Volatile Fatty Acids in the Rumen of the Sheep

EARLY workers in the field of ruminant physiology were aware of the presence in the rumen fluid of a number of the lower members of the saturated fatty acid series[1]; but accurate data were not reported until recently, when partition chromatography became available as a procedure for the analysis of complex mixtures of these acids. Acetic, propionic and butyric acids were then established as the main components of the mixture in the rumen fluid of sheep and other ruminants. It was claimed that traces of acid or acids higher than butyric acid were indicated, but that no formic acid was present[2].

We have now carried out a more detailed analysis by a combination of partition chromatographic and other methods and have shown the presence of formic, acetic, propionic, *n*-butyric, *iso*-butyric, valeric and caproic acids, and probably a trace of heptoic acid. An unbuffered 'Celite' column was used to separate the acids into formic, acetic and propionic acids, and a fraction containing higher acids. The latter was transferred to a buffered column to be separated into three parts, the first containing *n*-butyric and *iso*-butyric acids, the second valeric acid and the third caproic and higher acids. The third fraction was transferred again to a buffered column suitable for the separation of caproic and heptoic acids. Formic acid and *iso*-butyric were identified and estimated by separate methods. The valeric acid was in one instance separated into two fractions, the larger of which was identified as *n*-valeric acid by its *R*-value; the smaller fraction was probably one of its isomers, but this was not finally identified. The 'caproic' fraction contained a very small amount of acid corresponding approximately in *R*-value to heptoic acid.

A typical analysis of the acids in the rumen fluid of a sheep fed on wheat hay is:

Formic acid	1·0 per cent	*iso*-Butyric acid	0·7 per cent
Acetic acid	68·0 ,,	Valeric acid	2·6 ,,
Propionic acid	18·0 ,,	Caproic acid	0·7 ,,
n-Butyric acid	9·0 ,,	Heptoic acid (?)	trace

The substrates from which the fatty acids originate in the rumen and the intermediate steps leading to their formation have as yet received little attention. The production of acetic and propionic acids from pure cellulose was demonstrated *in vitro* by Elsden[2] and Marston[3], and the formation of acids by two organisms isolated from the rumen was studied in detail by Sijpestein[4]. One of these organisms produced propionic and acetic acids, and the other succinic and acetic. Decarboxylation of succinic to propionic acid was later shown to occur in the rumen[5,6].

It is reasonable to suppose that the dissimilation of other polysaccharides and of proteins may account for a considerable part of the three main acids produced in the rumen; and since *iso*-butyric and the isomers of valeric acid are known to occur as products of the amino-acid metabolism of a number of anaerobes of the *Clostridium* genus[7], these acids also may be derived from the protein fraction of the fodder.

We have made a preliminary investigation of the origins of some of the higher acids by including acetic and propionic acids labelled with carbon-14 in the rumen fermentation. Wheat hay was fermented *in vitro* as described by Gray and Pilgrim[8]; labelled acetic acid ($CH_3{}^{14}COOH$) or propionic acid ($CH_3CH_2{}^{14}COOH$) was added to the inoculum, and the distribution of the carbon-14 among the fatty acids was determined at the end of the 48-hr. fermentation period. The results indicated that acetic acid contributed to the formation of a large part of the butyric acid produced, since the specific (molar) activity of this acid was about the same as the mean molar activity of the acetic acid; it contributed also to the formation of the valeric acid and to the caproic acid (which in these analyses was not separated from the trace of higher acid). Propionic acid was shown to contribute to the formation of valeric acid and, to a slight extent, to the formation of the caproic acid fraction (caproic and higher acid). The mechanism of formation of these higher normal acids may well involve the addition of a 2 carbon compound to acetic, propionic or butyric acid as demonstrated by Barker and his colleagues[9,10] in the synthesis of volatile fatty acids by *Clostridium kluyveri*. Acetic acid also contributed to the formation of a small but significant proportion of the propionic acid produced in the fermentation.

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Length of the Period of the Periodic System

IF each period in the periodic system ends in a rare gas (H,He 1st period, Li—Ne 2nd, etc.), then the number of elements in a period can be found from the ordinal number of the period by the following formula:

$$L = \frac{(2n + 3 + (-1)^n)^2}{8},$$

where n is the number of the period and L is the number of elements in the period.

This formula also gives the relation between the number (n) of electron shells in an atom, and the number (L) of the elements in the period in which the particular atom is placed.

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