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Fermentation in the Rumen of the Sheep

IN recent years it has become widely accepted that acetic, propionic and butyric acids are important products of the fermentation which takes place in the rumen; but as yet the information most cogent to the nutritional physiology of the ruminant, namely, the quantities of these nutrients which become available to the animal, has not been obtained.

When it was shown by Barcroft and his colleagues[1] that the volatile fatty acids are readily absorbed through the rumen wall, it became evident that an *in vitro* approach would be the one most likely to provide a solution to the problem.

We have set up, *in vitro*, fermentations of wheaten hay and of lucerne hay inoculated with micro-organisms from the rumen, and have found that when the inoculum was sufficiently large, and the conditions similar to those in the rumen, the extent to which the various carbohydrates in these substrates were fermented corresponded closely to the extent of their breakdown in the rumen. Moreover, the amounts of methane formed were of the same order as the amounts formed *in vivo*. Between 30 and 50 per cent of both the cellulose and hemicellulose fractions of the fodders were broken down, and 15–20 litres of methane per kilogram of fodder were produced within forty-eight hours. The quantities of each of the fatty acids formed are shown in Table 1.

Table 1. FATTY ACIDS PRODUCED *in vitro*

Fodder	Acids (gm./kgm. fodder)			
	Acetic	Propionic	Butyric	Total
Wheaten hay	96	101	39	236
Lucerne hay	136	76	48	260

In Table 2 the differences between the composition of the mixture of acids formed *in vitro* and the composition of the mixture in the rumen fluid of animals fed on the same two fodders are shown. If the acids formed *in vitro* are indeed produced in the rumen, then these differences must throw some light on the problem of the relative rates of absorption of the acids through the rumen wall, for a comparison of the ratios of each pair of acids found *in vitro* and *in vivo* will indicate which acid is the more rapidly absorbed. For example, the ratio (acetic acid to propionic acid) in the case of wheaten hay fermented *in vitro* is $46/41 = 1{\cdot}1$, whereas the ratio in the rumen fluid is $71/17 = 4{\cdot}2$; indicating a more rapid absorption of propionic than acetic acid. From all of the data in the table, it may be concluded that when wheaten hay was fermented the relative rates of absorption of the acids fell in the series propionic > butyric > acetic, and in the case of lucerne hay the order was propionic > butyric ≈ acetic.

Table 2. COMPARISON BETWEEN THE ACIDS FORMED *in vitro* AND THOSE PRESENT IN THE RUMEN FLUID

Fodder	Fermentation	Fatty acids*		
		% Acetic	% Propionic	% Butyric
Wheaten hay	*In vitro*	46	41	13
	In the rument†	71	17	12
Lucerne hay	*In vitro*	59	27	14
	In the rument†	70	14	16

* Molecular proportions.
† Averages from numerous analyses.

When these fodders are fed to sheep, it is found that the concentration of acid in the rumen rises sharply, and later falls again, reaching its original level within twenty-four hours. If, then, the acids are formed in the proportions which we have found *in vitro*, it is clear that certain changes in the composition of the rumen fluid must take place during the day. For example, the ratio of acetic to propionic acid should decrease immediately after feeding when the rate of production of acid is increasing; and it should increase again later, when production is decreasing or has ceased, because the effect of the different rates of absorption of the individual acids will then predominate. We have examined the composition of the rumen fluid at frequent intervals after feeding both these fodders, and have found that, in general, the predicted changes do take place.

Details of these experiments will be reported elsewhere.

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Isolation of Sodium Hyaluronate

DURING recent investigations for the development of new methods for the isolation and purification of hyaluronic acid from human umbilical cords, the following interesting observation was made. The cords, stored in acetone, were extracted with hot water (65°) and the aqueous extract allowed to 'settle' overnight. The clear supernatant liquid was kept under toluene for fourteen days, when it deposited a precipitate. This was removed, and the clear liquid was acidified with glacial acetic acid. Half the resulting mucin clot was stored in acetone for several weeks. Upon attempting to dissolve it at *p*H 8·0, it was found to be incompletely soluble, whereas the original clot was soluble. The 'acetone-clot' was extracted with water at *p*H 8·0, the extract giving only a very faint cloudiness upon acidification to *p*H 4·0. It was poured into 3 vol. of alcohol and the precipitate re-extracted with water. Finally, a preparation of sodium hyaluronate was obtained