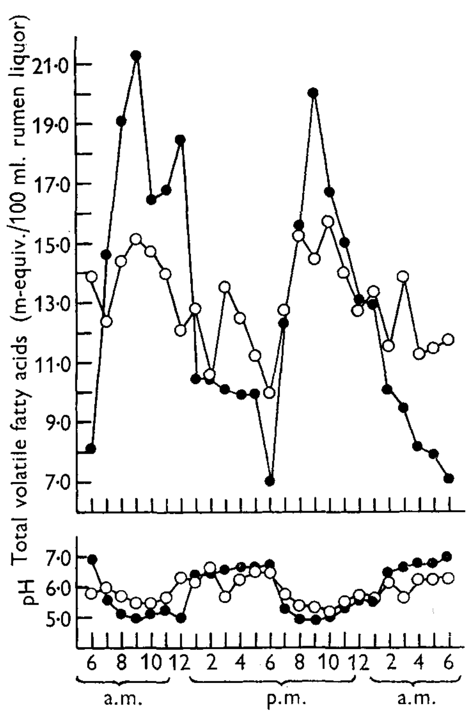

Fig. 3

Fig. 2. Exp. 9. pH of, and concentration of total volatile fatty acids in, rumen liquor of cows at hourly intervals over a 24 h period during the feeding of high-hay, concentrate and low-hay, concentrate diets. Feeding times: 6 a.m. and 6 p.m. ○—○, 16 lb. hay and 20 lb. concentrates; ●—●, 2 lb. hay and 24 lb. concentrates.

Fig. 3. Exp. 10. pH of, and concentration of total volatile fatty acids in, rumen liquor of cows at hourly intervals over a 24 h period during the feeding of long-hay, concentrate and ground-hay, concentrate diets. Feeding times: 6 a.m. and 6 p.m. ○—○, 16 lb. long hay and 20 lb. concentrates; ●—●, 18 lb. ground hay and 18 lb. concentrates.

Lactic acid

Only traces of lactic acid, less than 1 mg/100 ml. rumen liquor, were found in the rumen during Exps. 1–8, but in Exps. 9 and 10 there was a considerable accumulation shortly after each feed. The peak concentrations, which occurred 1–2 h after feeding, varied from 95 to 270 mg/100 ml. rumen liquor but showed no consistent variation with diet. The lactic acid was rapidly fermented, only traces being present 5 h after feeding.

Table 2. *Values for individual volatile fatty acids of rumen liquor of cows expressed as molar percentages of the total volatile fatty acids and arranged in ascending order of acetic-acid percentage*

Exp. no.	Cow	Acetic acid Range	Acetic acid Mean	Propionic acid Range	Propionic acid Mean	Butyric acid Range	Butyric acid Mean	'Higher' acids Range	'Higher' acids Mean
9, *b*	B	39·8–41·0	40·6	35·4–37·6	36·5	9·4–13·3	10·7	11·3–13·7	12·3
	C	39·7–41·6	40·7	36·5–40·7	39·1	6·8–7·8	7·5	11·8–14·4	12·7
9, *c*	C	44·9–47·8	45·8	29·1–32·1	31·0	13·1–14·4	13·8	8·7–10·4	9·4
	B	46·6–49·7	48·6	27·6–30·1	28·6	11·0–14·7	13·3	8·4–10·6	9·5
10, *b*	C	45·6–51·4	49·0	29·4–33·7	32·1	8·5–13·3	11·9	6·5–7·5	7·0
	D	47·4–57·8	52·1	25·2–36·9	30·6	9·4–15·0	11·6	5·2–6·3	5·7
9, *a*	C	56·1–59·3	57·1	21·8–25·1	23·7	11·3–12·8	12·0	5·6–8·4	7·2
	B	57·6–59·1	58·3	21·6–25·6	23·9	12·2–14·9	13·5	3·7–4·8	4·3
2	B	57·7–63·0	59·8	22·8–24·2	23·3	10·7–15·0	13·6	2·7–3·5	3·2
10, *a*	C	59·4–64·7	61·5	18·0–21·4	20·1	7·4–10·3	9·5	8·0–10·2	8·9
10, *c*	D	60·7–62·8	61·7	17·9–21·6	19·9	12·7–15·0	13·9	4·1–5·0	4·5
10, *a*	D	59·5–66·5	62·2	18·7–21·1	19·5	10·8–14·7	12·8	4·0–6·8	5·5
10, *c*	C	62·1–66·0	64·2	17·6–20·4	18·7	10·8–13·0	12·1	3·8–5·5	5·0
7	A	63·4–65·8	65·0	18·2–20·2	18·8	11·5–12·3	11·9	4·1–4·5	4·3
9, *d*	B	66·1–66·9	66·6	18·1–20·7	19·6	9·3–11·0	9·8	3·8–4·0	3·9
	C	64·6–69·8	67·1	17·4–20·1	18·8	9·0–11·9	10·4	3·4–4·2	3·7
7	B	66·6–68·2	67·5	17·4–18·8	18·2	10·2–11·5	11·1	3·0–3·3	3·2
6	A	65·1–71·5	68·4	15·2–17·4	16·5	10·5–14·5	12·6	1·9–3·4	2·5
1	A	67·0–72·3	68·9	15·1–18·4	17·6	7·9–11·8	9·9	2·6–4·1	3·6
5	B	68·5–73·2	70·3	16·2–20·0	17·9	7·5–9·1	8·5	2·1–4·9	3·3
4	A	70·2–73·4	71·9	17·4–19·4	18·3	6·6–7·1	6·8	2·9–3·4	3·0
3	A	70·3–73·9	71·9	15·8–17·9	16·8	7·6–9·5	8·6	2·0–3·0	2·7
8	A	72·7–75·2	73·7	15·7–17·7	16·8	5·0–7·4	6·6	2·1–4·2	2·9

Table 3. *Estimated range in weight (g) of fatty acids present in the rumen liquor of cows during Exps.* 8–10

Exp. no.	Cow	Acid: Acetic	Propionic	Butyric	'Higher'
8	A	248–371	70–104	33–49	17–25
9, *a*	B	240–358	121–181	81–121	30–45
	C	242–415	124–212	74–128	52–89
9, *b*	B	82–173	90–192	32–67	42–89
	C	63–225	75–266	17–61	34–114
9, *c*	B	162–268	128–194	71–108	59–90
	C	106–270	88–224	47–119	37–94
9, *d*	B	211–401	83–114	45–86	21–40
	C	269–462	92–158	61–104	25–43
10, *a*	C	248–389	100–157	56–88	61–96
	D	136–266	53–103	41–80	20–40
10, *b*	C	129–391	104–316	46–139	31–95
	D	102–202	74–147	34–66	19–38

DISCUSSION

The results of these experiments show clearly that the decrease and subsequent increase of pH of rumen liquor, observed by Phillipson (1942) with sheep, also occur in the rumen of the dairy cow. After a meal of silage, hay alone, or hay supplemented with mangolds or with small amounts of concentrates, the values were within the range of

6·0–7·1 observed by Monroe & Perkins (1939), Olson (1941), Hunt, Burroughs, Bethke, Schalk & Gerlaugh (1943) and Lardinois, Mills, Elvehjem & Hart (1944) with cattle fed on similar diets. The extremely low values observed with the diets low in hay and high in flaked maize (Exp. 9, *b*) were surprising. Similar low values have been recorded for lambs fed on diets high in flaked maize (Phillipson, 1952) and for sheep given grain or glucose directly into the rumen through a fistula (Hungate, Dougherty, Bryant & Cello, 1952). The constant feature of the diets causing such low pH values has been the high content of readily fermentable starch or sugar.

For a given diet the fluctuation in pH reflected the changes in concentration of volatile acids. The concentration of volatile acids in the rumen at a given time with a given diet depends on three factors: the rate of fermentation, the rate of absorption and the volume of rumen liquor. McClymont (1951) observed a high positive correlation between the concentration of volatile acids in the rumen and in the arterial blood of cows. This finding and the rapid changes of concentration of volatile acids in the rumen observed with high-concentrate diets suggest that such diets promote rapid fermentation and absorption. Popják, French & Folley (1951) found that 80% of the radioactive carbon of acetate injected into a goat was oxidized to carbon dioxide in 6 h. If a similarly rapid katabolism occurs in cows, it is possible that with high-concentrate diets at peak absorption the maximum utilization of acetate for energy purposes and for anabolic reactions may be exceeded, and some of the acetate may be wastefully oxidized.

The mixture of volatile fatty acids found in the rumen was qualitatively similar to that reported for sheep (see p. 288), but there was considerable variation with diet in the relative proportions of the individual acids. The percentage of acids higher than butyric increased with the weight of concentrates fed. El-Shazly (1952), with sheep, found that the percentage of such acids increased with protein intake. In Exp. 9 estimates of the extent of protein digestion in the rumen (Balch *et al.* 1955) were made and it was found that the increase in the higher acids coincided with an increase in the extent of protein breakdown. Rumen micro-organisms can synthesize higher acids from the lower (Gray *et al.* 1952; Elsden & Lewis, 1953). The possibility that the low-hay, high-concentrate diets, besides providing a greater intake of soluble protein, also promote the activity of the bacteria involved in such synthesis cannot be excluded. With the high-hay diets the values for acids higher than butyric were of the same order as those found by McClymont (1951) for cows and Gray *et al.* (1952) for sheep. The high values of up to 12·7% observed with the diet very low in hay have not been reported previously.

Except with the low-hay diet, the proportion of butyric acid also increased with protein intake. McNaught (1951) observed that the production of butyric acid during the in vitro fermentation of carbohydrates was sporadic, and Gray & Pilgrim (1952) found marked butyric fermentation only when protein was added in such incubations. There was no indication with the low-hay diet whether less butyric acid was formed or whether a portion was subsequently converted to other acids. With a similar diet high in flaked maize fed to lambs Phillipson (1952) also found low values.

The proportions of acetic and propionic acids varied inversely. Except with the ground-hay diet (Exp. 10, *b*), decreasing the ratio of fibrous to starchy carbohydrates in the diet caused a decrease in the ratio of acetic to propionic acid, especially in Exp. 9 where the concentrate carbohydrates were supplied mainly as flaked maize. The similarly low ratios found by Phillipson (1952) with diets high in flaked maize were accompanied by changes in the relative proportions of rumen micro-organisms (Masson, 1951). It is highly probable that the marked difference in carbohydrate intake when the cows changed from the high-hay diet in Exp. 9, *a* to the low-hay diet in Exp. 9, *b* would encourage the starch-digesting organisms at the expense of those digesting fibrous carbohydrates. However, in Exp. 10 the ratio of these acids was lowered by the feeding of ground hay in place of long hay without changing the ratio of fibrous to starchy carbohydrates. The low ratios of acetic to propionic acid arising from low- or ground-hay diets coincided with a marked increase in the range of pH in the rumen. With both diets there was a marked decrease in the time the cows spent ruminating, with a consequent decrease in the inflow of saliva to the rumen, and also rapid production of acid from the fermentation of the readily available starch. The failure to buffer the large amounts of volatile acids produced could lead to the selection of certain strains of the rapid acid-producing types of bacteria which become established with the diets high in starch and are, in part, the cause of the low pH values. The increased acidity of the rumen appears to be unfavourable to the development of the strains of bacteria that produce predominantly acetic acid. Such selection of bacteria could lead to the formation of acetic and propionic acids in ratios of the order of 1 to 1 instead of 2 or more to 1 observed with high-hay diets even when, as in Exps. 9, *a* and 10, *a*, the intake of starch from flaked maize was high.

Except with the diets containing flaked maize (Exps. 9 and 10) only traces of lactic acid were found. Lactic acid accumulates in the rumen only if the rate of fermentation to lactate exceeds its subsequent fermentation to volatile fatty acids. The failure to find lactic acid in the rumen after the feeding of silage of high lactic-acid content (Exp. 8) indicates that fermentation of lactate in the cow's rumen can be very rapid.

Only with the unusual low- or ground-hay diets did the molar percentage of acetic acid fall below 55. With the other diets values were within the range for sheep (see p. 288). Kiddle *et al.* (1951), working with sheep, found that no appreciable amounts of fatty acids were absorbed into the lymph draining the rumen, and that the major differences between the relative proportions of the volatile fatty acids in rumen liquor and blood draining the rumen were that the latter contained more acetic acid and considerably less butyric acid. If their results are applicable to cows, then acetic acid is the major metabolite from carbohydrate digestion in the cow. In Exps. 9 and 10, estimates made of the extent of carbohydrate digestion in the rumen (Balch *et al.* 1955) indicated that of the dietary carbohydrate that could be digested to glucose in the small intestine very little passed unfermented from the rumen. This observation emphasizes the importance also of the propionic acid absorbed from the rumen in supplying the glucose requirements of the cow by gluconeogenesis.

SUMMARY

1. The diurnal variation in the concentration of volatile fatty acids and lactic acid in, and in the pH of, the rumen liquor of four Dairy Shorthorn cows receiving diets

of hay, hay supplemented with mangolds or a variety of concentrate mixtures, finely ground hay with concentrates, silage, or herbage, has been investigated.

2. With hay alone there was little variation in the concentration of total volatile acids at hourly intervals after feeding, the values observed ranging from 8·1 to 12·1 m-equiv./100 ml. rumen liquor with different diets. On all other diets the concentration increased to peak values of 11·7–21·3 m-equiv. between 2 and 6 h after feeding. Diets containing small amounts of hay, or hay in a finely ground state, produced the greatest ranges of concentrations: 5·6–20·0 and 7·0–21·3 m-equiv./100 ml. respectively.

3. The pH varied inversely with the concentration of total volatile acids. The lowest pH and the greatest range (4·30–6·98) were observed with the low-hay diet, but the lowest sustained pH values (5·09–5·84) were observed with cows at pasture.

4. Only traces of lactic acid, less than 1 mg/100 ml. rumen liquor, were found with all diets except those containing large amounts of flaked maize, when peak concentrations of from 95 to 270 mg, which rapidly disappeared, were observed 2 h after feeding.

5. The production of a mixture of acetic, propionic, butyric and at least one higher acid was characteristic of fermentation in the cow's rumen. The mean molar percentage of these acids varied with the different diets as follows: acetic from 40·6 to 73·7, propionic from 16·5 to 39·1, butyric from 6·6 to 13·9 and higher acids from 2·5 to 12·7. In general, the percentages of butyric and higher acids increased with increase of protein in the diet. The values for acetic and propionic acids varied inversely. Except with the diet containing ground hay, the ratio of acetic to propionic acid decreased with decrease in the ratio of fibrous to starchy concentrates. Replacing long hay, in a diet of high flaked-maize content, by finely ground hay also decreased the ratio of acetic to propionic acid.

6. Low ratios of acetic to propionic acid were found when there was rapid production of fatty acids coincident with a reduced buffering capacity in the rumen liquor. It is suggested that highly acid conditions encourage the proliferation of organisms that produce lower proportions of acetic acid.

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REFERENCES

Balch, C. C. (1952). Unpublished.
Balch, C. C., Balch, D. A., Bartlett, S., Bartrum, M. P., Johnson, V. W., Rowland, S. J. & Turner, J. (1955). *J. Dairy Res.* **22**, 270.
Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
Carroll, E. J. & Hungate, R. E. (1955). *Appl. Microbiol.* **2**, 205.
Elsden, S. R. (1945). *J. exp. Biol.* **22**, 51.
Elsden, S. R. (1946). *Biochem. J.* **40**, 252.
Elsden, S. R., Hitchcock, M. W. S., Marshall, R. A. & Phillipson, A. T. (1946). *J. exp. Biol.* **22**, 191.
Elsden, S. R. & Lewis, D. (1953). *Biochem. J.* **55**, 183.
El-Shazly, K. (1952). *Biochem. J.* **51**, 640.
Friedemann, T. E. (1938). *J. biol. Chem.* **123**, 161.
Gray, F. V. & Pilgrim, A. F. (1951). *J. exp. Biol.* **28**, 83.
Gray, F. V. & Pilgrim, A. F. (1952). *Nature, Lond.*, **170**, 375.

Gray, F. V., Pilgrim, A. F., Rodda, H. J. & Weller, R. A. (1952). *J. exp. Biol.* **29**, 57.
Gray, F. V., Pilgrim, A. F. & Weller, R. A. (1951). *J. exp. Biol.* **28**, 74.
Hungate, R. E., Dougherty, R. W., Bryant, M. P. & Cello, R. M. (1952). *Cornell Vet.* **42**, 423.
Hunt, C. H., Burroughs, E. W., Bethke, R. M., Schalk, A. F. & Gerlaugh, P. (1943). *J. Nutr.* **25**, 207.
Kiddle, P., Marshall, R. A. & Phillipson, A. T. (1951). *J. Physiol.* **113**, 207.
Lardinois, C. C., Mills, R. C., Elvehjem, C. A. & Hart, E. B. (1944). *J. Dairy Sci.* **27**, 579.
McClymont, G. L. (1951). *Aust. J. agric. Res.* **2**, 92.
McNaught, M. L. (1951). *Biochem. J.* **49**, 325.
Masson, M. J. (1951). *Research*, **4**, 73.
Monroe, C. F. & Perkins, A. E. (1939). *J. Dairy Sci.* **22**, 983.
Moyle, V., Baldwin, E. & Scarisbrick, R. (1948). *Biochem. J.* **43**, 308.
Olson, T. M. (1941). *J. Dairy Sci.* **24**, 413.
Phillipson, A. T. (1942). *J. exp. Biol.* **19**, 186.
Phillipson, A. T. (1952). *Brit. J. Nutr.* **6**, 190.
Phillipson, A. T. & McAnally, R. A. (1942). *J. exp. Biol.* **19**, 199.
Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.
Schambye, P. & Phillipson, A. T. (1949). *Nature, Lond.*, **164**, 1094.
Troy, H. C. & Sharp, P. F. (1935). *Mem. Cornell agric. Exp. Sta.* no. 179.

The role of fat in the diet of rats

11. Influence of a small amount of ethyl linoleate on degeneration of spermatogenic tissue caused by hydrogenated arachis oil as the sole dietary fat*

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In earlier experiments (Aaes-Jørgensen, Funch, Engel & Dam, 1956; Aaes-Jørgensen, Funch & Dam, 1956) weanling rats reared on diets containing hydrogenated arachis oil as the only source of fat showed severe testicular degeneration and were sterile. The present experiment was undertaken to study the possible curative influence of a relatively small dose of ethyl linoleate (20 mg/male rat/day). This dose is about the same as the daily dose of linoleic acid (20 mg) that was sufficient for prophylaxis against testicular degeneration but insufficient for optimal growth in the previous experiments.

EXPERIMENTAL

Animals and their management. Six newly weaned male rats were fed on a diet consisting of 28% hydrogenated arachis oil (m.p. 40–42°),† 20% Vitamin Test Casein,‡ 46% sucrose, 5% salt mixture,§ 0·5% vitamin mixture,§ and 0·5% choline chloride. An aqueous colloidal solution containing vitamins A and D_2|| was given by

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† From Dansk Sojakagefabrik Ltd, Copenhagen.
‡ From Genatosan Ltd, Loughborough, England.
§ See Aaes-Jørgensen & Dam (1954).
|| Decamin aquosum, kindly furnished by Ferrosan Ltd, Copenhagen.