Production and Absorption of Organic Acids by the Perfused Goat Rumen.* (24418)

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It is well established that large quantities of volatile fatty acids (VFA) are produced within the rumen by microbial action on cellulose, starch and protein. One of the first requirements for an understanding of the biochemistry of ruminant metabolism is a knowledge of quantities and proportions of the VFA produced and absorbed and the form in which these rumen substances enter the blood of the host animal. Numerous technics have been devised to gain a measure of these values; however, these methods have measured only one part of this system, *i.e.*, either production or absorption(1). That variable results have been obtained is not unexpected in view of the dynamic nature of this system where production and absorption occur continuously and simultaneously. Rumen perfusion was selected as a technic for measuring simultaneously both total production and absorption of VFA.

Methods. The rumina of 2 mature goats weighing approximately 60 lb were perfused with heparinized blood by use of pump-oxygenator apparatus similar to one used previously in this laboratory(2). These animals had been fed a diet of mixed hay and concentrate *ad lib.* for 6 weeks prior to the perfusion studies. Labeled substrates were added to rumen contents immediately prior to perfusion, 50 μc of n-butyrate-1-C^{14}Na being used for perfusion 40 and 50 μc of propionate-1-C^{14} Na in perfusion 42. The 2 perfusions, 40 and 42, were conducted for 80 and 115 minutes respectively. Organic acids in blood and rumen fluid were measured by modification of the chromatographic technic of Wiseman and Irvin(3). Blood glucose was determined by the method of Somogyi(4). Analysis for total acetone bodies in blood was performed by procedure of Weichselbaum and Somogyi (5). Acetoacetic acid was isolated by method of Cavallini and Frontali(6). Measurements of radioactivity were made with a windowless flow tube used in the Geiger region. Osazones were prepared for estimating activity in blood glucose. The carboxyl carbon of β-hydroxybutyrate was removed by oxidation and counted as barium carbonate.

Results. Rates of blood flow through the isolated rumina were 65 ml/minute in perfusion 40 and 150 ml/minute in perfusion 42. These values are equivalent to 25 to 50% of the estimated *in vivo* rate(7).

Quantitative results for perfusion 40 are presented in Table I. The total increase in organic acids in the isolated system amounted to 2392 mg of which 31% is represented by increase in blood. Similarity in molar per cent of the organic acids in rumen fluid at beginning and end of perfusion, is indicative of maintenance of a nearly normal physiological state. Comparison of increases of individual acids in blood with increases in rumen fluid shows that, with the exception of formate and lactate, the increases in blood were a reflection of production of these acids.

The perfused rumen utilized glucose, blood concentration decreasing from 43.8 to 29.3 mg % during experiment. No change occurred in level of acetone bodies in the blood.

The quantitative results from perfusion 42 are presented in Table I. In an effort to remove vasaconstrictive substances blood was passed through the isolated liver of the goat for 10 minutes before attaching the blood supply to the vascular system of the rumen. This resulted in an increased concentration of certain blood metabolites.

The ruminal tissue utilized a total of 844 mg of glucose during perfusion, blood concentration decreasing from 265 mg to 171 mg/100 ml of blood. Blood levels of acetone

* Scientific Article No. A717, Contribution No. 2952 of Md. Agr. Exp. Station. Supported by U. S. Atomic Energy Comm.

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TABLE I. Changes in Blood and Rumen Metabolites during Perfusion of Rumina of Goats.

Blood and rumen metabolites	Organic acids in rumen liquor at beginning of perfusion		Organic acids in rumen liquor at end of perfusion		Increases in rumen organic acids during perfusion, mg	Total changes in blood metabolites during perfusion, mg
	mg/100 ml	Molar % of total	mg/100 ml	Molar % of total		
			Perfusion No. 40 (time, 80 min.)†			
Glucose						−145.0
Acetone bodies*						.0
Organic acids						
Valeric	27.8	2.4	41.3	2.9	107.2	24.7
Butyric	139.8	14.2	173.5	14.4	267.9	106.5
Propionic	142.0	17.2	180.5	17.8	305.7	117.2
Acetic	397.0	59.9	477.8	58.3	641.2	362.6
Formic	4.8	.9	6.1	.9	10.3	−32.9
Lactic	53.2	5.3	67.6	5.4	114.3	334.6
Total organic acids	764.5		946.7		1446.6	945.6
			Perfusion No. 42 (time, 115 min.)‡			
Glucose						−843.8
Acetone bodies*						− 87.8
Organic acids						
Valeric	10.1	1.1	11.2	1.0	18.4	18.0
Butyric	14.5	1.9	32.9	2.5	299.8	43.6
Propionic	152.2	23.3	229.5	21.0	1260.3	219.8
Acetic	391.4	73.8	669.2	75.7	4529.1	488.3
Formic	.0		.0		.0	−33.7
Lactic	.0		.0		.0	154.9
Total organic acids	568.2		942.9		6107.6	924.7

* Acetone bodies calculated as β-hydroxybutyric acid. † Total volume: rumen fluid = 794 ml, blood = 1000 ml. ‡ Total volume: rumen fluid = 1630 ml, blood = 900 ml.

bodies decreased from 55.2 to 45.5 mg % during perfusion. As in the previous perfusion there was marked production of organic acids in the isolated system. However, in this case formate and lactate were not present in the rumen fluid.

Distribution of recovered C^{14} label and specific activity (S.A.) of the various metabolites following perfusions 40 and 42 is shown in Table II. Distribution of label among the organic acids in rumen fluid at completion of perfusion 40, where labeled butyrate was added, (Table II) shows that there was recycling of the carboxyl carbon of butyric acid. Conversions of this nature have been reported previously(8,9). The greatest quantity of recovered label in blood was found in butyric acid. All blood acids measured, except formic acid, contained some of the C^{14} label. Comparison of the S. A. of acids in rumen fluid with the same acid in blood shows that the specific activities of blood butyrate and lactate were higher than those of these same acids in the rumen. On the other hand, specific activities of acetate, propionate and valerate were greater in rumen fluid. Only a trace of label was found in β-hydroxybutyrate in blood while none was found in acetoacetate or blood glucose.

Distribution of radioactivity in the rumen at completion of perfusion 42 where labeled propionate was added, (Table II) shows that a minor amount of the propionate was converted into acetic and valeric acids. Within blood 97.3% of the recovered label was in propionic acid. It is interesting to note that formate in the blood perfusate was labeled even though there was utilization of formate by the ruminal tissue.

Discussion. Large quantities of organic acids are normally produced in the rumen. The results reported herein, where total production and absorption were measured, show that on a diet of hay and grain, relative rates of production of the VFA are acetate>propionate>butyrate>valerate. With the exception of formate and lactate, absorption of or-

TABLE II. Distribution of C^{14} Label, and Specific Activity of Various Metabolites following Perfusion of Goat Rumina in Which n-Butyrate-1-C^{14} Na or Propionate-1-C^{14} Na Had Been Added to the Rumen Contents.

Metabolites	Distribution of activity in rumen fluid, %	Distribution of activity in blood, %	Specific activity Rumen fluid (CPM/μm)	Specific activity Blood (CPM/μm)
Exp. 40—Labeled butyrate added*				
Osazones				0
Acetoacetic acid				0
β-Hydroxybutyric acid		trace		31
CO_2		"		
Organic acids				
Valeric	1.8	1.3	185	104
Butyric	61.9	72.4	742	1083
Propionic	10.6	8.4	100	97
Acetic	25.8	7.9	59	21
Formic	.0	.0	0	0
Lactic	.4	9.7	14	25
Exp. 42—Labeled propionate added†				
Osazones		.0		
Organic acids				
Valeric	.7	.7	5396	4048
Butyric	.0	.0	0	0
Propionic	95.5	97.3	26,689	35,870
Acetic	3.8	1.3	294	139
Formic		.8		501
Lactic		.0		0

* 81% of added C^{14} recovered of which 6.3% was isolated in blood.

† 70% of added C^{14} recovered of which 43.7% was isolated in blood.

ganic acids appears to be a reflection of their production.

Utilization of blood formate by ruminal tissue is consistent with the findings of Annison(10) who reported that in ruminants portal levels of formic acid were no greater than peripheral blood concentrations.

Distribution of label in the 2 perfusions shows that recycling of the organic acids in the rumen may occur. Conversion of butyrate to other acids was much greater than conversion of propionate. It is of interest to note that butyrate was converted to propionate (a pathway which is obscure at this time) while propionate was not converted to butyrate.

Comparison of the distribution of label and S. A. of organic acids in blood with distribution and S. A. of the same acids in rumen fluid indicates direct absorption of the rumen acids *per se*. The S. A. of butyrate in blood was greater than the S. A. of butyrate in the rumen at completion of experiment. If butyrate was being absorbed *per se* this would be the expected result. Initially all activity was in rumen butyrate and therefore butyrate absorbed in the beginning of experiment had a high S. A. During perfusion the S. A. of butyrate in the rumen decreased due to production of unlabeled acid and conversion of butyrate to other acids. Thus after a period of time the S. A. of butyrate in blood would be greater than the S. A. of this metabolite in the rumen. We have shown previously that butyrate does not pass from blood perfusate into the rumen(11). The data in perfusion 42 show that propionate was also absorbed as such from the rumen.

Direct absorption of acetate, propionate and valerate in perfusion 40 is supported by the fact that the S. A. of these acids in blood was lower than the S. A. of the same acid in the rumen fluid. In this perfusion the amount of label found in lactate present in blood perfusate suggests that it arises from an unknown mechanism in addition to direct absorption. This concept is also supported by comparison of S. A. of lactate in rumen fluid with that in blood.

In perfusion 42 neither rumen nor blood butyric acid were labeled and the blood lactate was not labeled. However, in perfusion 40 where both blood and rumen butyrate were highly labeled the rumen and blood lactate were labeled with the latter having the higher S. A. The greater S. A. of blood lactate indicates that a part of the blood lactate arose by a mechanism other than direct absorption. Black *et al.*(12) estimated that one-half to two-thirds of glucose catabolism in ruminants occurs *via* the pentose cycle. The pentose cycle involves oxidation and ultimate decarboxylation of glucose-6-phosphate to form a pentose. The further catabolism of the pentose yields a 2 and 3 carbon fragment. The 3 carbon fragment is usually lactic acid. It can be speculated that the apparent appearance of a small amount of label from butyrate in lactate could result from butyrate providing a 4 carbon intermediate which is known to be involved in the pentose cycle.

The appearance of label in blood formate in perfusion 42, even though there was a utilization of formate by the ruminal tissue, indicates that the labeled formate probably resulted from metabolism of the ruminal tissue. This fact shows that the rumen acids can contribute to ruminal tissue metabolism.

The large utilization of glucose in perfusion 42 and the decrease in blood levels of acetone bodies in this same perfusion most likely resulted from a mass action effect since the passage of blood through the isolated liver resulted in a marked increase in blood concentration of these metabolites.

The fact that there was no increase in blood levels of acetone bodies in either perfusion is contrary to the belief of most ruminologists who have interpreted the work of Pennington (13) as indicating that butyrate is absorbed in the form of acetone bodies. The latter noted quantitative conversion of butyrate to acetoacetate by ruminal epithelial tissue slices. In perfusion 40 in which labeled butyrate was added to the rumen contents only a trace of label was found in β-hydroxybutyrate in blood. Even though there was no net increase in blood levels of acetone bodies a comparison of the S. A. of blood butyrate with the S. A. of β-hydroxybutyrate makes it doubtful that a direct conversion was occurring. In several rumen perfusions in this laboratory a moderate increase in blood acetone bodies was noted(11). Davis *et al.* (abstract) however, did not obtain an increase in blood acetone bodies during their perfusion experiments.

Extrapolation to a 24 hour basis shows that the organic acid production of perfusions 40 and 42 could provide 31 and 53% respectively of the estimated daily net energy requirements of the animal.

Summary. 1) Any inferences drawn from data of perfusion experiments must obviously be tempered by artificial conditions under which the tissue is operating. While recognizing that there are limitations it seems that a picture of the overall metabolism of the rumen may be formulated. 2) Large quantities of organic acids are normally produced in the rumen. On a diet of hay and grain relative rates of production of the VFA are in the order acetate>propionate>butyrate>valerate. Lactate may also be produced. Calculations based on data from perfusion experiments indicates that the organic acids are capable of providing up to 53% of the estimated net energy requirements of the animal. 3) Interconversion of organic acids may occur in the rumen. Butyrate in the rumen was converted to appreciable amounts of acetate and propionate and to lesser amounts of valerate and lactate. The interconversion of propionate in the rumen was negligible. 4) Acids produced in the rumen are absorbed as such into the blood of the animal, and relative quantities of these acids absorbed are, in general, a reflection of their production in the rumen. Rumen acids do not serve as the immediate precursors of blood acetone bodies. However, these acids apparently can contribute in part to the metabolism of the ruminal tissue.

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Received September 25, 1958. P.S.E.B.M., 1958, v99.