Onderstepoort Journal of Veterinary Research,
Volume 27, *Number* 1, *January,* 1956.

The Government Printer, Pretoria.

ALTERATIONS IN THE BLOOD SUGAR AND KETONE LEVELS CAUSED BY DOSING ACETATE, PROPIONATE AND BUTYRATE INTO THE RUMEN OF THE SHEEP.

R. CLARK AND J. R. MALAN, Onderstepoort Laboratory.

INTRODUCTION.

It is well established that by far the greater proportion of the carbohydrate in the diet of ruminants is absorbed as lower fatty acids of which acetic, propionic and butyric are the most important. (Elsden and Phillipson, 1948.) The study of the subsequent fate of these acids is now a major problem of ruminant physiology.

Acetate is known to act as a precursor of higher fatty acids and may be converted to ketone bodies by the liver. (Jarrett and Potter, 1950.) It may serve as a direct source of energy to the tissues as exemplified by the myocard. Propionate is converted to glycogen by the liver and its administration raises the levels of glucose, pyruvic acid and lactic acid in the blood, while at the same time reducing the ketone bodies. (Reid, 1950 and Jarrett and Potter, 1950.) Pennington (1952) has shown that butyrate is converted to ketone bodies especially by the epithelium of the fore-stomachs and by the liver.

In the experiments to be reported, solutions of sodium acetate, propionate and butyrate, singly and in combination, were run into the rumen of sheep and the effects on the blood sugar and ketone levels determined.

METHOD.

A team of 10 Merino sheep carrying permanent ruminal fistulae was used. The animals were fed exclusively on lucerne hay and the food was removed the evening before each trial.

In each trial 0·5 gram mol. of the acid to be tested was added to about a litre of water and neutralised with NaOH using phenolphthalein as indicator. The total volume was then brought up to 2 litres by the further addition of water. The sheep was bled immediately before dosing and the test dose was then run into the rumen through the fistula at a steady rate so that the total amount was given in exactly 2 hours. Blood samples were taken immediately after dosing and again at 3, 6, 7 and 24 hours after the commencement of dosing, 25 c.c. of blood being taken on each occasion. Individual sheep were rested for at least a week between trials. The blood sugar was determined by the amended Folin-Wu system of blood analysis (Graf 1933) and the ketones by the method described by Malan (1943).

A series of blank experiments, in which only water was dosed under identical conditions, was carried out to determine the effects of dosing and bleeding. As will be seen later, a significant rise in blood sugar was encountered at the 2nd, 3rd, 6th. and 7th hour readings. This effect, which was probably due to a sympatho-adrenal response to the bleeding, had to be taken into account in interpreting the final results. A second series of control trials using a 0·5 gram mol. NaCl in 2 litres water was carried out to test any possible effect of the Na ions present in the organic salt solutions but, as there was no difference between these results and those obtained with water alone, they will be ignored.

Received for publication on 24th November, 1954.—Editor.

RESULTS.

Blood Sugar.

The effects of the various salts on the average blood sugar levels are shown in Table 1.

TABLE 1.

The Effects of Dosing on Blood Sugar.

(Expressed as mgm. per cent.)

Treatment.	No.	Hours.					
		0	2	3	6	7	24
Water	8	49·8 46·0–51·6	58·0 47·5–66·7	56·7 48·8–66·7	55·2 50·0–60·0	55·3 50·0–63·3	52·3 48·8–56·7
Propionate	8	49·3 44·4–53·2	64·9 55·5–82·6	57·9 52·4–69·9	62·6 53·5–87·0	59·8 52·9–70·4	51·6 45·9–57·5
Butyrate	7	50·4 45·0–55·6	47·1 40·0–55·6	37·1 31·3–50·8	48·2 38·5–55·8	55·8 50·5–61·7	51·6 45·0–60·0
Acetate	12	45·1 32·5–67·5	47·5 39·2–65·4	48·2 40·0–63·3	51·1 43·5–62·1	50·2 41·3–56·2	48·0 43·5–55·0
Propionate + Butyrate	10	48·4 43·2–56·7	61·7 53·3–57·0	60·6 55·0–88·0	65·4 55·0–88·0	64·3 55·0–77·5	51·8 47·5–55·8
Butyrate + Acetate	12	47·3 41·9–55·0	47·4 36·3–55·0	45·2 31·3–56·7	51·7 46·2–58·3	53·0 45·0–60·0	50·5 45·0–60·0
Acetate + Propionate	6	44·5 38·8–53·3	59·9 53·3–70·0	59·9 53·3–72·5	56·1 50·0–65·0	54·9 50·0–62·5	47·9 43·8–54·1

The positive effect shown in the control (water dosing) experiments necessitated the correction of the other results and the corrected variations in blood sugar level are shown in Table 2.

TABLE 2.

The Effect of Dosing on Blood sugar after Correction for Blank Effect.

Salt.	Effect and Standard Error.				
	2	3	6	7	24
Propionate	+ **7·3**±2·8	+ 3·5±3·0	+ **6·8**±3·4	+ **4·9**±2·9	+ 1·0±2·4
Butyrate	−**11·6**±2·8	−**18·5**±2·8	− **7·4**±2·6	+ 0·8±2·6	0·0±2·2
Acetate	− **5·9**±1·7	− 2·0±1·9	+ 0·8±2·0	− 0·4±3·5	+ 1·6±2·8
Prop. + But.	+ **7·3**±2·3	+**10·5**±3·5	+**13·2**±1·8	+**11·2**±3·5	+ 1·0±1·8
But. + Acet.	− **8·2**±2·1	− **7·3**±3·2	− 0·8±1·8	+ 0·2+2·4	+ 1·9±2·0
Acet. + Prop.	+ **7·0**±2·5	+**10·2**±3·1	+ **6·4**±1·9	+ **4·8**±2·1	+2·0±1·5

Figures in heavy type indicate a statistically significant change at the 5 per cent level.

As will be seen, butyrate caused a significant depression in blood sugar whereas propionate caused a significant rise. When these two salts were given in equimolecular amounts the glucogenic action of propionate proved dominant. Acetate alone caused a transient depression in blood sugar but when given with butyrate it appeared to decrease the glucose depressing action of the latter salt. The addition of acetate to propionate had no demonstrable effect.

Ketone Bodies.

The effects of the various treatments on the circulating ketone bodies are shown in Table 3.

TABLE 3.

The Effects of Dosing on Blood Ketones.

(Expressed as mgm. per cent.)

Salt.	No.	Fraction.	Hours.					
			0	2	3	6	7	24
Butyrate.....	8	A	0·7	**1·7**	**2·0**	1·3	1·9	1·0
		B	1·1	**5·8**	**6·8**	**4·3**	**3·9**	**1·8**
		Tot.	1·8	**7·5**	**8·8**	**5·6**	**5·8**	**2·8**
Propionate.....	6	Tot.	1·8	1·7	2·1	1·6	2·2	2·1
Acetate.....	10	Tot.	1·6	2·0	1·9	**2·3**	**2·4**	**2·2**
Butyrate + Propionate	12	A	0·5	**0·8**	**0·7**	**0·6**	**0·6**	0·3
		B	1·5	**3·0**	**3·3**	**2·1**	**3·0**	1·7
		Tot.	2·0	**3·8**	**4·0**	**2·7**	**3·6**	2·2
Butyrate + Acetate.	10	A	0·7	**1·8**	**1·9**	**1·5**	**1·3**	0·7
		B	1·2	**7·1**	**6·8**	**5·4**	**4·7**	**1·9**
		Tot.	1·9	**8·9**	**8·7**	**6·9**	**6·0**	**2·6**
Propionate+Acetate	6	Tot.	1·7	1·8	1·7	1·7	1·7	2·5

Fraction A = Aceto-acetic acid + acetone.
Fraction B = Beta-hydroxybutyric acid.
Figures in heavy type show a statistically significant rise at the 5 per cent level.

The following facts may be noted:—

(i) Butyrate caused a significant rise in ketone bodies affecting especially the beta-hydroxybutyric acid fraction.

(ii) Acetate caused a smaller and more delayed rise which was however significant.

(iii) When acetate and butyrate were given together the ketogenic effect was slightly greater than with butyrate alone. Although this difference was not statistically significant, there is an indication that the two salts act synergistically.

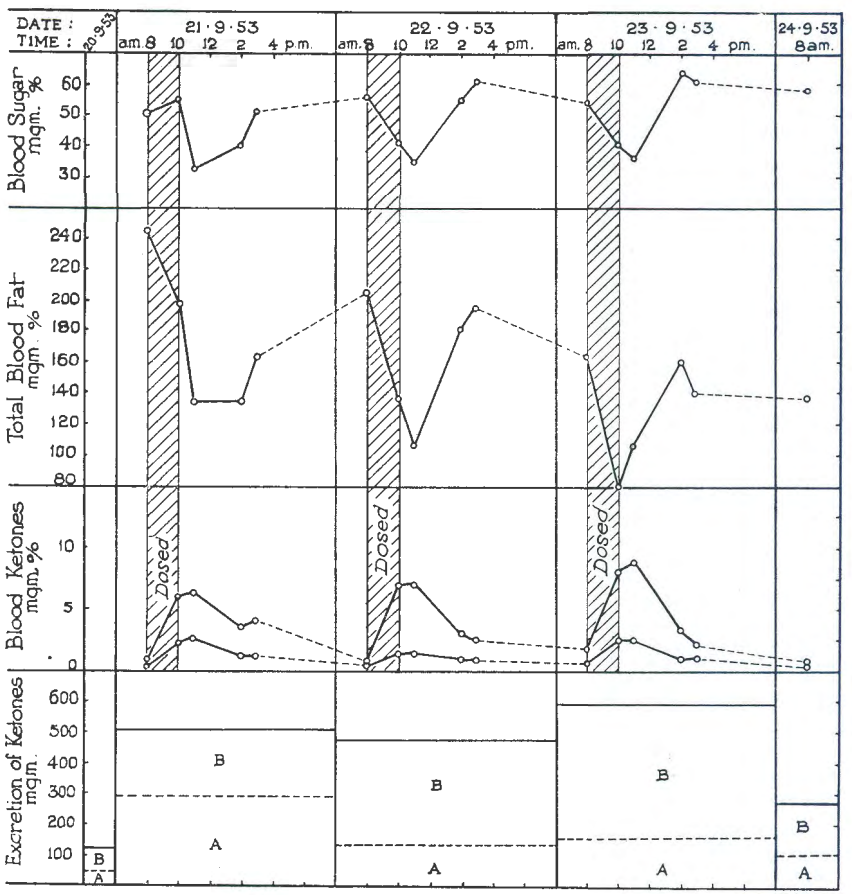
(iv) Propionate had no demonstrable effect on the blood ketone level of normal sheep but reduced the ketogenic effects of both butyrate and acetate.

Blood Fat.

The total titratable fatty acid content of the whole blood was also determined on all samples but no general trends in regard to this factor could be determined except that butyrate frequently, but not consistently, caused a drop in blood fat. This is well shown in Graph 1.

GRAPH 1.

The Effect of Daily Dosing with Butyrate.



The upper curve for blood ketones represents the beta-hydroxybutyric acid fraction and the lower curve the aceto-acetic acid fraction. For explanation see text.

The sheep was kept in a metabolism cage for the collection of urine and dosed 0·5 gram mol. sodium butyrate by the standard method described for three consecutive days.

Note the recurrent hypoglycaemia and ketonaemia with recovery and the cumulative depression of blood fat. The relatively small amount of ketones excreted through the urine indicates a high level of utilisation.

DISCUSSION.

The Glucose Depressing Action of Butyrate.

Conflicting reports on the effect of butyrate dosing on the blood sugar level have been reported. In 1951, Johnson stated—"*When equal amounts of propionic acid and butyric acid are received by the animal the rise in blood glucose is far less than if the same amount of propionic acid alone is received.*" In 1953 the same author qualified this statement as follows:—

> "*It has since been observed that this is not always the case. In some goats butyric acid seems to show no glucose depressing action when given alone or in conjunction with propionic acid.*" In the same article he states "*Our understanding of the effects of butyric acid on the blood glucose is indeed in a state of confusion*".

Schultz and Smith (1951) obtained a sharp drop in blood glucose following the oral administration of butyric and other ketogenic acids to goats.

Jarrett, Potter and Filsell (1952) reported a rise in blood sugar when butyrate was injected intravenously but it must be remembered that the normal route of absorption of butyrate is via the epithelium of the fore-stomachs and the liver where a large scale conversion to ketones takes place. Its direct injection to the blood would therefore probably not give rise to a normal physiological response. Discussing their results these authors state—"*The possibility arises that the response following the injection of butyrate may have been due to glycogenolysis mediated by the sympatho-adrenal system*". The effects of dosing water and bleeding reported in the present experiments emphasise how such reactions may cause confusion.

Although the present authors found a significant drop in blood sugar after butyrate dosing in most cases, individual animals varied considerably in their reaction and some even showed a rise in blood sugar. Furthermore butyrate did not reduce the glucogenic action of propionate.

The Effect of Ketosis on Blood Sugar.

Schultz and Smith (1951) also administered the ketone bodies to goats but could not demonstrate any consequent drop in blood glucose. They concluded that the blood sugar depression observed after fatty acid feeding was not due to the ketone bodies themselves but to some mechanism operating during their formation. These authors, however, did not succeed in producing an appreciable ketonaemia with beta-hydroxybutyric acid, the main ketone body found after butyric feeding. Their observation that the blood sugar level was not influenced by a high concentration of acetone may be of little physiological significance as this fraction does not normally occur to any great extent in the blood.

As the question of a possible relationship between the sugar and ketones of the blood did not appear to have been settled, three sheep were given intravenously 5 mg. of the sodium salt of beta-hydroxybutyric acid in 200 c.c. of saline. Another two sheep received the same amount of saline as controls. The results are shown in Table 4.

TABLE 4.

The Effect of the Intravenous Injection of Beta-hydroxybutyric Acid on the Blood Sugar.

Time after Injection.	Blood Sugar, mgm per cent.		Blood Ketones, mgm per cent.	
	B.hydr. but.	Controls.	Fraction A.	Fraction B.
Before.....	43·8	44·0	0·7	1·5
3 minutes.....	43·8	51·4	1·5	13·7
10 minutes.....	42·2	55·6	1·1	10·8
30 minutes.....	41·9	60·5	0·7	6·7
1 hour.....	50·4	62·6	0·7	3·3
2 hours.....	49·5	59·2	0·7	2·3
5 hours.....	37·1	43·2	0·7	1·5
24 hours.....	43·8	45·0	0·8	1·5

Fraction A = Aceto-acetic acid + acetone.
Fraction B = Beta-hydroxybutyric acid.

As the controls showed no change in blood ketones, these figures are omitted.

It will be noted that again the control group showed a marked rise in blood sugar probably in response to the bleeding. Assuming that this factor played an equal role in the experimental group, the beta-hydroxybutyric acid can be said to have suppressed the blood sugar.

An inverse relationship between the blood sugar and blood ketones, as alternative sources of energy, would appear to be logical but the reverse reaction does not appear to occur as Schultz and Smith (1951) found that prolonged insulin induced hypoglycaemia in goats was not accompanied by any rise in blood ketones.

The Interconversion of the Ketone Bodies.

According to Wright (1952), aceto-acetic acid is the parent substance of the ketone bodies formed from the dissimilation of long chained fatty acids and the ketogenic amino-acids. Beta-hydroxybutyric acid is the reduction product of aceto-acetic acid and the two acids are freely interchangeable. Acetone arises from aceto-acetic acid by spontaneous and irreversible decarboxylation, a reaction which occurs chiefly in the lungs and bladder.

Pennington (1952) showed that butyrate is converted to ketone bodies, mainly aceto-acetic acid, by the epithelium of the rumen, reticulum and omasum and by the liver *in vitro*. In the present experiments butyrate mainly caused a rise in the beta-hydroxybutyric acid fraction. This apparent discrepancy can be explained by interconversion.

In one experiment a sheep was given intravenously 10 c.c. of aceto-acetic ethyl ester diluted in an equal amount of saline. The effects on the blood ketones are shown in Table 5.

TABLE 5.

The Effect of the Injection of Aceto-Acetic Ester on the Blood Ketones.

Time.	Fraction A. mgm. per cent.	Fraction B. mgm. per cent.
Before	0·5	1·4
3 minutes	26·5	1·9
5 minutes	14·5	2·9
30 minutes	1·5	4·3
60 minutes	1·5	2·4

The conversion of aceto-acetic acid to beta-hydroxybutyric acid was also demonstrated in sheep's blood *in vitro* by adding 0·5 c.c. aceto-acetic ethyl ester to 220 c.c freshly drawn citrated blood and incubating the mixture at 37° C. The calculated concentration of this mixture was 101 mg. per 100 c.c. The results of analysis of samples taken at intervals are shown in Table 6.

TABLE 6.

The Conversion of Aceto-Acetic Acid to Beta-hydroxybutyric Acid by Blood in *vitro*.

Time.	Fraction A. mgm. per cent.	Fraction B. mgm. per cent.	Total.
Before	0·5	1·5	2·0
Immediately after	99·9	1·9	101·8
1 hour after	97·0	3·9	100·9
4 hours after	89·7	7·8	97·5
24 hours after	75·2	14·0	99·2

Reverting to Table 4 it will be noted that the injection of beta-hydroxybutyric acid caused only a very small rise in the circulating aceto-acetic acid, indicating that the conversion in this direction is not rapid.

The Possible Significance of the Metabolism of the Lower Fatty Acids in the Aetiology of Ketoses in Ruminants.

This aspect of the problem has been discussed fully by Johnson (1953) and need not be enlarged on here. A few remarks on the cause of the nervous symptoms, such as dullness and blindness, usually associated with ketoses may,

however, be added. Jasper (1953) describes clinical symptoms typical of Pregnancy Disease in sheep suffering from prolonged insulin hypoglycaemia and states further—"*Severity of symptoms was dependent not only upon depth of hypoglycaemia but to a large degree on duration as well. Little or no difference in glycaemia levels was apparent between the different stages of symptoms observed, duration of hypoglycaemia being the determining factor. Marked symptoms often persist for several hours or days following spontaneous or induced return to normal or hyperglycaemic levels*".

None of the sheep in the present experiments ever showed any such symptoms despite the fact that many of them exhibited levels of ketonaemia and hypoglycaemia of an order found in Pregnancy Disease. This would be explained by the short duration of the hypoglycaemia.

SUMMARY.

Solutions of acetate, propionate and butyrate, alone and in combinations, were dosed into the rumen of sheep and the effects on blood sugar and ketones determined.

Acetate was found to cause a slight and delayed rise in ketone bodies without affecting the blood sugar.

Propionate caused a marked rise in blood sugar and had a strong antiketogenic effect when given with butyrate.

Butyrate produced a sharp rise in ketones, mainly beta-hydroxybutyric acid, together with a fall in blood sugar. The latter effect, however, was not constant.

The intravenous injection of beta-hydroxybutyric acid appeared to reduce the blood sugar level.

Aceto-acetic acid injected intravenously was partially converted to beta-hydroxybutyric acid.

These results are discussed in relation to the more recent literature.

LITERATURE.

ELSDEN, R. S. AND PHILLIPSON, A. T. (1948). Ruminant digestion. *Annual Rev. Biochem.*, Vol. 17, p. 705.

GRAF, H. (1933). Chemical Blood Studies. *Onderstepoort Jnl.*, Vol. 1, p. 269.

JARRETT, I. G. AND POTTER, B. J. (1950). Metabolism of acetate and propionate in the ruminant. *Nature*, Vol. 166, p. 515.

JARRETT, I. G., POTTER, B. J. AND FILSEL, O. H. (1952). Lower fatty acids in the intermediary metabolism of sheep. *Aust. Jl. Exp. Biol. and Med. Sci.*, Vol. 30, p. 197.

JASPER, D. E. (1953). Prolonged insulin hypoglycemia in sheep. *Am. Jl. Vet Res.*, Vol. 14, p. 209.

JOHNSON, R. B. (1951). The relative rates of absorption of the volatile acids from the rumen and their relationship to ketosis. *Cornell Vet.*, Vol. 41, p. 115.

JOHNSON, R. B. (1953). A theoretical discussion of ruminant ketosis. *Am. Jl. Vet. Res.*, Vol. 14, p. 366.

MALAN, J. R. (1943). Chemical Blood studies IX. The fractional determination of the acetone bodies in blood and urine of sheep suffering from domsiekte. *Onderstepoort Jl.*, Vol. 18, p. 311.

PENNINGTON, R. J. (1952). The metabolism of short-chain fatty acids in sheep. I. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. *Biochem. Jl.*, Vol. 51, p. 251.

REID, R. L. (1950). Utilization of acetic and propionic acids in sheep. *Nature*, Vol. 165, p. 448.

SCHULTZ, L. H. AND SMITH, V. R. (1952). Experimental alteration of the sugar and ketone levels of the blood of ruminants in relation to ketosis. *Jl. Dairy Sci.*, Vol. 34, p. 1191.

WRIGHT, SAMSON (1952). Applied Physiology. (9th edit.) Oxford University Press. London, New York, Toronto.