FERMENTATION IN THE RUMEN OF THE SHEEP

III. INTERMEDIATE STAGES IN THE FERMENTATION OF WHEATEN HAY *IN VITRO* BY MICRO-ORGANISMS FROM THE RUMEN

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(With One Text-figure)

A previous communication in this series of papers (Gray, Pilgrim & Weller, 1951) gave an account of the fermentation of wheaten hay and lucerne hay by organisms from the rumen of the sheep, in which the overall production of each of the main volatile fatty acids was determined. In view of the complex nature of both the substrate and the mixed population of organisms involved, it was decided to investigate the intermediate stages of the fermentation to determine whether any important changes take place in the composition or in the rate of production of the mixture of acids.

EXPERIMENTAL

Groups of eight fermentations were set up in flasks, each of which contained the same quantity of a uniform sample of wheaten hay; the fermentations were initiated by the addition of equal volumes of rumen fluid.

Fermentation flasks. 250 ml. Erlenmeyer flasks fitted with inlet tubes reaching nearly to the bottoms of the flasks and outlet tubes arranged for the escape of gas.

Substrate. 5 g. ground wheaten hay chaff.

Inoculum. 1500 ml. rumen fluid were drawn 1½ hr. after feeding the sheep on wheaten hay chaff. To this was added a solution of 6 g. $(NH_4)_2CO_3$ in 200 ml. of tap water. Each flask was inoculated with 150 ml. of the resulting liquor.

Anaerobiosis. Immediately after inoculation a stream of CO_2 was passed through each flask to displace air, and the flasks were sealed by means of a water trap.

Incubation. 38–40° C.

Samples for analysis of volatile fatty acids. 100 ml. of the inoculum were retained for analysis. At intervals during the period from 0 to 48 hr. after inoculation a flask was taken from the incubator, the solids removed by filtration, and the remaining liquor used for the analysis.

Analyses. The methods for the separation and determination of the individual fatty acids have been described previously (Gray *et al.* 1951).

Results. The analytical data from one typical experiment are summarized in Table 1. The composition of the acid mixture at various stages, after allowing for

Table 1. *Fatty acids present in the inoculum and in the fermentation flasks*

	Time (hr.)	ml. N/100 acid/ml. of fluid			
		Acetic	Propionic	Butyric	Total
Inoculum	0	3·02	1·10	0·65	4·77
Flask 1	2	3·33	1·47	0·69	5·49
Flask 2	4½	4·21	2·44	0·91	7·56
Flask 3	7½	5·77	3·28	1·13	10·18
Flask 4	12½	6·43	3·68	1·39	11·50
Flask 5	19½	6·68	3·83	1·38	11·89
Flask 6	28½	7·21	4·20	1·54	12·95
Flask 7	37½	7·80	4·54	1·74	14·08
Flask 8	48	8·50	4·77	1·96	15·23

the acids added with the inoculum, is given in Table 2. The changes in composition of the acid mixture, and the progressive total amounts of acid formed, are illustrated graphically in Fig. 1.

DISCUSSION

In the first place it can be seen that the overall production of fatty acids, as illustrated by the 48 hr. sample, followed a course similar to that taken by the fermentations on a larger scale previously described (Gray *et al.* 1951). Secondly, although the composition of the fatty acid produced showed a change from a higher to a lower proportion of propionic acid during the period of maximum rate of production, it is important to note that in all stages of the fermentation the composition differed from the composition of the mixture present in the rumen fluid in that it contained a significantly greater proportion of propionic acid. Thirdly, it is clear that the rate of production of fatty acid was rapid at first (60% of the total acid was formed in the first quarter of the fermentation period) and thereafter declined to a lower but fairly steady level.

Table 2. *Fatty acids formed during various periods of the fermentation*

Period (hr.)	ml. N/100 acid/ml. of fluid				Percentages of individual acids		
	Acetic	Propionic	Butyric	Total	Acetic	Propionic	Butyric
0–2	0·31	0·37	0·04	0·72	43	51	6
0–4½	1·19	1·34	0·26	2·79	43	48	9
0–7½	2·75	2·18	0·48	5·41	51	40	9
0–12½	3·41	2·58	0·74	6·73	51	38	11
0–19½	3·66	2·73	0·73	7·12	52	38	10
0–28½	4·19	3·10	0·89	8·18	51	38	11
0–37½	4·78	3·44	1·09	9·31	51	37	12
0–48	5·48	3·67	1·31	10·46	52	35	13

SUMMARY

In an investigation of the intermediate stages of the rumen fermentation of wheaten hay *in vitro*, the proportion of propionic acid in the fatty acid products was shown to decrease during the first few hours, when the rate of production of acid was at

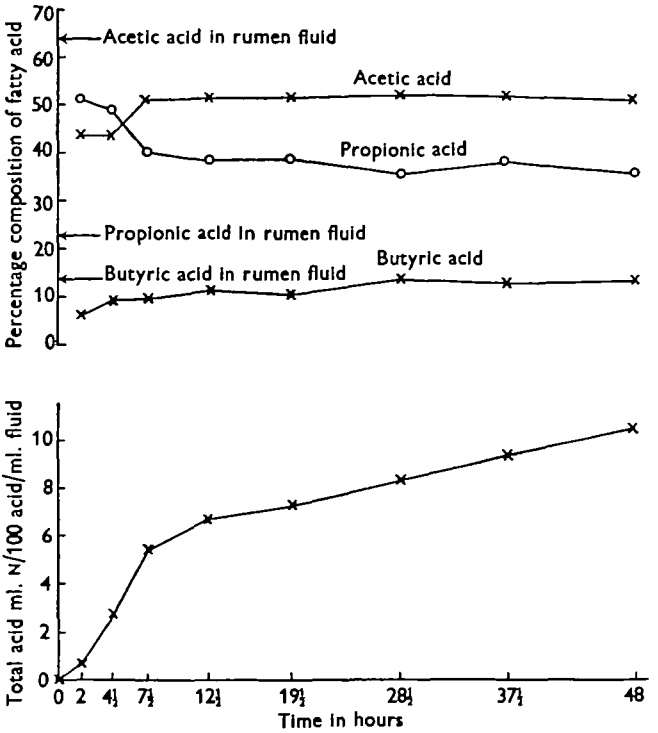

Fig. 1. Composition and total amount of the fatty acids formed at various stages of the fermentation *in vitro*.

a maximum. The composition and the rate of production were uniform over the rest of the fermentation period. Throughout the fermentation the proportion of propionic acid was considerably higher than in the rumen fluid.

REFERENCE

GRAY, F. V., PILGRIM, A. F. & WELLER, R. A. (1951). Fermentation in the rumen of the sheep. I. The production of volatile fatty acids and methane during the fermentation of wheaten hay and lucerne hay *in vitro* by micro-organisms from the rumen. *J. Exp. Biol.* **28**, 74–82.