THE ABSORPTION OF LACTIC ACID FROM THE RETICULO-RUMEN OF THE SHEEP

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Summary

A series of acute experiments has been carried out on sheep to study some of the factors which influence the rate of absorption of lactic acid from the ligated washed-out forestomachs.

The rate of absorption of lactic acid was faster from an 0·12M than from an 0·06M solution and faster from a solution at pH 4 than from a solution at pH 5. The absorption rate was less from a solution with a tonicity of 440 m-osmoles than from a solution with a tonicity of 300 m-osmoles. The absorption rate of lactic acid was also decreased with the concomitant absorption of a mixture of acetic, propionic, and butyric acids from acid solutions but not from alkaline solutions.

The rate of absorption of lactic acid was slow relative to the volatile fatty acids from solutions which were either acid or alkaline.

Anaesthetization of the sheep with sodium pentobarbitone did not decrease the rate of lactic acid or volatile fatty acid absorption from the non-ligated, washed-out reticulo-rumen.

The results are in conformity with the hypothesis that the diffusion barrier of the reticulo-rumen wall to nutrients in the digesta is a lipid membrane containing water-filled pores.

I. Introduction

Lactic acid is sometimes found to be a constituent of the rumen digesta of sheep and cattle.

Pfander *et al.* (1956) found that lactate absorption was slow and less than volatile fatty acid absorption from the washed-out isolated rumen. Very little detail is given in this report.

Hueter, Shaw, and Doetsch (1956) observed a rapid increase in the concentration of lactate in the blood after the administration of sodium lactate into the rumen of dairy cows. They considered that the rise was due to fast absorption of lactate from the alimentary tract. This could have taken place from either the forestomachs or lower parts of the tract.

Dobson (1961) considered that the results he reviewed on lactate absorption from the rumen were equivocal and suggested that more data was required.

The presence of the hydroxyl group on the α-carbon in the lactic acid molecule gives it different physicochemical properties from those of the volatile fatty acids. Its lipid solubility is less. Camien, Fowler, and Dunn (1959) have published partition ratios for lactic acid and the volatile fatty acids between isopropyl ether and 1N hydrochloric acid. The ratios found were L-lactic acid 0·05, acetic acid 0·2, propionic acid 1·0, and butyric acid 2·8. These ratios indicate only approximately the relative

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solubility of the acids in other organic solvents. Lactic acid is also a stronger acid than the volatile fatty acids (pK_a's 3·86 and approximately 4·7 respectively).

It is probable that the lactic acid anion is smaller in size than the propionic acid anion in aqueous solution. This is indicated by their respective values for λ_0, the limiting equivalent conductivity of ions in water. The greater the value of λ_0 the relatively smaller the effective hydrated radius of an ion. Robinson and Stokes (1959) show values for λ_0 which include acetate 40·9, propionate 35·8, butyrate 32·6, and sodium 50·1. Martin and Tartar (1937) calculated λ_0 of sodium lactate to be 88·86 from conductance measurements. λ_0 for lactate is therefore 38·76 which is greater than λ_0 for propionate but less than that for acetate.

A basic model for the structure of the plasma membrane of cells was proposed by Danielli and Davson (1943). Although undoubtedly an oversimplification of the selective barrier to the passage of metabolites into and out of the cell, the model has proved useful in interpreting data from permeability studies. They suggested that the plasma membrane can be considered as a double layer of lipids with the polar groups outermost and with an adsorbed layer of protein forming a mesh over the surface. They also considered that the membrane had areas permeable to small lipid-insoluble molecules and ions. These areas were referred to as water-filled pores. Estimates of the pore size in a variety of tissues have been made (Mullins 1960; Solomon 1960; Lindemann and Solomon 1962).

Danielli *et al.* (1945) gave detailed consideration to the properties of the plasma membranes of the rumen epithelial cells from a study of the absorption of acetic, propionic, and butyric acids from the ligated washed-out rumen. The data supported the hypothesis that the anions are absorbed by simple diffusion through water-filled pores and the free acids by diffusion through water-filled pores and lipid membranes. Thus the rates of absorption of the individual acids were very slow from alkaline solutions relative to their rates from acid solutions. Also, the rates of absorption of the individual acids were very roughly proportional to their concentrations in the absorption fluid under alkaline conditions but from acid solutions the data indicated that the rates of absorption were butyrate > propionate > acetate, which is in the order of the relative solubilities of the free acids in fat solvents.

From consideration of the work of Danielli *et al.* (1945) and the relative solubilities of lactic acid and the volatile fatty acids in fat solvents, it seemed probable that the report of Pfander *et al.* (1956) is correct and that free lactic acid is absorbed from the rumen at a very much slower rate than the free volatile fatty acids.

The experiments reported here were carried out to study the effects of a number of factors on the absorption rate of lactic acid from the reticulo-rumen.

II. Experimental Procedure and Methods

(*a*) *Animals*

Mature Merino ewes and wethers were used.

(*b*) *Feed*

All sheep except No. 30 were fed 700 g lucerne chaff once per day, and feed was withheld for 48 hr prior to the absorption periods. Sheep No. 30 was fed 100 g maize grain and 600 g lucerne chaff once per day, and feed was withheld for 24 hr prior to the absorption periods.

(c) *Animal Preparation*

(i) *Establishment of a Rumen Fistula.*—A permanent rumen fistula was established in all sheep at least 14 days prior to the absorption periods by a one-stage operation using the rubber cannulae of Jarrett (1948).

(ii) *Preparation for the Absorption Periods.*—On the morning of the absorption periods the sheep were weighed and anaesthetized with sodium pentobarbitone. The trachea was exposed and cannulated; the oesophagus was ligated with cotton tape; the carotid artery was freed from surrounding tissues and separated from the vagus by blunt dissection.

The reticulo-rumen was emptied of most of its digesta via the fistula and the body cavity was entered by an incision about 3 in. long on the right side of the animal, parallel with the last rib and starting from the point of the xiphoid cartilage. The omasal–abomasal orifice was ligated with cotton tape. Care was taken not to tie-off any large blood vessels supplying or draining the area. The manipulation of the viscera was kept to a minimum. The incision was closed with Michel clips.

(iii) *Procedure during the Absorption Periods.*—The reticulo-rumen was washed free of digesta via the fistula with warm tap water and a final wash with warm physiological saline. A test solution of 2500 ml was then introduced into the rumen and it was allowed to remain in the organ for 2 hr (this is referred to as absorption period 1). At the end of this time the rumen was emptied via the fistula and washed-out twice with warm physiological saline. Absorption period 2 was then carried out in an identical manner except that the test solution had a different composition. After the final wash at the end of adsorption period 2 the animal was killed with an overdose of sodium pentobarbitone. The positions of the ligatures were then checked.

The general procedure was modified in experiments performed to study the effect of anaesthesia on absorption from the rumen. The rumen was washed-out without anaesthetizing the sheep or ligating the oesophagus or omasal–abomasal orifice. The sheep was laid on its right side and the test solution run into the rumen. At the end of the first absorption period of 1 hr the animal was anaesthetized and the rumen emptied in the normal way. A similar solution was then introduced into the rumen for the second absorption period of 1 hr.

By analyses of the original test solutions and the solutions recovered after the absorption periods, plus the washings, an estimate was made of the amount of the test substance which had been absorbed. The order, within one experiment, in which different solutions were introduced into the washed-out rumen was reversed for alternate animals. This was done to show any consistent differences in the rate of absorption occurring during period 1 compared with period 2 due to deterioration in the preparations with time. Masson and Phillipson (1951) found that the rate of absorption of volatile fatty acids from the washed-out rumen decreased with time.

(d) *Test Solutions*

The details of the composition of each solution used are presented under each table of results.

Sodium lactate was obtained by hydrolysing a solution of lactic acid by gentle boiling with a slight excess of sodium hydroxide. Enough distilled water was added so that the concentrations of the solutions were less than 1M.

The buffers used were either disodium hydrogen citrate or sodium dihydrogen phosphate.

The required pH of each solution was obtained by the addition of sufficient concentrated hydrochloric acid or solid sodium hydroxide.

The volatile fatty acid mixture was approximately 60% acetic, 20% propionic, and 20% butyric acid on a molar basis.

In experiments designed to study the effect of volatile fatty acid on lactic acid absorption from a solution at pH 5 the buffering capacity of the solution had to be lowered or the osmotic pressure of the solution made hypertonic with respect to blood. It was decided that it was most important to maintain a high buffering capacity in these solutions. Thus the solutions containing the volatile fatty acids had a greater buffering capacity at pH 5 and a greater osmotic pressure than solutions without volatile fatty acid.

Phenol red was added to all solutions as a marker to check the fluid volume recoveries.

(*e*) *Collection of Samples*

(i) *Test Solutions.*—Samples were taken of the solutions added to, and recovered from, the rumen and of the washings.

(ii) *Blood.*—Venous blood was obtained from the jugular vein. Arterial blood was obtained directly from the carotid artery. Blood samples from sheep Nos. 1, 2, 3, 7, 8, 9 were allowed to stand until the end of the second absorption period (i.e. up to 5 hr for the first venous sample) before the proteins were precipitated with an equal volume of 6% w/v perchloric acid. The proteins were precipitated immediately in the remaining samples.

(*f*) *Chemical Determinations*

The following methods of analysis were used:

(i) *pH.*—This was determined with a Radiometer pH-meter 22 with a glass electrode. No attempt was made to prevent changes in bicarbonate content of solutions recovered from the rumen.

(ii) *Osmotic Pressures.*—These were measured with a Fiske Osmometer model G-62.

(iii) *Phenol Red.*—Phenol red was determined by the colorimetric method of Mixner and Anderson (1958).

(iv) L(+)-*Lactic Acid.*—The enzymatic method of Barker and Britton (1957) was used.

(v) *Total Lactic Acid.*—The colorimetric method of Barker and Summerson (1941) was used.

(vi) *Total Volatile Fatty Acid.*—The total volatile fatty acid was determined by the steam-distillation method of Briggs, Hogan, and Reid (1957).

(vii) *Individual Volatile Fatty Acids.*—The gas chromatographic method of James and Martin (1952) and the liquid-liquid partition chromatographic method of Brown (unpublished data) were used for these determinations. In the latter method the acids are eluted from a silicic acid column by hexane–n-butanol mixtures. The acids are titrated with 0·01N KOH in alcohol using bromthymol blue as an indicator. Good agreement was obtained between this method and the steam-distillation method for the determination of total volatile fatty acids.

(viii) *Optical Density Measurements.*—All measurements of optical density were made with a Bausch and Lomb Spectronic 20 spectrophotometer.

III. Results

(*a*) *Blood Lactate Levels and Rumen–Blood Lactate Gradients* (*Table 1*)

In a number of the earlier blood samples the proteins were not precipitated until the end of the day and it is possible that the lactate concentration obtained by

Table 1

CONCENTRATION OF LACTIC ACID IN WHOLE BLOOD BEFORE, DURING, AND AT THE END OF TWO CONSECUTIVE ABSORPTION PERIODS

Table No.*	Sheep No.	Blood Lactate Concentration (mg/100 ml)							
		Absorption Period No. 1			Wash-out Period 15–20 Min	Absorption Period No. 2			
		Jugular Blood				Jugular Blood			Carotid Blood
		Start	1 Hr	2 Hr		Start	1 Hr	2 Hr	2 Hr
2	11	63·5					34·5		
	12	83·0	55·5			31·0	20·0		
3	24	21·0		11·5		12·0		16·1	15·2
	25	12·4		14·2		17·5		15·5	14·4
4	6	25·0		10·0		10·0		10·0	10·0
5	1†	28·5		24·0		25·0		25·5	24·0
	2†	18·0		19·5				17·0	11·5
	19	22·4		9·8		12·0		7·2	6·8
	20	36·5		13·5		10·0		7·3	6·5
	21	15·5		16·5		16·5		11·8	10·3
	3†	22·0	13·5	13·0		21·5	14·5	16·0	14·0
	4	10·0						14·0	12·5
6	7†	18·5		24·0				17·5	13·5
	8†	18·5		19·5		23·5		22·0	19·0
	9†	25·5		27·5				25·0	23·5
7	17	14·0		15·0		13·4		13·4	12·0
	18	63·8	34·5	20·5		28·0		25·8	22·4
8	29	13·4		9·1		8·2		9·3	9·3
9	22	21·8		15·2		18·9		16·8	12·6
	23	15·1		10·2		11·3		10·8	10·8
	26	30·6		17·6		15·4		9·4	7·7
	28	21·2		13·6		14·0		14·8	12·7

* For the details of the solutions used and the absorption results, see the relevant table. The minimum concentration of lactic acid added to the rumen was 540 mg/100 ml.

† Not immediately deproteinized after removal from the animal.

analysis of these samples would have been lower if the blood proteins had been precipitated immediately.

Jugular vein blood was either equal to, or greater than, carotid artery blood in lactate concentration when the two samples were obtained within a few minutes of one another.

In every experiment the concentration gradient from the rumen to the arterial blood was high.

High blood lactate levels were measured in sheep Nos. 11 and 12 after the rumen had been washed out while they were conscious. There was a substantial fall after the animals were anaesthetized.

(*b*) *Phenol Red Determinations*

High total recoveries of phenol red were obtained (Tables 2–9). However, the percentages of the total amounts recovered in the washings were higher for phenol red than for lactic acid. This indicated that phenol red was being adsorbed on to the rumen wall. Adsorption tended to be greater from solutions at low pH.

TABLE 2

EFFECT OF ANAESTHESIA ON THE ABSORPTION RATES OF LACTIC ACID AND A MIXTURE OF VOLATILE FATTY ACIDS FROM SOLUTIONS ADDED TO THE NON-LIGATED WASHED-OUT RUMEN

Sheep were anaesthetized at the end of period 1 and were laid on their right sides. The only surgery experienced by these animals was the establishment of a rumen fistula 2 weeks previously

Sheep No.	Liveweight (kg)	Absorption Period	pH of Test Solution*		Lactate Absorption (m-moles/kg/hr)	Volatile Fatty Acid Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
			In	Out			
11	41	1	5·25	5·70	0·133	1·95	95·5
		2	5·25	5·80	0·186	1·71	98·3
12	43	1	5·25	5·55	0·209	1·06	96·5
		2	5·25	5·50	0·244	1·43	100·4

* Test solutions: 0·06M sodium lactate, 0·045M acetic acid, 0·015M sodium butyrate, 0·015M sodium propionate, 0·1M disodium hydrogen citrate, 0·01% phenol red.

Phenol red was detected in the urine of all the sheep, except No. 26, from which urine samples were collected (Table 14). The total amount of phenol red measured in the urine tended to be greater when the solutions at pH 5 were added to the rumen than when solutions at pH 7·5 were used.

Although phenol red was not measured in the urine of any of the sheep in which solutions at pH 4 were used it is suggested that the low total recovery of phenol red measured in some of these sheep was due to a relatively high rate of absorption.

(*c*) *Effect of Anaesthesia on the Absorption of Lactic Acid and Volatile Fatty Acid from the Washed-out, Non-ligated Rumen* (*Table 2*)

The results show that lactic acid was absorbed from the rumen of the sheep. Similar results have been obtained with all other sheep used in this series of experiments.

The results also indicate that the anaesthetic did not decrease lactic acid or volatile fatty acid absorption. High phenol red recoveries were obtained indicating very little passage of fluid on to the abomasum which had not been tied off from the forestomachs.

(d) Effect of Increasing the Osmotic Pressure of a Test Solution on the Absorption of Lactic Acid from the Solution (Table 3)

The osmotic pressure of the solution was increased by approximately 140 m-osmoles by increasing the concentration of disodium hydrogen citrate in the

TABLE 3

EFFECT OF INCREASING THE OSMOTIC PRESSURE OF A TEST SOLUTION OF LACTIC ACID ON THE ABSORPTION OF LACTIC ACID FROM THE SOLUTION WHEN ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Lactic Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out		
24	37	A*	1	5·25	5·45	0·212	96·7
		B†	2	5·30	5·35	0·196	98·5
25	39	B	1	5·30	5·50	0·200	92·1
		A	2	5·25	5·54	0·223	97·7

*Test solution A: 0·06M sodium lactate, 0·1M disodium hydrogen citrate, 0·01% phenol red.
†Test solution B: 0·06M sodium lactate, 0·16M disodium hydrogen citrate, 0·01% phenol red.

solution. Increasing the osmotic pressure caused an approximately 10% decrease in the rate of lactic acid absorption. Low recovery of phenol red was obtained from sheep No. 25 and more than twice as much phenol red was found in the bladder of this sheep than in that of sheep No. 24.

(e) Effect of Lactic Acid Concentration on its Rate of Absorption (Table 4)

This table shows that the absorption rate was increased when the lactic acid concentration in the solution was increased. The increase in absorption rate was more

TABLE 4

EFFECT OF LACTIC ACID CONCENTRATION ON ITS RATE OF ABSORPTION FROM TEST SOLUTIONS ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Absorption Period	Lactic Acid Concn. in Test Solution	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out		
5	32	1	Low*	5·15	5·35	0·189	100·1
		2	High†	5·15	5·25	0·493	99·2
6	43	1	High	5·15	5·40	0·306	102·0
		2	Low	5·05	5·20	0·182	97·6

*0·06M sodium lactate, 0·1M disodium hydrogen citrate, 0·01% phenol red.
†0·12M sodium lactate, 0·06M disodium hydrogen citrate, 0·01% phenol red.

than doubled in sheep No. 5 with double the concentration but in sheep No. 6 the increase was less than the proportional increase in lactic acid concentration in the solution.

The estimated osmotic pressure of the high lactic acid solution was 40 m-osmoles greater than that of the solution containing a low lactic acid concentration.

(*f*) *Effect of Decreasing the pH of the Test Solution from approximately pH 5 to approximately pH 4 on the Absorption Rate of Lactic Acid* (*Table 5*)

The rate of absorption of lactic acid from the rumen was faster from a test solution at pH 4 than from a test solution at pH 5. This was so irrespective of

TABLE 5

COMPARISON OF THE RATE OF LACTIC ACID ABSORPTION FROM LACTIC ACID SOLUTIONS AT APPROXIMATELY pH 5 AND pH 4 WHEN ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Lactic Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out		
1	34	A*	1	5·10	5·55	0·114	98·9
			2	4·00	4·35	0·173	97·7
2	34	A	1	5·15	5·65	0·163	101·0
			2	3·95	4·35	0·185	99·2
19	33	B†	1	4·30	4·45	0·234	96·1
			2	5·30	5·30	0·208	97·0
20	33	B	1	4·00	4·05	0·319	90·4
			2	5·10	5·15	0·185	95·9
21	37	C‡	1	5·15	5·30	0·189	97·3
			2	4·00	4·30	0·425	88·8
3	36	C	1	5·10	5·30	0·182	96·2
			2	4·05	4·25	0·287	96·6
4	43	C	1	5·15	5·40	0·157	97·5
			2	4·10	4·30	0·337	96·6

* A: 0·06M sodium lactate, 0·1M sodium dihydrogen phosphate, 0·01% phenol red, sufficient concentrated HCl to reach required pH.

† B: 0·06M sodium lactate, 0·1M disodium hydrogen citrate, 0·01% phenol red, sufficient concentrated HCl to reach required pH.

‡ C: 0·12M sodium lactate, 0·06M disodium hydrogen citrate, 0·01% phenol red, sufficient concentrated HCl to reach required pH.

whether the lactic acid concentration was 0·06M or 0·12M. The ratios of the rates of absorption at pH 4 and pH 5 varied between 1:0·44 and 1:0·89 for individual sheep and averaged 1:0·65.

The ratios of the concentrations of acid to salt changed from 1:14 at pH 5 to approximately 5:7 at pH 4. Thus the amount of free acid present was much greater at the lower pH.

The solutions at pH 4 had a higher osmotic pressure than those at pH 5 because of the addition of concentrated hydrochloric acid to the more acid solution. Thus for sheep 19 the more acid solution had an osmotic pressure 68 m-osmoles higher than the solution at pH 5.

(*g*) *Effect of a Volatile Fatty Acid Mixture in a Test Solution of Lactic Acid at pH 5 on the Absorption Rate of Lactic Acid* (*Table 6*)

This table shows that volatile fatty acid absorption was much more rapid than lactic acid absorption from the isolated rumen and that lactic absorption was decreased in the presence of volatile fatty acids. The ratios of the rates of absorption of lactic acid in the absence of volatile fatty acid to that in the presence of volatile fatty acid varied between 1:0·28 to 1:0·63 for individual sheep and averaged 1:0·49.

TABLE 6

EFFECT OF THE ADDITION OF A MIXTURE OF VOLATILE FATTY ACIDS TO A TEST SOLUTION OF LACTIC ACID AT APPROXIMATELY pH 5 ON THE ABSORPTION RATE OF LACTIC ACID FROM THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Lactic Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Volatile Fatty Acid Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out			
7*	34	A†	1	5·20	5·90	0·187		100·3
		B‡	2	5·10	6·60	0·117	1·40	99·2
9	41	A	1	5·20	5·40	0·148		95·9
		B	2	5·15	5·35	0·063	1·35	97·8
8	33	B	1	5·25	5·80	0·211	1·79	99·6
		A	2	5·15	5·35	0·348		96·0
10	32	B	1	4·95	5·45	0·064	1·36	101·7
		A	2	5·05	5·25	0·230		99·1

* Sodium dihydrogen phosphate replaced the disodium hydrogen citrate in both solutions A and B.

† A: 0·06M sodium lactate, 0·1M disodium hydrogen citrate, 0·01% phenol red.

‡ B: 0·06M sodium lactate, 0·045M acetic acid, 0·015M sodium propionate, 0·015M sodium butyrate, 0·1M disodium hydrogen citrate, 0·01% phenol red.

At pH 5 the ratios of acid to salt for volatile fatty acid were approximately 5:10 but only 1:14 for lactic acid. Thus for approximately the same molar concentrations of lactic and volatile fatty acid there was about 5 times more free volatile fatty acid than free lactic acid in the solution.

The osmotic pressures of similar solutions were measured and the presence of volatile fatty acid caused an increase of approximately 150 m-osmoles in the osmotic pressure.

(h) Effect of Raising the pH of the Test Solution from pH 5 to pH 7·5 on the Absorption Rate of Lactic Acid and Volatile Fatty Acid (Table 7)

Increasing the pH of the solution from pH 5 to 7·5 resulted in a marked depression in volatile fatty acid absorption. Lactate absorption was not affected. Within treatments there was a tendency for lactate absorption to be slightly more rapid during absorption period 1 than during absorption period 2.

TABLE 7

COMPARISON OF THE RATE OF ABSORPTION OF LACTIC ACID AND VOLATILE FATTY ACID FROM SOLUTIONS AT APPROXIMATELY pH 5 AND pH 7·5 ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Lactic Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Volatile Fatty Acid Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out			
17	41	A*	1	7·55	7·35	0·144	0·562	100·0
		B†	2	5·10	5·35	0·138	1·391	97·4
18	38	B	1	5·00	5·15	0·107	1·154	95·9
		A	2	7·20	7·15	0·091	0·194	99·6

* A: 0·06M sodium lactate, 0·1M sodium dihydrogen phosphate, 0·036M sodium acetate, 0·012M sodium propionate, 0·012M sodium butyrate, 0·01% phenol red.

† B: 0·06M sodium lactate, 0·1M disodium hydrogen citrate, 0·036M acetic acid, 0·012M sodium propionate, 0·012M sodium butyrate, 0·01% phenol red.

The change in pH caused the ratio of the concentration of free acid to salt for lactic acid to change from 1:14 at pH 5 to 1:4365 at pH 7·5 and for volatile fatty acids to change from 1:2 to 1:631.

(i) Effect of a Volatile Fatty Acid Mixture in a Test Solution of Lactic Acid at pH 7·5 on the Absorption Rate of Lactic Acid (Table 8)

The presence of volatile fatty acid did not greatly affect the rate of lactic acid absorption.

TABLE 8

EFFECT OF THE ADDITION OF A MIXTURE OF VOLATILE FATTY ACIDS TO A TEST SOLUTION OF LACTIC ACID AT APPROXIMATELY pH 7·5 ON THE ABSORPTION OF LACTIC ACID FROM THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Lactic Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Volatile Fatty Acid Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out			
29	36	A*	1	7·65	7·35	0·110		99·0
		B†	2	7·70	7·30	0·082	0·487	98·1
30	32	B	1	7·70	7·45	0·102	1·057	97·4
		A	2	7·70	7·35	0·087		101·8

* A: 0·06M sodium lactate, 0·1M sodium dihydrogen phosphate, 0·01% phenol red.

†B: 0·06M sodium lactate, 0·036M sodium acetate, 0·012M sodium propionate, 0·012M sodium butyrate, 0·04M sodium dihydrogen phosphate, 0·01% phenol red.

Volatile fatty acid absorption from sheep No. 30 was very rapid when compared with absorption from similar solutions in sheep No. 29 and in the sheep for which the results are shown in Tables 7 and 9. Sheep No. 30 was fed maize and lucerne.

This table indicates that within treatments lactate was more slowly absorbed during absorption period 2 than during absorption period 1.

(j) Effect of Lactic Acid in a Test Solution of Volatile Fatty Acids at pH 7·5 on the Absorption Rate of Volatile Fatty Acids (Table 9)

Volatile fatty acid absorption during period 2 was slower than during period 1 irrespective of treatment.

TABLE 9

EFFECT OF THE ADDITION OF LACTIC ACID TO A TEST SOLUTION OF A VOLATILE FATTY ACID MIXTURE AT APPROXIMATELY pH 7·5 ON THE ABSORPTION OF VOLATILE FATTY ACID FROM THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Liveweight (kg)	Test Solution of Acid	Absorption Period	pH of Test Solution		Lactate Absorption (m-moles/kg/hr)	Volatile Fatty Acid Absorption (m-moles/kg/hr)	Phenol Red Recovery (%)
				In	Out			
22	35	A*	1	7·55	7·30		0·275	95·7
		C‡	2	7·40	7·10	0·052	0·140	99·4
23	32	B†	1	7·75	7·40	0·103	0·613	97·0
		A	2	7·75	7·45		0·503	98·0
26	32	B	1	7·50	7·30	0·086	0·577	96·6
		A	2	7·65	7·40		0·471	99·0
28	33	A	1	7·55	7·45		0·476	98·0
		B	2	7·50	7·35	0·280	0·340	97·9

* A: 0·036M sodium acetate, 0·012M sodium propionate, 0·012M sodium butyrate, 0·1M sodium dihydrogen phosphate, 0·01% phenol red.

† B: 0·06M sodium lactate, 0·036M sodium acetate, 0·012M sodium propionate, 0·012M sodium butyrate, 0·04M sodium dihydrogen phosphate, 0·01% phenol red.

‡ C: Solution A + 0·06M sodium lactate.

Absorption of volatile fatty acid in sheep Nos. 28 and 22 in the presence of lactic acid during period 2 was 29% and 49% slower respectively than absorption during period 1. In both sheep Nos. 23 and 26 absorption of volatile fatty acid during period 2 in the absence of lactic acid was only 18% slower than absorption during period 1. This indicates that lactic acid may have caused a slight inhibition of volatile fatty acid absorption.

The solution used in absorption period 2 for sheep No. 22 had an osmotic pressure approximately 100 m-osmoles greater than the solution used in period 1. This accounts for some of the 50% inhibition of volatile fatty acid absorption observed in period 2 for this animal.

(*k*) *Relative Rates of Absorption of Acetic, Propionic, and Butyric Acids from Solutions containing Lactic Acid at pH 5 or pH 7·5 (Table 10)*

From solutions at pH 5 the order of absorption, expressed as the total number of moles of each acid absorbed, was acetic > butyric > propionic. Expressed as a percentage of the molar amount of each individual acid added, the absorption rates were butyric > propionic > acetic, i.e. in order of the length of the carbon chain and their lipid solubilities.

TABLE 10

RELATIVE RATES OF ABSORPTION OF THE INDIVIDUAL FATTY ACIDS FROM SOLUTIONS CONTAINING LACTIC ACID AT APPROXIMATELY pH 5 AND pH 7·5 WHEN ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Table No.	pH	Absorption Period	Volatile Fatty Acid	Amount Added (m-moles)	% of Total	% of Total Recovered	Amount Absorbed (m-moles)	Amount Absorbed (%)
7	6	5·10	2	Acetic	113·4	61·3	74·1	58·1	51·2
				Propionic	37·6	30·3	16·1	25·6	68·1
				Butyric	34·0	18·4	9·8	26·7	78·5
9	6	5·15	2	Acetic	99·7	59·9	69·8	49·4	49·6
				Propionic	35·5	21·3	19·4	21·5	60·6
				Butyric	31·3	18·8	10·8	23·5	75·1
17	7	5·10	2	Acetic	91·8	61·2	83·4	61·9	67·4
				Propionic	30·3	20·2	12·3	25·9	85·4
				Butyric	27·9	18·6	4·3	26·4	94·5
18	7	5·00	1	Acetic	91·7	62·8	78·2	46·1	50·3
				Propionic	29·2	20·0	14·2	20·9	71·6
				Butyric	25·1	17·2	7·6	20·7	82·4
17	7	7·55	1	Acetic	91·8	61·2	64·1	25·2	27·5
				Propionic	30·3	20·2	19·2	10·4	34·2
				Butyric	27·9	18·6	16·7	10·6	37·8
18	7	7·20	2	Acetic	91·7	62·8	65·2	6·1	6·7
				Propionic	29·2	20·0	19·3	3·9	13·2
				Butyric	25·1	17·2	15·5	2·6	10·5
29	8	7·65	2	Acetic	97·1	62·1	62·5	21·3	21·9
				Propionic	31·8	20·3	20·2	7·2	22·8
				Butyric	27·5	17·6	17·3	6·5	23·7
30	8	7·70	1	Acetic	93·5	61·2	60·9	41·8	44·7
				Propionic	31·5	20·6	19·1	15·3	48·5
				Butyric	27·8	18·2	20·0	10·8	39·0

The order of absorption from the solutions at pH 7·5, expressed as the total number of moles of each individual acid absorbed, was acetic > propionic > butyric. Expressed as a percentage of the amount of each individual acid added, the results were variable. However, there was a trend for the percentage absorption rates of the individual acids to be equal. Table 11 shows similar results.

(l) Effect of the Addition of Lactic Acid to a Test Solution of a Volatile Fatty Acid Mixture at pH 7·5 on the Relative Rates of Absorption of Acetic, Propionic, and Butyric Acids (Table 11)

The presence of lactate did not influence the relative rates of absorption of the volatile fatty acids.

TABLE 11

EFFECT OF LACTIC ACID ON THE RELATIVE RATES OF ABSORPTION OF THE INDIVIDUAL VOLATILE FATTY ACIDS FROM SOLUTIONS AT APPROXIMATELY pH 7·5 WHEN ADDED TO THE LIGATED, WASHED-OUT RUMEN

Sheep No.	Table No.	Lactic Acid (m-moles)	Absorption Period	Volatile Fatty Acid	Amount Added (m-moles)	% of Total	% of Total Recovered	Amount Absorbed (m-moles)	Amount Absorbed (%)
22	9	0	1	Acetic	95·1	62·8	62·6	12·3	13·0
				Propionic	30·8	20·3	20·0	4·3	14·0
				Butyric	25·6	16·9	17·4	2·6	10·2
		60	2	Acetic	93·7	62·8	62·9	6·7	7·1
				Propionic	30·3	20·3	20·7	1·6	5·4
				Butyric	25·2	16·9	16·4	2·5	10·0
23	9	60	1	Acetic	93·7	61·3	60·3	24·6	26·2
				Propionic	35·5	23·2	22·7	9·4	26·6
				Butyric	23·7	15·5	17·0	4·2	17·7
		0	2	Acetic	93·7	61·3	61·5	18·9	20·2
				Propionic	35·5	23·2	22·8	7·7	21·8
				Butyric	23·7	15·5	15·7	4·6	19·4
26	9	60	1	Acetic	96·3	61·6	60·3	24·3	25·2
				Propionic	30·5	19·5	19·7	7·0	22·8
				Butyric	29·5	18·9	20·0	6·7	22·6
		0	2	Acetic	96·3	61·6	60·2	20·3	21·1
				Propionic	30·5	19·5	20·1	5·1	16·8
				Butyric	29·5	18·9	19·7	4·6	15·6
28	9	0	1	Acetic	96·0	61·3	60·2	20·6	21·5
				Propionic	30·8	19·7	19·6	6·3	20·5
				Butyric	29·7	19·0	20·2	4·5	15·0
		60	2	Acetic	96·0	61·3	61·1	14·0	14·6
				Propionic	30·8	19·7	19·8	4·3	13·9
				Butyric	29·7	19·0	19·1	4·1	13·9

(m) Relative Rates of Absorption of D(−)- *and* L(+)-*Lactic Acid (Table 12)*

An analysis of variance of the results shown in Table 13 showed that there was no significant difference between the two methods of determination of lactic acid in the solutions added to and recovered from, the rumen. However, the statistical analysis showed a significant interaction ($P < 0{\cdot}05$) between the methods of determination and the solutions added to the rumen and recovered from the rumen. The cause of this variation was that the colorimetric method measured more lactic acid in the

solutions added to the rumen than the L-lactic dehydrogenase method and less in the solutions recovered from the rumen. This would indicate that D(−)-lactic acid was absorbed more rapidly than L(+)-lactic acid.

IV. Discussion

The isolated washed-out rumen technique of Danielli *et al.* (1945) has a number of obvious advantages in studying the absorption of nutrients from the rumen, but its disadvantages may be less easily discerned.

Table 12

DETERMINATION OF LACTIC ACID IN SOLUTIONS ADDED TO, AND RECOVERED FROM, THE WASHED-OUT RUMEN BY TWO METHODS

The two methods used were the L-lactic acid dehydrogenase method and the optically non-specific colorimetric method of Barker and Summerson (1941)

Solutions Added to the Rumen		Solutions Recovered from the Rumen	
L-Lactic Dehydrogenase (mg/ml)	½ × Colorimetric Value* (mg/ml)	L-Lactic Dehydrogenase (mg/ml)	½ × Colorimetric Value (mg/ml)
2·73	2·72	2·50	2·41
2·70	2·72	2·50	2·34
2·73	2·70	2·40	2·42
2·73	2·63	2·40	2·45
2·50	2·63	4·80	4·82
5·23	5·50	2·40	2·30
5·30	5·38	4·60	4·63
5·20	5·38	2·50	2·38
5·00	5·28	2·45	2·48
2·50	2·63	2·30	2·35
		2·50	2·40
		2·15	2·25
		4·70	4·50
		4·20	4·25

* In order to obtain comparable results, the colorimetric value has been divided by two as it determines both the D- and L-forms of a racemic mixture.

Reduction in blood flow to the portal area after abdominal surgery in ruminants has been reported by Fegler and Hill (1958), Fries and Conner (1961), and Bensadoun and Reid (1962). The degree of reduction will depend on the extent of the trauma. Effects on the absorption rates of nutrients from the alimentary tract will thus be variable and this could have been a major source of between-sheep variation in these experiments.

It was necessary to starve the animals for 48 hr prior to an experiment before the rumen could be readily emptied and washed clean of digesta. Pfander and Phillipson (1953), and Armstrong, Blaxter, and Graham (1957) obtained data that indicated reduced volatile fatty acid absorption from fasted animals. Sutton, McGil-

liard, and Jacobson (1963) have produced quantitative data for the reduction in the absorption of acetic acid after replacement of the rumen contents for 24 hr with saline. The reduction was at least 25% and in some cases more than 70%. Part of this reduction was alleviated by the introduction of volatile fatty acids into the saline in approximately physiological concentrations. Reduced absorption after fasting may be the result of reduced blood flow through the rumen wall (Waldern, Johnson, and Blosser 1963).

TABLE 13

ANALYSIS OF VARIANCE OF THE LACTIC ACID CONCENTRATIONS IN SOLUTIONS ADDED TO AND RECOVERED FROM THE RUMEN, AS MEASURED BY THE L-LACTIC DEHYDROGENASE AND COLORIMETRIC TECHNIQUES

Source of Variation	Degrees of Freedom	Mean Square	*F*
Methods of estimation	1	0·0058	0·13
Solutions added *v.* solutions recovered	1	5·6504	2·05
Samples within solutions	22	2·7505	
Estimation × solutions	1	0·0455	7·71*
Estimation × samples	22	0·0059	

* $P < 0·05$.

The results (Table 14) indicate that phenol red was a suitable soluble marker of fluid recovery for the purposes of these studies. High total recoveries were obtained in the majority of experiments. However, its usefulness is limited by the absorption

TABLE 14

AMOUNTS OF PHENOL RED FOUND IN THE URINE OF SHEEP AFTER TWO 2-HR ABSORPTION PERIODS

Sheep No.	Approximate pH of the Solutions in Rumen	Amount of Phenol Red Found (mg)	% of Phenol Red Added to Rumen
22	7·5	1·20	0·20
23	7·5	0·73	0·13
26	7·5	0	0
29	7·5	0·70	0·15
30	7·5	0·14	0·03
24	5	4·71	0·94
25	5	11·90	2·56

and adsorption which was observed. It cannot be used as a marker of water movement into, or out of, a test fluid by its determination in small samples removed serially. It is also not suited for use in very acid solutions as both its absorption and adsorption appeared to increase at pH 4 relative to pH 5.

If it is assumed that blood lactate concentrations reflect the intensity of trauma of a preparation, the decline in the blood lactate concentration which followed

anaesthetization indicated that anaesthetized sheep are probably better suited to the study of lactic acid absorption than the unanaesthetized animal when the technique involves washing out the rumen contents.

In the present experiments the net absorption of lactic acid may be considered as the difference between the flux from the blood into the rumen, and the flux from the rumen into the blood. The flux into the rumen contents was relatively slow as indicated by the small accumulation of lactic acid of less than 2 mg per 100 ml in lactic acid-free solutions added to the rumen. Similar results have been obtained by Dobson (1959).

The data in Table 12, and the analysis of the data in Table 13, would indicate that D(−)-lactic acid may be slightly more rapidly absorbed than L(+)-lactic acid. The interaction was significant only at the 5% level, and requires further investigation. A low concentration of D(−)-lactic acid in the blood relative to the L(+)-isomer may explain the differences in absorption rates. It is unlikely that the D(−)-isomer was actively absorbed.

Slight inhibition of lactate absorption resulted from an increase in the hypertonicity of the solution added to the rumen (Table 3). This was possibly due to the opposing movement of water through the water-filled pores of the rumen wall. Parthasarathy and Phillipson (1953) found slight inhibition of volatile fatty acid absorption from hypertonic solutions.

An increase in the concentration of lactic acid should have resulted in an approximately equi-proportional increase in its rate of absorption from the rumen, if it was absorbed by simple diffusion. Table 4 shows results that fall rather widely on either side of the expected result, but they are probably not contrary to the hypothesis.

The rate of absorption of the volatile fatty acids from acid solutions was much faster than lactic acid and this can be explained by their relative lipid solubilities.

There was no effect on lactic acid absorption of a decrease in pH from 7·5 to 5 (Table 7) probably because the increase in the amount of free lactic acid in the solution was small. However, decreasing the pH of the solution from 5 to 4 markedly increased the amount of free lactic acid present and increased the rate of absorption of lactic acid (Table 5). These data suggest that free lactic acid is absorbed a little faster than the anion, probably because the slight lipid solubility of the free acid allows it to diffuse slowly through the lipid membrane as well as diffusing through the water-filled pores.

Table 6 shows that the concomitant absorption of volatile fatty acid from a solution in the rumen at pH 5 decreased the absorption rate of lactic acid. This suggests that the volatile fatty acid competed more successfully than lactic acid for the available absorption sites. This could have been the result of the greater amount of free volatile fatty acid present compared with free lactic acid and the greater lipid solubility of the free volatile fatty acids.

Volatile fatty acid was more rapidly absorbed than lactic acid from a solution at pH 7·5 (Tables 7, 8, and 9). Ash and Dobson (1963) have suggested that as much as 50% of the volatile fatty acid absorbed from alkaline solutions is associated with

carbon dioxide diffusion into the rumen and that this volatile fatty acid is absorbed as free acid. Lactic acid would not compete equally for the hydrogen ion produced by the carbon dioxide mechanism because it is a stronger acid than the volatile fatty acids, and, in any case, as shown by other data, the free acid is only slowly absorbed relative to the free volatile fatty acids.

There was probably some partial inhibition of volatile fatty acid absorption by lactic acid from a solution at pH 7·5 (Table 9). The reason for this is not known.

The marked reduction in volatile fatty acid absorption rate due to a decrease in the acidity of the solution from acid to alkaline (Table 7) was expected from the results of other workers (Danielli *et al.* 1945; Masson and Phillipson 1951).

The results for the relative rates of absorption of the individual volatile fatty acids from acid solutions within the rumen are in agreement with those of Danielli *et al.* (1945), namely butyric > propionic > acetic when compared by expressing the moles of each acid absorbed as a percentage of the moles of that particular acid added to the rumen.

The relative rates of absorption from alkaline solutions were approximately equal to each other, and thus equal to the overall rate of absorption of the mixture. However, there were some results at pH 7·5 from individual sheep which showed considerable variation in absorption rates between acids (see, for example, sheep Nos. 17 and 18, Table 10), but it is considered that this variation was most likely the result of experimental error. A small error in the measurement of the proportion of an acid in the recovered solution would have had a large effect on the calculated percentage absorption because of the relatively low concentrations of propionic and butyric acids to acetic acid in the test solutions.

The data presented in this paper show that the factors controlling the absorption of lactic acid from the rumen are in conformity with the hypothesis that the diffusion barrier of the rumen wall consists of lipid membrane with water-filled pores.

If the results from these acute experiments can be extrapolated at all quantitatively to normal animals, they also indicate that an animal is unlikely to suffer from high blood lactic acid concentration, with accompanying acidosis, through the absorption of lactic acid from the forestomachs.

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