FERMENTATION IN THE RUMEN OF THE SHEEP

II. THE PRODUCTION AND ABSORPTION OF VOLATILE FATTY ACIDS DURING THE FERMENTATION OF WHEATEN HAY AND LUCERNE HAY IN THE RUMEN

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(With Two Text-figures)

INTRODUCTION

Although the composition of the mixture of volatile fatty acids in the rumen of the sheep may be determined, it can throw no light on the composition of the mixture actually formed in the fermentation unless the relative rates of absorption of the acids are known, for it has been established that they are readily absorbed through the rumen wall into the blood stream (Barcroft, McAnally & Phillipson, 1944). Some attempts have been made (Barcroft *et al.* 1944; Danielli, Hitchcock, Marshall & Phillipson, 1945; Gray, 1947, 1948) to determine these relative rates of absorption, but it seems certain that the results must remain of doubtful significance in view of the fact that the experimental conditions under which they were obtained were too far removed from the normal physiological state of the animal. In fact, while Gray (1947) concluded that propionic acid was more rapidly absorbed than the other acids, the English School, whose work is summarized by Elsden & Phillipson (1948) arrived at the opposite conclusion, namely that acetic acid is more rapidly absorbed than either propionic or butyric acid. The only practicable approach to the problem of the amounts of the fatty acids actually formed in the rumen is by an examination of the fermentation *in vitro*. The results of experiments in this field have been reported in Part I of the present studies, and it is clear that the findings imply certain relative rates of absorption of the acids *in vivo*. It is important that these rates should be checked, if possible, by evidence derived from the animal in its normal state.

It is known that during the digestion of the natural fodders in the rumen there may be a considerable increase in the concentration of fatty acid there immediately after feeding, followed later by a decrease to the original level. It might be expected that if fatty acids are produced in the proportions found *in vitro*, proportions very different from those in the rumen fluid, then the composition of the rumen fluid should undergo certain changes during the fermentation. In the period of an increasing rate of production of acid these changes should reflect the difference in composition between the mixture formed and the mixture previously present in the

rumen fluid. During the period of decreasing rate of production of acid, however, the changes should reflect the effects of the different rates of absorption of the individual acids. In short, if the rate of production of acid is great enough to bring about a temporary, but marked, increase in the concentration of acid in the rumen, then the composition of the acid there should change towards the composition of the mixture being formed, and later should gradually revert to the composition of the original rumen fluid. In the experiments described here, such changes in the composition of the rumen fluid have been observed, and it is considered that they provide strong evidence in support of the findings reported in Part I of these studies.

EXPERIMENTAL

Sheep in which permanent rumen fistulae had been established were fed a constant ration for a period of 7 days. On the last day samples of rumen fluid were withdrawn at intervals after feeding, and analyses of the fatty acids were made by the procedure, including the chromatographic method, described in Part I of this work (Gray, Pilgrim & Weller, 1951).

Series 1. One of two sheep was fed on wheaten hay and the other on lucerne hay. On the day before sampling the fodder was removed at 5 p.m. and on the day of sampling it was offered at 10 a.m. and again removed at 5 p.m.

Series 2 was a repetition of series 1 except that fodder was offered at 10 a.m. and removed at 5 p.m. throughout the whole week, and more frequent samples of rumen fluid were taken. The results of the analyses are given in Tables 1 and 2.

Table 1. *Volatile fatty acids present in the rumen fluid, series 1*

Fodder	Time after feeding (hr.)	Fatty acids mmol./100 ml. rumen fluid				Percentages of individual acids		
		Acetic	Propionic	Butyric	Total	Acetic	Propionic	Butyric
Wheaten hay:	0	3·40	0·82	0·68	4·90	69·4	16·7	13·9
	½	3·91	1·14	0·76	5·81	67·3	19·6	13·1
	1	4·46	1·38	0·92	6·76	66·0	20·4	13·6
	4	5·72	2·28	1·34	9·34	61·3	24·4	14·3
	7	6·68	2·54	1·58	10·8	61·9	23·5	14·6
	12	7·28	2·14	1·48	10·9	66·8	19·6	13·6
	24	5·70	1·40	1·15	8·25	69·1	17·0	13·9
Lucerne hay:	0	3·58	0·76	0·80	5·14	69·6	14·8	15·6
	½	7·75	2·08	1·34	11·2	69·4	18·7	11·9
	1	9·38	2·78	1·61	13·8	68·1	20·2	11·7
	4	12·3	3·32	1·94	17·6	70·1	18·9	11·0
	7	13·8	3·61	1·88	19·3	71·5	18·7	9·8
	12	13·4	3·30	1·97	18·7	71·8	17·7	10·5
	24	5·17	1·22	1·18	7·57	68·3	16·1	15·6

DISCUSSION

The data in Tables 1 and 2 show that the rate of production of fatty acid was sufficient to bring about a considerable increase in the concentration of acid in the rumen after feeding. By the end of the day the concentration had returned more or

Table 2. *Volatile fatty acids present in the rumen fluid, series 2*

Fodder	Time after feeding (hr.)	Fatty acids mmol./100 ml. rumen fluid				Percentages of individual acids		
		Acetic	Propionic	Butyric	Total	Acetic	Propionic	Butyric
Wheaten hay:	0	5·87	1·58	1·24	8·69	67·6	18·1	14·3
	½	6·04	1·89	1·43	9·36	64·5	20·2	15·3
	1	6·95	2·50	1·77	11·2	61·9	22·3	15·8
	2	8·39	3·54	2·19	14·1	59·4	25·1	15·5
	3	8·48	3·67	2·22	14·4	59·1	25·5	15·4
	4	10·6	4·76	2·84	18·2	58·2	26·2	15·6
	6	12·1	5·48	2·98	20·5	58·8	26·7	14·5
	8	8·26	3·52	1·86	13·6	60·5	25·8	13·7
	12	9·64	3·57	1·97	15·2	63·5	23·5	13·0
	16	8·82	2·76	1·62	13·2	66·8	20·9	12·3
	20	7·76	2·13	1·53	11·4	67·9	18·7	13·4
	24	6·28	1·49	1·22	8·99	69·8	16·6	13·6
Lucerne hay:	0	6·48	1·38	1·43	9·29	69·8	14·8	15·4
	½	8·93	2·13	1·48	12·5	71·2	17·0	11·8
	1	11·2	2·83	1·80	15·8	70·7	17·9	11·4
	2	14·6	4·08	2·28	21·0	69·7	19·4	10·9
	3	17·4	4·78	3·03	25·2	69·0	19·0	12·0
	4	17·6	4·93	3·00	25·5	68·9	19·3	11·8
	6	15·0	4·10	2·45	21·6	69·7	19·0	11·3
	8	15·9	4·15	2·28	22·3	71·2	18·6	10·2
	12	16·6	3·95	2·23	22·8	72·9	17·3	9·8
	16	13·3	2·91	2·04	18·3	73·0	15·9	11·1
	20	9·56	2·22	1·75	13·5	70·5	16·5	13·0
	24	6·90	1·65	1·42	9·97	69·3	16·5	14·2

less to its original value. It is clear that quite appreciable changes occurred in the composition of the mixture of acids during the day.

It has been pointed out that, to be consistent with the production in the rumen of a mixture of acids similar in composition to that already found *in vitro* (Part I), these changes should proceed in a certain direction when the rate of production of acid is increasing, and in the reverse direction when the production declines. It is not possible to know precisely when the rate of production of acid reached its peak in these experiments, because the concentration of acid may have continued to rise for a considerable time after the rate of production began to decline. However, in the sheep which were fed on wheaten hay it may be said that the rate of production began to decrease within 12 hr. after feeding in series 1, and within 6 hr. in series 2. When lucerne hay was fed the maximum rate of production must have occurred earlier than in the case of wheaten hay.

The changes in the composition of the rumen fluid are reviewed more clearly in the graphs drawn in Figs. 1 and 2, where the ratio of each pair of acids is considered separately. As an example, changes in the ratio [acetic acid]/[propionic acid] when wheaten hay was fed, may be considered (Fig. 1). It can be seen that in the period when the rate of production of acid was increasing this ratio decreased in value—a change consistent with the production of a mixture of acids containing a smaller proportion of acetic acid and a larger proportion of propionic acid than was originally present in the rumen fluid. Furthermore, in the latter part of the day (12–24 hr. after feeding) when the production rate was low and the effects of absorption

predominated, the ratio [acetic acid]/[propionic acid] increased to approximately its original level. It is a reasonable inference that this change was due to the more rapid absorption of propionic than acetic acid.

Any other interpretation of these changes would seem to involve the postulation of very considerable alterations in the relative rates of production and/or absorption

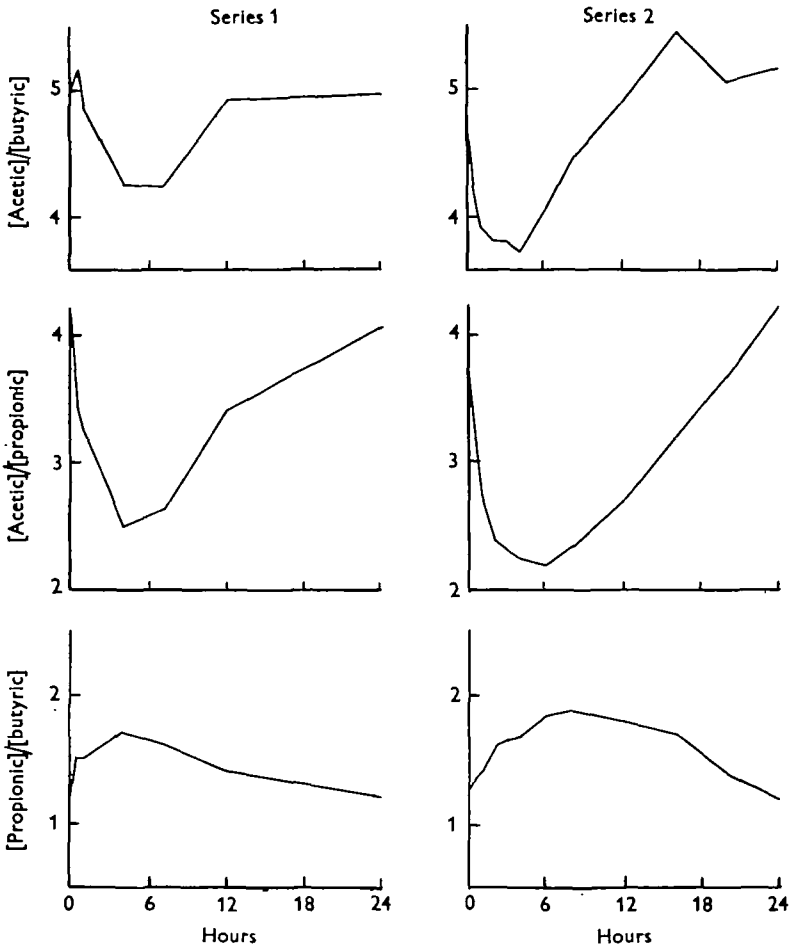

Fig. 1. The ratios [acetic]/[butyric], [acetic]/[propionic] and [propionic]/[butyric] in the rumen during the fermentation of wheaten hay.

of the fatty acids during the fermentation and, while there is no *a priori* reason why this should not be so, the interpretation given not only accounts directly for the changes, but is also consistent with the findings which have been discussed in the *in vitro* studies of the rumen fermentation (Part I). There it was predicted that changes very similar to those which have been observed should take place in the composition of the rumen fluid after the feeding of these fodders. The remaining data, examined and interpreted in the same way, suggest that when wheaten hay was fermented the

relative rates of absorption of the acids fell in the series propionic acid > butyric acid > acetic acid, and that in the case of lucerne hay the order was propionic acid > acetic acid > butyric acid. In view of these conclusions it is necessary to make a critical survey of the earlier work in the field.

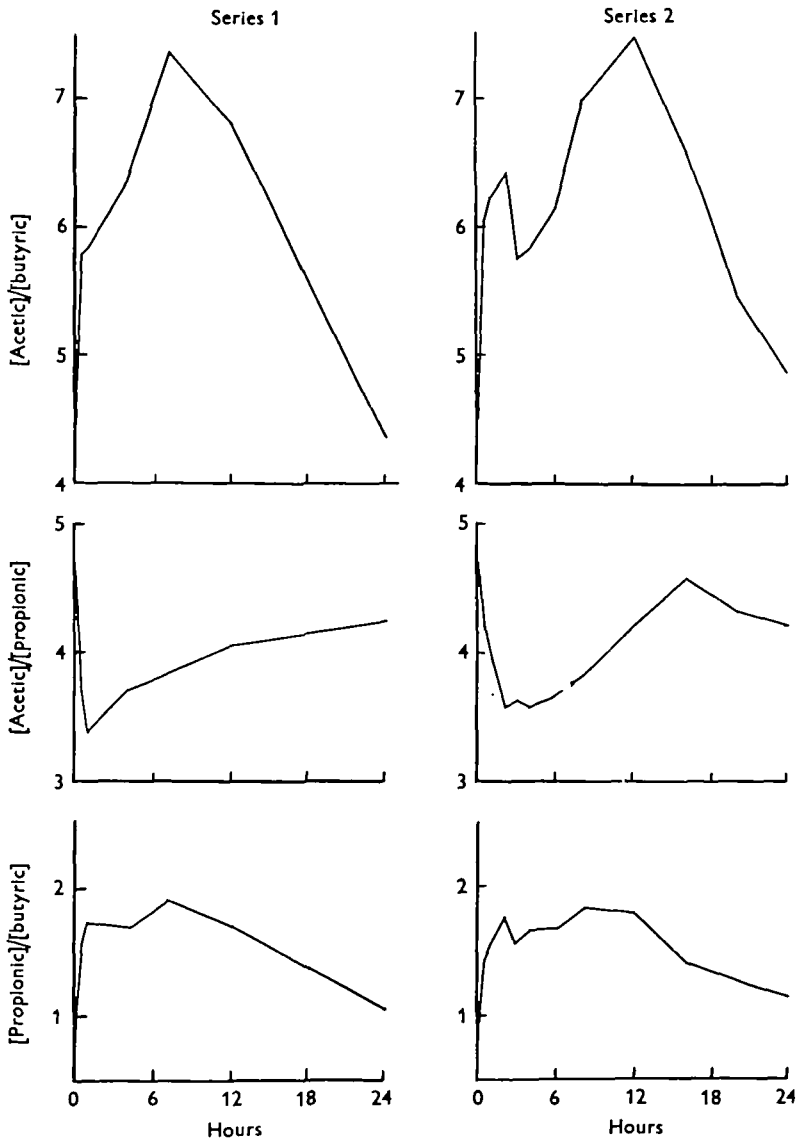

Fig. 2. The ratios [acetic]/[butyric], [acetic]/[propionic] and [propionic]/[butyric] in the rumen during the fermentation of lucerne hay.

The absorption of fatty acids from the rumen was first studied in England by Barcroft and his colleagues (Barcroft *et al.* 1944) who approached the problem from two different aspects. First, they compared the proportions of the individual acids

present in the fatty acid of the rumen fluid with those in the fatty acid of the venous blood draining the rumen wall. Because the latter contained the greater proportion of acetic acid they concluded that acetic acid was absorbed more rapidly than were the higher acids. Secondly, they introduced solutions of the sodium salts of each of the three acids (undoubtedly at a reaction higher than pH 7) into an isolated, empty rumen, and measured the concentration of volatile acid developed in the rumen venous blood in each case. They found the concentration to be highest after the introduction of sodium acetate and lowest after the introduction of sodium butyrate. The results then, appear to confirm their first finding.

The work of the English School was continued (Danielli *et al.* 1945) by examining further the absorption of pure acids or their salts when introduced into the empty rumen. The three fatty acids were introduced simultaneously and the mixture remaining in the rumen was analysed at intervals. In one group of experiments the reaction was maintained at pH 6·5, and under these conditions it was established that the relative rates of absorption fell in the series butyric > propionic > acetic. In the other group of experiments the reaction was maintained at pH 7·5, and it was claimed that this led to a reversal of the relative rates of absorption, acetic > propionic > butyric. It has been pointed out, however (Gray, 1948), that the data indicated rather that under these alkaline conditions the rates of absorption were equal.

Meanwhile, work in this laboratory (Gray, 1947, 1948) had covered some of the same ground. From the loss of acids in mixtures introduced into the rumen at pH 6·5 it was found that propionic acid was absorbed more rapidly than acetic acid, but the only experiment in which butyric acid was included suggested that the relative rates of absorption of the three acids fell in the series propionic > butyric > acetic. Furthermore, it was claimed that under alkaline conditions no absorption took place.*

In discussing this earlier work it is necessary to bear in mind that a most important factor in the normal physiological state of the rumen is the reaction of its contents.

The early investigations in this subject, in which material from slaughtered animals was used, may be disregarded. The examination *in vitro* of material taken from living animals has yielded more consistent results. Thus Hale, Duncan & Huffman (1932) found an average reaction of pH 6·8 for animals fed on lucerne hay and Kick, Gerlaugh, Schalk & Silver (1938) gave a range of pH 5·5–7·7 also for lucerne hay; Phillipson (1942) found a range of pH 5·7–6·7 for a sheep fed a mixture of oats, bran, hay and straw. The most reliable values, however, must be obtained by measurements made within the rumen itself. Smith (1941), using a glass electrode *in vivo*, found averages of less than pH 6·27 and 6·0 for cattle fed on lucerne, and on beet pulp plus lucerne, respectively. Gray (1946), also using a glass electrode *in vivo* found a range of pH 5·5–6·8 for sheep fed on lucerne hay, the lowest values occurring

* This latter claim has since been found only to be correct in certain circumstances. Under alkaline conditions absorption takes place to a varying degree, as found by Danielli *et al.* (1945). A number of factors have been shown to influence the extent of absorption when the rumen contents are alkaline (Jarrett, Gray & Rodda, 1949).

soon after feeding. When the animal is starved the reaction rises to pH 7 or higher after the first 24 hr. (Phillipson, 1942; Gray, 1946).

It may be concluded that the reaction of the normal rumen is decidedly acid and that, therefore, any experimental work carried out with the rumen contents alkaline can have no direct bearing on the problem of absorption from the rumen. Thus one part of the evidence from Barcroft *et al.* (1944) in favour of the more rapid absorption of acetic acid than of the higher acids is disposed of.

The evidence relating to the fatty acids in the blood may be criticized on two grounds. In the first place no account was taken of the acids present in the arterial blood supplying the rumen wall. The experimental data included analyses of blood from the carotid artery which showed acid concentrations ranging from 0 to 13 ml. N/100 volatile acid/100 ml. blood, while the rumen venous blood showed a range of 17–75 ml. N/100 volatile acid. The actual proportions of the individual acids in each case were not stated and so, even if the methods of analysis were sound, it is not possible to decide that their final conclusion was justified.

A more important criticism concerns these methods of analysis. It has already been pointed out (Gray, 1947) that the interpretation given to the results of the distillation procedure could not be justified. But the analyses were also checked by the chromatographic method of Elsden (1946). In this method, however, acetic acid is determined as the difference between the total acid and the sum of the propionic and butyric acids, because acetic acid is not eluted from the column.

Now it has been found (Gray, Rodda & Weller, 1949) that the mixture of 'volatile acids' derived from blood by the methods used by Barcroft *et al.* (1944) contains a considerable proportion of acid which is not acetic, propionic or butyric acid. Moreover, this extra fraction consists of at least three acids which all move more slowly down the column than does acetic acid. It is possible then that they were included in the 'acetic' fraction in the work under discussion. To approach the problem of absorption of fatty acids by examination of the fatty acids of the blood would therefore require methods for the separation of the acids from blood which avoided these difficulties. It is clear that the evidence for the absorption of acetic acid at a greater rate than the other acids cannot at present be considered sound.

On the other hand, the work of Danielli *et al.* (1945) suggested that at the reaction of the rumen contents the rates of absorption of the acids should be in the order of butyric > propionic > acetic. The work has been checked in this Laboratory and there is no doubt that, under the particular experimental conditions, the acids are absorbed at these relative rates. But the conditions differ greatly from the normal conditions which obtain in the rumen.

The approach to the problem which has been adopted in the present work avoids the difficulties associated with the animal being in an unphysiological state. Furthermore, the findings are strongly supported by the results of the *in vitro* fermentations reported in Part I of these studies. The difference in the order of the relative rates of absorption of the acids from the two fodders used in these experiments points to the necessity for investigation of the relationship between the concentrations of the acids in the rumen and their rates of absorption from it.

SUMMARY

1. Analyses of the rumen fluid of sheep fed on wheaten hay and on lucerne hay showed that characteristic changes take place in the composition of the mixture of volatile fatty acids in the rumen throughout the day.

2. The changes conform closely to those predicted from the composition of the mixture of fatty acids produced from the same two fodders *in vitro*. They support the view that propionic acid is relatively more rapidly absorbed than either acetic or butyric acid, and that the fermentation of these fodders in the rumen produces a mixture of the acids in which propionic acid forms a larger proportion than it does in the rumen fluid.

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REFERENCES

BARCROFT, J., MCANALLY, R. A. & PHILLIPSON, A. T. (1944). *J. Exp. Biol.* **20**, 120.
DANIELLI, J. F., HITCHCOCK, M. W. S., MARSHALL, R. A. & PHILLIPSON, A. T. (1945). *J. Exp. Biol.* **22**, 75.
ELSDEN, S. R. (1946). *Biochem. J.* **40**, 252.
ELSDEN, S. R. & PHILLIPSON, A. T. (1948). *Ann. Rev. Biochem.* **17**, 705.
GRAY, F. V. (1946). Thesis. University of Adelaide.
GRAY, F. V. (1947). *J. Exp. Biol.* **24**, 1.
GRAY, F. V. (1948). *J. Exp. Biol.* **25**, 135.
GRAY, F. V., PILGRIM, A. F. & WELLER, R. A. (1951). *J. Exp. Biol.* **28**, 74.
GRAY, F. V., RODDA, H. J. & WELLER, R. A. (1949). Unpublished data.
HALE, E. B., DUNCAN, E. W. & HUFFMAN, C. F. (1932). *Proc. Amer. Soc. Anim. Prod.* **32**, 389.
JARRETT, I. G., GRAY, F. V. & RODDA, H. J. (1949). Unpublished data.
KICK, C. H., GERLAUGH, P., SCHALK, A. F. & SILVER, E. A. (1938). *Bull. Ohio Agric. Exp. Sta.* no. 592.
PHILLIPSON, A. T. (1942). *J. Exp. Biol.* **19**, 186.
SMITH, V. R. (1941). *J. Dairy Sci.* **24**, 569.