

Studies on the Alimentary Tract of the Merino Sheep in South Africa. XIX.—The Influence of Sulphanilamide on the Activity of the Ruminal Flora of Sheep and Cattle.

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INTRODUCTION.

IN ruminant animals the activity of the ruminal flora is of vital importance in digestion and the nutrition of the host animal. The administration of drugs affecting this flora may, therefore, have serious repercussions. The advent of the sulpho-namides has resulted in large numbers of ruminants being dosed with highly active bacteriostatics, yet no investigations have apparently as yet been carried out into the effect of such treatment on the process of digestion. Kon and Porter (1947) state: "Sulphonamide drugs are used for the treatment of disease, but no investigation of the effect of these or other antibiotics on the composition or activity of the rumen micro-organisms of the adult ruminant has yet been made".

Stableforth and Hignett (1942) dosed cattle with sulphanilamide mainly with a view to determining the concentrations attained in the blood and milk. No work was done on the effect on the ruminal flora but mention is made of diarrhoea and inappetence following dosing.

It was therefore decided that this matter required urgent attention and this paper reports the findings so far made.

In the present series of experiments only sulphanilamide was used as a preliminary investigation. Similar tests with other sulphonamide preparations will have to be conducted later.

The work was carried out both *in vitro* and *in vivo*.

IN VITRO TESTS.

The Fermentation of Glucose.

Various amounts of sulphanilamide were added to 20 c.c. samples of ruminal ingesta and incubated at 38° C. for two hours. Glucose (1 c.c. of a 20 per cent. solution) was then added and Durham tubes inverted in the liquid to trap the gas formed during incubation for one further hour.

* Work done while on a study visit to Onderstepoort.

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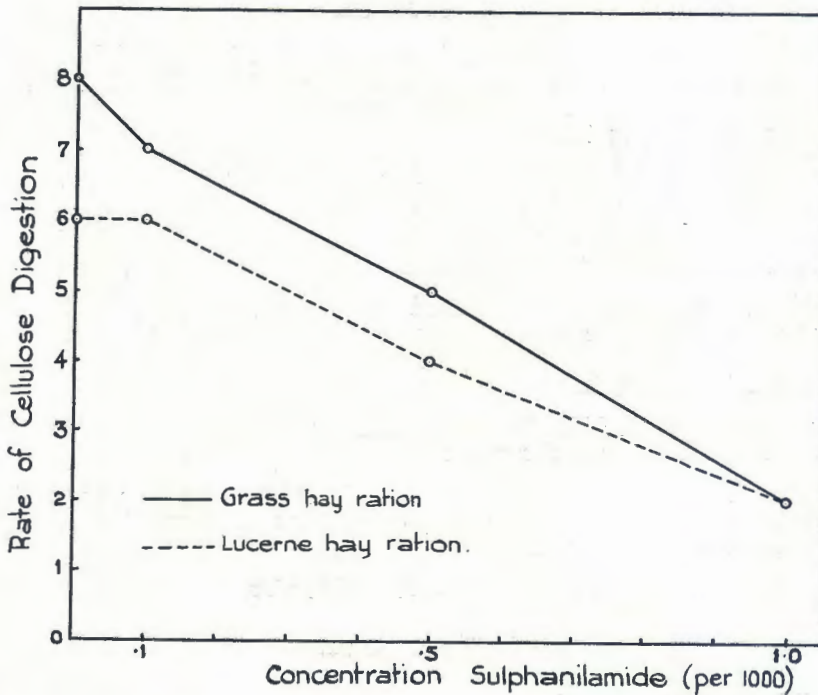
In this way it was found that the gas formation from sugar was significantly retarded by concentrations of sulphanilamide of 0.1 per cent. and over, in ruminal ingesta from sheep fed on either grass or lucerne hay.

This figure of 0.1 per cent. may appear high as compared with the standard concentration aimed at in the blood (.01 per cent.) but it must be remembered that the rapid evolution of gas from glucose in ruminal ingesta is mainly caused by yeast-like types which are presumably much more resistant to sulphonamides than bacteria.

Assuming the therapeutic dose of sulphanilamide to be 0.2 gm. per kilo body weight, the dose for a sheep weighing 100 lb. would be 10 gm. per day divided into two equal doses. As the ruminal capacity of such an animal would be approximately 3 litres, a dose of 5 gm. would give a concentration of about 0.17 per cent. This would therefore be sufficient to materially inhibit the fermentation of sugar.

GRAPH 1.

The Effect of Sulphanilamide on the in vitro Digestion of Cellulose.



Digestion of Cellulose.

The *in vitro* tests on the digestion of cellulose were carried out by the cotton thread technique of Hoflund, Quin and Clark (1948).

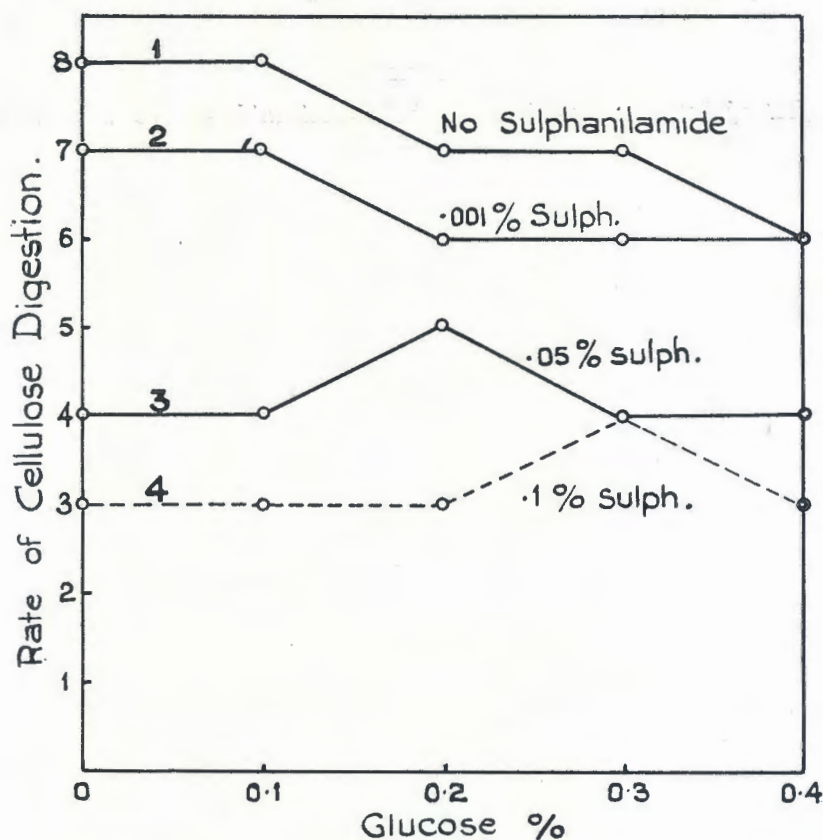
Trials were made with various concentrations of sulphanilamide added to ingesta of sheep fed on both grass hay and lucerne hay. The results are given in Graph 1.

As will be seen from the above graph the addition of sulphanilamide to ruminal ingesta caused a retardation of cellulose in almost linear relation to the amount added. This effect was very definite in concentrations of 0.5 per 1,000 and over.

Hoflund *et al* (1948) showed that the *in vitro* digestion of cellulose was retarded by concentrations of glucose of over 0.2 per cent. On the other hand it is well known that the higher the energy value of the bacterial medium, the greater the concentration of sulphonamides necessary to produce bacteriostasis. In view of these facts it was considered advisable to study the effects of combinations of glucose and sulphanilamide on cellulose digestion. The results are given in Graph 2.

GRAPH 2.

The Effect of Varying Concentrations of both Glucose and Sulphanilamide on the in vitro Digestion of Cellulose.



The following points may be noted from the above graphs:—

- (1) In the absence of sulphanilamide, cellulose digestion was suppressed by the presence of glucose in concentrations of 0.2 per cent. and over (line 1).

- (2) Sulphanilamide alone suppressed cellulose digestion in proportion to the concentration present.
- (3) When sulphanilamide and glucose were both present the glucose had an antagonistic action to that of sulphanilamide. This is seen in lines 3 and 4 where concentrations of glucose of 0.2 and 0.3 per cent. respectively showed beneficial effects.

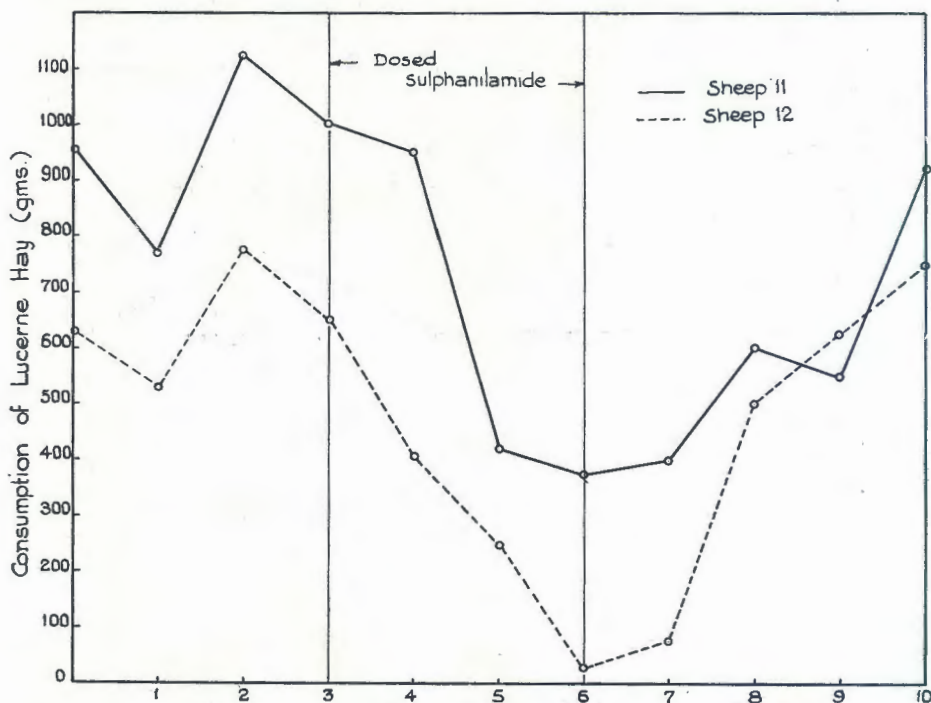
These results were obtained using ruminal ingesta from sheep fed on grass hay. They could not be repeated in ingesta from sheep fed on lucerne hay owing to the rapid removal of the glucose by fermentation.

THE EFFECT OF SULPHANILAMIDE DOSING ON HAY CONSUMPTION.

Two sheep on a ration of lucerne hay were dosed 5 gm. sulphanilamide each twice daily through the ruminal fistula. Dosing was carried out at 8 a.m. and 3.30 p.m. and the amount given represented approximately 0.2 gm. per kilo live weight which is the recognised therapeutic dosage. The effect of this treatment on the daily hay consumption is shown in Graph 3.

GRAPH 3.

The Effect of Intra-Ruminal Dosage of Sulphanilamide on the Consumption of Lucerne Hay.



The above graph clearly shows that the dosing with sulphanilamide caused a prompt and marked drop in the food consumption but that progressive recovery commenced 24 hours after the dosing was stopped.

Samples of ruminal ingesta and blood were analysed for sulphanilamide at intervals after dosing. The method used was that of Bratton and Marshall (1939). The ruminal contents were centrifuged and the supernatant fluid cleared by adding an equal volume of 10 per cent. NaOH solution. After filtration the fluid was neutralised with 2N. HCl, diluted to ten times its volume and then treated in the same way as blood. The results of these analyses are given in Table 1.

TABLE 1.

Time of Collection.	Dosage of Sulphanilamide.	SULPHANILAMIDE. (m.gm. per 100 c.c.).	
		Rumen Ingesta.	Blood.
8.00 a.m.....	gm. 5	—	—
9.30 a.m.....	—	116	2.3
11.00 a.m.....	—	80	4.6
12.30 p.m.....	—	66	6.1
4.00 p.m.....	5	—	—
8.00 a.m.....	—	23	1.9

As has already been shown *in vitro*, the concentrations of sulphanilamide found in the rumen were sufficient to depress cellulose digestion and this in turn was reflected in the decreased appetite. It will also be noted that none of the blood samples showed a sulphanilamide concentration of 10 mgm. per cent. which is generally accepted as the required level for successful therapeutic action.

THE EFFECT OF RUMINAL MOTILITY ON THE ABSORPTION OF SULPHANILAMIDE.

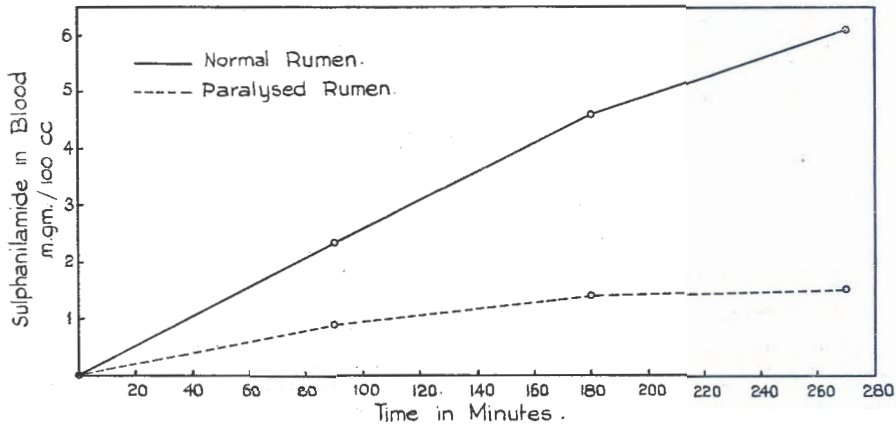
Hoflund (1940) stated that the reticulo-omasal orifice was closed in the static rumen. If this were so all absorption from the rumen during stasis would have to take place through the ruminal wall and consequently could be expected to be slower. In order to study the effect of ruminal stasis on absorption of sulphanilamide, the above experiment was repeated on two sheep after the rumen had previously been paralysed by atropin given subcutaneously. The findings are given in Table 2 and Graph 4.

TABLE 2.

The Effect of Ruminal Paralysis on the Absorption of Sulphanilamide.

Time in Minutes after Dosing.	SULPHANILAMIDE CONCENTRATION IN THE BLOOD (mgm. per 100 c.c.).		
	Normal.	Rumen Paralysed.	
	Sheep 3.	Sheep 3.	Sheep 10.
90.....	2.3	0.9	1.3
180.....	4.6	1.3	1.5
270.....	6.1	1.5	1.7

GRAPH 4.



As will be seen the absorption of sulphanilamide was greatly retarded when the rumen was paralysed.

DISCUSSION.

In the light of these findings the advisability of dosing sulphonamides to ruminants is open to question. If, on dosing, the drug passes into the rumen normal therapeutic amounts will cause marked retardation of cellulose digestion. This in turn will aggravate any inappetence which may already exist. Furthermore absorption was so slow that the required blood concentration could not be attained. This applies with even greater force in cases showing ruminal stasis.

Stableforth and Hignett (1942) found the maximum concentration of sulphanilamide in the blood some 5 to 6 hours after dosing. With a dosage of 1 gm. per 20 lb. body weight the highest figure obtained was 4.5 gm. per 100 c.c. blood, while with double that dose the concentration rose to 9.5 gm. per 100 c.c.

As will be seen these figures correspond very well with those found by the present authors, where the dose corresponded to 1 gm. per 15 lb. body weight and the maximum blood concentration was 6 mgm. per 100 c.c. blood attained in 15 hours.

It is realised that these findings may not apply equally to the water-soluble sulphonamides but this work has emphasised the necessity for further work on the effect of these commonly-used drugs on ruminant digestion. In the absence of precise knowledge in this respect it would appear advisable to administer sulphonamides parentally to these animals whenever practicable.

SUMMARY.

(1) It has been shown that therapeutic doses of sulphanilamide depress cellulose digestion and appetite in ruminants.

(2) The fermentation of sugar and gas formation are also suppressed but only by higher concentrations.

(3) The absorption of sulphanilamide after intra-ruminal dosing is very slow and effective blood concentrations are not attained with recognised therapeutic dosage.

(4) The absorption of sulphanilamide is further retarded by paralysis of the rumen induced by atropin.

(5) These findings raise the question as to the advisability of dosing sulphoamides to ruminants in view of their deleterious effects on cellulose digestion and appetite and the low blood concentration of the drug achieved by dosing.

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