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THE INFLUENCES OF THE NATURE OF THE DIET AND OF STARVATION ON THE CONCENTRATION CURVE OF SULPHANILAMIDE IN THE BLOOD OF SHEEP AFTER ORAL DOSING.

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INTRODUCTION.

Stableforth and Hignet (1942) investigated the concentration of sulphanilamide in the blood of horses and cattle after oral dosing but did not take the diet or stage of digestion into consideration in their experiments. The present authors considered that these factors might well influence the absorption of any drug introduced into the rumen through their known effects on both ruminal pH and motility. Previously, for example, Oyaert, Quin and Clark (1951) had shown that the absorption of sulphanilamide was markedly retarded when the rumen was paralysed with atropine.

METHOD.

Merino sheep were used throughout the experiments. Each trial was conducted on four to six sheep and the animals were kept on the selected diet for at least two weeks prior to dosing. The sulphanilamide was administered per stomach tube at a standard rate of 1 gm. per 7 kilo body weight. The test animals were then either fed *ad lib.* or starved for 48 hours subsequent to dosing. Blood samples were taken every three hours for the first twelve hours after dosing and then at six or twelve hour intervals. The sulphanilamide concentration in the blood was determined according to the method of Bratton and Marshall (1939).

Appropriate statistical methods for the analysis of this type of data were developed by the biometrician, Mr. D. van der Reyden. In his study * a formula is suggested to express the efficiency of the standard dose under the varying conditions.

The desired characteristics of a blood concentration curve are that absorption should be rapid and the maintenance of an efficient concentration prolonged. Taking the two curves in Graph 1 as examples, the time taken to reach the

* "Statistical Treatment of the data of blood concentration of a Drug", *Biometrics* 7 (3), 1951.

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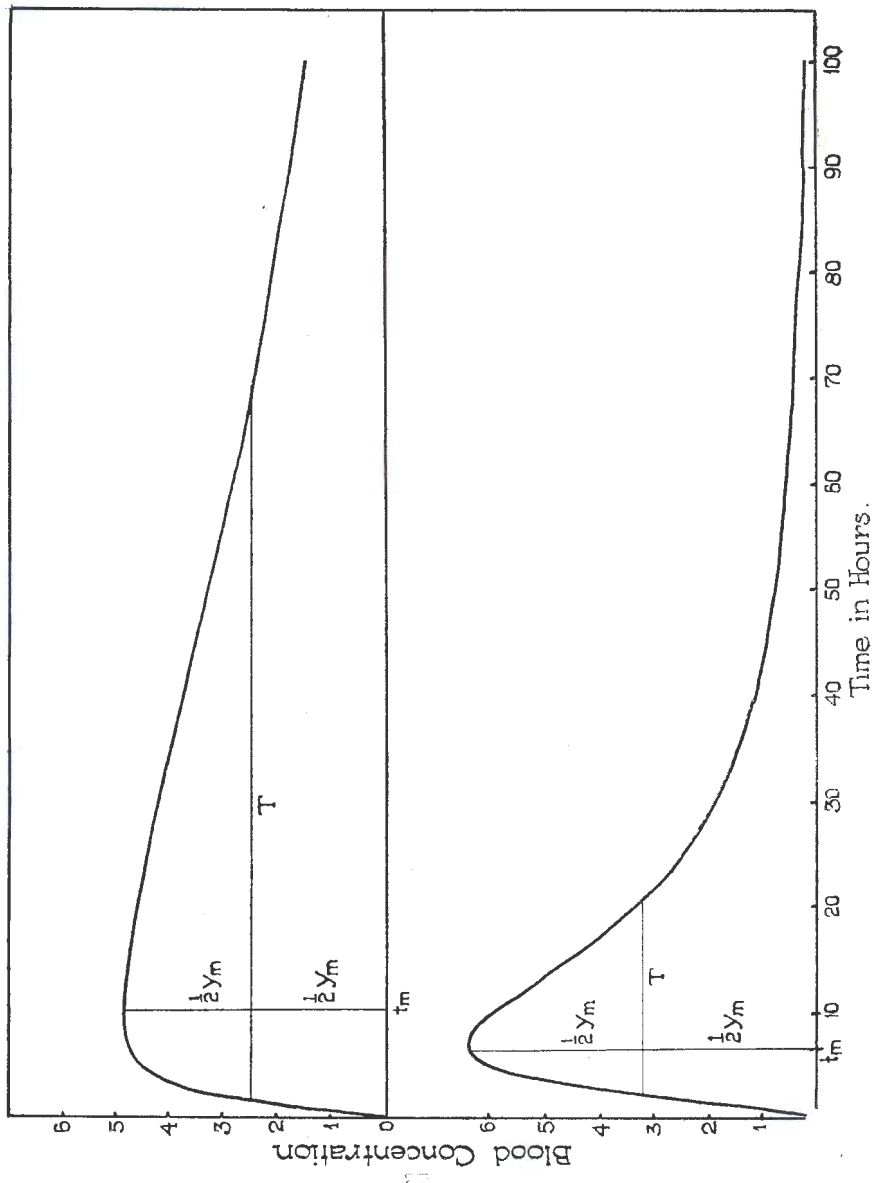
maximum concentration (tm) should be short and the time over which a concentration equal to half the maximum (T) should be long. The efficacy of a given curve can then be expressed as a percentage of the maximum possible efficacy by the formula

$$\text{Treatment Efficiency} = 100\ (1 - \tfrac{2tm}{T}).$$

It must be remembered that this formula expresses not only the shape of the curve but also the maximum height in that the position of the line T is determined by the maximum concentration reached.



GRAPH 1.—*Characteristics of Blood Concentration Curves.*

RESULTS.

Effect of Diet.

As will be seen from Table 1, the diet had a profound effect on the sulphanilamide blood concentrations. The calculated curves are shown in Graph 2. On a lucerne diet, whether green or in the form of hay, the maximum blood concentration was reached in six to nine hours, whereas on a diet of poor quality grass hay absorption was retarded and the maximum was not reached till up to 24 hours after dosing. The excretion on a diet of lucerne hay was more rapid than when green lucerne was fed.

TABLE 1.

Average Experimental Results.

Free Sulphanilamide in the Blood (mg. per 100 c.c.).

Hours after Dosing.	3	6	9	12	$\frac{24}{27}$	30	$\frac{47}{51}$	E%.	tm.	T.
FED.										
Green Lucerne.....	3·6	5·4	4·7	5·0	4·2	3·0	1·4	45·6	8·3	21·0
Lucerne Hay and Maize	3·9	5·5	6·1	5·7	4·0	3·0	1·5	41·3	8·7	23·1
Lucerne Hay.....	3·6	5·4	5·6	4·5	2·6	—	0·7	23·8	7·3	16·7
Grass Hay.....	1·5	2·6	4·6	5·7	6·6	5·0	2·9	28·8	15·9	47·0
STARVED.										
Green Lucern.....	3·8	5·0	5·1	4·8	4·7	—	2·7	62·0	10·3	55·1
Lucerne Hay and Maize	5·4	5·5	6·5	5·6	4·9	—	2·5	57·5	7·8	38·0
Lucerne Hay.....	3·0	5·2	5·6	5·8	5·4	4·6	2·2	44·9	11·2	27·0
Grass Hay.....	1·6	3·2	4·2	6·2	6·2	4·9	3·5	36·2	15·6	31·2

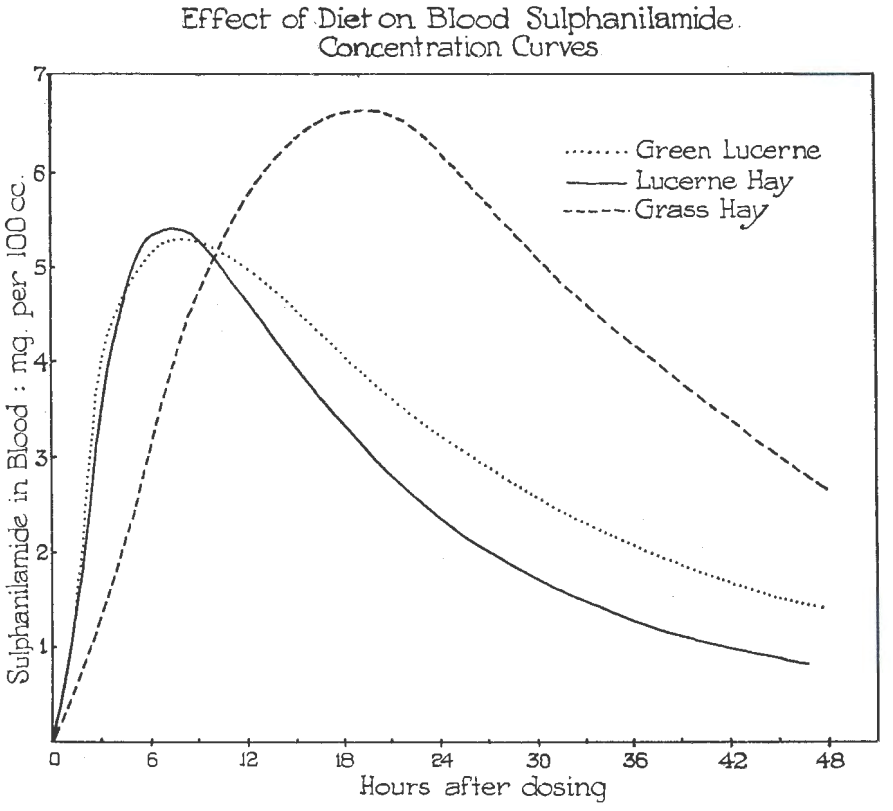
For detailed experimental data see appendix.

Effect of Starvation.

Reverting to Table 1 it will be seen that the cessation of feeding greatly increased the Efficiency Coefficient of a standard dose of the drug, mainly by decreasing the rate of excretion. This applied to all the different experimental diets. Contrary to expectation, starvation did not affect the absorption.

The effect of starvation on the sulphanilamide blood concentration curve is well shown in Graph 3. In this experiment a second dose was given after 24 hours in order to study the cumulative effect. As will be seen starvation greatly retarded the rate of excretion.

GRAPH 2.



In two trials on a lucerne hay ration, the animals were kept in metabolism cages for the collection of urine and the determination of sulphanilamide excretion. The amount of drug recovered over the first 24 hours after dosing, expressed as a percentage of the amount dosed, is shown in Table 2.

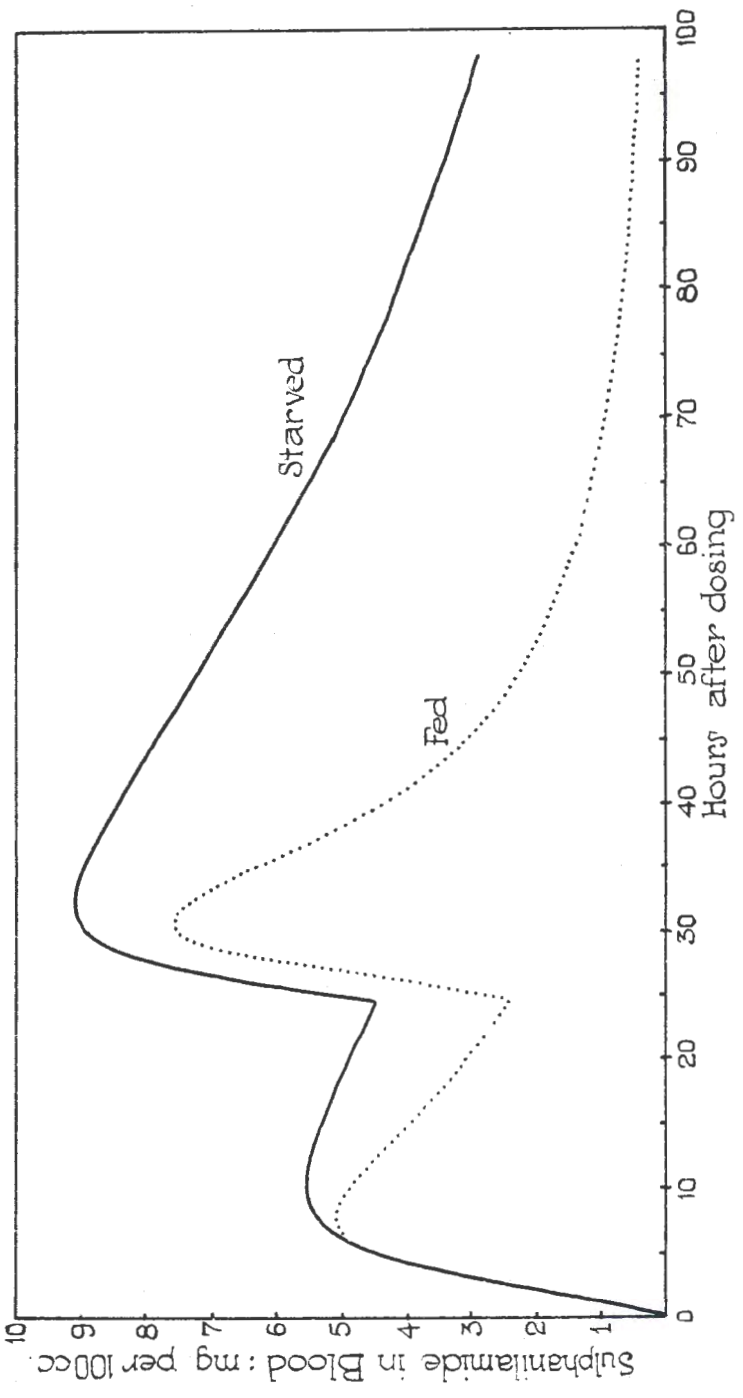
TABLE 2.

Percentage of Sulphanilamide dosed recovered in the Urine after 24 hours.

	Individual Sheep.					Average.
Fed.....	38	77	80	28	45	53·6
Starved.....	33	30	32	28	26	29·8

Conjugation of Sulphanilamide.

In the foregoing data on blood concentration only the free sulphanilamide is taken into account. In several of the trials the total sulphanilamide present was also determined. It was found that, from the sixth hour onwards, an almost



GRAPH 3.—*Effect of Starvation on the Blood Sulphanilamide Concentration Curve.*

constant proportion of approximately 25 per cent. of the drug was in the conjugated form. Alexander (1943) found that some 50 per cent. of the drug was present in the blood of sheep in the conjugated form. In the present experiments it was found that the inclusion of the conjugated drug did not affect the basic shape of the curves and so only the free active drug was taken into account.

DISCUSSION.

The retarded absorption of sulphanilamide by sheep on a poor quality grass hay diet is probably a reflection of the slower passage of ingesta from the rumen and generally retarded assimilative capacity. No physiological explanation can be advanced for the effects of diet and starvation on the urinary excretion of the drug. This phenomenon raises the interesting hypothesis as to whether there is, in ruminants at least, a direct correlation between the functional activity of the alimentary tract and that of the kidneys.

The results again emphasise the essentiality of considering the basic diet and the state of digestion in any pharmacological or toxicological investigations where ruminants are concerned. Clark and Quin (1945) demonstrated that the dose of potassium cyanide required to cause ruminal paralysis can be reduced by a quarter if the sheep are starved for 14 hours previous to dosing. Likewise Sapiro, Hoflund, Clark and Quin (1949) showed that the lethal dose of nitrate to sheep on grass hay is half that for sheep on lucerne hay.

The present experiments have shown that the blood concentration curve of sulphanilamide after oral dosing is profoundly influenced by the basal ration and by starvation.

This probably explains the variable results obtained from the oral dosing of sulphonamides to ruminants especially in the treatment of Heartwater and emphasises the necessity for the parenteral administration of drugs to this class of animal where success of treatment depends on the attainment of a certain blood concentration.

It is, therefore, clear that in the ruminant, where chemical compounds can be altered or destroyed by microbial action in the forestomachs and where the whole physiological tempo of the body is so dependent on ruminal activity, no pharmacological or toxicological investigations can be interpreted without full consideration of the basic diet and feeding regime.

SUMMARY.

It has been shown that the concentration curve of sulphanilamide in the blood of sheep after oral dosing is profoundly influenced by the basic diet and by starvation.

In view of this and other similar findings, it is emphasised that, especially in the ruminant, no pharmacological or toxicological findings can be correctly interpreted without due regard being given to the basic diet and the feeding regime.

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APPENDIX (See next page).

APPENDIX.

Summary of Experimental Results: Sulphanilamide Concentration in the Blood (mg./100 c.c.)

DIET.	FED.									
	HOURS AFTER DOSING.							E%.	tm.	T.
	3.	6.	9.	12.	24.	30.	48.			
Green Lucerne.....	2·7	3·6	4·1	5·2	5·5	4·4	1·9	64·9	10·5	21·0
	3·4	5·2	3·5	4·1	4·4	2·1	0·8	31·9	7·0	—
	3·0	5·2	5·2	5·5	4·2	3·1	1·1	46·8	9·1	18·2
	4·4	5·3	4·4	5·0	3·4	2·6	1·2	50·7	6·5	12·9
	4·4	6·5	5·7	5·9	3·8	2·9	1·1	33·4	7·4	14·8
	3·5	5·3	4·8	4·4	4·0	—	1·9	47·1	9·2	34·6
	3·7	5·6	5·1	4·8	4·0	—	1·9	44·4	8·7	31·4
AVERAGE.....	3·6	5·2	4·7	5·0	4·2	3·0	1·4	45·6	8·3	21·0
Lucerne Hay and Maize	4·6	6·1	6·9	6·1	4·0	—	1·7	41·4	8·1	27·6
	5·6	6·4	7·2	6·4	4·2	—	1·7	46·8	7·2	26·9
	4·8	6·4	6·6	6·1	4·2	—	1·9	45·7	8·1	29·8
	4·4	5·8	6·9	6·4	4·4	—	2·1	44·5	8·8	31·7
	2·8	4·2	4·7	5·2	4·4	2·0	1·1	49·5	10·5	20·9
	3·0	4·8	4·8	4·4	2·7	2·0	0·8	27·5	7·8	15·7
	3·0	4·4	4·7	4·5	3·1	2·9	1·3	43·5	8·9	17·7
	3·6	6·5	6·5	6·9	5·3	4·2	1·5	29·9	9·4	18·7
	3·7	5·2	6·5	5·7	3·8	3·1	1·5	47·5	9·2	18·6
AVERAGE.....	3·9	5·5	6·1	5·7	4·0	3·0	1·5	41·8	7·7	23·1
Lucerne Hay.....	3·5	5·1	5·6	4·2	2·5	—	0·4	21·0	7·0	17·6
	4·4	6·4	6·6	4·4	2·4	—	0·8	19·5	6·4	15·9
	4·4	6·6	6·4	4·8	3·3	—	0·8	23·9	6·8	17·9
	2·5	4·2	4·4	4·2	2·5	—	—	27·7	8·4	16·6
	3·1	4·9	5·0	4·8	2·4	—	—	27·1	7·7	15·5
AVERAGE.....	3·6	5·4	5·6	4·5	2·6	—	0·7	23·8	7·3	16·7
Grass Hay.....	2·1	4·5	6·3	6·9	6·9	5·9	3·4	50·4	18·4	74·2
	1·3	1·8	4·1	5·7	6·9	4·8	2·5	22·7	16·0	41·3
	1·2	2·1	3·8	5·5	7·2	5·2	3·0	22·7	16·0	41·3
	1·6	2·8	5·2	5·5	5·5	4·1	2·0	15·8	12·7	30·2
	1·2	1·8	3·5	4·7	6·5	5·2	3·5	32·1	16·2	47·6
AVERAGE.....	1·5	2·6	4·6	5·7	6·6	5·0	2·9	28·8	15·9	47·0

APPENDIX—(Continued).

DIET.	STARVED.									
	HOURS AFTER DOSING.							E%.	tm.	T.
	3.	6.	9.	12.	24.	30.	48.			
Green Lucerne.....	4·2	5·6	5·3	4·8	4·8	—	3·1	61·5	9·9	51·2
	3·7	5·1	5·1	4·8	4·8	—	2·4	57·0	10·1	47·2
	4·0	4·8	4·8	4·8	4·8	—	3·3	69·6	10·3	67·6
	3·3	4·4	4·8	4·6	5·1	—	2·6	63·8	11·8	65·1
	4·0	5·1	5·6	5·1	4·2	—	2·0	58·2	9·3	44·4
AVERAGE.....	3·8	5·0	5·1	4·8	4·7	—	2·7	62·0	10·3	55·1
Lucerne Hay and Maize	5·1	5·8	6·9	5·8	4·4	—	3·3	55·1	7·5	33·3
	5·6	5·6	6·9	5·8	4·2	—	1·5	52·7	7·0	29·5
	5·4	5·1	5·6	5·3	5·1	—	2·6	64·8	9·0	51·3
AVERAGE.....	5·4	5·5	6·5	5·6	4·9	—	2·5	57·5	7·8	38·0
Lucerne Hay.....	3·0	5·3	5·5	5·7	5·2	4·1	1·5	36·0	10·0	20·1
	4·2	6·5	6·7	6·9	5·3	3·9	1·7	37·1	9·5	19·0
	3·0	4·7	5·7	6·3	6·5	5·7	3·2	67·0	15·3	30·5
	2·7	4·4	4·5	4·5	4·2	3·5	1·4	41·3	9·6	19·6
	2·9	5·3	6·1	6·1	6·5	5·9	3·0	46·3	13·0	25·9
	3·1	5·2	5·7	5·6	4·6	—	—	40·4	10·2	34·2
	3·1	5·0	5·2	5·4	5·3	—	—	46·5	10·7	39·9
AVERAGE.....	3·0	5·2	5·6	5·8	5·4	4·6	2·2	44·9	11·2	27·0
Grass Hay.....	1·8	3·8	4·5	5·0	6·3	5·2	3·6	38·2	15·1	30·3
	1·3	2·6	3·8	4·8	6·7	5·3	3·5	34·4	16·7	33·5
	1·8	3·9	5·2	5·2	5·9	4·5	3·0	26·0	13·5	27·1
	1·4	2·2	3·4	3·9	5·9	4·7	3·8	46·2	16·9	33·9
AVERAGE.....	1·6	3·2	4·2	6·2	6·2	4·9	3·5	36·2	15·6	31·2