

There is a further point to be made as to the origin of the large amounts of acetic acid which make it the predominating acid in the rumen (Marshall and Phillipson, 1945), and I must state at once that I do not know how it is formed. It is probable to my mind that it arises from the polysaccharides other than cellulose in feeding stuffs and, in particular, from the so called hemicelluloses and pentosans, about which we know little or nothing. Until, however, a detailed study of these substances has been made, and until they are available in a state of purity and in large quantities, the mode of production of volatile fatty acids from them in the rumen is a matter of speculation.

### Summary

1. The diet of the ruminant conditions the type of flora in the rumen. This may be of practical importance because it is well known that a sudden change of diet is not to the animal's advantage.

2. Three types of organism have now been associated with the fermentation of carbohydrate in the rumen: a yeast like form which becomes dominant when the animal consumes a diet rich in readily fermentable carbohydrate; a coccus isolated in pure culture by Van der Wath (1942) which, while fermenting glucose or starch stores a polysaccharide which stains blue with iodine; members of the genus, *Propionibacterium*, which have been shown to be responsible for the production of the propionic acid and part of the acetic acid found in the rumen.

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## The Utilization of Volatile Acids

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Dr. Phillipson has told of researches which were initiated by him and which cast quite a new light on the possibilities of ruminant digestion, the gist of which was that the carbohydrate of the diet was largely converted into volatile fatty acids and was absorbed as such (Marshall and Phillipson, 1945) and, in this connection, Dr. Elsdén has discussed the chemistry of the digestion of cellulose and other carbohydrates by the flora of the rumen (Elsden, 1945). My part is to take the matter up where they have left it off and to give you the information now available about the fate of the volatile acids actually absorbed.

The first question was to estimate the actual amount of acids which found their way from the gut into the blood. Here let me emphasize the fact that, though our introduction to the subject came from the study of the ruminant, production of volatile fatty acids in the alimentary

canal and their absorption into the blood is a phenomenon of much wider occurrence, probably extending to the herbivora generally and even to some omnivorous animals such as the pig and rat. Table 1 gives an indication of the average quantities of volatile fatty acid found in samples of blood obtained from various vessels in the sheep, rabbit, pig and pony. The range of error for one determination is about 3 ml.

TABLE 1

AVERAGE AMOUNT OF VOLATILE FATTY ACID IN BLOOD FROM VARIOUS BLOOD VESSELS OF DIFFERENT ANIMALS, EXPRESSED AS ml. N/100 ALKALI NEEDED TO NEUTRALIZE THE VOLATILE FATTY ACID IN 100 ml. BLOOD

Animal	No. of experiments	Situation of vessel from which blood was taken				
		General circulation, carotid	Portal	Vein draining		
				Rumen or stomach	Small intestine	Large intestine
Sheep ..	14	6	—	38	4	22
Rabbit ..	4	4	9	—	4	23
Pig ..	4	5	9	—	11	28
Pony ..	1	7	—	7	5	49

There appears to be a trace of volatile fatty acid as a normal constituent of the blood in the general circulation but the blood which comes from the large intestine and the rumen, if there is one, is quite outstanding in the amount of volatile acid which it contains. The question now comes as to the extent of the daily absorption of volatile fatty acid. The information comes principally from the sheep. Numerous experiments have been carried out on the blood flow from the rumen of these animals. It is possible to measure the blood flow from only about one-third of the rumen at a time and, as this is not the part from which the absorption is greatest, the results, if taken as representing the whole, give an underestimate of the total volatile acid carried away. Typical results are given in Table 2. I have already said that for several reasons the values

TABLE 2

RATE OF ABSORPTION OF VOLATILE ACIDS FROM THE RUMEN INTO THE BLOOD STREAM OF THE SHEEP

Sheep no.	Blood flow per minute from posterior ruminal vein ml.	Concentration of volatile acid expressed as ml. N/100 NaOH per 100 ml. rumen blood	Amount of volatile acid absorbed per hour g.
13	87	43	3.9
15	59	55	3.7
16	107	35	3.5
18	126	44	5.3
19	67	35	2.6
29	92	26	2.3

shown are probably an underestimate, and about 100 g. a day is a probable figure for the amount of volatile acid in terms of acetic acid.

The above estimate is given in terms of equivalent of acetic acid but what are the actual acids involved? Actually about 80 per cent. of the volatile acid in blood coming from the rumen is acetic acid and most of the remainder is propionic, so that when these two have been considered little remains for discussion. Neglecting for the moment the trifling quantities of volatile acid other than acetic and propionic, let us address ourselves to the fates of these two. Here we are on ground as yet not fully explored. Certain statements can, however, be made. The first is that acetic acid at all events does not appear to be immediately excreted as such. Unpublished experiments of Carlyle may be cited in this connexion. One hundred g. of acetic acid in 2 l. of artificial saliva were put into the rumen of a sheep which had been without food for 60 hours and of which the rumen had been washed out 36 hours previously. After 24 more hours 20 g. were found in the rumen and less than 1 g. in the urine, so that roughly 75 g. remained unaccounted for. Table 3 gives the results of these experiments.

TABLE 3  
FATE OF ACETIC ACID INTRODUCED INTO THE RUMEN OF THE SHEEP

Sheep no.	Duration of period before administration of acetic acid		Amount of acetic acid put into rumen g.	Acetic acid recovered		
	After withholding of food hours	After washing out of rumen hours		Time since administration hours	From rumen g.	From urine g.
769/1	60	36	100	24	24.9	0.71
770/2	60	36	100	24	20.1	1.62
770/6	80	60	150	48	4.5	0.33

The next step was to see whether either acetic or propionic acid disappeared in the blood vessels of some organ. The organ which we selected was the beating heart. The method of perfusion used was substantially that of Locke and Rosenheim (1904, 1907), with the improvement that, instead of using a heating system to maintain the temperature of the perfusion fluid, we worked in a room at 30° C. The results are shown in Tables 4 and 5.

In view of the fact that acetic acid seemed to be destroyed on a much greater scale than propionic it seemed desirable to compare the rates of disappearance with that of glucose. Comparable observations of the rate of disappearance of glucose are given in Table 6. The values obtained are substantially the same as those found by Locke and Rosenheim (1904, 1907) for the quantities of glucose removed from Ringer's solution, and for the equivalent of the CO<sub>2</sub> produced if all the carbon in the acetic acid appeared as CO<sub>2</sub>. In Table 7 is set out a summary comparing the rates of removal of glucose, acetic acid and propionic acid.

It seems clear that the case for the utilization of acetic acid by the heart is as good as it was for that of glucose, when Locke and Rosenheim performed their classical experiments. This however is not enough. The question is complicated by the fact that there is always a certain

amount of glycogen initially present in the heart. This varies greatly but there is always less in perfused than in unperfused hearts. All we could say at this stage is that the greatest amount of glycogen we have ever found in a heart is 3.9 mg. per g. of heart. If the heart weighing 2.8 g. in experiment 1 of the acetic acid series (Table 4) is credited with that concentration of glycogen it would contain about 11 mg. but, in the course of the experiment lasting over 6 hours, 130 mg. acetic acid were removed from the perfusion fluid.

TABLE 4  
PERFUSION OF THE RABBIT HEART WITH A FLUID CONTAINING ACETATE

Serial no. of experiment	Weight of heart g.	2 hour period	Acetate introduced at commencement of each 2 hour period mg.	Acetate disappearing in each 2 hour period mg.	Remarks
1	2.80	1st	134	56	—
		2nd		52	—
		3rd		28	—
2	5.36	1st	28	Heart not beating	
3	4.60	1st	66		
		2nd	54		
4	5.20	1st	36	1.7 mg. acetate recovered from heart	
		2nd	34		
5	4.60	1st	141	54	0.8 mg. acetate recovered from heart
Control experiments in which the heart was replaced by a piece of rubber tubing					
1	—	—	134	5	—
2	—	—	„	4	—

TABLE 5  
PERFUSION OF THE RABBIT HEART WITH A FLUID CONTAINING PROPIONATE

Serial no. of experiment	Weight of heart g.	No. of 2 hour period	Propionate introduced at commencement of each 2 hour period mg.	Propionate disappearing in each 2 hour period mg.
1	4.4	1	181	12
		2		5
2	3.6	1	17	
		2	13	
		3	15	
3	5.9	1	22	
4	4.3	1	7	
5	7.2	1	9	
		2	5	

In order to discover whether acetic acid was utilized, as opposed to being destroyed, 3 courses presented themselves, all of which are being followed up.

TABLE 6

PERFUSION OF THE RABBIT HEART WITH A FLUID CONTAINING GLUCOSE

Serial no. of experiment	Weight of heart g.	Experimental period		Glucose introduced at commencement of each period mg.	Glucose disappearing in each period mg.
		Serial no.	Duration hours		
1	4.4	1	2	149	20
		2	2½	"	11
2	5.8	1	2	"	21
		2	1½	"	9
3	4.1	1	2	142	29
		2	2	"	16
4	2.9	1	2	144	30
		2	2	"	30
		3	2½	"	17

TABLE 7

AMOUNT OF GLUCOSE AND OF ACETIC AND PROPIONIC ACIDS DESTROYED PER g. OF PERFUSED RABBIT HEART PER HOUR

Substance	Amount destroyed mg.
Glucose . . . .	2.4
Acetic acid . . . .	5.5
Propionic acid . . . .	1.1

The first was, by treating the experimental animal with phloridzin, to reduce it to a condition in which the ratio of dextrose to nitrogen was constant, then to administer acetic or propionic acid and ascertain the effect on the ratio. This has been done, with the preliminary result that with acetic acid no commensurate rise in the dextrose to nitrogen ratio has so far been obtained; with propionic acid, however, there was a definite rise and the excess sugar recoverable from the urine was in terms of carbon 87 per cent. of the propionate injected. These experiments are not yet complete.

The second approach was through experiments by indirect calorimetry and the third was by the use of acetic acid containing labelled carbon. Researches with these last 2 methods are in progress but neither has yet reached the stage at which a report can be made.

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Discussion

Dr. J. Stewart (Moredun Institute, Gilmerton, Midlothian), opener: In the papers we have just heard three findings, important enough to change all textbook theories of ruminant digestion, have been described. (1) Digestible cellulose and other carbohydrates are for the most part

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converted into volatile fatty acids. (2) A large proportion of such fatty acids is absorbed from the rumen itself. (3) The perfused heart can utilize acetic acid to as great an extent as glucose.

Absorption from the rumen has been suggested to occur by other workers in this country and on the Continent, but it has never been so firmly established as by the experiments we have just heard described. It is an important point since the theory of the breakdown of cellulose to fatty acids cannot be accepted unless it can account for the high nutritive value of these acids to the animal. At this stage of the work we must not forget the careful experiments of Kellner in 1900 which showed the nutritive value of 1 lb. of digestible cellulose to be equal to that of 1 lb. of starch which in turn has been shown to have a net energy equal to 1071 Calories. Thus the amount of energy is large which must be accounted for by the combustion of the fatty acids or by any other theory of cellulose breakdown in the ruminant. As Sir Joseph Barcroft has said, the ultimate proof of any such theory must depend on calorimetric experiments, and these are in progress.

I do not think that the Cambridge workers have made any direct comparison between cellulose and starch. So many calorimetric and metabolism experiments have established beyond doubt the nutritive value of starch that if it was found that equal amounts of starch and digestible cellulose were converted to the same amount of fatty acids in the rumen some proof would be afforded that these fatty acids possessed a high nutritive value to the animal organism and that cellulose had indeed the high nutritive value ascribed to it by Kellner.

We have heard today two different schools of thought on the subject of cellulose breakdown. The first holds the view that the cellulose splitting organisms in the rumen build up within their bodies polysaccharides which are digested by the animal in another part of the alimentary tract. The second school is of the opinion that cellulose is broken down to volatile fatty acids which are absorbed and used by the animal. Surely the former would be one of the most uneconomic processes in nature, involving the breakdown of a complicated substance like cellulose, to simpler substances, the re-synthesis of these substances to polysaccharides, and the subsequent breakdown of the polysaccharides to elementary substances which the animal can utilize.

Sir Joseph Barcroft mentioned that in phloridzinized sheep after doses of propionic acid there was an immediate rise in the dextrose : nitrogen ratio, indicating the immediate conversion of propionic acid to carbohydrate. In similar experiments carried out by myself in 1934 on phloridzinized sheep, there was a rise in the ratio of dextrose to nitrogen within 24 hours after feeding with soda treated straw. This immediate rise in the ratio may be important in considering the two theories, since Mr. Baker mentioned a time lag in the appearance of the iodophile organisms in the rumen and their passage through the alimentary tract. To my mind the more attractive theory is the breakdown of the cellulose to the volatile acids and their absorption from the rumen.

The subject is so important, not only for the understanding of the exact physiological processes involved in rumination but also for the practical feeding of livestock generally, that we hope that Sir Joseph

Barcroft and his colleagues will be able quickly to give us the results of the experiments upon which they are at present engaged.

Mr. F. Baker (County Technical College, Guildford, Surrey): There are certain points in connexion with Dr. Elsden's and Dr. Stewart's statements which deserve close attention. In the first place it is pleasant for me to corroborate Dr. Elsden's findings in regard to the micro-organisms developing during the incubation of the rumen contents of the sheep. With well fed animals, as I have stated earlier, the predominant species is the so called *Schizosaccharomyces ovis*, a fact noted some time ago by Quin (1943). I have, however, tried to make clear, and cannot sufficiently emphasize, that each species of herbivor tends normally to maintain a distinctive type of microbial population. Conclusions drawn from the sheep need not, therefore, be, and in point of fact are not, valid for the ox. In the sheep glycogen is the main product of polysaccharide synthesis. In the ox, starch is formed, the agents of synthesis being here the macro- and micro-iodophile types that I have recently described. A fuller acquaintance, therefore, with the characteristics of the microbial populations concerned entirely resolves any discrepancy on this point between Dr. Elsden's statements and my own.

Dr. Stewart has, however, attributed to me a statement which rests upon a misapprehension, due no doubt to a failure on my part to make certain points clear. Decomposition of cellulose occurs in both the ox and sheep at a later phase in the rumen process than the decomposition of starch and soluble carbohydrates. In both animals, and at each phase, decomposition is accompanied by synthesis of polysaccharide (Baker, 1942). Quin (1943) showed that in the sheep decomposition of soluble carbohydrate, as evidenced by evolution of CO<sub>2</sub>, commenced within 15 minutes of incubation. I can confirm this result and add to it the observation that, over this period, the number of reactant yeast cells showed a parallel increase, *i.e.*, there was synthesis of glycogen (unpublished work). In the rabbit I found that evolution of gas, production of acid and synthesis of bacterial starch were demonstrable after 30 minutes. Similar considerations are valid for the ox. It is clear, therefore, that the agents of decomposition are the agents also of synthesis, and that these processes are simultaneous so that the distinction which Dr. Stewart has sought to draw is groundless.

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Dr. S. R. Elsden: Glycogen may well be synthesized during decomposition of carbohydrate, but it is rapidly autolysed in the absence of fermentable sugar.

Mr. F. Baker: It is once again a happy circumstance that Dr. Elsden and I find ourselves in complete agreement over matters of fact. It is admittedly the case that in the absence of carbohydrate the polysaccharide synthesized is eventually autolysed. Indeed, I have repeatedly emphasized the possible significance of this process for the animal (Baker, 1939, 1942). We have to recognize that several alternative routes are in principle available for the assimilation of the products of microbial synthesis, and that, in addition to the digestive enzymes, a

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number of factors may co-operate in this process. One of these factors is the recurrence of autolysis; another is the ingestion of bacteria by protozoa, which are themselves digested. A third is coprophagy and a fourth bacterial phagocytosis. The extent to which these several routes are in fact used in particular species under known dietetic conditions requires investigation.

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Dr. A. C. Thaysen (Chemical Research Laboratory (Department of Scientific and Industrial Research), Teddington, Middlesex): How can it be explained that cellulose is, according to Kellner, equivalent to starch in food value when only one-third of its carbon is recovered during digestion in the form of organic acids? Obviously part at least of the remaining two-thirds of the carbon must be used in the production of other nutritionally important substances such as protein and fats, formed during the growth of the intestinal microflora and subsequently utilized by the host.

Dr. A. T. Phillipson gave the following reply: The output of nitrogen from the rumen in the form of bacteria cannot exceed the input of nitrogen into the rumen in the form of food. The ratio of protein to carbohydrate in grass is in the region of 1 : 4, this indicates that even if all the nitrogen of the food is converted into bacterial nitrogen the quantity of carbohydrate that is converted to other substances during the process is substantially larger. Part of the carbohydrate so digested can be stored as polysaccharide by the bacteria as Baker has shown while part is converted to volatile acid. The relative quantities of polysaccharide and volatile acid formed in this way are not known but, in formulating estimates of the probable quantities of bacteria and of any substance contained in the bacterial cell, the significance of the limiting factor, namely of the amount of nitrogen available, must be taken into account.

### Chairman's Summing Up

Dr. W. R. Wooldridge (London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1): The subject we have been discussing today is a wide one and indeed the contributions of speakers have covered a wide range. Nevertheless, it seems to me that the breadth of the discussion could have been much greater. I am not going to try and summarize the detail of the discussion put before us, for speakers themselves have been precise, but I take this opportunity to try to place the present work in its wider setting so that we shall not get lost in immediate experimental detail and forget the very great difficulties of the problem as a whole.

There are 3 major factors concerned in the problem of "The Nutritional Role of the Microflora in the Alimentary Tract". They are the nature of the host animal itself, of the ingested material or food, and of the microflora.

The nature of the animal itself affects our problem in that the shape of the alimentary tract varies considerably within the animal kingdom,