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BLOOD TRANSFUSION IN CATTLE WITH SPECIAL REFERENCE TO THE INFLUENCE OF BLOOD GROUPS

I. Single transfusions into young animals and pregnant cows

K. VAN DER WALT* AND D. R. OSTERHOFF**

SUMMARY

The results failed to identify a single blood factor or phenogroup which appeared to be responsible or relatively more important for transfusion reactions than others.

One out of seven single transfusions given to young animals resulted in a transfusion reaction, but in only one out of eighteen transfusions should one be concerned that real danger might be involved.

The incidence of transfusion reactions is significantly higher in pregnant animals; in 41 per cent of such transfusions reactions were noted and of 22 animals obtaining a large amount of blood four aborted within eight days after transfusion.

INTRODUCTION

In South Africa blood transfusions are mainly given to cattle suffering from severe babesiasis or anaplasmosis where it is often the only means of saving the animal's life. The benefit derived from this form of therapy is well established in a number of other conditions but extensive clinical application of blood transfusions is unfortunately limited by the occurrence of transfusion reactions in some recipients.

Neimann-Sorensen [1] believed that the clinical reaction in the recipient of foreign blood was the result of an antigen-antibody reaction, and that in general there was a correlation between the level of antibody and the severity of the reaction. He was unable, however, to demonstrate antibodies in 28 animals showing reaction whilst 33 animals with a substantial level of antibodies showed no clinical reaction. Ferguson [2] stated that cattle with no previous history of transfusion could be given blood with little danger of post transfusion reaction. He recommended the use of donors negative for J antigens to reduce the likelihood of undesirable reactions. Braend [3] considered the likelihood of getting anaphylactic shock by transfusing J positive blood cells into animals having anti-J to be about one in 16 transfusions in summer, but much less in winter. Schmid [4] recorded the loss of two animals immediately after transfusions of 25 and 70 ml blood respectively; he believed that a vasomotor-traumatic rather than anaphylactic shock was the reason for death.

Rendel [5] studied the effect of small blood transfusions on pregnancy and conception but was unable to demonstrate any deleterious effects. Tolle [6] observed a considerable drop in milk production following similar transfusions.

Many authors in the clinical field believe the cause of transfusion reactions to be "incompatible" blood but this incompatibility is never defined. Some workers [7-10] describe tests to detect incompatibilities before transfusion but the agglutination test, the cross matching test and the larger cross test served only the purpose of detecting strong naturally occurring iso-antibodies, mainly anti-J, and the reaction between these antibodies and the corresponding J antigen on the red cells of the donor or *vice versa*. Not one of these *in vitro* tests appears sufficiently reliable to avoid transfusion reaction. and their value is rightly debated [8, 11].

Otte [7] considered the only practical solution to the problem of blood transfusion in animals is the replacement of full blood by plasma and the establishment of plasma banks.

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In view of the above conflicting opinions and the unknown role of blood groups in producing transfusion reactions, this study was undertaken.

MATERIAL AND METHODS

The apparatus consisted of a 2 or 3 litre flask closed by a rubber stopper with two perforations. Into one hole a glass tube acting as an air inlet was inserted and into the other a Baxter filter drip apparatus. The latter consists of a short, wide bore glass tube with narrow ends for the inlet and outlet and contains a filter made of exceedingly fine wire mesh, which prevents any small clots from entering the circulation. A transparent latex tube about one metre long with an internal diameter of 5 mm and fitted with a male attachment for insertion into the cannula, completed the set.

With strict aseptic precautions under local anaesthesia, the *vena jugularis* was exposed by a skin incision of 1 cm. A trocar (10 cm long) and cannula (4 mm internal diameter) was used to draw blood from the distended vein. In all experiments an anticoagulant consisting of 100 ml of a 2.5 per cent freshly prepared solution of sodium citrate was mixed with 900 ml of blood.

The recipient was prepared in the same way as the donor, and a similar trocar and cannula inserted into the vein. After elimination of air bubbles from the delivery tube, the speed of infusion was regulated by an adjustable clamp.

As the majority of transfusions were carried out on untamed Afrikaner and Afrikaner crosses, the necessary restraint could only be attained by using a crush and securing the heads with halters and ropes. In most of the trials reciprocal transfusions were performed on a pair of selected animals, using two sets of apparatus.

Except for the lactating cows, all the animals grazed on natural veld consisting mainly of grass with sparse bush and trees. A salt-phosphate lick was provided, and during the winter months grazing was supplemented with maize silage.

All the animals were clinically normal and none had previously received an injection of blood. All transfusions and subsequent collection of blood samples were done in the forenoon, during different seasons of the year.

The experimental animals were selected according to their blood groups, their soluble J substance, and their naturally occurring iso-antibodies. Donor-recipient combinations were made in order to obtain the greatest possible variety.

From the first day after transfusion, serum samples were collected daily for the first eight to ten days, with the exception of Sundays. Thereafter it was done every third or fourth day in order to obtain as complete a picture as possible of both qualitative and quantitative antibody (haemolysin) formation. As soon as the first reactions were obtained in the haemolytic test, doubling dilutions of each reacting serum were made to determine the titre reached at different stages of haemolysin formation.

For haematological studies 10 ml jugular blood was drawn into tubes containing 0.15 ml of a 20 per cent sodium oxalate solution evaporated at room temperature.

Haemoglobin was determined in a Leitz colorimeter and bilirubin estimations were done on the same apparatus (Malloy & Evelyn [12]). A Bürker counting chamber with Hayem's diluting fluid was used for the red cell counts and packed cell volume was determined in Wintrobe haematocrit tubes centrifuged at 3,000 r.p.m. for an hour.

Simultaneous clinical observations were made to check the differences in behaviour, respiratory frequency, pulse rate and rectal temperature before and after transfusion. The animals were watched for about an hour following the transfusion; in cases of shock observations were made for a longer period.

Transfusion reactions were arbitrarily classified into mild, severe, and very severe reactions according to clinical symptoms.

Mild reactions were characterised by signs of discomfort accompanied by a moderate (10-15) increase in respiratory rate, but very little change in pulse rate. The animal was usually disinclined to move, stood with the neck slightly extended and occasionally gave a soft, moist. suppressed cough. These symptoms lasted for 40-60 minutes, and were only noticeable if the animal was not disturbed.

In severe reactions the respiratory rate increased by more than fifteen respirations per minute and a slight but constant increase in the heart rate was noticed. The animal would move stiffly, show signs of bloat,

lachrymation and salivation. If not disturbed it would stand with an arched back, extended neck and the head lowered. Coughing was more frequent and the breathing more laboured. Muscle tremors were frequently noticed.

In the few very severe reactions observed, the animal was hardly able to move and there was profuse salivation from an open mouth with protruding tongue. Lachrymation and muscular tremors were marked and diarrhoea was often present. There were frequent attempts at urinating; in some of the fatal cases a reddish brown urine was passed within an hour. The symptoms lasted for many hours. The animal usually lay down after two hours and showed difficulty in rising. Marked arrhythmia, sometimes associated with heart block, were noticed.

RESULTS

Autologous blood infusions

The first trial consisted of five single infusions performed on animals of different ages with known blood types. Two litres of their own blood were collected and reinfused to test the toxicity of the anticoagulant used.

In two animals the blood was infused immediately after bleeding; in the remaining three animals the infusions were delayed two and a half hours. In the last mentioned cases the temperature of the transfused blood was considerbaly lower than in the first two cases. The infusion time varied between eight and ten minutes.

No clincal "transfusion" reactions whatsoever could be observed. The average respiratory rate for the whole group deminished from 32.8 before to 28.0 after transfusion, the pulse rate from 111.2 to 100.0, while the rectal temperature remained constant at 102.4°F.

Single transfusions given to young animals

In this experiment 55 animals, mainly Afrikaner or Friesian-Afrikaner crossbred heifers were used. The average age of the group was 13 months and the average weight 626 lbs.

As transfusions were given reciprocally the weights of the respective partners were matched as closely as possible. In this way the same amount of blood collected from one animal could be replaced by the blood of its partner. (In one case the already collected blood could not be transferred to the partner because of the intractibility of the latter). The blood groups of the donor and recipient were carefully scrutinised and as many different combinations as possible were employed.

The influence of naturally occurring isoantibodies in the serum of either the donor or the recipient was studied separately. From the whole group ten pairs could be selected where animals, showing strong or medium strong antibodies in their serum could be matched with recipients with no naturally occurring antibodies and *vice versa*. On the one hand, donor blood with normal antibodies was given to partners which had the J-substance either on the cells or on both cells and in the serum. On the other hand, the blood of the latter was transferred to the recipients with normal antibodies of varying titre.

After the transfusions the antibodies against the red cell antigens (immune haemolysins) were studied, i.e. the time of appearance, the type of antibodies, the concentration and the variation of the antibody level until final disappearance. The scores for the titres were calculated according to the method described by Osterhoff [13]. In spite of the fact that donor and recipient differed in many red cell antigens (up to eight different factors) only eight transfusion reactions were observed in 55 procedures. Five recipients appeared slightly uncomfortable while three showed symptoms of severe shock with heavy panting and coughing.

A comparison of the transfusion rate between the reacting and non-reacting recipients revealed that the transfusion was actually performed more slowly in the reacting animals (201.6 ml blood/min) than in the non-reactors (210.1 ml blood/min.) No influence could be established between the total amount of blood given and transfusion reactions. (See Table 1).

Table 1: THE RELATION BETWEEN THE AMOUNT OF BLOOD GIVEN AND TRANSFUSION REACTIONS

Amount of blood transfused	No. of recipients with		
	No reaction	Mild reaction	Severe reaction
1½—2 litres	20	2	1
2—3 litres	11	0	0
3—4 litres	8	1	1
4—5 litres	3	2	1
more than 5 litres	5	0	0
Total	47	5	3

In Table 2 the possible relationship between antibody formation and transfusion reaction is demonstrated.

Table 2: THE RELATION BETWEEN ANTIBODY FORMATION AND TRANSFUSION REACTIONS

Transfusion reaction	No. of recipients	Antibody-type
Severe reaction	3	E′, R, W, –*)
Mild reaction	5	C, R, Y_1, **)
No reaction	47	E′, R, W, C, Y and others

*) One recipient had no antibodies at all.
**) One type of antibody could not be identified.

Antigenic differences, as judged by subsequent antibody formation did not play the expected role in transfusion reactions; specific antibody stimulation occurred in non-reacting as well in the mildly or severely reacting groups. Conversely, one severely reacting recipient developed no antibodies at all.

The influence of naturally occurring isoantibodies on blood transfusion. performed once only, is demonstrated in Table 3.

Table 3: THE INFLUENCE OF NATURALLY OCCURRING ISOANTIBODIES ON BLOOD TRANSFUSIONS

No. of transfusions performed	Naturally occurring antibodies in		Transfusions reactions
	Donor	Recipient	
10	strong—medium strong*)	none**)	2 (mild)
10	none**)	strong—medium strong*)	1 (severe)
35	none**)	none**)	5 (3 mild, 2 severe)

*) Strong = titre higher than 1/16; medium strong = titre varies from 1/4—1/16 at the transfusion time.
**) These animals could have the J substance either on the red cells and in the serum, or in the serum only, or had no J substance at all.

The concentration of naturally occurring haemolysins reaches its peak in autumn [13] and the influence of season should, therefore, be considered as a possible contributory cause of reaction. Of the 55 transfusions, 32 were performed in Spring (September/October). Five transfusion reactions occurred; three were mild and two of a more severe nature. Of the remaining 23 transfusions given in autumn (Mach/April), two mild and one severe recation resulted.

Immune haemolysins occurred in 41 of the 55 animals. The appearance of these haemolysins did not have the slightest, clinically observable effect on the health of the animals. Where the recipients had naturally occurring haemolysins in their serum they responded by producing additional immune antibodies against the foreign antigenic substances introduced into their systems.

The recipients were grouped according to the concentration of antibodies produced:
Group I: No immune haemolysis produced (14 recipients);
Group II: Weak haemolysins produced (17 recipients);
Group III: Strong haemolysins produced (24 recipients).

The difference between these groups, in regard to the variation of antibody is clearly demonstrated in Fig 1, where averages of three or more animals were used for the calculations of titre values. The variation of the titre values towards the end of both curves can be explained by the fact that at different stages after transfusion, the values were calculated from different recipients.

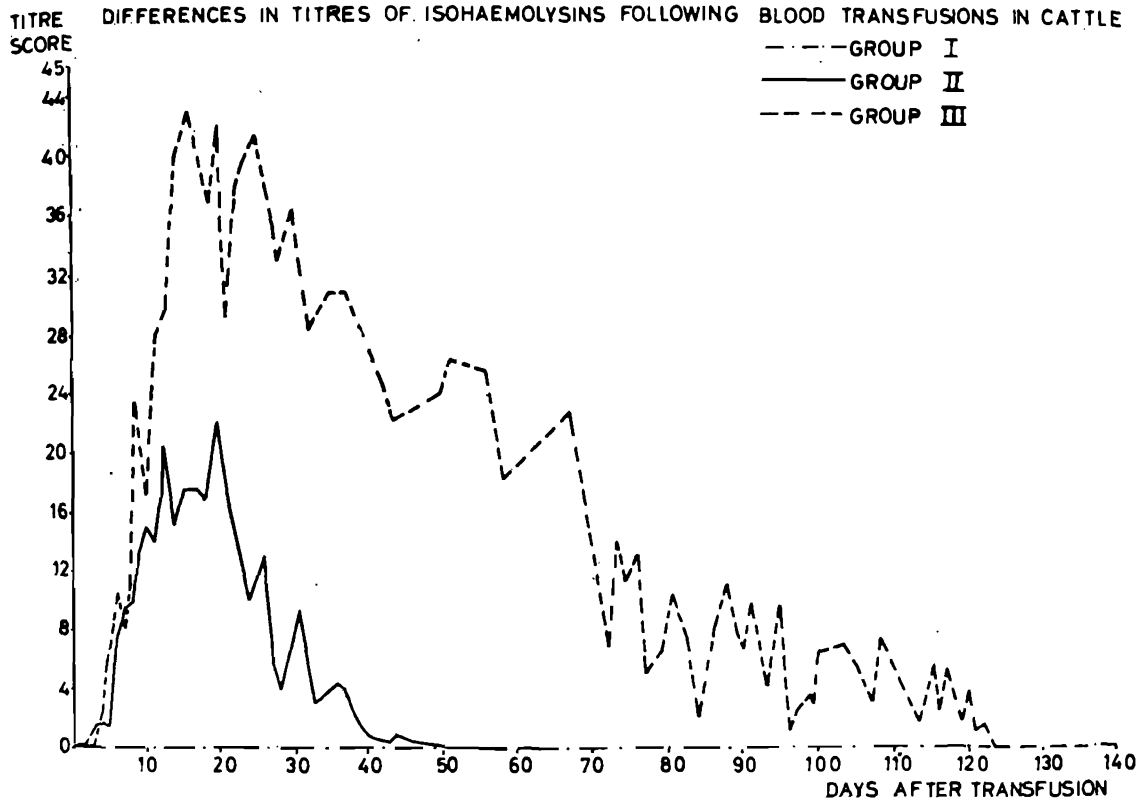
It was expected that all the animals would respond with antibody formation against transfused antigens. Fourteen animals, however, did not respond at all (Group I in Table 4).

Table 4: THE EFFECT OF BLOOD TRANSFUSION ON THE FORMATION OF ISOHAEMOLYSINS

	Haemolysins produced against factors:	Interval in days before haemolysins appeared	Day after transfusion of disappearance of haemolysins
Group I	—	—	—
Group II	A, G, E′, X, R, F, S, Z′, and two types not identified	5.9	36.4
Group III	A, G, D′, I′, C, W, X, R, F, V, S, and Z	5.9	116.0

In group II the highest titre score of 35 for antibodies against blood factor F was reached by one animal on the ninth day after transfusion. In group III the score of 57 was attained for antibodies against factor R by one animal on the twenty-fifth day after transfusion.

FIGURE 1



It was assumed that antibody formation might play an important role especially if transfusions were repeated to save the lives of severely ill animals. Therefore, all possible experimentally known factors which might be involved in the stimulation of antibody formation were analysed and the results subjected to the X^2 method of Snedecor [14].

The following conclusions were arrived at:—

1. No difference between breeds with regard to haemolysin production could be established.
2. The age of the recipients and the production of haemolysins were not related.
3. The weight of the animals has no influence on the production of haemolysins.
4. There were no seasonal differences in the production of haemolysins.
5. The amount of blood given had no effect on the production of haemolysins.
6. The duration of the transfusion had no effect on the production of haemolysins.
7. The amount of blood per unit body weight did not influence the production of haemolysins.
8. The rate of transfusion (speed) did not effect the production of haemolysins.

None of these X^2-tests was significant at any level, but the difference in the respiratory frequency before and after transfusion in group III could be significant (Table 5), but it is not believed that respiratory frequency is related to antibody production which starts only on the sixth day after transfusion (see Fig. 1 and Table 4).

Table 5: THE RESPIRATORY FREQUENCY, PULSE AND BODY TEMPERATURE OF THE THREE RECIPIENT GROUPS

	Group I	Group II	Group III
Respiratory Frequency			
Before Transfusion	42.5	40.6	46.7
After Transfusion	51.7	49.6	61.9
Pulse			
Before Transfusion	94.0	86.5	96.3
After Transfusion	103.5	92.6	96.1
Temperature (°F)			
Before Transfusion	103.5	102.8	103.2
After Transfusion	102.9	102.9	103.1

It could be demonstrated that in the greater percentage of the recipients the red cell count, the packed cell volume and the haemoglobin content of the recipients blood decreased immediately after transfusion (Table 6).

Table 6: THE RED CELL COUNT, RED CELL VOLUME AND HAEMOGLOBIN CONTENT OF THE THREE RECIPIENT GROUPS

	Group I	Group II	Group III
Red Cell Count (10^6/mm^3)			
Before transfusion	7.51	7.60	7.57
After transfusion	7.04	7.26	7.26
Red Cell Volume (ml/100 ml)			
Before transfusion	33.84	35.03	35.08
After transfusion	31.60	32.96	32.30
Haemoglobin Content (gm/100 ml)			
Before transfusion	11.47	11.44	11.15
After transfusion	10.71	10.75	11.04

This reduction in blood values occurring immediately after the transfusion is due to haemodilution caused by shifts of body fluid following the withdrawal of blood. After 24 hours the picture returned to pretransfusion levels in groups II and III but remained slightly below normal in group I. On the second day there was a significant rise in the P.C.V. of group III. This rise was accompanied by a very slight rise in the R.C.C. and in haemoglobin content which led to the belief that a temporary increase in cell size might have been responsible for the increased P.C.V.

The haematological values remained within the normal limits in 28 out of 55 animals receiving transfusions, the percentage in group I, II and III being 71, 53 and 38 per cent respectively (Table 7).

In the remaining animals the haematological values started decreasing from the end of the second day. Between the ninth and the thirteenth day the reduction in values averaged 30 per cent. From this point a

FIGURE 2

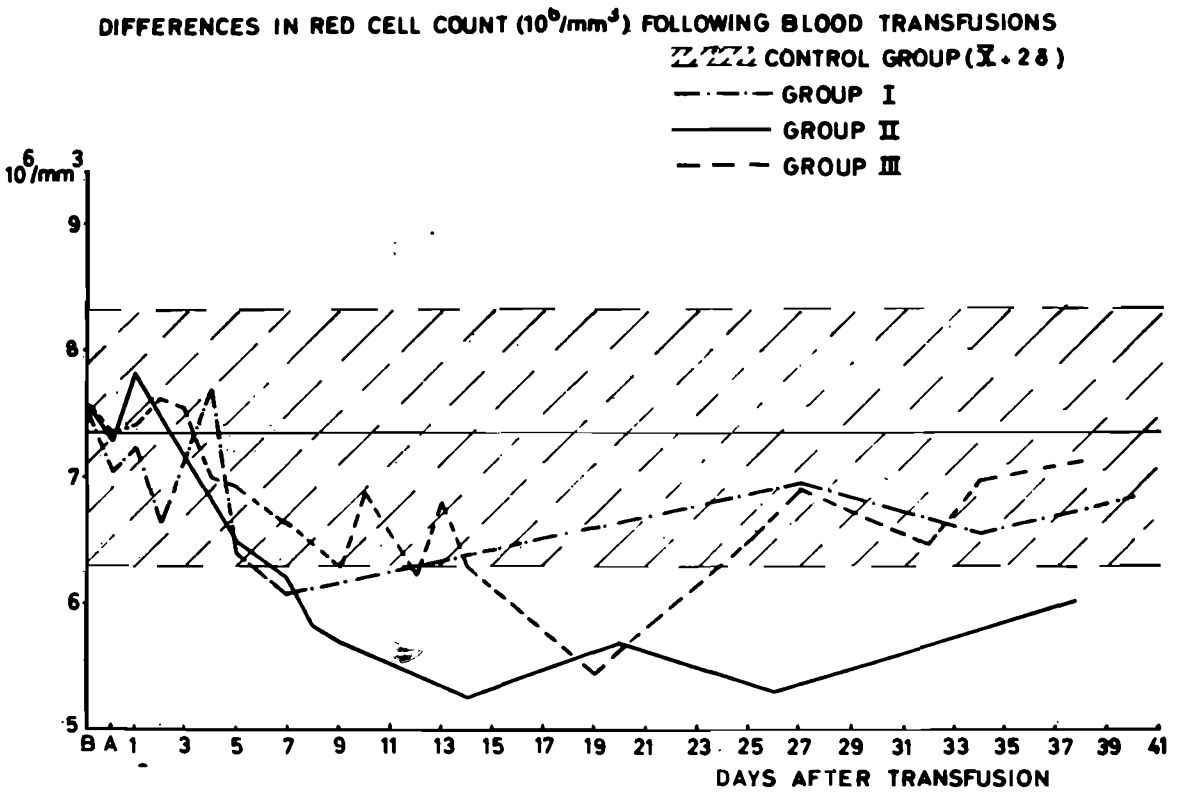


Table 7: THE SUBSEQUENT RETURN TO NORMAL HAEMATOLOGICAL VALUES IN RELATION TO THE PRODUCTION OF HAEMOLYSINS

R.C.V., Hb., R.C.C.	Group I No Haemo-lysins	Group II Weak Haemo-lysins	Group III Strong Haemo-lysins	Total
Immediate return to normal haematological values	10	9	9	28
Delayed return to normal haematological values	4	8	15	27
Total	14	17	24	55

$x2 = 4.114$ · 2 d.f. 0 $.20 > P > 0.10$

steady recovery took place to reach pre-transfusion levels at about the fortieth day.

In group I the decrease was only slight and hardly exceeded the lower limits of normal haematological values. The decline was most marked in group II where a moderate level of antibodies appeared after transfusion, and less noticeable in group III.

Each point in Fig. 2—Fig. 4 represents the composite value obtained for the group before transfusion (B), immediately after transfusion (A), and on a particular day after transfusion.

On the assumption that the foreign red blood cells might be destroyed rapidly in the circulatory system of the recipient, the van den Bergh test was performed daily on blood of the recipients. The results were inconclusive; weak positives were found in some cases up to the seventh day after transfusion. In many animals the test remained negative throughout. These results indicated that rapid lysis of transfused blood did not occur, some of the weak positive results were probably due to subcutaneous haematomata produced during the procedures.

Blood transfusions in pregnant cows

FIGURE 3

DIFFERENCES IN PACKED CELL VOLUME (ml/100 ml) FOLLOWING BLOOD TRANSFUSIONS

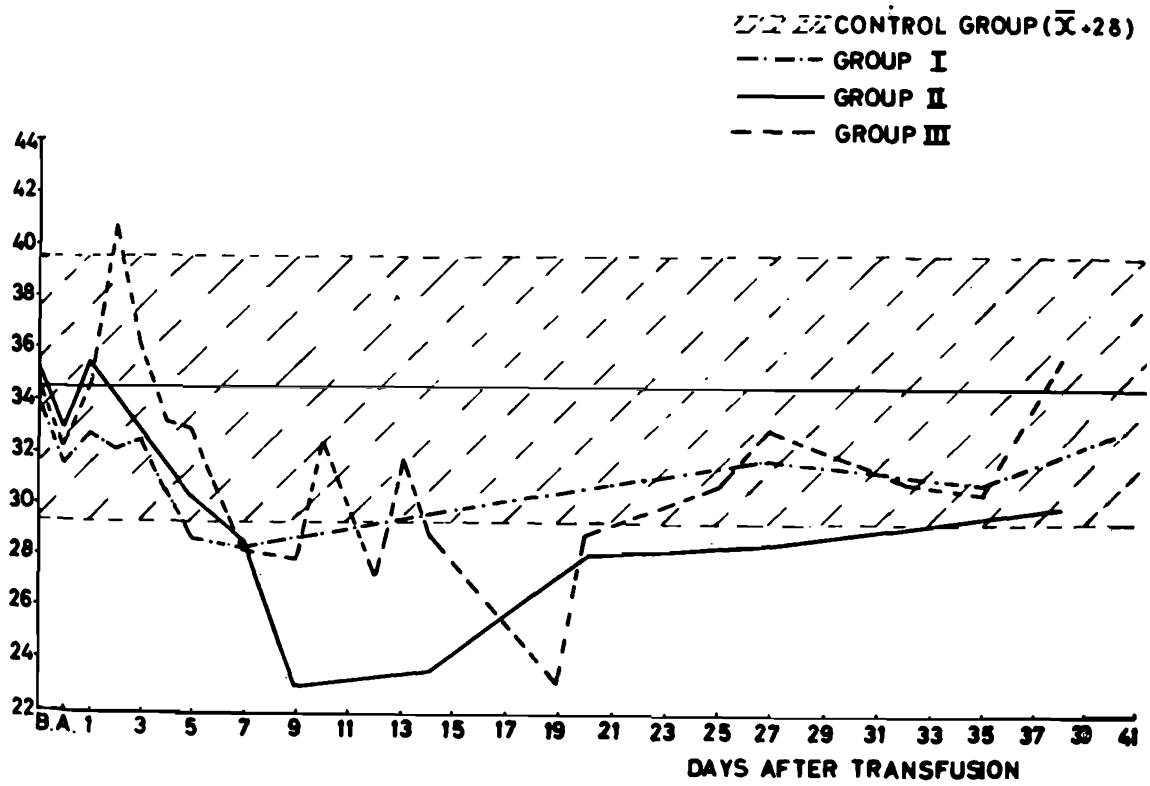
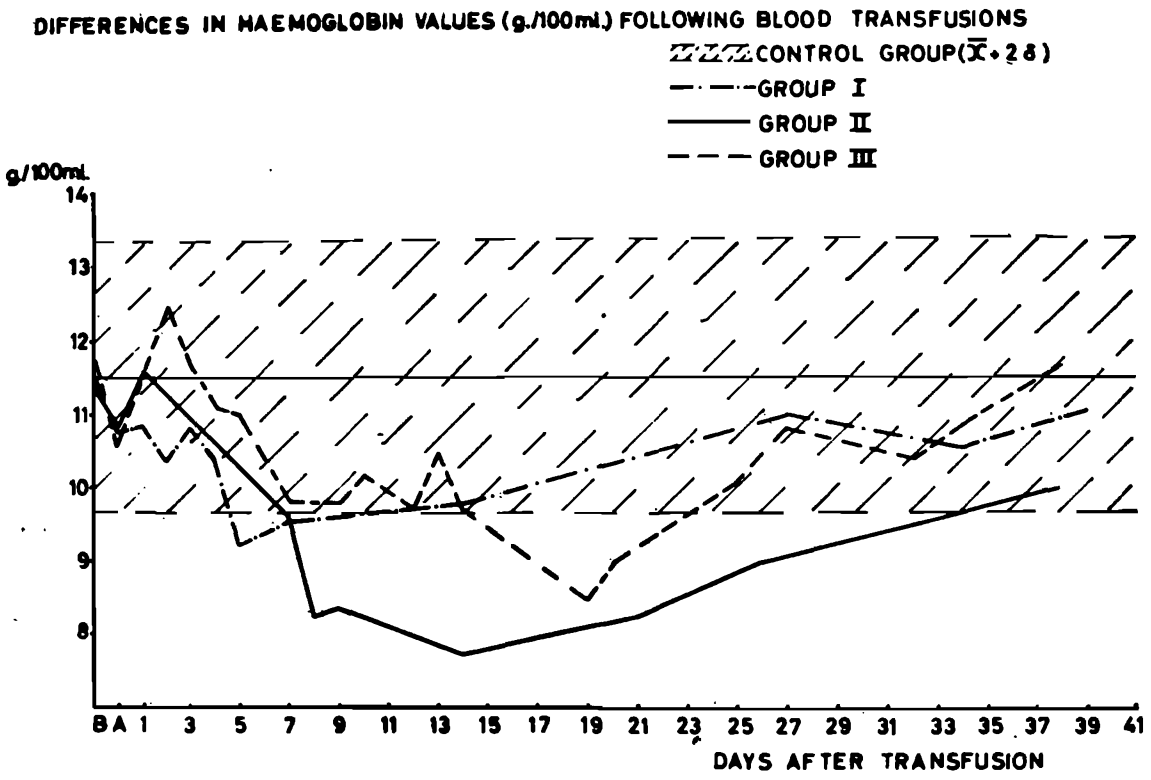


FIGURE 4



Twenty-two pregnant cows were used, one group was three to four months pregnant and the other group seven to eight months. Three litres of blood were exchanged between pairs selected for the greatest difference in their blood types.

Nine of the twenty-two cows showed transfusion reaction (see Table 8) of which four aborted after six to eight days. Of the latter only two showed a moderate reaction at the time of transfusion but the remaining two appeared quite comfortable.

Great care was exercised during the transfusion to ensure that the handling would not cause abortion. The abortions which occurred could, therefore, be attributed to the transfusions, although shock at the time of transfusion did not appear to be the only cause. Abortions only occurred in animals pregnant for four months or longer but the series is too small to draw any significant conclusions in this respect.

The relation between blood transfusion reaction and haemolysin production in pregnant animals is shown in Table 9.

After the unfavourable results obtained in this series it was decided to discontinue this part of the experiment. No further blood was collected and consequently no serum titre studies were performed. Whether particular incompatibility of blood factors in the cases of shock and abortion existed was not established.

The average transfusion time for non-reacting recipients was 17.5 minutes and in the animals showing transfusion reactions. 15.4 minutes (Table 9). This difference is significant at the 0.5 level. In the previous series of young animals, however, the speed of transfusion was not significant and the fact that abortions occurred only after a week casts some doubt on the possible influence of transfusion speed as a cause of shock or abortion. From clinical observations (see Table 10) it was however evident that shock reactions in pregnant cows, as in the

Table 8: EFFECT OF TRANSFUSION ON GESTATION, AGE, BREED AND NUMBER OF GESTATIONS

Cow No.	Breed	Age Yrs.	Age Mths.	No. of calves	Days in calf at the transfusion time	Remarks
8982	Friesian ×	6	2	3	108	
8335	Friesian	4	4	2	114	
8046	Friesian	4	6	2	114	
9358	Friesian	3	11	2	119	Secondary shock reaction later on
8793	Friesian	3	6	1	120	
8889	Friesian	3	4	1	124	Aborted after transfusion
60	Friesian	4	1	2	165	Mild shock
8732	Afrikaner	6	2	2	210	
9879	Afr. × Fries	2	0	0	211	Shock
6731	Afrikaner	7	1	3	215	
9126	Afr. × Fries	3	4	0	220	No immediate reaction, aborted 6 days later
8710	Afrikaner	9	2	3	220	
6289	Afr. × Red Poll	8	0	3	226	Shock, aborted 8 days later
8718	Red Poll ×	9	2	4	230	
9331	Afr. × Heref.	2	11	1	242	
9123	Afr. × Fries	3	4	1	243	Shock
8227	Afrikaner	3	1	1	243	
9140	Afr. × Fries	3	4	1	251	
8704	Hereford ×	9	2	3	254	
9799	Afrikaner	3	1	1	256	
8627	Afr. × Heref.	4	3	1	264	Shock, aborted 7 days later
6734	Afrikaner	7	0	4	265	Shock

other groups, can be predicted during the actual transfusion by the rise in respiratory rate.

DISCUSSION

A detailed discussion concerning all aspects investigated will follow the second part of the report presented.

It can be stated that the results given here failed to identify a single blood factor or a phenogroup which appeared to be responsible or relatively more important for transfusion reactions than others. Such reactions are expected to occur when the donor possesses strong naturally occurring antibodies and the recipient strong antigens on the red cells alone, or on the red cells and in the serum, or *vice versa*. Also in this connection the present studies failed to yield conclusive results.

Otte[7] observed no reactions in 40 cases of transfusion, while Schmid[9] reported 18 cases of shock (two were fatal) in over 500 transfusions of small amounts of blood. Bohn[14] obtained four cases of shock in 25 transfusion of 0.5 to 6 litres of blood. Summarising the results of single transfusions reported here it can be said that about one out of seven single transfusions is likely to result in a transfusion reaction, but in only one out of eighteen transfusions would one be concerned that danger might be involved.

Reports on haematological events shortly after blood transfusions are scant. The drop in values in about 50 per cent of recipients after the first transfusion also shown by

Wujanz & Rittenbach [15] is difficult to explain. In all three groups some animals remained normal while those showing a delayed return to normal haematological values constituted 29, 47 and 62 per cent respectively. The individual reacting animals in group II however had a more severe anaemia than those in other groups.

Table 9: THE RELATION BETWEEN HAEMOLYSIN PRODUCTION AND BLOOD TRANSFUSION REACTION

Recipient No.	Expected Haemolysins	Produced Haemolysins	Duration of Transfusion (min.)
No reaction after transfusion			
8982	D′E′$_1$ X$_2$ F M	F	13
8335	C V J	C	12
8046	GO$_1$	G*	11
8793	BYD′I′MZSA$_3$	Y	13
8732	O$_1$WU$_1$ SA$_3$	W	—
6731	W X$_2$ Z	W	32
8710	Y$_2$E′$_1$I′ U$_1$	Y$_2$I′	12
8718	IY$_2$A′D′I′R S	I′ S	22
9331	B Q U$_1$ Z	B Z	22
9227	I Q W	W	—
9140	C$_1$ W L′	C$_1$W	20
9799	BPQY$_2$A′E′$_3$I′ LMU$_1$ZSA$_3$	Y$_2$I′ Z	13
Shock reaction after transfusion			
9358	H BO$_1$A′I′X$_2$L′L	I′ L*	10
8889**	C	—*	12
60	—	unidentified	13
9479	X$_2$ZZ′	Z	12
9126**	Z I′SU$_1$Z SA$_2$	AS	14
6389**	GQL′	—	18
9123	W V Z	V	20
8627**	PE′$_1$Y′L′ L SA$_1$	?	28
6734	B Q A′E′$_1$R U$_1$	R U$_1$	12

*Naturally occurring haemolysins in the serum of recipients.

**Animals aborted after transfusion.

The most feasible explanation for this drop is that the red cells are destroyed within the first 9 to 14 days after transfusion and subsequent return to normal follows haemopoietic response by the blood forming organs. It was anticipated that this theory would be supported by a positive v. d. Bergh test on the serum but the results obtained were erratic and inconclusive.

The fact that a drop in haematological values did occur in some cases means that in practice a sufficiently big transfusion to save the animal's life still entails a possible anaemia up to 14 days after the transfusion.

Braend [3], Rendel [5], Kiddy *et al* [16] and Neimann-Sorensen [1] reported on series of pregnant animals injected repeatedly for isoimmunization. Although some reactions were noticed, none of the animals aborted and it was concluded that pregnant animals were not necessarily more prone to mishap than non-pregnant cows.

On the other hand, Laing *et al* [17], Ferguson [2] and Haring [18] reported abortions up to 8 days following transfusions. Wright [10] believes that abortions are caused by pyrogens contained in the anti-coagulant mixture and not by incompatible blood.

In the present series of pregnant animals reactions were noticed in 41 per cent of first transfusions and, of the 22 animals transfused, 4 aborted within 8 days.

The incident of transfusion reaction thus is significantly higher in pregnant animals and there is a very real danger of abortion when larger amounts of blood are transfused.

Table 10: CLINICAL OBSERVATION ON PREGNANT COWS BEFORE AND AFTER TRANSFUSION

	Respiratory Frequeicy		Pulse Rate		Body Temperature	
	Before	After	Before	After	Before	After
Cows 3—4 months pregnant (av. of 7)	34.6	42.6	75.1	77.1	101.2	101.3
Cows 7—8 months pregnant (av. of 15)	39.9	53.9	84.7	89.6	101.8	102.7
Cows showing no reactions after transfusion (av. of 13)	36.0	42.5	87.7	83.7	101.3	102.0
Cows showing shock reactions after transfusion (av. of 9)	41.3	60.3	74.7	86.7	102.0	102.4

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Jl S. Afr. vet. med. Ass. 40 (2): 121-128 1969

THE FUNCTIONAL ADVANTAGE OF HAEMOGLOBIN TYPE A IN HAEMOLYTIC SYNDROMES IN SHEEP

Phenylhydrazine, organic selenium and partial exsanguination as external agents in the production of anaemias

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SUMMARY

Haemoglobin AA and AB phenotypes have been shown to be able to resist the harmful effects of severe haemolytic episodes better than BB types. The artificial production of haemoglobin C in the sheep is discussed. The relevance of these findings to studies on the aetiology of geeldikkop and enzootic icterus in sheep is noted.

INTRODUCTION

In an earlier paper[1] we reported the association of an abnormal haemoglobin type, designated haemoglobin C, with the haemolytic syndromes, geeldikkop and enzootic icterus. The present paper is a report of its experimental production and significance in relation to the problems of selenosis and haemolytic anaemias in general.

MATERIALS AND METHODS

Adult or young Merino sheep of both sexes and including wethers were used throughout this work. Sheep maintained at Onderstepoort were drawn from the pool of available animals and were fed a diet of green lucerne hay, crushed maize and water *ad libitum*. The sheep examined and bled in the Karoo were raised and kept on the farms mentioned below and at the time of examination were grazing on the late summer vegetation of the areas concerned[2, 3].

All blood samples were collected by jugular venupuncture using heparin as anticoagulant. Selenium was determined on blood samples by non-destructive neutron-activation analysis as described earlier[4]. Other biochemical and chemical pathological determinations reported here were done by the methods set out in Table 1. Haematological studies were done using standard procedures[5].

Table 1: BIOCHEMICAL METHODS USED IN THESE STUDIES

Method	Reference
Haemoglobin phenotypes	Neethling, Brown, Oosterhoff, de Wet & Ward-Cox[1]
Haemoglobin	Drabkin cited by King & Wootton[6]
Red cell fragility	Brown[7]
Albumin bound plasma copper	Brown[8]
Caeruloplasmin	Houchin[9]
Plasma iron	Marrack cited by King & Wootton[6]
Total plasma proteins	Weichselbaum[10]
Plasma aldolase	Sibley and Lehninger[11]
Plasma glutamic oxalacetic transaminase	King[12]
Plasma glutamic pyruvic transaminase	King[12]
Plasma lactic dehydrogenase	Wroblewski & La Due[13]

Statistical analyses of raw data, where mentioned, were performed using standard procedures.

RESULTS

1. *Correlation between erythrocyte selenium levels,* and *haemoglobin phenotype distribution.*

Four farms were selected for study in parts of the Karoo where enzootic icterus

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and geeldikkop occur regularly. Two of these farms, numbers 1 and 2, were located in the Hofmeyr district. Losses due to either syndrome on these farms were minimal during the season 1966—67. The other two farms, numbers 3 and 4 were situated near Murraysburg. Stock losses due to the two diseases mentioned were heavy during the last two seasons. A fifth farm, number 5, was selected as a control farm. Losses due to the disease complex were minimal over a period of a number of years. The sheep studied on all these farms were 15—34 month old Merino ewes. Very few of these animals had had a prior clinically detectable attack of either disease. Heparinized blood samples were obtained from these animals during the first week of December in 1967. At this time no cases of either syndrome were reported in the areas concerned.

Erythrocyte selenium levels and haemoglobin types were determined on blood samples obtained from all sheep. The results relating to these determinations are presented in Tables 2 and 3.

Table 2: SELENIUM LEVELS IN THE ERYTHROCYTES OF SHEEP FROM THE FIVE FARMS STUDIED

(Values for selenium are expressed as mcg Se per g of dried erythrocytes. To obtain mcg Se per g intact red cells divide by 3.2)

Locality	Number of samples	Mean selenium level (Standard deviation)	Statistical significance when compared with control farm
Farm No. 1 (Hofmeyr)	50	1.68 (±0.37)	Highly significant
Farm No. 2 (Hofmeyr)	64	1.29 (±0.38)	Highly significant
Farm No. 3 (Murraysburg)	33	1.10 (±0.26)	Significant
Farm No. 4 (Murraysburg)	67	1.26 (±0.28)	Highly significant
Farm No. 5 (Middelburg)	12	0.85 (±0.37)	

2. *The experimental production of haemoglobin C.*

Sheep were rendered anaemic for this purpose by the administration of phenylhydrazine, selenocystine and selenomethionine or by partial exsanguination over a long period.

Table 3: HAEMOGLOBIN PHENOTYPES AND GENE FREQUENCY

Farm No.	Total No. of observations	Hb Phenotypes			Frequency of Hb^A
		AA	AB	BB	
1	102	4	35	63	0.201
2	137	7	40	90	0.197
3	135	14	54	67	0.304
4	49	9	22	18	0.408
5	42	2	22	18	0.310

(i) *Phenylhydrazine induced haemolytic anaemia:* Six sheep were each given ed in physiological saline at the dosage rate of 40 mg/Kg body weight. Blood samples were taken from them four days before injection, at the time of injection and then at various intervals for the next 10 days. Three of the sheep were haemoglobin AB phenotypes and three were BB phenotypes. A marked anaemia developed within two days of injection and as shown in figure 1 became progressively worse over the nine days following injection after which recovery commenced. Figures 1A, B and C represent the mean values obtained for haemoglobin, packed red cell volume and red cell counts for each group of sheep at the various sampling times. Haemoglobin C appeared in the erythrocytes of all the AB phenotypes animals five to six days after injection but not in those of the BB group.

(ii) *Anaemia induced by intravenous injection of seleno-amino acids.* The particular experiments concerned here are described fully elsewhere [14]. In brief this work entailed the following: One adult sheep was given two single doses of 100 mg of selenomethionine (equivalent to 1 mg Se/Kg bodyweibht) intravenously, a period of three months being the interval between doses; a ram lamb aged two months was given a total of 45 mg of selenocystine over a period of three months in the form of an initial dose of 12.5 mg of the amino acid (equivalent to 0.9 mg Se/Kg), followed one week later by a similar dose and two and

three quarter months later by a final dose of 20 mg of the compound (equivalent to 1 mg Se/Kg body weight). Three months after the start of each experiment each sheep showed the presence of haemoglobin C.

(iii) *Anaemia induced by partial exsanguination.* Twenty-three adult Merino wethers were used for this work. Six of them possessed haemoglobin AB types, one was a haemoglobin AA type and the remaining 16 were haemoglobin BB types. The animals were bled once a week for a period of eight weeks, the average amount of blood removed at each bleeding, being 740—765 ml.

Packed red cell volumes fell in all cases from initial values of 33—37% to 10—27%. Six weeks later the mean percentage decrease in packed cell volumes for the two groups of animals six weeks after the start of the experiment was 27.1% and 27.2% respectively.

No difference was evident in the degree of anaemia produced in the three different groups of haemoglobin phenotypes. Haemoglobin C failed to appear in the red cells of any of the BB animals, but developed in the erythrocytes of all the AB and AA haemoglobin types by the 16th day after the start of the experiment. The concentration of haemoglobin C as revealed by the intensity of the stained bands on starch gel electrophoretograms was directly proportional to the amount of blood removed.

Haemoglobin C was still present in the erythrocytes of three animals 150 days after the initial appearance of this pigment indicating its persistence in the new population of erythrocytes, formed as a response to the exsanguination, for their entire lifespan.

3. *Differences in reaction of phenotypes to an acute haemolytic crisis.*

The six sheep given phenylhydrazine hydrochloride intravenously ((i) above) were used to obtain this information. The chemical pathology of the haemolytic state induced by this compound will be described in full elsewhere and only the most striking changes encountered in this study are recorded here for the sake of the present discussion. The reader is referred back to figure 1 for a reminder of the equal severity of the anaemic state in both groups of animals. Figures 1 E—L are a graphic portrayal of the plasma levels of activity of aldolase (Ald), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LD); the plasma levels of iron, caeruloplasmin and total bilirubin and absolute eosinophile counts on whole blood throughout the course of the experiment in the two groups of animals. Units of plasma enzyme activity are as defined in the original procedures.

It is obvious from these figures that there is a marked difference in the effects of an anemia of the same severity in the two groups of animals. Plasma Ald, LD and GOT activity levels are noticeably higher and rise sooner in the BB types than in their AB counterparts. A less marked difference is seen in the case of plasma GPT activity levels. The recovery from tissue damage as revealed by plasma Ald, GOT and LD activity levels is longer in the BB animals than in those of the AB group. An early rise of greater magnitude in plasma caeruloplasmin and iron levels is also seen in the BB animals. This is associated with changes in the total plasma protein figures which are similar in both groups.

The curves for plasma total bilirubin levels and absolute eosinophile counts are most interesting. In the first instance it is apparent that there is a very marked difference in the ability of the two groups of animals to clear bilirubin, formed under identical circumstances, from the blood. The BB sheep show clear evidence of insufficiency in this regard. The eosinophile counts show that in the early stages of the haemolytic syndrome the adrenals of the AA types are able to rise to occasion better than their less fortunate BB counterparts and the general reaction to the stress of severe intravascular haemolysis is also far less severe throughout the course of the syndrome in the former group.

We believe that this is the first time that a differing response to the stress of a severe acute disease process has been demonstrated in the two haemoglobin groups of sheep noted above. The significance of this difference is discussed below.

FIGURE 1

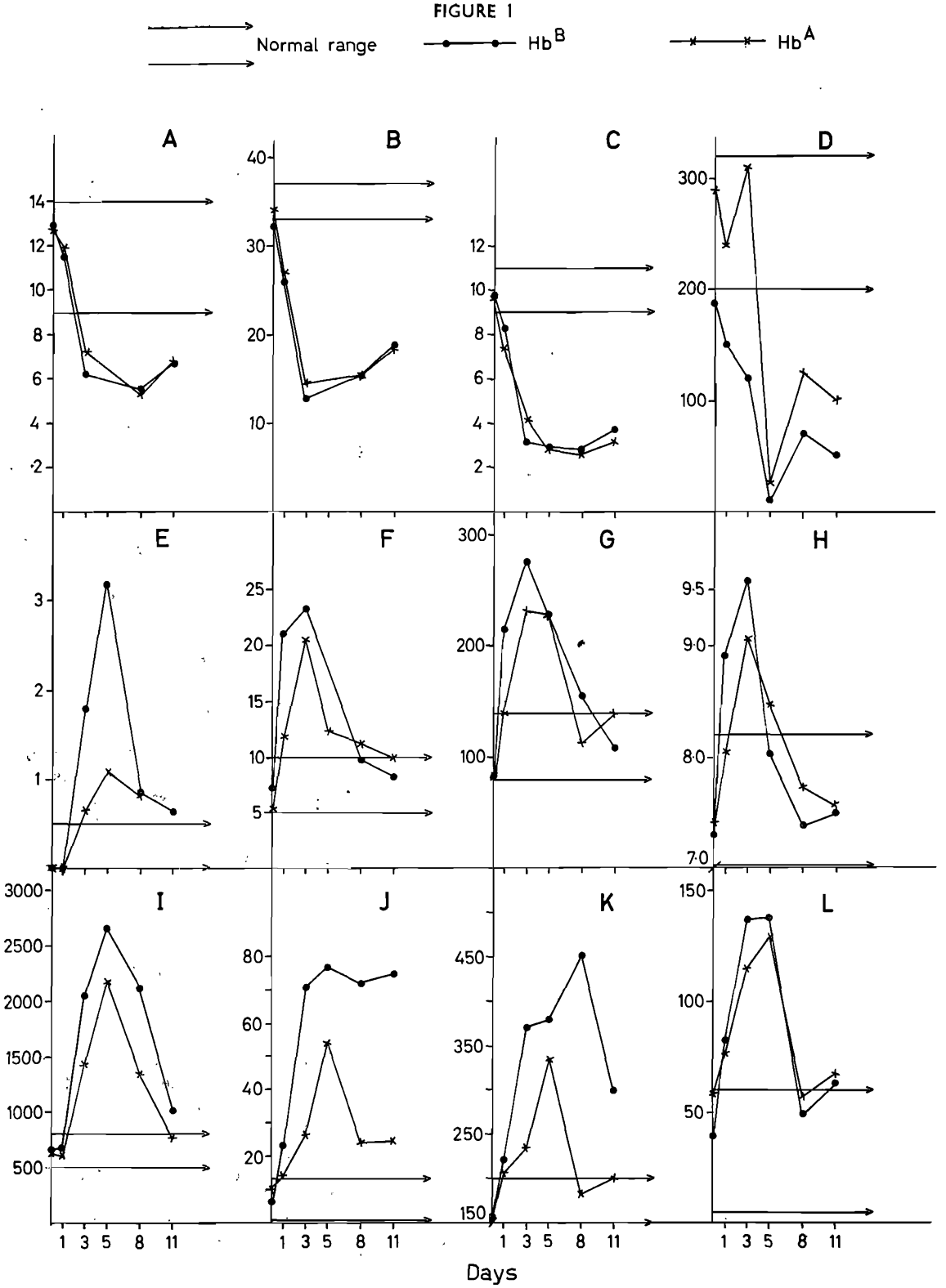

Figure 1: The differing response of haemoglobin phenotypes to the stress of acute haemolysis (A = haemoglobin, B = packed cell volume, C = red cell count, D = absolute eosinophile count, E = total plasma bilirubin, F = plasma caeruloplasmin, G = plasma iron, H = total plasma proteins, I = plasma lactic dehydrogenase, J = plasma aldolase, K = plasma glutamic oxalacetic transaminase, L = plasma glutamic pyruvic transaminase.)

DISCUSSION

It is clear from the foregoing results that haemoglobin C can apparently only be formed by AA or AB phenotypes. This confirms the published findings of Huisman and others [15, 16, 17]. This haemoglobin has been produced before in sheep by partial exsanguination [18]. It is clear from the work reported in the present paper that it occurs in haemolytic disease (chronic selenosis must be included here [14, 19]) as well as in post-haemorrhagic states. The concentration of the pigment in erythrocytes seems to be directly proportional to the severity of the anaemia. It is presumably synthetised in the erythrocyte at the time of erythropoiesis and apparently remains in these cells for their entire life span. This has been calculated to be in the order of 150 days in the sheep. This thus provides a convenient method of establishing the lifespan of erythrocytes in animals capable of producing haemoglobin C.

Fechter and Myburgh [20] have reported a gene frequency of Hb^A of 0.38 for South African Merino sheep. while Evans and Blunt [21] have given a corresponding frequency of 0.45 for Australian Merinos.

Our data in Table 3 show that on farms considered to be free from haemolytic syndromes like geeldikkop or enzootic icterus, e.g. farm 5, the frequency of Hb^A is for some or other reason below that considered normal elsewhere. This phenomenon is even more accentuated on farm 1 and 2 where the diseases have been quiescent during the last two seasons. Large scale outbreaks of either syndrome, such as occurred recently on farms 3 and 4 seem to shift the frequency distribution of Hb^A towards the more generally accepted value.

The data presented in this paper show marked differences between the haemoglobin phenotypes in the chemical pathology produced in the various experiments. The BB types obviously showed a more severe reaction to the harmful effects of phenylhydrazine than did their AB counterparts. Their failure to produce haemoglobin C is also interesting. The ability to do this as in the AB animals, may be one of the manifestations of the way in which the bodies of A type animals can successfully limit the harmful effects of acute stresses like haemolytic crises.

There is evidence that a more severe temporary liver insufficiency developed in the BB sheep than in the AB types. This is shown to be the case by the higher plasma levels of bilirubin in the former group. The elevations of Ald, GOT and LD levels in the plasma of both groups may be a further indication of hepatic dysfunction in these sheep. Here again the BB animals showed themselves to be at a disadvantage. In this particular regard it must be remembered that GPT activity is known to be low in the sheep liver and high in ovine erythrocytes [19, 22]. Similarly Ald, GOT and LD activity is also high in these cells [19]. Haemolysis could therefore have contributed in no uncertain manner towards the observed elevations of the plasma levels of these enzymes.

The raised plasma caeroloplasmin and iron levels in both groups can be expected in an acute haemolytic crisis. The fact that these elevations are greater once more in the BB types may be further evidence of embarrassment of hepatic storage, biliary excretion or both, with consequent regurgitation of protein bound iron or copper into the blood.

The observations of Huisman and Kitchens [23] indicate that the presence of haemoglobin C is advantageous in compensating for a potential relative tissue hypoxia of sheep during severe anaemic states. Furthermore sheep of the Hb^A phenotype have a larger blood volume, erythrocyte mass and splenic reserve than the Hb^B counterparts [24]. These findings are in agreement with the results presented in the present paper and stress the functional advantages of Hb^A in haemolytic syndromes.

It is difficult to explain our findings in Table 3, unless we assume largescale survival of Hb^A types on farms 3 and 4, as could be expected from what is known of the functional advantage of this type, and restocking of farms 1 and 2 with a population of predominantly Hb^B phenotypes. It is felt that far more work on the gene frequency of Hb phenotypes amongst sheep in the Karoo is required before any definite interpretation of Table 3 can be attempted.

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BOOK REVIEW

THE VETERINARY ANNUAL

EDITED BY W. A. POOL, M.R.C.V.S.

Ninth Issue 1968. John Wright and Sons Ltd., Bristol. Pp XXX+311 18 plates. United Kingdom price: 65 shillings.

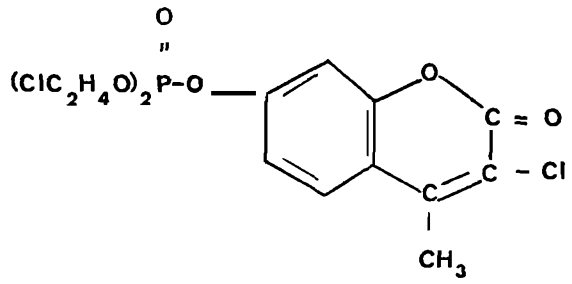
The latest edition of this useful reference work contains contributions from 34 authoritative contributors who, between them, have presented an immense amount of newer knowledge in concise, lucid reviews covering a wide variety of fields. The following titles caught the eye as being of general interest:

"Infection and resistance of the bovine mammary gland", "Mastitis Control"; "Scrapie"; "Helminthology"; "Physiology and Pathology of Reproduction"; "Ruminant Ketosis"; "Nervous Diseases of Animals"; "Poisons and Poisoning"; "Animal Husbandry"; and "Future Developments in Animal Feeding." Other sections of interest to pathologist, physiologist and parasitologist have not been specified here. Lists of new drugs, appliances and publications appear in the back of the book, which also contains an elaborate subject index where the more important articles are listed in heavy type.

Amidst today's intimidating publication explosion this annual provides valuable aid to all who remain dedicated to the practise of continuing education.

R. K. L.

HALOXON



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J. 4790

Jl S. Afr. vet. med. Ass. 40 (2): 129-136 1969

TREATMENT OF *SCHISTOSOMA MATTHEEI* INFESTATION IN CATTLE

J. A. LAWRENCE* AND W. O. H. SCHWARTZ**

SUMMARY

Investigations into the treatment of cattle naturally infested with *Schistosoma mattheei* revealed that Stibophen at 7.5 mg/kg daily for six days was very effective in removing the parasites. Lucanthone, administered as a 30 mg/kg dose on each of three alternate days, was also apparantly effective. Cattle showing clinical evidence of infestation with *S. mattheei* improved following removal of the parasites.

INTRODUCTION

Schistosoma mattheei was originally described as a parasite capable of pathogenic effects in sheep [1]. Although infestation of cattle was well recognised the first outbreak of disease in this species attributable to the parasite was not reported until 1963 [2]. In Rhodesia outbreaks of clinical schistosomiasis have been more commonly recognised in sheep than in cattle.

S. mattheei is very widely distributed amongst the cattle population of Rhodesia. A survey by Condy [3] in 1960 revealed an overall incidence of 69% in 2,509 slaughter cattle. The incidence at the Salisbury abattoir, which draws stock for slaughter mainly from the well watered highveld was 92%. At what level of infestation this parasite becomes pathogenic to the bovine remains to be established. It is probably largely dependant on the nutritional and physiological status of the animal. On several farms clinical illness and death have been associated with very heavy infestations of the parasite, and clinical signs and post-mortem lesions have indicated that the parasite was responsible for the disease.

Clinically affected cattle show emaciation and weakness, with a moderate anaemia. In the later stages the eyes are sunken, indicating some degree of dehydration. There is often diarrhoea, and blood is sometimes noted in the faeces. Post-mortem examination reveals a grey discolouration of liver and lungs, and irregularities of the liver surface due to portal fibrosis. The intestinal wall is often thickened due to the deposition of eggs in the mucosa and submucosa, and the wall of the bladder may show granulomatous lesions containing eggs. Schistosomes are readily seen in mesenteric and hepatic portal veins.

It appears that on occasion it may be necessary to remove *S. mattheei* from cattle, or at least to reduce its numbers, to enable the animals to survive an overwhelming infestation but little seems to be known of the drugs that are effective.

The drug recommended for the treatment of *S. mattheei* in cattle in the standard textbooks is antimosan [4]. This drug was not readily available in Rhodesia but stibophen, the sodium salt, was found to be available at a reasonable price.

Lucanthone is widely used in the treatment of human schistosomiasis in Rhodesia and has been found to be moderately effective in sheep [5]. Its cost was not considered to be excessive for use in cattle.

Trichlorphon has been shown to be effective in the treatment of *S. bovis* in cattle [6], and is cheap and readily available.

Niridazole has been shown to be effective in sheep [7], but its present cost is prohibitive for the herd treatment of cattle.

In this article are described trials which were carried out to investigate the use of stibophen, luncanthone and trichlorphon in the treatment of natural infestation with *S. mattheei* in cattle.

MATERIALS

Animals—Cattle were selected by the owners on two farms as being clinically affected. On Farm A the animals were Africaner cross. Twenty-six cows were made

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available for a controlled trial, and in addition the owner treated 100 head of varying ages with varying drug regimes and reported his findings. On Farm B twenty Angus × Mashona cows were made available for a controlled trial, and the owner treated two bulls and reported his findings. On Farm B it appeared that not all the illness could be attributed to schistosomiasis. Several animals died from a hepatitis of unknown origin, and a change in grazing management and supplementary feeding produced a striking clinical improvement even in untreated controls. However, the trial on this farm did successfully reveal the effect of the drug on the parasites.

We were dependent on the owners of the cattle to administer the drugs, but all evidence indicated tha this was done according to our recommendations.

DRUGS

Stibophen—Injection of Stibophen B.P.I, which is a 6.4% solution containing the equivalent of 10.3 mg Antimony Trioxide per ml, was administered intramuscularly. No dosage rate for this product is cited in the British Veterinary Codex. Lapage [4] recommends ten doses each of 1.6 g of antimosan, the potassium salt, administered on alternate days. In this trial stibophen was used at an equivalent dosage rate on cattle weighing 5—600 lb (ten doses of 6.5 mg/kg). It was also used at a total dosage rate of 45 mg/kg, either administered in ten doses of 4.5 mg/kg on alternate days or in six daily doses of 7.5 mg/kg. Finally a more intensive course of three daily doses of 10 mg/kg was administered to two animals.

Lucanthone—Lucanthone Hydrochloride[II] was administered orally as a powder suspended in water. The normal human dosage rate is 60 mg/kg divided into six doses over three days. Cattle were dosed at an arbitarily chosen rate of 90 mg/kg divided into three doses of 30 mg/kg administered on alternate days. This interval was selected as it appeared to reduce the severe depression caused by the drug.

Trichlorphon — Dimethyl - hydroxy - trichloro-ethyl phosphonate[III] was administered orally as a powder suspended in water at a rate of 75 mg/kg. Dinnik [6] recommends four such doses at intervals of three days.

EXPERIMENTAL METHODS

Farm A—The owner treated 70 head with three doses of 30 mg/kg of lucanthone on alternate days and 20 head with ten doses of 4.5 mg/kg of stibophen also on alternate days. He reported that both groups showed a clinical response to treatment.

In order to confirm his observations a herd of cows which he considered required treatment was subjected to a controlled trial. Twenty cows received three doses of lucanthone at 30 mg/kg on alternate days. Six cows remained untreated as controls. The animals were weighed at the commencement of the course of treatment, and were reweighed seven weeks later.

Farm B—When the opportunity arose to carry out further investigations into the treatment of cattle a more critical method was adopted. Twenty cows in poor condition were selected and divided into three groups of seven, seven and six head. The severity of disease in each animal was assessed by reference to the following criteria: number of schistosome eggs in the faeces as estimated by direct examination of sediment, haemoglobin determined by cyanmethaemoglobin mehod, packed cell volume by microhaematocrit, degree of eosinophilia, plasma protein by the biuret method and albumin/globulin content by precipitation of globulin with sodium sulphite. The animals were then distributed among the three groups so that each group contained a good cross-section of of the whole.

Group I (7 head) received 10 doses of 6.5 mg/kg stibophen on alternate days.

Group II (7 head) received six daily doses of 7.5 mg/kg.

Group III (6 head) remained untreated but was held under the same conditions as the treated groups.

Response to treatment was assessed by comparing body weight, haematological findings and plasma protein levels at the commencement of treatment and ten weeks afterwards. The number of eggs in the faeces was not found to be a consistent index of

I—"Fantorin"—Glaxo-Allenburys.
II—"Nilodin"—Burroughs Wellcome.
III—"Dylox"—Bayer Agro-Chem.

infestation. Two animals showing an average response for the group were then slaughtered from each of Groups I and II, and the number of parasites found compared with the number found in two animals from Group III, one slaughtered at the same time and the other slaughtered in extremis in the early stages of the trial.

Two cows from the same farm were subsequently treated at the Laboratory with three doses of 10 mg/kg/day and the number of parasites assessed at slaughter eight weeks later.

RESULTS

Stibophen—

On Farm A the owner reported that the twenty head he treated showed a satisfactory clinical response as evidenced by improvement of appetite, cessation of diarrhoea, and improvement in condition.

On Farm B one of a group of three Angus bulls in very poor condition died of congestive heart failure possibly associated with schistosomiasis. The other two received stibophen and made a marked clinical improvement in condition and strength.

In the controlled trial at slaughter both controls showed a very heavy infestation estimated by counting *in situ* to be in excess of 1000 parasites. The treated animals showed:

Group I—one no parasites, one a slight infestation.

Group II—both no parasites.

An evaluation of the clinical response was confined to cows found subsequently to be not-in-calf. Two cows were found to be in calf in each group and although they showed a similar response to the not-in-calf cows there was a marked difference in degree. They are therefore not included in the results which appear in Table I.

As already mentioned all animals showed a marked improvement in condition and there was no significant difference in the weight response between any of the groups. There was no significant difference in the improvement in blood and plasma protein levels between Groups I and II but the levels in these groups were significantly higher than those in Group III and this can be attributed to the removal of the parasites. It is possible that had the observation period continued longer, the improvement in blood and plasma protein levels might have been reflected in the weight gain.

Of the two animals receiving 10 mg/kg/day one was free from parasites at slaughter and the other had only a very light infestation.

No untoward side-effects were reported by the owners in any of the animals that they treated with stibophen. A clinically normal animal in the laboratory that received 8 mg/kg/day showed after the second dose some abdominal discomfort, depression, diuresis and increased water intake, but these signs disappeared after treatment was discontinued. A second normal animal that received 10 mg/kg/day showed only a slight redution of appetite on the day of the third and final dose, but one of the infested animals treated at this rate showed a marked loss of appetite, rapid pulse and respiration and evidence of discomfort on the two days following the last dose.

Lucanthone—

On Farm A the owner reported a satisfactory clinical response in the 70 head he treated and was firmly of the opinion that a number of animals would have died but for the treatment.

Table 1: RESPONSE OF CATTLE CLINICALLY INFESTED WITH **S. MATTHEEI** TO TREATMENT WITH STIBOPHEN COMMENCED ON 5.3.68

Group	No. of Animals	Date	Average weight (Lb)	Average Haemoglobin (g/100 ml)	Average P.C.V. (%)	Average Plasma Protein (g/100 ml)	Average Albumin (g/100 ml)	Average Globulin (g/100 ml)	Average A/G Ratio
I	5	5.3.68	581	7.3	24	8.6	1.4	7.2	0.19
(65 mg/kg)		17.5.68	659	12.8	36	10.2	4.1	6.1	0.67
II	5	5.3.68	524	7.4	24	9.0	1.2	7.8	0.15
(45 mg/kg)		17.5.68	608	12.4	36	10.3	3.6	6.7	0.53
III	3	5.3.68	557	7.3	25	8.6	1.2	7.4	0.16
(control)		17.5.68	633	10.3	28	11.4	2.6	8.8	0.30

In the controlled trial the initial weight of the cows ranged from 415 to 770 lb. Six untreated controls all lost weight, an average of 36.6 lb/head. The twenty treated animals gained an average of 6.8 lb/head though there was a wide variation and six cows actually lost weight. The mean difference between untreated and treated groups after 49 days was 43.4 lb in favour of the treated group and this difference was shown to be significant. This result was considered to confirm the owner's observations.

Only one animal treated with lucanthone was examined post-mortem after treatment and it was free from schistosomes. All untreated animals examined during the outbreak were heavily infested.

Lucanthone at three doses of 30 mg/kg on alternate days caused a marked depression and loss of appetite but this did not persist after treatment was completed. Seven animals treated in error with three doses or 60 mg/kg survived.

Trichlorphon—

Our experience with this drug was catastrophic. Two clinically affected cows received 75 mg/kg and close observation revealed very little side effects. Four days later these two and three others were treated again. All showed very severe side effects and two died in spite of treatment with atropine, one from the group receiving its second dose and one of the others. It must be confessed that we did not carry out the 17 hours predosing and 4 hours postdosing starvation observed by Dinnik [6] and the animals did receive a small supplement of corn and cob meal in addition to veld grazing. However, in our opinion unpredictable reaction to the high dosage rate of this drug by the clinically affected animal makes it unsafe for the treatment of this condition.

DISCUSSION

Within the limits of these trials the following conclusions can be drawn.

Stibophen appears to show a high efficiency against *S. mattheei* in cattle at a dosage rate of 7.5 mg/kg/day administered for six days. Toxic side-effects are negligible. Administration by intramuscular injection is convenient for herd treatment and the cost is within reason. A preliminary trial of a more intensive course of 10 mg/kg/day for three days showed that it was equally effective but in view of the side-effects in one animal this trial would need to be repeated on a large scale in the field before its use could be recommended. Such an intensive course would make treatment even more conveient and less expensive.

Lucanthone at three doses of 30 mg/kg on alternate days also appeared to have a good effect against the parasite but further investigations are necessary to confirm this. Disadvantages in comparison to stibophen are the higher cost, oral administration and severe side-effects.

From the results of our trials we are satisfied that it is necessary on occasions to treat cattle for schistosomiasis to save their lives and that they do respond to effective removal of the parasites. In this respect the situation appears to differ somewhat from that experienced in sheep [5]. Stibophen appears to be the drug of choice. In addition to treatment measures should be taken to maintain a high nutritional status, especially in animals under the stress of pregnancy, lactation and growth, and to prevent a repetition of the circumstances leading to an overwhelming infestation with the parasite.

Future work may be expected to reveal that subclinical infestations have an economically detectable effect by lowering productivity but with the drugs available at present there can be no justification for recommending treatment of any but the clinically affected animal.

ACKNOWLEDGEMENTS

We are extremely grateful to Mr. T. M. Lambert and to Col. F. Harpur for their enthusiastic co-operation, without which these studies would not have been possible. We thank Burroughs Wellcome Central Africa (Pvt) Ltd for making available bulk supplies of Nilodin. and A. S. Ruffel (Pvt) Ltd. for supplies of Dylox. Miss D. Beak, Miss S. Salter and Mr. R. Hill provided valuable laboratory assistance and Mr. A. Waller cared for the cattle at the Laboratory. The Director of Veterinary Services has given permission for the publication of this account.

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BOOK REVIEW

EGG QUALITY: A STUDY OF THE HEN'S EGG

EDITED BY T. C. CARTER

1968 Oliver & Boyd, Edinburg. pp vlll & 336, Tabs. 41, Figs. 34 & Plates 11.

This is the proceedings, and includes the discussion, of the fourth of a series of symposia organised by the Scientific Advisory Committee of the British Egg Marketing Board and held in Sept. 1967. Acknowledged experts in their respective fields, emanating from the United Kingdom, Canada, the United States of America, and Australia, were responsible for the ten chapters, and there were over 150 participants from all over the world.

The symposium was divided into four parts, and the scope of the papers and discussions is perhaps best indicated by the titles of the papers preesnted:

1. The structure and formation of the shell membrances; 2. The gross composition, chemistry and physico-chemical basis of organisation of the yolk and the white; 3. The proteins of egg white; 4. Macromolecular components of egg yolk; 5. Microbiology of the egg; 6. Disease and egg quality; 7. Eggs in Virology; 8. The measurement of certain egg quality characteristics (a review); 9 Storage of eggs; and 10. The science and technology of egg products manufacture in the U.S.A.

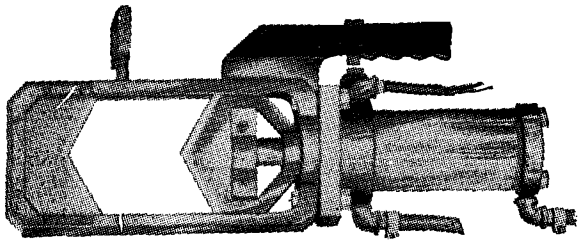
The papers are a clear reflection of the great scientific interested which is centred in the hen's egg, a remarkable biological entity which has long been taken for granted and which is frequently only known as a breakfast item. The contributors deal expertly with the formation, structure, composition, chemistry and physical chemistry of the egg, then with the macromolecular components and microbiology of its contents. Attention then moves to the effects of disease on egg quality and the uses of eggs in virology; finally, matters such as measuring egg quality, storage and the preparation of egg products are covered.

The book is a unit of the most up-to-date scientific and technological information, presented in a most readable form and well illustrated. The printing and reproduction are a credit to the publishers, and the book is very well indexed in respect of both authors and subjects. Authors have also provided extensive lists of references.

This book is surely the best of its kind and will undoubtedly become a standard reference work for all who are directly or indirectly interested in the egg, whether it be as an item of food or as a complex biological unit of infinite scientific use.

L. W. v. d. H.

POWERTOOLS for ABATTOIRS



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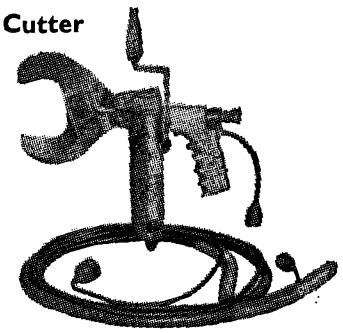

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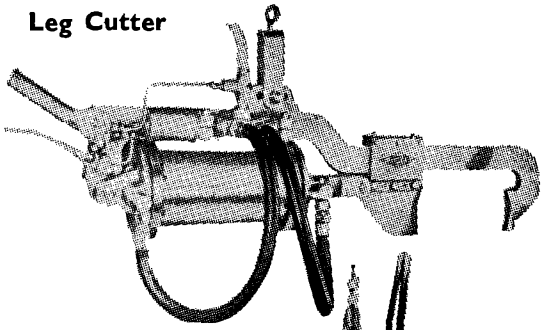
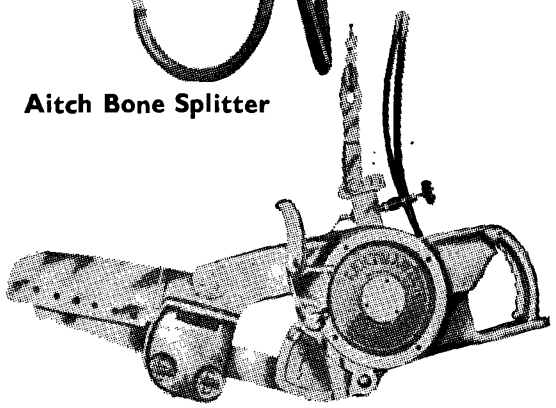
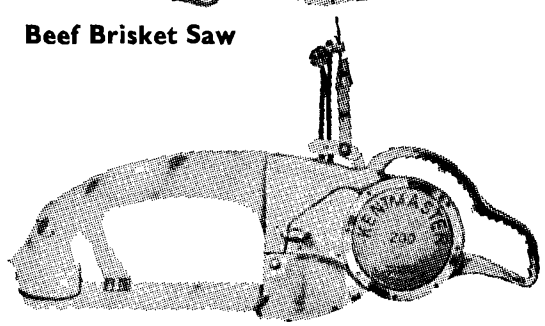


Aitch Bone Splitter



Beef Brisket Saw



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Jl S. Afr. vet. med. Ass. 40 (2): 137-152 1969

THE DEVELOPMENT OF ANTIBIOTICS FOR THE TREATMENT OF MASTITIS IN CATTLE*

O. UVAROV**

INTRODUCTION

During the early stages in the development of some chemotherapeutic substances such as acridine dyes, sulphonamides, tyrothricin and penicillin, it became obvious that these substances could be of use in the treatment of mastitis in cattle. It was soon found, however, that some of these preparations removed the infection, but were irritant to the udder tissue, so the cure became worse than the disease. Thus, all of the above substances except penicillin were discarded. The real advances in the treatment of mastitis stem from the development of penicillin, which remains the most predictable and the best evaluated antibiotic so far evolved. The development of penicillin was rapidly followed by the introduction of several other antibiotics prepared singly or in combination with other chemotherapeutic agents, thus leading to a very widespread use of these substances. In some countries, the distribution of antibiotics is direct to the farmer who uses them without any diagnosis, or at times to remove the signs of the disease but not its cause. Occasionally, opinions have been expressed that the use of antibiotics has not reduced the incidence of mastitis. It is pertinent to ask, however, if the antibiotic preparations so used had been properly evaluated and applied; was it taken for granted that all antibiotics would control infections as penicillin can control and eradicate *Streptococcus agalactiae* infection, and had full therapy always been carried out? From practical experience of evaluating drugs in cattle over the last 15 years, it is the author's opinion that the use of antibiotics has reduced the losses previously caused by this disease considerably, and provided that modern drugs are used early in the disease in a correct manner, efficient weapons are available to control mastitis. Moreover, in the early days of antibiotic development for mastitis there were some omissions in the knowledge on the antibiotics themselves, the pharmaceutical requirements for their formulations, and the criteria for their evaluation[1]. Co-incidental with the increased knowledge of antibiotics and their action in the udder there is now more recognition of the fact that mastitis is a problem of the herd and not of the individual cow, that it is a man-made disease[2] and that different infections[3] produce pathological changes of different severity. The importance of management, including hygiene and good milking technique, is also recognised as an essential part in the overall control of mastitis[4-7].

At present in some countries, the control of mastitis is in part also complicated by the multiplicity of drugs available for use in the disease, and the farmer has a free choice of drugs but insufficient guidance on their uses. In other countries the necessity of keeping antibiotics out of the nation's bulk milk may well lead to a reduction in treatment or restricting the choice of the drug to its excretion time from the udder rather than for its efficacy. The latter requirement was in part responsible for directing a more extensive use of antibiotic during the dry period, and is discussed in another section.

In this paper an attempt is made to:—

(1) Briefly mention the disease—The Disease.
(2) Describe the development of antibiotics for use in mastitis—The Development.
(3) Describe treatment of mastitis—The Treatment.
(4) Make a few recommendations for the further control of mastitis—Recommendations.

(1) THE DISEASE

The definition of mastitis varies between different workers[6, 8, 9]. In the U.K. the disease is defined as clinical or sub-clinical, and

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**Glaxo Laboratories Limited, Greenford, Middlesex, England.

it occurs in acute, sub-acute and chronic forms. The British Veterinary Association publication "Controlling Bovine Mastitis" [10] lists the most predominant bacteria causing the disease. These are:

A. *Staphylococci*—These are the most common single pathogens at present encountered. Their habitat is widespread and they can live on the skin of the udder, the worker's hands etc. It is thought that the increased use of milking machines causes some damage to the teat sphincter, so enabling predisposing organisms to establish themselves in the teat canal.

B. *Streptococci*—

a. *Streptococcus agalactiae* — These live only in the udder and do not multiply on the intact skin. They can be fully controlled by penicillin. It appears that the incidence of this infection is again on the increase.

b. *Streptococcus dysgalactiae* and *Streptococcus uberis* — These can live and multiply on the teats and the skin of the udder. They are rarely associated with a mastitis herd problem. *Str. dysgalactiae* can be recovered from cases of dry cow mastitis either in association with *Corynebacterium pyogenes*, or by itself.

C. *Corynebacteria*

a. *Corynebacterium pyogenes* — This is frequently associated with so-called "summer mastitis" or dry cow mastitis, but may also be found in very acute cases of the disease during lactation. It causes severe necrosis of the udder tissue and is generally incurable, leading to the loss of the quarter or, in very severe cases, to the death of the animal.

b. *Corynebacterium ulcerans* and *Corynebacterium bovis* — Both are being more widely recognised and can be associated with a herd problem of the disease. *C. bovis* had in the past been regarded as a normal inhabitant of the teat canal, but in recent years it has been recovered from severe cases of mastitis [10].

D. *Escherichia coli* — It appears that the incidence of this infection is being more widely diagnosed in several countries and has been associated with enteric conditions in housed animals.

E. *Pseudomonas aeruginosae* — This infection is one of the most difficult to control. It has been thought that the use of quaternary ammonium compounds as a disinfectant in milking sheds, predisposed animals to this infection. The author has worked in several herds where other disinfectants were being used and where the infection has persisted for a long time. One of the bad characteristics of this organism is that it is a chance excretor, disappearing for periods of time and reappearing at others.

F. *Other organisms and infections* — In many cases of mastitis there are mixed infections of staphylococci and streptococci. There are also several yeasts, mycoplasma species, acid fast organisms, and some have been suspected as being secondary to some intramammary treatments. The incidence of these infections is difficult to assess.

In discussing mastitis, it is as well to remind ourselves that the disease is a reaction to bacterial invasion. Pattison [3] in an excellent review on the progressive pathology of mastitis, indicates that mastitis pathogens enter the udder via the teat canal and penetrate duct walls causing an immediate neutrophilic reaction resulting in an increased cell count. He differentiates between:—

A. Low grade organisms such as streptococci which only produce patchy inflammation leading to fibrosis and involution, and

B. More pathogenic organisms such as haemolytic staphylococci which are potentially more dangerous than for example *Streptococcus agalactiae*.

Staphylococci can produce toxins, and many strains elaborate enzymes which increases their pathogenicity and can cause severe necrotic tissue damage. These necrotic areas get walled off by fibrous tissue so that no treatment can penetrate such a fibrosed lesion. The extent of the damage will depend on the invasiveness of the organism and the resistance of the animal.

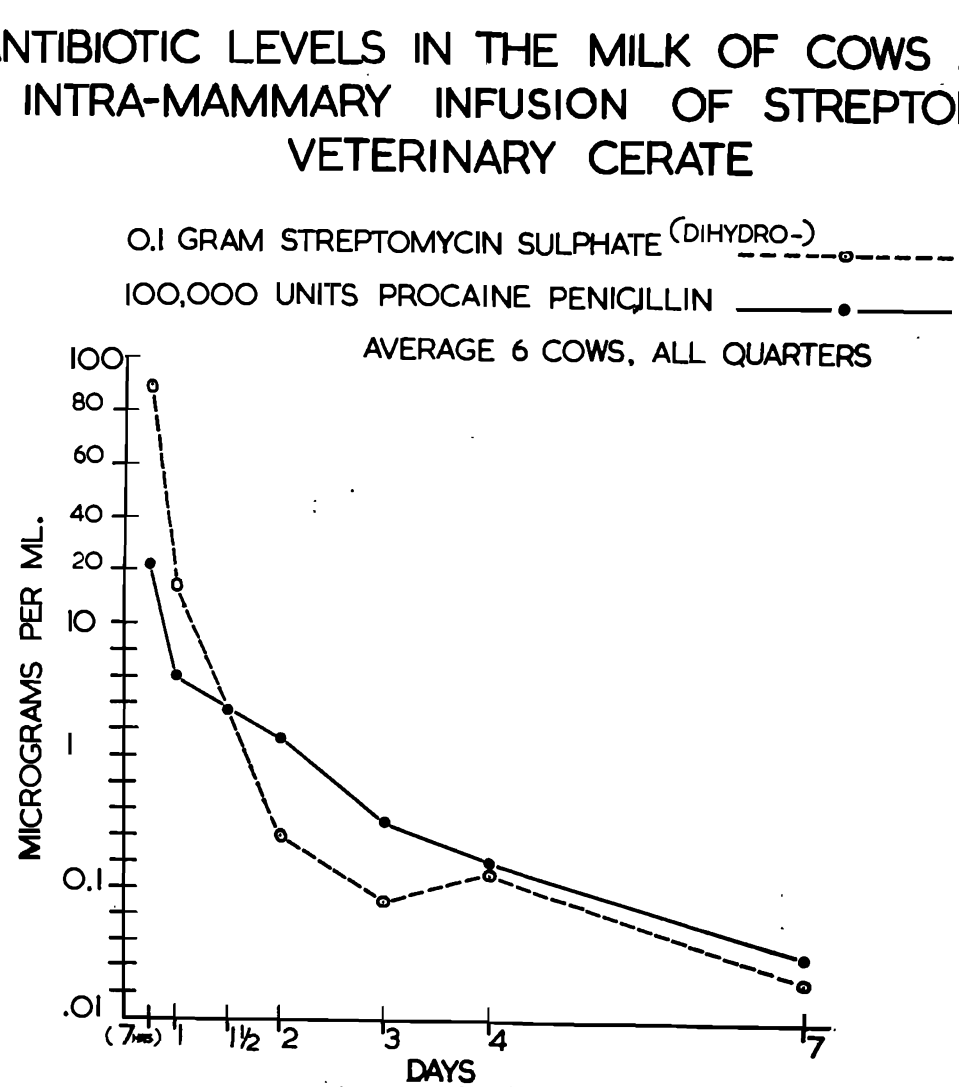
Although many cases of mastitis can be looked upon as local lesions in the udder, requiring treatment via the teat canal, in acute cases of the disease the conditions may be different. There can be congestion of the whole udder with scant secretion, swelling of the interstitium and closure of smaller ducts by inflammatory products which act as a potential barrier to intramammary treatment, as no galoctogenic distribution of the drug can occur if it is used via the teat canal. It is, therefore, essential to recognise that in mastitis, different organisms are responsible for dissimilar pathological changes in the udder, and that different treatments may be necessary.

(2) THE DEVELOPMENT OF ANTIBIOTICS

Penicillin, the first successful antibiotic developed for mastitis therapy, was at first used as an aqueous solution. It was soon recognised that this method had many disadvantages and the individual single dose tube presentation was evolved. There is now an extensive literature on the use of penicillin and its excretion from the udder when different doses and bases were used [11,23].

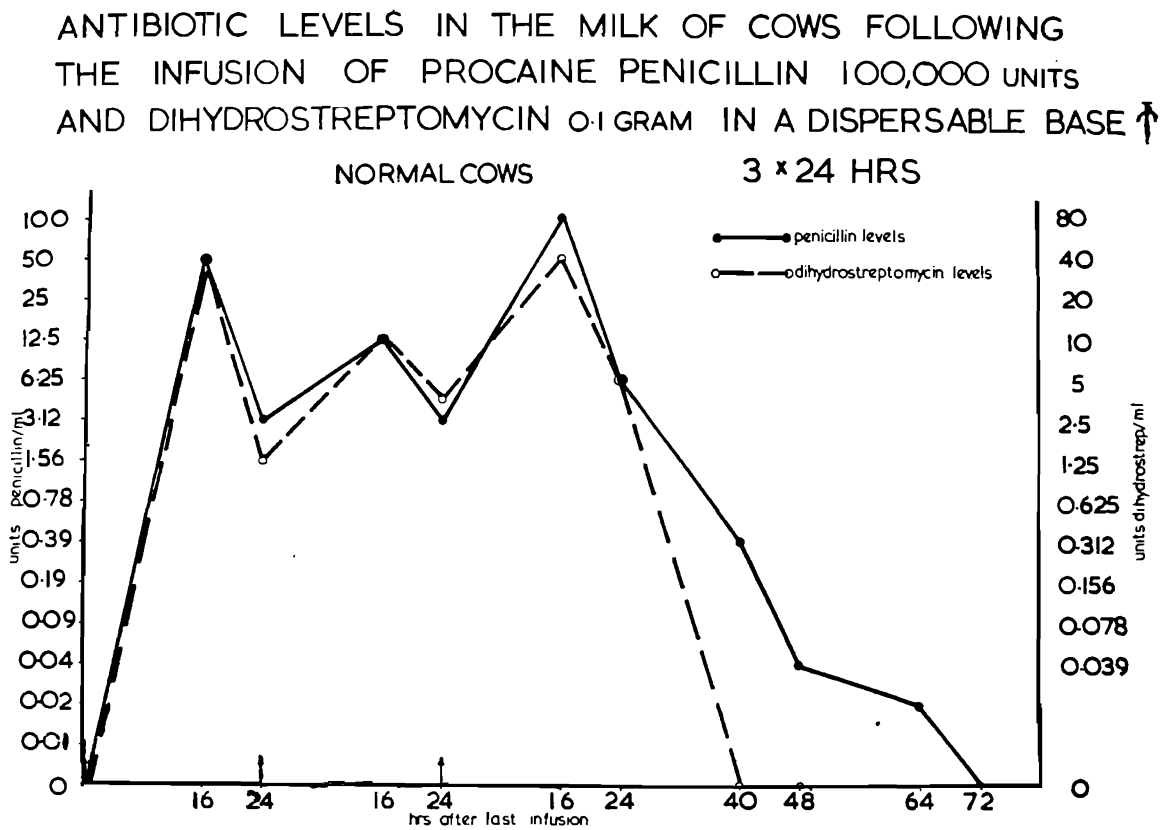
In the development of intramammary preparations it is essential to know a great deal about the antibiotic i.e. its action,—bactericidal or bacteriostatic; its spectrum of activity against the common pathogens of mastitis; the minimum inhibitory concentra-

FIGURE I. (Uvarov O.[22])



*(Fig. 1)—Streptopen Veterinary Cerate, Glaxo Labs. Ltd., Greenford.

FIGURE 2



†(Fig. 2)—Streptopen Q.R. Veterinary Cerate, Glaxo Labs. Ltd., Greenford.

tion required for different organisms, and the sensitivity or resistance to the antibiotic in the cattle population of the country. It is also necessary to study bases or vehicles for incorporating autibiotics for intramammary use. The author's studies have been carried out with bactericidal antibiotics in different bases [21-23]. The bases studied included vegetable and mineral oils, and aqueous suspension with or without wetting agents. Considerable care is required to control the production conditions to obtain a uniform release rate of antibiotics. The physical properties of such bases are also subjected to extensive investigation. Studies on viscosity are included to obtain physically stable preparations suitable for administration even when stored at different temperatures. The excretion of an antibiotic from the udder is chiefly governed by the base, the milk yield of the cow, antibiotic characteristic, and state of the disease. It has been possible to evolve three types of bases allowing long action (L.A.), medium retention (M.R.) or quick releasing (Q.R.) bases. One of the major advances in the development of a long acting preparation (L.A.) was the development of a mineral oil base with 3% aluminium monosterate [22]. This base has enabled certain antibiotics now to be used in the dry period treatment. The retention of antibiotics has already been studied in lactating and dry cows. Examples of some of these bases are shown in Figures 1, 2, 3 and 4.

It is possible to increase the persistence of penicillin, in certain bases, by increasing its dose. Yet with other antibiotics, i.e. streptomycin, this persistence is not achieved, see Figures 5 and 6. Having established the pharmaceutical properties of intramammary preparations, the number of treatments and the clinical efficacy of these substances are

then evolved through field trials. During the course of this work, standards have been evolved for evaluation of drugs in the udder —see Table 1.

There are many different preparations containing large volumes of base. The author has mostly used tubes containing a 3.3 g-volume of base, since it has been established that diffusion in the udder of this amount is good and the concentration of antibiotic is not influenced by the volume of the base provided there is no obtsruction, i.e. chronic fibrosis or induration as found in chronic cases of mastitis.

Available Antibiotics—In several countries there are many antibiotic preparations. The average number excluding the U.S.A. appears to be in the region of 30 to 50. Many

FIGURE 3 (Uvarov O.[22])

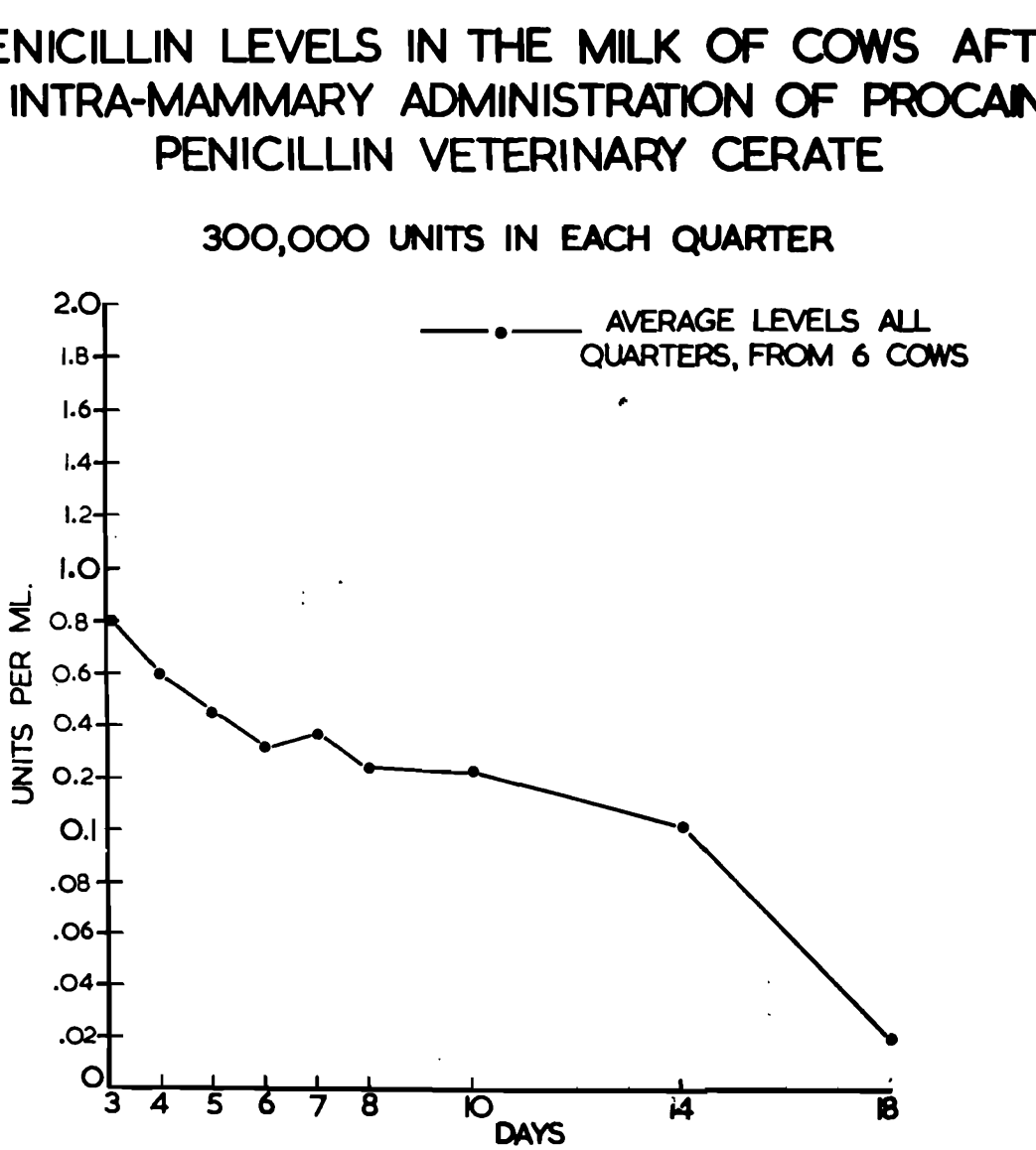
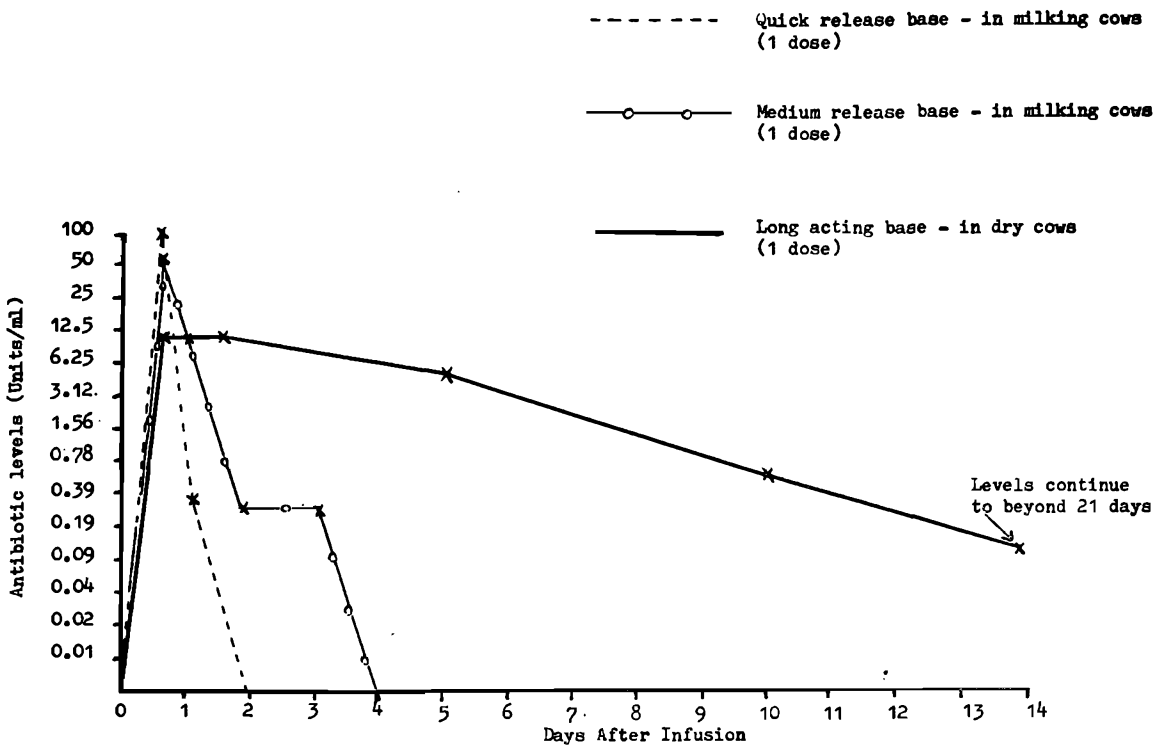


FIGURE 4

REPRESENTATION OF ANTIBIOTIC LEVELS IN THE MILK AFTER THE INFUSION OF DIFFERENT CERATE PREPARATIONS



From: Glaxo Veterinary Research News Letter "The Importance of the base in intramammary Preparations, September 1968.

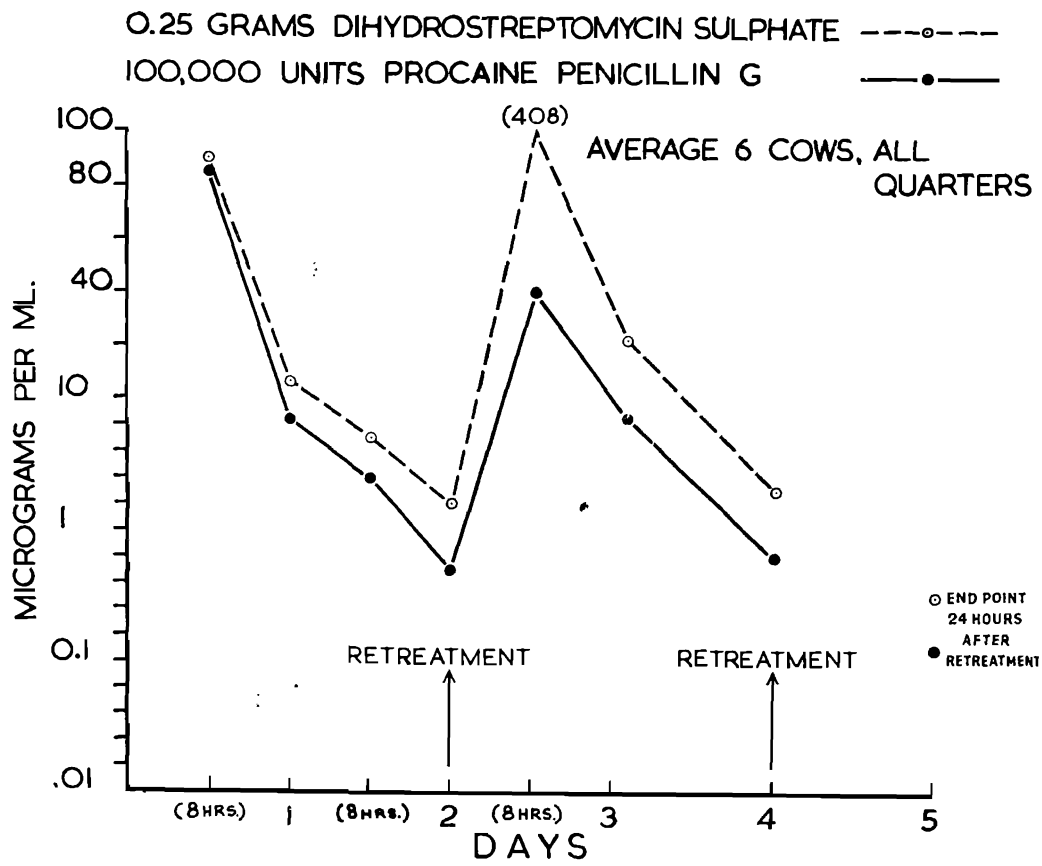
are prescribed in combination with other antibiotics, suphonamides and/or nitrofurazone, as well as other additives including cobalt, corticosteroids and enzymes. The scientific reasons for the inclusion of the last three preparations are not substantiated. The inclusion of corticosteroids is at times justified on the grounds that these substances can remove an alleged chemical irritancy of the formula itself, or could be of use in suppressing the inflammatory response in the udder. Wilson [19] did not produce any different results when using a penicillin plus streptomycin preparation with and without hydrocortisone. Keller and Boller [24] found that leucocyte count was not affected when prednisone was used. Swarbrick [25] found that corticosteroids were not of value in intramammary preparations. At a time when extraneous substances in milk are unacceptable to the dairy industry and the medical authorities, and the treatment of mastitis is already complicated by other factors, the use of corticosteroids in mastitis should be reserved only as parenteral therapy and should be accompanied by appropriate antibiotic coverage.

(3) THE TREATMENT OF MASTITIS

One of the main objectives must be early treatment initiated as soon as symptoms of the disease become apparent. Ideally, milk samples for bacteriological examination

FIGURE 5 (Uvarov O.[22])

ANTIBIOTIC LEVELS IN THE MILK OF COWS AFTER INTRA-MAMMARY INFUSION OF STREPTOPEN VETERINARY CERATE



should be taken, but treatment must not be delayed whilst waiting for the laboratory results. Thus the initial treatment can be regarded as being in the nature of first aid and may tend to be empiric. Alternatively, if the veterinarian in charge of the herd is able to take samples of bulk milk from the herd from time to time, a general indication on the most prevalent organisms in the herd will be known. It is necessary to carry out the prescribed course of treatment and to supervise general management factors. In countries where antibiotics are freely available to farmers, veterinarians have less opportunity to exercise professional control over the disease to the detriment of the animal and the farmer. Treatment can be:—

A. *During Lactation* — a. Intramammary; b. Parenteral.

B. *During the Dry Period* — Intramammary preparation infused immediately after last milking.

A. *During Lactation*

a. *Intramammary Treatment* — Antibiotics administered via the teat canal diffuse rapidly to the top of the udder provided there is no obstruction or blockage by inflammatory debris. as can occur in acute cases of the disease; some of the antibiotic is absorbed from the udder into the blood stream — see Figure 7.

Streptococci — These organisms are always sensitive to penicillin; as this is a bactericidal antibiotic it has remained the first choice for the control of this group of organisms. In particular, *Streptococcus agalactiae* is amenable to eradication and this had been previously achieved.

Staphylococci — If these are resistant to penicillin, combined antibiotics such as penicillin and streptomycin, penicillin and novobocin, rovamycin, cloxacillin and the broad spectrum antibiotics are all used. Prognosis in the treatment is uncertain in part due to the ubiquitous nature of the pathogen, high risk of reinfection and its progressive pathology producing localisation and fibrosis.

Gram Negative Organisms — Streptomycin, either locally or parenterally and broad spectrum antibiotics and their various mixtures are used. Streptomycin appears to produce the best results.

Sub-clinical Mastitis—Ideally this should be a blitz herd treatment, provided the

FIGURE 6. (Uvarov O.[22])

STREPTOMYCIN LEVELS IN THE MILK OF COWS AFTER INTRA-MAMMARY INFUSION OF 0.5 GRAMS DIHYDROSTREPTOMYCIN CERATE

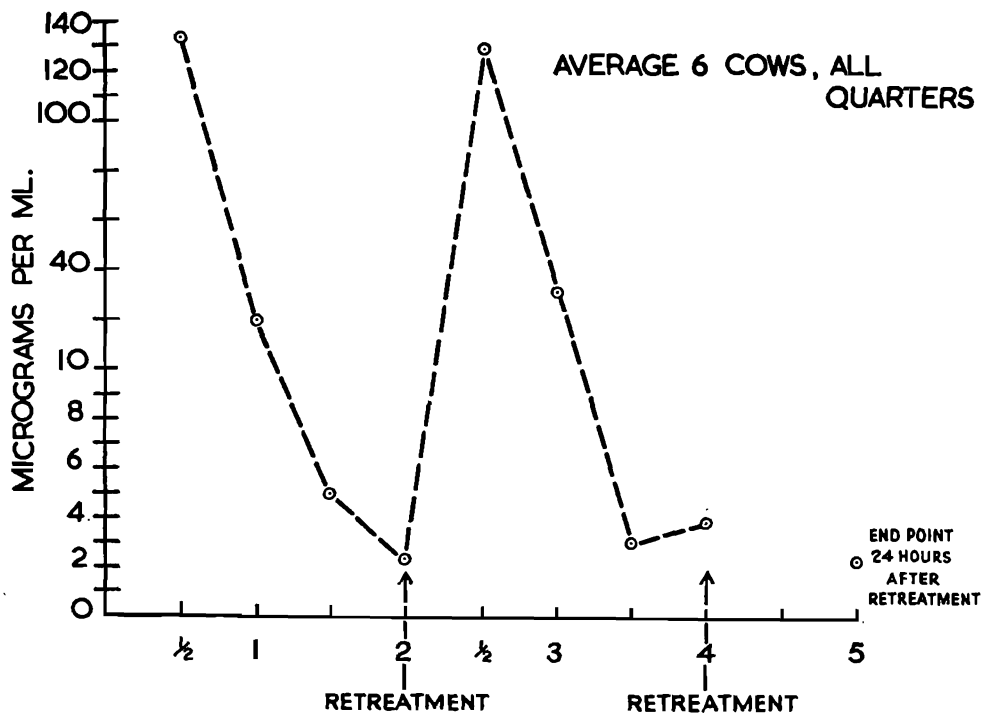
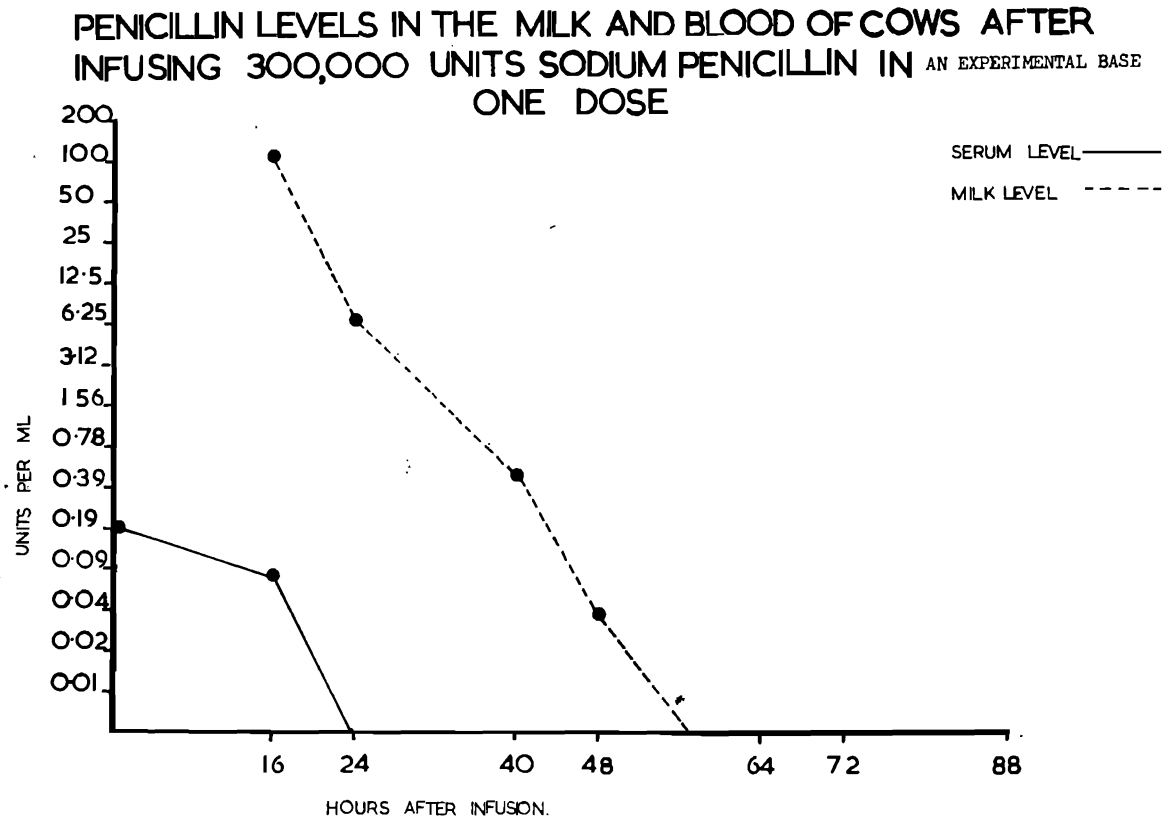


FIGURE 7



economies of this method are acceptable. In sub-clinical mastitis there may not be obvious symptoms of the disease, but the occurrence of a number of clinical cases of mastitis indicates that a herd problem exists. Diagnosis is established if the bulk milk contains a cell count of over 500.000/ml and is accompanied by heavy growth of pathogenic bacteria. Ideally, all animals are then sampled individually for bacterialogical examination, treated, and re-sampled after an appropriate interval. Re-treatment is carried out if necessary and chronic cases are ultimately culled. Such rigorous treatment is mainly feasible on an experimental trial basis, as farmers are often unwilling to undertake mass investigations and treatment.

b. *Parenteral Treatment*—Several antibiotics and some sulphonamides when injected in large doses are excreted in milk. This type of therapy is specially indicated in acute cases when congestion, oedema or blockage by inflammatory debris restricts diffusion from intramammary therapy. The excretion

Table 1: EVALUATION OF DRUGS IN THE UDDER

Tests include:

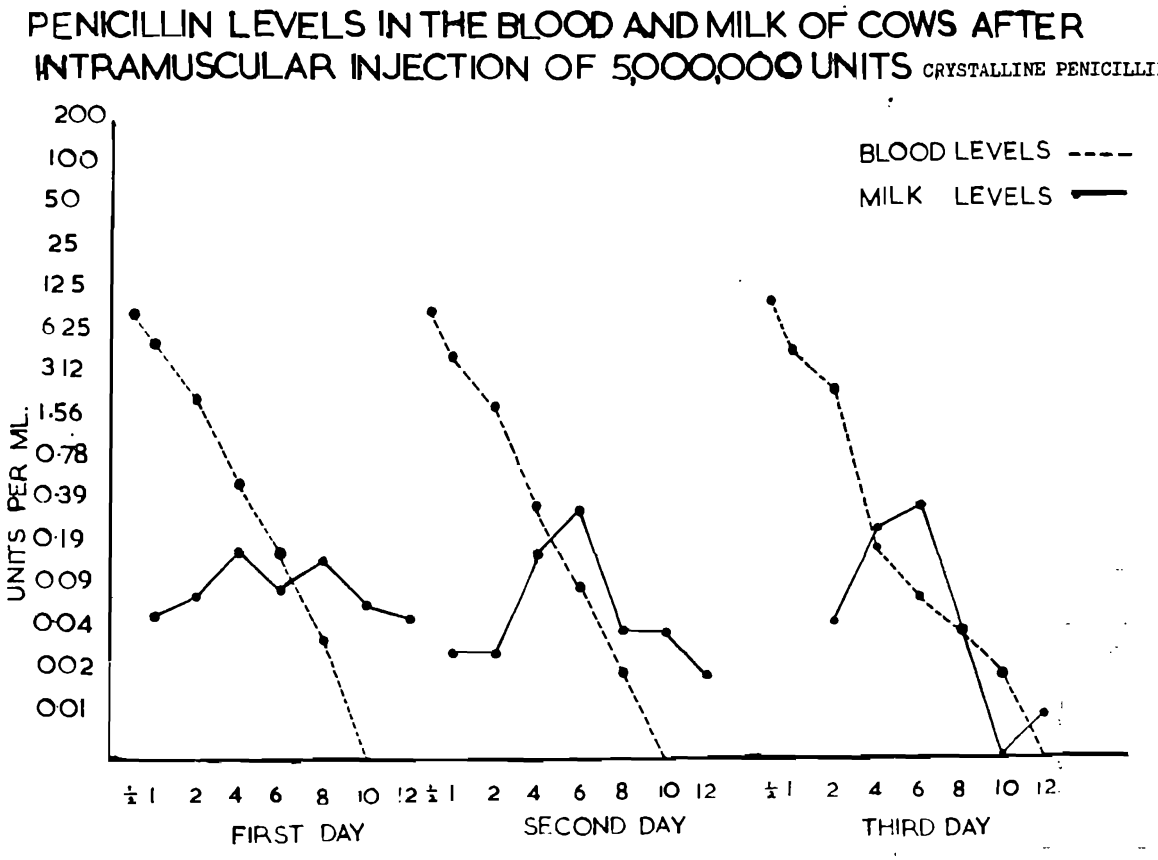
(1) Acceptability in normal udders: This is judged by comparing the total cell count of milk, taking samples 2 days before treatment and throughout treatment.
(2) Excretion of antibiotic from normal udders.
(3) Acceptability in mastitis udders.
(4) Excretion of antibiotics from mastitis udders.
(5) Therapeutic efficacy, using a standard treatment as a control. Clinical and bacteriological results are considered necessary in these tests.
(6) Milk samples from treated cows must also be free of infection from at least 21 days after treatment.

Table 2: MEAN CONCENTRATION OF ANTIBIOTIC IN BLOOD AND MILK OF COWS AFTER INTRA-MUSCULAR INJECTION OF A DOSE OF 11 MG/KG

Antibiotic	Daily milk yield (lbs)	Sample tested	Concentration (ug./ml) in sample at intervals (hours)									
			1	2	3	4	6	8	12	24	36	48
Penicillin	13—29	blood	3.84	1.92	0.96	.48	.06	0	0	0	—	—
		milk	0	.03	.06	.12	.12	.12	.06	0	—	—
Streptomycin	13—29	blood	—	20.48	—	20.48	10.24	2.56	0.64	0.08	0	0
		milk	—	0	—	0.16	2.56	2.56	2.56	0.32	0	0
Aureomycin	20	blood	—	0.4	—	0.4	0.2	0.2	0.2	0.2	0.2	0.2
		milk	—	0.2	—	0.4	0.4	0.8	1.6	0.8	0.8	0.4
	3	blood	—	0.4	—	0.4	0.4	0.2	0.1	0.1	0.1	0.05
		milk	—	3.2	—	3.2	3.2	6.4	6.4	6.4	3.2	3.2

By: S. J. Edwards and Mary D. Haskins [26].

FIGURE 8



of antibiotics from this route was illustrated by Edwards and Haskins [25] — see Table II — and by Uvarov (Fig. 8). Streptomycin is particularly effective for this therapy.

B. *Treatment of the Dry Cow*

Pearson [27] introduced this form of therapy for the control of dry cow mastitis, mostly associated with *C. pyogenes* and other infections. This treatment consisted of infusing penicillin into cows during the dry period; the antibiotic was retained throughout the dry period if more than one treatment was used. The development of the mineral oil plus 3% aluminium monosterate base [21, 22] with 300 mg of procaine penicillin incorporated (see Table III), resulted in a preparation which is widely used in dry cows [27-30]. More recently, control of other infections is also being carried out. The aims of dry cow treatment are:—

a. to cure existing infections;

b. to prevent new infections;

c. to save milk by avoiding treatment during lactation.

A number of drugs have been, or are being, evaluated, i.e. penicillin, penicillin plus novobiocin, penicillin plus streptomycn, cloxacillin and rovamycin. Broad spectrum antibiotics appear to be unsuitable for this purpose. Most preparations eliminate approximately 90% of existing streptococcal

Table 3: PENICILLIN RETENTION IN THE DRY COW AFTER INFUSION OF PROCAINE PENICILLIN
Procaine penicillin—300,000 units

1959

Herd	Cow No.	Dry Period (days)	Concentration I.U./ml 21 days (Average 4 Qtrs.)		Interval to Calving (days)	Concentration I.U./ml Post-Calving (Average 4 Qtrs.)
A	1	6	0.40	RE-INFUSION	126	nil
	2	30	0.05		118	
	3	44	0.12		82	
	4	52	0.63		100	
	5	69	1.35		13	0.04
	6	72	0.93		90	nil
	7	101	1.30		18	0.01
	8	133	—		—	0.04
	9	150	0.13		30	nil
B	1	24	0.11		—	—
	2	37	0.24		123	nil
	3	72	0.43		—	—
	4	77	0.05		134	nil
	5	84	4.76		61	0.05
	6	93	0.64		73	nil
	7	105	0.03		114	|
	8	111	0.43		36	
	9	122	0.19		39	
	10	133	0.18		109	

No. of Animals	Group	Dried Off	Sampled	Average Penicillin Concentration I.U./ml. 21 days
1	1	March		0.13
4	2	April	All	0.21
4	3	May	Cows	1.68
5	4	June	sampled	0.68
4	5	July	18.8.59	0.13
1	6	August		0.40

infection, and the percentage control of staphylococcal infections is in the region of 70% to 80%. Attention to hygiene, the method of drying off, and teat dipping with a suitable disinfectant, all play a part in this treatment [31-35]. This method, whilst it is fairly widely used at present, is not without criticism. From the farmer's point of view, infected, uninfected and at times chronic quarters are indiscriminately treated, in the hope that mastitis will be reduced at the subsequent lactation. The treatment of uninfected quarters leads to unnecessary expense and cows with chronic fibrosed quarters should be culled.

(4) RECOMMENDATIONS

Certain constant reminders are necessary when dealing with mastitis:—

A. The disease is a herd problem, not of the individual cow;

B. Management, including hygiene, is very important;

C. Treatment should be started as early as possible. It should be aimed at removing the cause and not the symptom of the disease;

D. Differential diagnosis on a herd basis should be aimed for;

E. A programme of mastitis control should consist of:—

a. diagnosis

b. treatment

c. culling of chronic cases

d. education of the farmer

F. Intramammary treatment using correctly evaluated preparations is suitable for many cases of mastitis;

G. Parenteral treatment should be considered for acute cases of the disease and for those caused by gram negative organisms;

H. Dry cow therapy must be assessed in individual herds, remembering the economics of such treatment;

I. Sub-optimal treatment should be avoided if the correct course of treatment is not carried out;

J. A mastitis control policy should be devised which is suitable for each country, depending on availability of trained personnel to implement such methods.

Many methods and drugs are now available for the control of mastitis, and yet it remains one of the most devastating diseases of the dairy industry. This suggests that other avenues of research are yet to be explored.

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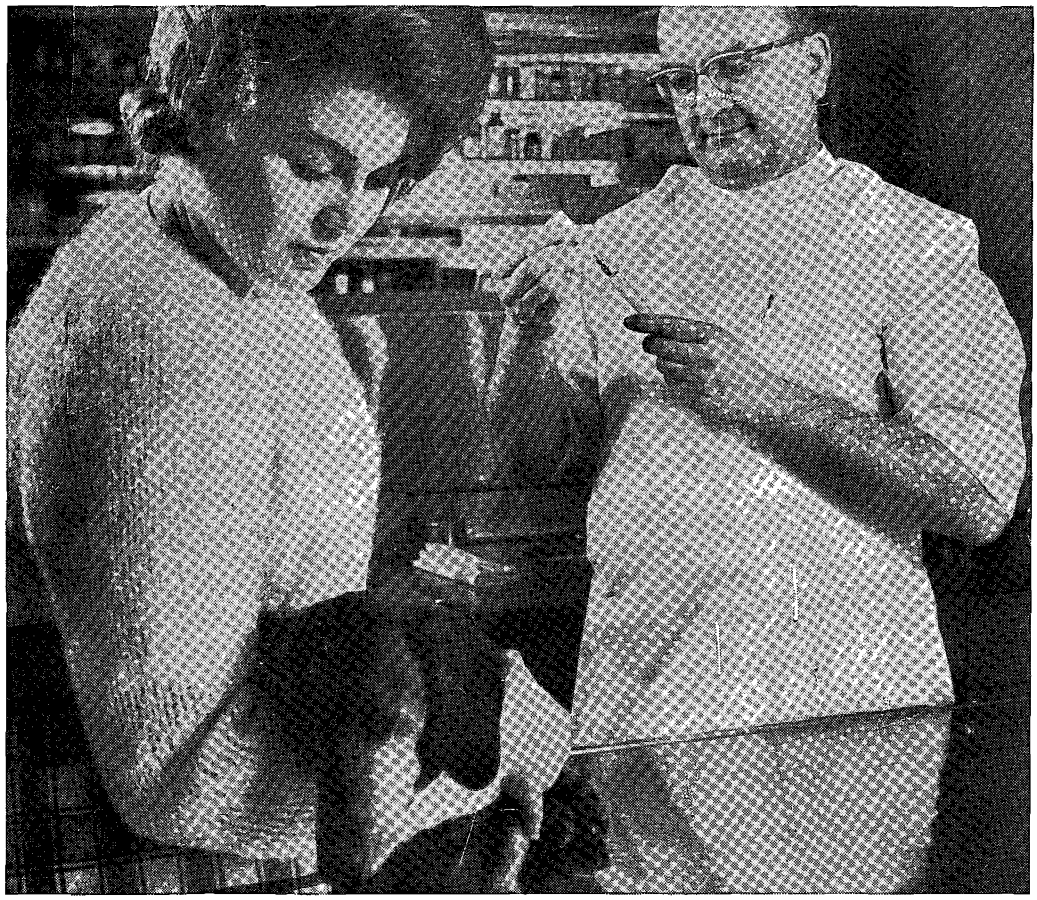
from a single injection—intrasynovial or intramuscular



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Jl S. Afr. vet. med. Ass. 40 (2): 153-158 1969

THE USE OF ANTIBIOTICS IN THE CONTROL OF MASTITIS*

G. C. BRANDER**

If we accept that subclinical mastitis is the major problem, with spasmodic clinical cases acting as indicators of latent infection within a herd, than our aim should be to use antibiotics to reduce the overall incidence of "mastitis-causing" bacteria within a herd.

It has been possible in co-operation with the National Institute for Research in Dairying (Reading) to record the organisms found at regular herd sampling, those found in clinical cases, and those present at drying off and calving. These examinations have shown that staphylococci and streptococci predominate, and that other organisms such as *E. coli* and *Pseydomonas* tend mainly to be found in clinical cases, and under special conditions. I consider that *E. coli* and *Pseudomonas* tend to establish themselves in tissues which have previously been colonised and damaged by other organisms. This phenomenon is recognised in ear and kidney infections both in man and animals.

In our experience, an antibiotic which is bactericidal and highly active against gram positive organisms has proved to be effective when used for treatment both during the dry period and in lactation.

Dry period therapy. Therapy during the dry period has been recorded in detail (Smith *et al.*[1,2]) and it is clear that as we gain more experience of this form of treatment the best results are obtained with an antibiotic which will persist in the quarters for at least three weeks, and that the most successful results are obtained against relatively new infections.

Lactation therapy. It has been possible, as a result of large scale trials carried out in co-operation with the National Institute for Research in Dairying to record the effect of therapy for a period of over 1½ years during lactation in approximately 30 herds, and these results, which have not yet been published, indicate that a long acting formulation of cloxacillin† will give about a 60% cure of new staphylococcal infections, but that the effectiveness tends to fall in quarters with long established infections[3].

Streptococcal infections on the other hand, respond well to treatment even in relatively well established cases. The results of our trials indicate that to be effective, therapy must be related to the cell count history of infected herds. Treatment is most effective when applied immediately infection is recognised, so that it is important to develop an agreed system of supply of antibiotics between the farmer and the veterinary surgeon.

FORMULATIONS

The use of antibiotics for the control of mastitis dates back to the late 1940's when penicillin was first examined. The initial formulations were aqueous solutions, and they proved particularly effective against streptococcal infections. Aqueous solutions only provided significant levels of antibiotic in the udder for a short time, so that it was decided to develop a cream formulation which would provide a stable material, and which could be prepared in individual metal tubes for quarter therapy. This method would avoid the danger of cross infection from syringe treatment, and provided a sterile pack.

The initial formulations were based on oil of arachis, but to deal with staphylococcal infections and persistent streptococcal cases, more long acting formulations of antibiotics were prepared based on mineral oil plus aluminium monosterate. When it was decided to re-investigate the value of dry cow

*Paper read at Veterinary Research Institute, Onderstepoort.
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†Orbenin, Beecham Research Laboratories.

therapy, the work of Funke[4] indicated that dry cow treatment could well provide a high degree of success.

Funke[4], in a study involving the use of S35 labelled benzyl penicillin in lactating and dry cows, found that there was a somewhat better distribution of antibiotic in the interlobular interstitium in the dry mammary gland. He also found that penicillin is distributed throughout the whole gland, whether introduced in an aqueous or oil base, as the penicillin once in the gland leaves the oil phase and passes into the secretions. Oil drops serve as depots from which the penicillin is gradually liberated.

This work of Funke's could well be extended in the dry cow so that we could discover more about the long term behaviour of an antibiotic in the inactive udder, and thus be able to formulate material which could achieve the ideal of long activity, associated with quick release when milking starts.

By milk sampling aqueous, quick release and long acting formulations in the dry cow we have found it possible to predict how long detectable levels of an antibiotic will remain in the average treated gland, but we do not know exactly in what forms and where it is retained within the gland.

Smith *et al.*[1] showed that using benzathine cloxacillin in a long acting formulation which persisted for three weeks in the udder, it was possible to obtain approximately 80% control of staphylococcal infections, whereas sodium cloxacillin, a more soluble salt which was present in the udder for three days, only gave 60% control of infection. This work was repeated using 500 mg benzathine cloxacillin in both quick release and long acting formulations, and it was shown quite clearly that persistence in the udder of an antibiotic was more important than the concentration of antibiotic used as far as staphylococcal infections were concerned. This finding is consistent with similar results following lactation therapy.

The dilemma of the formulation, therefore, is that if he is to provide a formula which will be of real bacteriological value, it must persist both in the lactating and dry udder. Public health authorities, however, require formulations which will have the minimum persistence so that the milk does not contain antibiotic when sold to the public.

It is important, therefore, that treatment when carried out should be the most effective possible, and carried out at the most favourable time, both from the point of view of the farmer and the public health authorities.

RESULTS OF THERAPY

(a) Lactation therapy

In a trial in which subclinical infections were treated, the results using three doses of 0.2 gm sodium cloxacillin in a slow release given at intervals of 48 hours were as follows:

	Treated	Cured	% cure
Haemolytic staphylococci	61	34	56
Strep. agalactiae	11	11	100
Strep. dysgalactiae	6	6	100
Strep. uberis	14	10	71

Three doses of 0.2 gm sodium cloxacillin in a quick release base given at intervals of 48 hours gave the following results:

	Treated	Cured	% cure
Haemolytic staphylococci	71	29	41
Strep. agalactiae	8	8	100
Strep. dysgalactiae	8	7	87
Strep. uberis	8	6	75

These results are repeatable so that it will be seen that a long acting base with its greater persistence gives a better control of subclinical staphylococcal infection. A cure indicates that no organisms were recovered from milk samples taken from treated quarters up to 21 and 28 days after treatment.

In a treatment trial of clinical cases of mastitis recorded during lactation in 14 herds, the following results were obtained:—

Trial	Antibiotic dose	No. of doses	Time between doses (hours)	Base	No. of cases treated	Pathogen eliminated per cent
1	100 000 units penicillin 100 000 units streptomycin	3	48	Slow release	133	37
	100 000 units penicillin 350 000 units streptomycin	3	48	Quick release	100	26
2	Orbenin 0.375 g	3	48	Slow release	51	63
	Orbenin 0.375 g	3	48	Quick release	95	23

Treatment	Pathogen	Present at drying off	Same infection at calving	Percentage original infection eliminated
0.5 g	Haemolytic Staph.	185	39	78
	Str. agalactiae	18	0	95
	Str. dysgalactiae	28	1	
	Str. uberis	58	4	
	Other	9	1	
1.0 g	Haemolytic Staph.	136	39	74
	Str. agalactiae	29	0	97
	Str. dysgalactiae	34	0	
	Str. uberis	46	3	
	Other	18	0	

Dry Cow Therapy

The result of a large scale field trial were reported by Kingwill *et al.*[5] where 0.5 gm benzathine cloxacillin in a slow release base was compared with 1 gm benzathine cloxacillin in a slow release base:—

A comparison was made between 0.5 g benzathine cloxacillin used in a variety of ways, Smith *et al.*[6].

Treatment 1. 0.5 g cloxacillin as benzathine salt in quick release base infused at drying off.

Treatment 2. 0.5 g cloxacillin as benzathine salt in long acting base infused at drying off.

Treatment 3. 0.5 g cloxacillin as benzathine salt in quick release base + 0.5 g in long acting base both infused at drying off.

Treatment 4. 0.5 g cloxacillin in benzathine salt in long acting base infused at drying off + 0.5 g benzathine salt in long acting base infused three weeks later.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
No. of cows calved	102	104	97	95
% infected at calving	34	15	14	6
% quarters infected at calving	11	4	5	2
Staphylococcal infections treated	55	53	51	49
% eliminated	47	82	82	90

This result confirmed that a long acting formulation had significant advantages over a quick release preparation, even when such a relatively insoluble salt as benzathine cloxacillin was used.

DISCUSSION

In my introduction, I suggested that subclinical mastitis was the major problem in any mastitis control programme. Workers at the N.I.R.D. have shown that a system of hygiene can reduce the spread of new infections by 50%. But this benefit can be enhanced if we reduce the existing infection—usually carried by 50% of the cows in a herd, by the use of therapy during the dry period.

The advantages of applying therapy at the end of lactation are many, but one very important one is that instead of half the cows starting a new lactation with udder infection in one or more quarters, 80—90% of them will have a much better chance of being free of udder disease at this critical period.

The application of an efficient hygiene system, plus dry cow therapy, should lead to a progressive reduction of infection, and reduce the necessity for continual lactation therapy.

Further work is still necessary, particularly in the formulation of antibiotics for lactation therapy, so that it may be possible to evolve a formulation with a relatively low level of antibiotic, but with more penetration, so that the antibiotic can reach the main centres of infection. At present the levels of antibiotic reached in the milk are well above the minimum inhibitory concentration required for successful activity, yet the success in therapy is still relatively low against established infections.

CONCLUSIONS

Subclinical mastitis is the major problem

in mastitis infections, and field investigations involving both antibiotic therapy and hygiene have shown that a marked reduction in mastitis within a herd can be achieved by the use of hygiene measures and treatment of all cows during the dry period.

It must be emphasised that the problem will vary from herd to herd, and that the success of any measures is dependent on full co-operation by the milking staff.

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BOOK REVIEW

REPAIR OF THE RUPTURED CRANIAL CRUCIATE LIGAMENT IN THE DOG

ANDERS STRANDE*

The Williams and Wilkins Company, Baltimore, 1967. Pp 143, Figs. about fifty. Price not stated.

It is now just over 40 years since the clinical picture of rupture of the cranial cruciate ligament in the dog was first described. Little progress in the treatment was made up to 1952, since when different operative methods followed each other at an accelerated speed. As a variety of materials has been used to reconstruct the ligament (fascia lata, tendon, skin, plastics) the time has arrived for a critical review and investigation of the divergent techniques and materials. This monograph was written in an attempt to fill this gap.

After a historical survey and summary of earlier experimental surgery on the stifle, a detailed anatomy of the stifle is presented. Strande then describes his experimental investigations followed by a report on clinical cases. The text is concluded with a general discussion and conclusions.

The author concludes that trauma is the main cause of rupture of the cranial cruciate ligament, followed by osteo-arthritis. The mean age of cases was 4.6 years and the very suggestive finding is reported that in about one third of cases the same ligament in the other stifle eventually ruptured.

Very good results were obtained in dogs where the tendon of *m. peroneus longus* was used as transplant. This was followed by cialit-preserved tendon, skin and taflon braided tape in that order. After meniscectomy the results were much less satisfactory.

Under general discussions and conclusions interesting post-operative histopathological studies are reported. Detailed information in tabular form is presented in 21 tables. The plates, several of which are in colour, are clear and informative.

This book is recommended for the surgeon, experimental surgeon and practitioner practising advanced surgery.

C. F. B. H.

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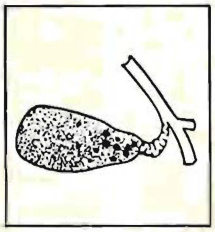

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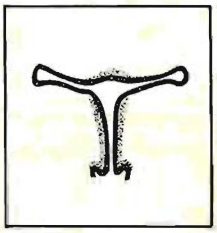

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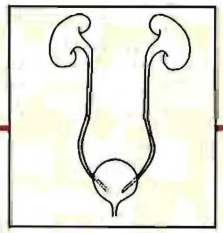

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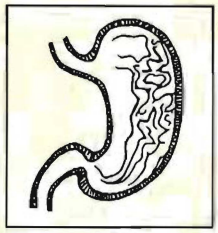

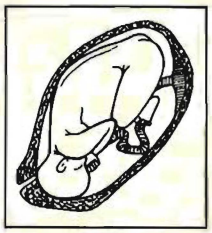

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STUDIES ON THE EFFECTS OF THE INTRARUMINAL ADMINISTRATION OF SULPHADIMIDINE TO ADULT SHEEP*

W. L. JENKINS**

SUMMARY

The effects of the intraruminal administration of sulphadimidine to adult sheep maintained on high and low nutritional planes were studied under three sets of experimental conditions. In general the severity of the effects observed was a function of the concentration of sulphadimidine attained in the rumen. Temporary inappetance and reduction of water intake, transient depression of ruminal motility, diminished glucose fermentation rate, cessation of cellulolytic activity and transient reduction of volatile fatty acid concentration within the ruminal fluid, were the most notable features recorded. However, spontaneous recovery occurred in most cases within variable time intervals.

Differences between the blood concentration curves of sulphadimidine in the two nutritional groups were demonstrated. Furthermore when ruminal motility was reduced accumulation of the sulpha-compound within the rumen was observed and markedly decreased blood levels were recorded.

INTRODUCTION

In the Republic of South Africa the age limit of domesticated ruminants to which any antimicrobial compound may be administered orally for therapeutic purposes is generally regarded to be about three months. However in most other countries it is an accepted procedure in the practice of clinical veterinary medicine to dose sulphonamide preparations orally to ruminants of all ages [1-6]. Nevertheless a review of previous studies on the effects of sulphonamides on rumen microfloral activity and ruminal function revealed an apparent paucity of conclusive evidence as to how serious any detrimental interference with the normal rumen milieu might be, especially under non-physiological conditions. This impression was supported by Warner [7] who noted that only a few of the drugs commonly administered orally to ruminants have been examined for their effects on the rumen microbiota.

Oyaert *et al* [8] demonstrated that therapeutic doses of sulphanilamide depress cellulose digestion and appetite in sheep. Furthermore the fermentation of sugar was also suppressed but only by higher concentrations of the drug. Subsequently Gilchrist & Clark [9] observed a depression of the total volatile fatty acid concentration and a fall of the propionic/butyric acid ratio in the rumen liquor of a sheep which had received sulphadimidine orally for five consecutive days. In addition this animal developed a marked ketonaemia. These authors suggested that although the recovery of flora after dosing sulphonamides is rapid, provided the animal has a normal flora initially, in the case of sick animals, where the flora is already affected by diminished intake, serious disturbances may be produced. The importance of such considerations was well illustrated by Procos & Gilchrist [10] who, during the experimental induction of ovine ketosis, found the treatment most conductive to clinical signs was one in which the original green lucern diet was substituted by poor hay with simultaneous dosing of sulphadimidine. It is

*Based on a dissertation submitted to the Faculty of Veterinary Science, University of Pretoria, in partial fulfilment of the requirements for the degree of M.Med. Vet. (Pharmacology and Toxicology).

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noteworthy, however, that in this study the dosing of sulphadimidine without any alteration of diet was ineffective in precipitating ketosis.

The effect of three sulphonamides, including sulphadimidine, on the activity of rumen flora in dairy cows was studied by Egetoft & Rasmussen[11] and although the condition of the animals was not affected by these treatments, the cellulolytic activity of the rumen flora was reduced by all three sulphonamides but spontaneously returned to normal levels two to three days after cessation of the treatment. The glycolytic activity and pH of the rumen fluid was unchanged in these studies. Nesic & Ibrovic[12] dosed sulphaguanidine and sulphadimidine per os at 0.1 gm per kilogram body weight to fully grown ruminants for five days without apparently altering digestion in the rumen.

Following an investigation on the effect of sulphonamides on the composition of the long chain fatty acids in rumen fluid of cattle, Viviani, Gentile & Di Michele[13] concluded that the sulphonamides appeared to block a fundamental metabolic sequence either for the branched chain fatty acids' biosynthesis or for the utilization of cellulose.

An additional fundamental aspect of great importance in the oral use of sulphonamides and other compounds in ruminants is the question of absorption and the consequent blood concentrations which are obtained. Many features peculiar to the ruminant in this regard have been reviewed by Dobson[14].

Oyaert *et al*[8] recorded the slow absorption of sulphanilamide following intraruminal administration and in addition demonstrated that absorption is even further retarded by paralysis of the rumen induced by atropine. These findings posed the query as to be advisability of dosing sulphonamides indiscriminately to ruminant animals particularly if any tendency towards ruminal stasis existed. This consideration was further supported by Clask & Wessels[15] who emphasized that the basic diet and especially starvation had a profound influence on the concentration curve of sulphanilamide in the blood of sheep. Nesic & Ibrovic[12] also suggested that care should be taken when dealing with an atonic rumen as sulphonamide may accumulate.

The biochemical mode of action of the sulphonamide group of drugs is identical[16], but their absorption, distribution and excretion patterns may vary considerably depending primarily on individual pKa values[17].

In an endeavour to elucidate some of the problems which seemed to exist regarding the oral use of sulphonamides in ruminants, studies were conducted using sheep on two different dietary regimens. Three major experimental approaches were adopted in an effort to simulate practical sitations as they might occur in every day farm-yard therapeutics. A single popular representative of the sulphonamide group of drugs, viz., sulphadimidine, was selected for these investigations.

MATERIALS AND METHODS

Four Merino wethers, aged four to six years, with permanent ruminal fistulae were utilized for the major investigations on the administrations of the standard 0.5 gm tablet form of sulphadimidine B.P. 2-(aminobenzenesulphonamide) 4 : 6-dimethylpyrimidine.

Based on the plane of nutrition two groups of experimental animals were established, viz.,

(a) A "good ration" group which received lucern hay *ad libitum* plus 200 g crushed maize per sheep daily. These sheep will be referred to as Group A.

(b) A "poor ration" group which received teff hay *ad libitum*. These sheep will be referred to as Group B.

Each set of sheep were kept on their respective diets for six weeks before the following investigations were initiated:

Physiological Investigations

Body weight: The body weight of each experimental sheep was recorded weekly under standard conditions.

Feed intake: The daily food consumption of the two sheep in an experiment was measured; all sheep were fed daily at 9 a.m. and the feed remaining was removed at 4 p.m.

Water intake: The daily water intake was recorded after correction for evaporation loss.

Ruminal motility: A connection between the free gas space in the rumen and a water manometer was established. Using this system the number and pressures of the primary mixing movements during a ten minute period were noted. The product of the number of contractions per minute (expressed

to the nearest tenth) and the mean of the recorded pressures was used as an index of functional rumen motility. When it was found necessary a membrane tambour was incorporated into the system and tracings were made using a kymograph and smoked drum [18].

Rumen Fluid Investigations

Ruminal fluid: Representative samples of ruminal fluid were collected before feeding by aspirating ruminal ingesta from different levels and areas of the rumen. The rumen liquor was strained through a double layer of muslin and the following determinations were then carried out:

Glucose fermentation raise: The method of Quin as modified by Grosskopf & Briel [19] was employed to measure the rate of gas evolution following the addition of glucose to ruminal fluid.

Total volatile fatty acid concentration: The method of Schambye [20] was used to establish the concentration of the total volatile fatty acids (v.f.a.) present.

Ammonia nitrogen concentration: The aeration method of van Slyke & Cullen as modified in Hawk. Oser & Summerson [21] was utilized for the determination of the concentration of ammonia nitrogen. Aeration of a sample was commenced within a minute of collection.

Lactic acid concentration: After removal of soluble carbohydrates and protein by precipitation with copper-lime [22] lactic acid was determined by the method of Barker & Summerson as described in Hawk, Oser & Summerson [21].

pH: The pH of the strained ruminal fluid was measured immediately after collection at 39°C using a Radiometer pH meter PHM27 fitted with a glass (G202C) and calomel (K401) electrode.

Eh: The Eh value was established following pH measurement by using a platinum (P1011) electrode and the calomel electrode as the reference electrode. At 39°C the calculation was thus Eh=E+234 mV. On each occasion the platinum electrode was cleaned by electrolysis immediately before use.

Rate of cellulose digestion: As a parameter of cellulolytic activity of the ruminal flora the cotton thread digestion technique of Grosskopf [23] was utilized. The cotton strands were suspended in the rumen for 21, 24, 26 and 30 hours and their loss in weight was determined. A graph of the percentage of the original weights lost on each set of four strands plotted against time in hours allowed the time required for a 50% reduction of the original weight to be established. The cellulose digestion index [19] was then calculated by multiplying the reciprocal of the "half life" of the cotton threads in the rumen by 1000. An index figure of greater than 50 represented a very high rate of cellulose digestion while the figure of 10 or lower indicated poor cellulolytic activity. This procedure was repeated at 40 hourly intervals.

Investigational Monitors

Blood and rumen sulphadimidine levels: The method of Bratton & Marshall [24] was used to establish the total sulphadimidine levels in the blood and the concentration of sulphadimidine in the rumen.

Blood glucose: This was determined by the Folin-Wu principle as described in Varley [25].

Blood ketone bodies: The total blood ketone body levels were established using the method of Malan [26].

Blood urea nitrogen: This was determined by the method of Hench & Aldrich [27].

Plasma pH: The instrument utilized was the Radiometer Micro Electrode unit (E 5021) used with the Radiometer pH thermostat (VTS13).

Plasma carbon dioxide content: A Natelson Microgasometer (Model 600) was used and the results recorded as millimoles per litre.

Urine examination: The pH of daily urine specimens was established and a full urine examination was carried out when it was considered necessary. Standard clinical laboratory procedures where employed for this examination.

RESULTS

Group A.

Sheep on high nutritional plane

(Sheep Nos. 9 and 11)

1. Normal Rumen function

The initial experiments were conducted on sheep which were clinically healthy and which were regarded as having normal rumen

Table 1: EFFECTS OF ADMINISTRATION OF SULPHADIMIDINE TO GROUP A SHEEP WITH UNIMPAIRED RUMEN FUNCTION

Day	Food consumption Gm				Water Intake ml		Rumen Motility Index		Glucose Ferment. ml gas/ 15 min.		Cellulose Digestion Index		Total V.F.A. mM/L		Ammon. Nitrogen mg/100 ml		Lactic Acid mg/100 ml		Rumen pH		Rumen Eh –mV	
	9		11		9	11	9	11	9	11	9	11	9	11	9	11	9	11	9	11	9	11
	Lucern	Maize	Lucern	Maize																		
1	800	200	620	200	3180	2800	13.3	9.3	2.1	5.8			76.4	74.2	16.4	19.6	15.0	19.5	7.12	6.92	338	324
2	690	200	750	200	1740	1760	11.5	10.5	2.0	6.6			79.8	77.4	13.9	16.7	11.0	17.0	6.98	6.98	317	308
3	650	200	630	200	5360	3100	12.4	10.3	2.3	7.3	24.2	26.8	85.6	91.9	15.5	21.8	11.7	14.7	6.91	6.88	33.8	341
4	820	200	790	200	4040	3000	16.1	11.1	1.2	5.9			63.5	70.4	17.9	19.2	16.5	17.0	7.08	6.99	347	351
5	740	200	710	200	1480	1430	—	—	—	—	18.7	23.6	—	—	—	—	—	—	—	—	—	—
6*	790	200	710	200	4360	3010	12.0	10.3	1.3	6.8			64.5	90.0	17.4	21.7	21.2	19.5	7.07	6.86	376	308
7*	660	200	550	200	3620	1820	16.1	11.5	2.4	5.8	0.0	0.0	80.6	84.4	12.3	17.9	13.2	16.2	6.98	7.01	358	7.01
8*	860	200	480	200	2230	1690	13.9	9.5	1.2	4.8			67.8	78.8	12.7	15.6	12.5	19.5	7.12	6.99	404	371
9*	780	200	520	200	2060	1340	20.7	4.0	1.6	2.0	2.8	1.0	98.1	90.2	16.7	18.7	7.0	21.0	6.96	6.83	366	356
10	710	200	500	200	4400	3180	23.2	10.6	1.1	2.7			84.0	94.8	10.7	12.7	4.7	18.7	7.03	6.75	354	341
11	790	200	620	200	1600	1600	16.9	14.7	0.4	1.9	8.9	4.3	71.0	79.6	14.1	12.8	7.0	16.2	6.98	6.85	348	416
12	900	200	680	200	4990	3420	—	—	—	—			—	—	—	—	—	—	—	—	—	—
13	720	200	680	200	1700	1850	16.5	11.8	0.1	1.4	16.1	10.3	66.5	87.3	11.9	15.2	7.7	22.7	7.05	6.84	394	406
14	880	220	710	200	3230	2910	14.8	13.6	0.0	0.8			56.3	83.5	10.8	15.8	7.0	17.7	7.22	6.68	398	364

* = Dosage days

function. The sulphadimidine dosage regimen instituted was as follows: initial dose 0.2 mg/Kg, then 0.1 gm/Kg daily for the three succeeding days.

Table 1 presents the results obtained from the rumen function parameters studied while Figure 1 demonstrates the blood and rumen sulphadimidine levels from sheep no. 11. The pattern in sheep no. 9 was very similar.

The body weight of both sheep declined by approximately 1.5 Kg during the rather stringent experimental period but returned to the pre-dosage weights within a few weeks. Their habitus, however, remained unchanged throughout this study.

The blood glucose, total ketone body and blood urea nitrogen levels remained within accepted normal limits. The acid: base status of both sheep as reflected by plasma pH, plasma CO_2 content and urine pH, remained unchanged.

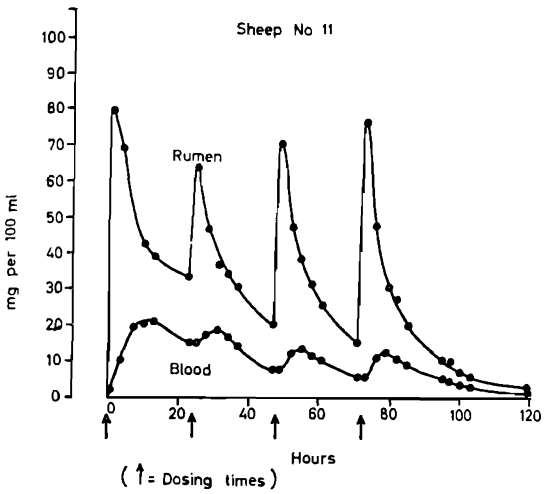

Figure 1—Blood and rumen sulphadimidine levels in a Group A sheep with normal rumen function.

2. Induced Ruminal Stasis

In an attempt to simulate the ruminal condition which would possible exist during any illness, the parasympatholytic alkaloid hyoscine hydrobromide was administered subcutaneously at 5—8 hourly intervals for 48 hours. The dose employed was 70 mg initially followed by 40 to 60 mg depending upon the response to the drug. Ruminal motility was monitored during this period and the inhibition which resulted and which was subsequently maintained was regarded as being sufficiently severe to represent a clinical case with primary or secondary ruminal stasis. The dosage regimen of sulphadimidine was as recorded previously. During the period of induced ruminal stasis the sheep did exhibit anticipated mild signs of hyoscine overdosage. The symptoms observed inluded initial excitement with hyperaesthesia and coarse tremor followed within one to two hours by depression, moderate tympany and anorexia. Furthermore they remained rather dull for 24—28 hours after the final hyoscine dose but then quickly returned to their normal habitus.

It was necessary in this case to establish control values when only hyoscine was injected and Table 2 presents the results obtained in sheep no. 11 under the above conditions. Figure 2 illustrates the blood and rumen sulphadimidine levels. Once again the pattern observed in sheep no. 9 was virtually identical to that in sheep no. 21.

The body weight loss which occurred under these conditions was somewhat more severe (approx. 2.0 Kg) than in the basic investigation but recovery again occurred within a few weeks.

No significant deviation in acid: base balance or in blood sugar, total blood ketone body or blood urea nitrogen levels occurred in either sheep under both ontrol and sulphadimidine-dosed conditions.

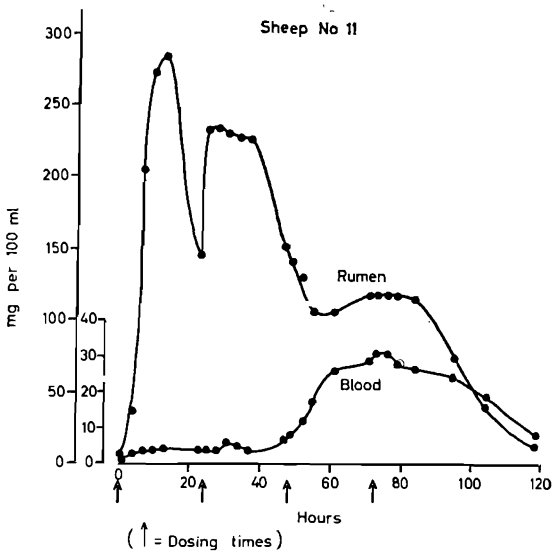

Figure 2—Blood and rumen sulphadimidine levels in a Group A sheep with induced ruminal stasis.

Table 2: EFFECTS OF ADMINISTRATION OF SULPHADIMIDINE TO A GROUP A SHEEP WITH INDUCED RUMINAL STASIS

Day	Food consumption Gm				Water Intake ml		Rumen Motility Index		Glucose Ferment. ml gas/ 15 min.		Cellulose Digestion Index		Total V.F.A. mM/L		Ammon. Nitrogen mg/100 ml		Lactic Acid mg/100 ml		Rumen pH		Rumen Eh -mV	
	11		Control		11	C	11	C	11	C	11	C	11	C	11	C	11	C	11	C	11	C
	Lucern	Maize	Lucern	Maize																		
1	810	200	—	—	2360	—	—	—	—	—	18.1		—	—	—	—	—	—	—	—	—	—
2	610	200	—	—	1740	—	16.4	—	5.2	—			81.6	—	17.8	—	7.0	—	6.69	—	426	—
3	800	200	870	200	2420	2500	13.4	15.4	6.9	5.2	30.7	17.2	74.0	93.8	18.9	10.7	6.2	12.2	6.89	6.79	395	458
4	750	200	740	200	2370	2180	—	—	—	—			—	—	—	—	—	—	—	—	—	—
5	760	200	1003	200	2370	2010	15.6	15.3	6.2	4.7	23.9	22.2	79.8	87.2	16.2	12.8	5.7	9.7	6.78	6.93	436	454
6*	60	50	0	0	0	200	16.0	14.1	7.7	4.6			96.8	105.1	16.8	16.1	6.2	16.5	6.61	6.69	456	416
7*	20	0	0	0	1130	310	4.0	8.4	0.0	0.2	0.0		210.6	65.2	44.5	21.7	29.7	14.8	5.85	7.06	416	486
8*	230	200	460	200	2830	2890	6.5	2.3	0.0	0.0			111.1	35.8	42.2	14.8	8.1	16.5	6.35	7.22	458	321
9*	110	30	250	135	730	1900	8.4	12.6	0.5	0.0	3.1	7.3	91.2	118.0	5.6	2.4	7.2	16.5	5.75	5.80	206	426
10	240	100	730	200	1480	2210	13.5	10.5	0.9	0.0			42.7	81.9	12.3	3.4	7.0	13.5	6.94	6.48	381	364
11	490	170	—	200	2200	2360	—	13.3	—	0.5	5.7	21.3	—	84.8	—	2.2	—	15.0	—	6.62	—	366
12	810	200	—	—	2670	—	11.2	—	3.4	—			71.5	—	7.8	—	6.2	—	6.71	—	456	—
13	740	200	—	—	2320	—	12.8	—	4.4	2.7	15.2		114.2	—	6.7	—	7.5	—	6.40	—	461	—
14	780	200	018	—	2120	—	18.6	—	4.7	3.1			—	—	12.7	—	6.7	—	—	—	—	—

*=Dosage days.

C=Control levels.

Table 3: EFFECTS OF OVERDOSAGE OF SULPHADIMIDINE TO GROUP A SHEEP WITH NORMAL RUMEN FUNCTION

Day	Food consumption Gm				Water Intake ml		Rumen Motility Index		Glucose Ferment. ml gas/ 15 min.		Cellulose Digestion Index		Total V.F.A. mM/L		Ammon. Nitrogen mg/100 ml		Lactic Acid mg/100 ml		Rumen pH		Rumen Eh -mV	
	9		11		9	11	9	11	9	11	9	11	9	11	9	11	9	11	9	11	9	11
	Lucern	Maize	Lucern	Maize																		
1	1020	200	1040	200	4500	3650	—	—	—	—			—	—	—	—	—	—	—	—	—	—
2	890	200	1100	200	2780	2260	18.9	15.2	2.6	3.6	22.7	17.8	109.4	108.8	18.9	12.1	9.8	16.5	6.69	6.69	388	382
3	880	200	830	200	2040	2280	22.4	14.4	2.1	2.7			90.0	93.0	18.4	17.9	12.8	14.8	6.80	6.78	361	366
4	1010	200	850	200	2670	1640	—	—	—	—	31.3	22.8	—	—	—	—	—	—	—	—	—	—
5*	600	200	520	200	2360	2250	19.4	13.3	2.8	4.0			96.2	80.1	18.0	15.7	9.0	13.5	6.79	6.81	341	318
6*	180	55	80	60	660	420	4.0	8.0	0.2	1.9	<1.0	1.0	161.9	120.3	30.0	11.3	12.0	12.3	6.00	6.41	196	254
7*	30	35	0	45	890	790	8.6	3.2	0.0	0.9			95.8	67.0	23.2	21.4	10.5	13.5	6.68	6.85	374	315
8*	0	0	0	0	260	390	2.7	1.3	0.0	0.0	0.0	0.0	100.2	43.3	33.3	14.7	15.5	13.3	6.72	7.02	248	334
9	0	0	0	0	1100	540	1.7	0.3	0.0	0.0			43.6	27.7	13.6	16.8	12.8	13.3	7.06	7.06	146	211
10	0	0	0	0	730	420	4.2	0.6	0.0	0.0	<1.0	<1.0	18.1	20.9	—	—	12.3	18.5	7.20	7.11	254	376
11	460	0	120	0	1400	650	—	—	—	—			—	—	—	—	—	—	—	—	—	—
12	730	0	20	0	3100	540	16.6	2.0	0.3	0.0	2.5	13.6	46.8	19.1	11.2	10.4	11.8	12.0	6.91	6.88	356	186
13	950	145	50	0	2980	250	—	—	—	—			—	—	—	—	—	—	—	—	—	—
14	850	200	0	0	3500	760	21.6	1.8	3.2	0.0	12.7	13.0	85.2	9.8	16.5	11.3	17.0	19.0	6.70	6.92	358	166
15	800	200	0	10	3180	480	27.0	2.6	3.6	0.0	(14.7)	(0.0)	83.2	10.3	11.9	12.1	7.0	13.8	6.74	6.96	354	216

* = Dosage days.

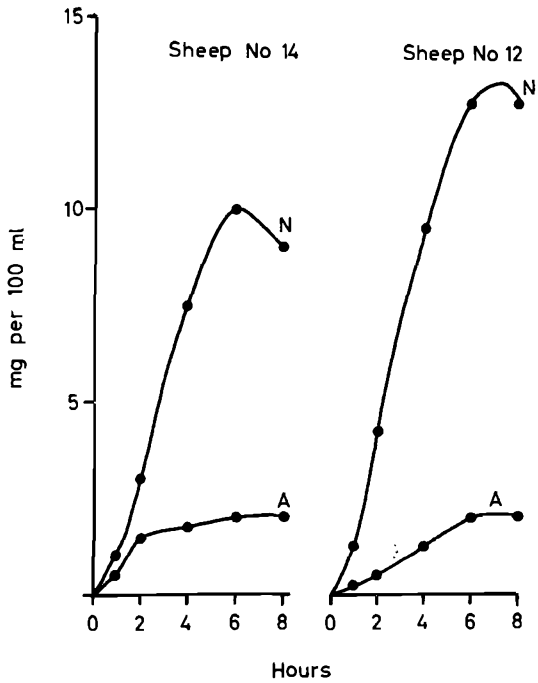

Figure 3—Blood sulphadimidine levels in normal (N) and atropinized (A) sheep.

In order to establish the approximate degree of maximum reduction of absorption of sulphadimidine with absolute ruminal paralysis, absorption studies were conducted on a sheep from each nutritional group (sheep no 14 from the "good ration" group and sheep no. 12 from the "poor ration" group). In this case 20 mg atropine sulphate was administered subcutaneously every hour which ensured the absence of any significant ruminal movement during the eight hour collection period. Feed and drinking water were not made available. Figure 3 illustrates the blood levels of sulphadimidine obtained compared with the control values. The dose in each case was 0.2 mg/Kg administered intraruminally.

3. Gross Sulphadimidine Overdosage

The administration of a frank overdose of the sulphonamide compound was undertaken to establish to what extent rumen flora function could be eliminated by such an extraordinary high dose. Besides the practical possibility of accidental or miscalculated overdosage, the question of drug accumulation during stasis of the rumen had to be considered.

The dosage regimen of sulphadimidine employed in this case was 1.0 gm/Kg as the initial dose, followed by 0.5 gm/Kg daily for the three succeeding days.

The results obtained in both sheep are shown in Table 3 while Figure 4 illustrates the blood and rumen sulphadimidine levels in sheep no. 11 under the above conditions. The levels observed in sheep no. 11 were somewhat higher than in sheep no. 9, but the pattern over the course of the experiment was similar in both except that the rumen concentrations in sheep no. 9 decreased to very low levels within 167 hours of the last dose while in sheep no. 11 the concentration remained high throughout.

Apparent elimination of functional microflora in the rumen of sheep no. 11 occurred following this treatment and this animal had lost 8.8 Kg within two weeks when therapeutic measures were applied to re-establish its rumen function. The weight loss in sheep no. 9 was not as severe (approx. 3.8 Kg) and its pre-experimental body weight was attained within six weeks.

Again no significant deviation in acid : base balance, blood sugar or blood urea nitrogen levels were recorded in either sheep. In the ase of total blood ketones, however, higher values (up to 8.9 mg percent in sheep no. 11) were obtained than in the two previous experiments.

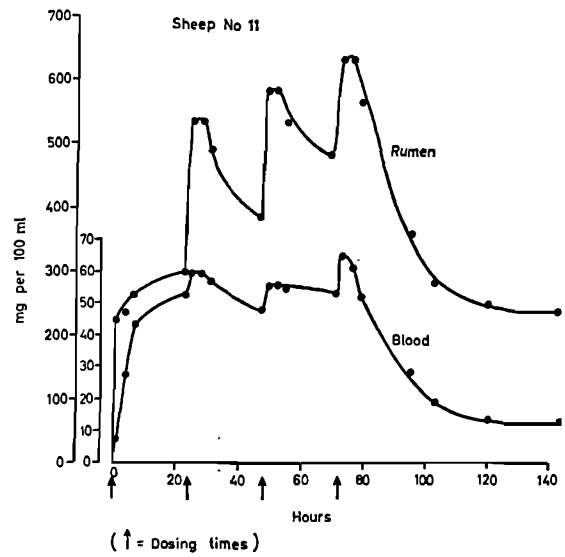

Figure 4—Blood and rumen levels in a Group A sheep following overdosage (5×) with sulphadimidine.

Table 4: EFFECTS OF ADMINISTRATION OF SULPHADIMIDINE TO GROUP B SHEEP WITH UNIMPAIRED RUMEN FUNCTION

Day	Food Consumption Teff (Gm)		Food Consumption Hay (Gm)		Water Intake (ml)		Rumen Motility Index		Glucose Ferment. (ml gas/15 min.)		Cellulose Digestion Index		Total V.F.A. (mM/L)		Ammon. Nitrogen (mg/100 ml)		Lactic Acid (mg/100 ml)		Rumen pH		Rumen Eh (−mV)	
	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13
1	770	630	1620	1210	12.3	10.1	Values very low and disregarded.		Values very low and disregarded.				60.8	52.7	1.8	1.9	4.2	—	6.95	6.88	374	353
2	650	680	1340	900	13.9	12.4							64.8	66.9	3.3	3.5	7.1	2.6	6.72	6.95	347	327
3	700	590	1760	2190	13.8	16.9					13.2	16.2	75.6	58.8	1.9	0.1	3.6	3.3	6.76	6.69	354	321
4	660	510	1760	1060	12.0	13.3							60.8	57.6	2.4	0.3	4.7	4.4	6.80	6.86	384	361
5	660	660	1480	1580	—	—					12.9	15.8	—	—	—	—	—	—	—	—	—	—
6*	570	440	2250	760	13.8	12.0							68.2	60.1	1.4	1.4	4.2	1.4	6.82	7.03	353	330
7*	590	310	1180	1570	9.2	14.3					0.0	0.0	48.2	45.6	4.4	1.2	4.4	1.2	6.96	7.05	301	236
8*	620	530	2030	1240	7.5	14.8							37.6	32.0	2.2	1.5	2.2	1.5	7.16	7.19	266	256
9*	600	500	1410	710	8.1	17.3					5.6	0.0	53.0	54.2	2.7	3.4	2.7	3.4	6.93	7.00	231	296
10	430	220	2820	2420	3.2	12.2							54.2	45.6	3.0	2.7	3.0	2.7	6.88	7.05	291	258
11	510	510	580	200	2.6	5.7					10.0	7.1	33.0	31.6	4.0	2.8	4.0	2.8	6.99	7.16	246	346
12	580	570	970	1120	—	—							—	—	—	—	—	—	—	—	—	—
13	510	730	980	1500	4.7	8.6							57.1	45.0	1.7	0.5	6.5	1.9	6.68	6.81	318	349
14	700	810	1580	1920	8.8	11.8					23.8	19.6	54.4	43.0	1.8	0.6	3.6	4.6	6.72	6.85	326	326
15	780	740	1950	2110	12.4	17.5							62.3	54.9	—	—	—	—	—	—	—	—

* = Dosage days.

At this dosage level evidence of mild renal involvement in both sheep became apparent from the routine urine examinations. Following the administration of the sulpha compound the urine specific gravity increased, the albumin test was noted as ++ and the sediment revealed red blood cells, renal tubular epithetial cells, granular casts and numerous crystals. These changes were reversed within a week and the final urine examinations produced an apparently normal picture.

Group B.

Sheep on low nutritional plane

(Sheep Nos. 7 and 13)

1. *Normal Rumen Function*

The experimental approach and sulphadimidine dosage was identical to that employed for the Group A pair of sheep. Table 4 presents the results of the rumen function parameters investigated and Figure 5 illustrates the blood and rumen levels of the drug from sheep no. 7. The results in sheep no. 13 resembled those in sheep no. 7 very closely and once again only a single representative dosage curve is presented.

The body weight of both sheep declined by approximately 2.0 Kg during the experimental period but returned to pre-dosage weights within a few weeks. Their habitus remained unchanged throughout this trial.

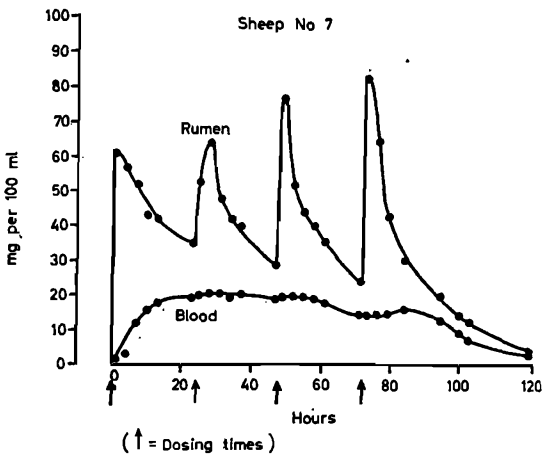

Figure 5—Blood and rumen sulphadimidine levels in a Group B sh.ep with normal rumen function.

No aberration of blood glucose, total blood ketone body, blood urea nitrogen levels or of acid : base balance occurred in either sheep.

2. Induced Ruminal Stasis

The procedure followed in the case of the Group B sheep was identical in every respect to that described for the Group A sheep. Very similar signs of hyoscine overdosage were manifested. Once again the results presented in Table 5 are those of a single animal (sheep no. 7) with the control values established when hyoscine was injected without subsequent sulphadimidine dosage. Figure 6 illustrates the blood and rumen sulphadimidine levels.

The loss of weight recorded was about 2.5 Kg in each sheep with recovery occurring within three to four weeks.

The blood and urine values showed no deviations from the normal. The apparent maximum reduction of absorption of sulphadimidine in a "poor ration" sheep (no. 12) with absolute ruminal stasis produced by atropine is illustrated in Figure 3.

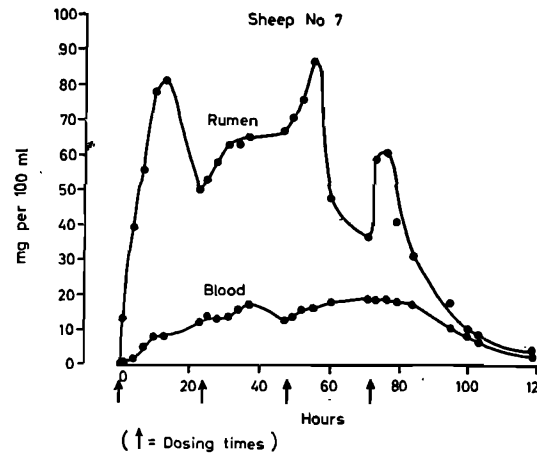

Figure 6—Blood and rumen sulphadimidine levels in a Group B sheep with induced ruminal stasis.

3. Gross Sulphadimidine Overdosage

The overdosage investigation in this group was undertaken in an identical manner to the Group A study i.e., the dose being five times that normally administered.

The results obtained in both sheep are shown in Table 6 and the blood and rumen sulphadimidine levels of sheep no. 7 are presented in Figure 7. The experimental procedure resulted in a body weight loss of 2.1 Kg for sheep no. 7 and 4.9 Kg for sheep no. 13 but the habitus of both remained unaltered throughout the period.

Table 5: EFFECTS OF ADMINISTRATION OF SULPHADIMIDINE TO A GROUP B SHEEP WITH INDUCED RUMINAL STASIS

Day	Food Consumption Teff Gm	Hay Gm	Water Intake ml		Rumen Motility Index		Glucose Ferment. ml gas/ 15 min.		Cellulose Digestion Index		Total V.F.A. mM/L		Ammon. Nitrogen mg/100 ml		Lactic Acid mg/100 ml		Rumen pH		Rumen Eh -mV	
	7	C	7	C	7	C	7	C	7	C	7	C	7	C	7	C	7	13	7	13
1	950	—	3410	—	—	—	—	—			—	—	—	—	—	—	—	—	—	—
2	910	—	1470	—	11.1	10.6	0.9	0.2	23.8	26.0	77.6	68.3	1.3	2.0	2.4	—	6.91	6.84	324	434
3	810	850	3600	2460	11.2	—	0.8	0.4			72.3	—	2.0	—	1.5	3.3	6.81	—	334	434
4	890	1180	1360	2400	11.2	12.8	0.7	0.2	34.5	38.6	76.6	66.6	4.1	2.3	4.2	4.4	6.82	6.81	284	428
5	820	1000	1870	2460	—	—	—	—			—	—	—	—	—	—	—	—	—	—
6*	390	70	0	160	8.5	12.0	0.6	0.0	0.0	27.0	66.1	72.4	6.4	3.3	3.2	1.9	6.96	6.82	300	394
7*	50	0	1930	190	6.8	5.8	0.6	0.1			63.4	48.8	4.5	7.3	3.4	4.6	6.88	7.20	306	366
8*	560	780	2160	3540	3.3	2.6	0.2	0.0	0.0	23.5	31.3	23.3	11.3	5.4	1.3	2.8	7.12	7.35	251	371
9*	590	680	1950	1810	5.0	11.0	0.7	0.0			52.0	80.6	4.2	0.6	1.0	2.7	6.80	6.48	324	456
10	820	880	1810	2560	6.9	9.3	0.5	0.2	15.6	24.1	51.4	70.5	4.6	0.5	1.2	3.4	6.90	6.66	308	381
11	710	950	3720	2330	12.4	—	1.0	0.7			72.2	68.2	1.2	1.1	1.6	5.0	6.66	6.72	326	403
12	650	—	540	—	—	—	—	—			—	—	—	—	—	—	—	—	—	—
13	740	—	1930	—	10.4	—	0.6	—	25.0	—	58.4	—	1.3	—	3.8	—	6.92	—	328	—
14	970	—	2390	—	11.3	—	0.6	—			69.7	—	1.6	—	1.8	—	6.85	—	332	—

C = Control levels.
* = Dosage days.

Table 6: EFFECTS OF OVERDOSAGE OF SULPHADIMIDINE TO GROUP B SHEEP WITH NORMAL RUMEN FUNCTION

Day	Food Consumption Teff Gm		Food Consumption Hay Gm		Water Intake ml		Rumen Motility Index		Glucose Ferment. ml gas/ 15 min.		Cellulose Digestion Index		Total V.F.A. mM/L		Ammon. Nitrogen mg/100 ml		Lactic Acid mg/100 ml		Rumen pH		Rumen Eh -mV	
	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13	7	13
1	880	810	2560	1810	11.3	—	0.2	—					70.5	—	0.5	—	—	—	6.66	—	381	—
2	1040	770	2600	2040	—	13.2	—	0.4	25.6	21.7			—	72.4	—	0.6	—	9.5	—	6.75	—	388
3	960	830	1320	940	—	—	—	—					—	—	—	—	—	—	—	—	—	—
4	780	680	1900	1560	13.3	20.9	0.2	0.8	11.1	16.1			65.1	77.4	0.7	0.3	4.3	9.8	6.84	6.72	361	436
5*	800	630	2660	2160	11.4	14.7	0.3	0.3					57.4	74.0	0.3	0.7	6.3	7.0	6.83	6.69	402	384
6*	260	150	1110	910	9.8	19.7	0.7	0.2	7.3	8.9			75.1	85.4	5.5	2.4	7.5	9.0	6.72	6.30	314	326
7*	570	390	1430	1430	4.2	8.4	0.7	0.5					37.6	49.2	6.4	3.8	8.3	7.5	7.23	6.81	285	384
8*	540	310	1920	1690	9.6	10.0	0.6	0.7	±2.6	21.2			54.0	59.2	2.8	1.5	6.0	8.0	6.92	6.76	356	391
9	310	370	630	1400	11.7	11.5	0.7	0.7					55.6	49.2	4.6	1.2	9.2	11.0	6.78	6.79	469	404
10	480	610	1079	1130	—	—	—	—	4.3	32.2			—	—	—	—	—	—	—	—	—	—
11	790	570	2280	2040	9.4	13.6	0.5	0.4					47.2	53.8	2.6	1.0	12.2	8.8	6.95	6.80	386	376
12	790	630	2270	1940	10.7	16.5	0.4	0.7	22.7	34.4			50.4	54.1	1.9	0.8	8.2	8.0	6.68	6.82	334	394
13	700	520	1780	1950	13.8	16.9	0.5	0.3					60.0	56.3	0.8	0.5	11.0	10.5	6.73	6.79	336	371
14	770	690	1310	1760	—	—	—	—					59.4	65.2	—	—	—	—	—	—	—	—

* = Dosage days.

No significant alteration of blood sugar, total blood ketone body or blood urea nitrogen levels occurred and the acid : base balance remained steady. However, as in the Group A study, evidence of renal involvement became evident as albumin. renal tubtular cells, red blood cells and granular casts were demonstrated on urine examination.

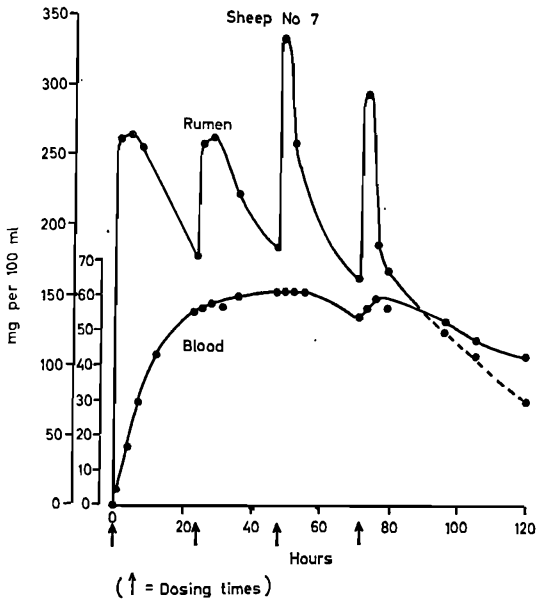

Figure 7—Blood and rumen levels in a Group B sheep following overdosage (5×) with sulphadimidine.

DISCUSSION

The dosage regimens followed produced graded concentrations of sulphadimidine in the rumen liquor which allowed a fairly practical evaluation of the influence of the compound on rumen function especially as these values seemed to be influenced by the quality of ration which was fed. The dosing of the controlled groups with unimpaired rumen function resulted in levels of approximately 80—100 mg/100 ml, while accumulation of sulphadimidine due to induced ruminal stasis led to concentrations from 100—300 mg/100 ml rumen fluid. Overdosage by five times the calculated therapeutic dose produced levels of 360—650 mg/100 ml.

These investigations were conducted through a summer, autumn and most of a winter season. The minor variations of feed and water intake in individual animals from one study to the next can probably be explained by these seasonal changes.

In the majority of the investigations on both nutritional groups there was a consistent depression of appetite (in 14 of 16 experiments) which also reflected, in most instances, a decreased water intake. The regulation of appetite in ruminants must ultimately depend on an intact "feeding centre" in the diencephalon [28]. However, it is known that many dietetic factors do influence food intake and examples of these include amount of feed ingested [29], bulk of the digesta [30], digestibility of feed [31] and extent of cellulose digestion [32]. Although conclusive evidene is lacking, several observations suggest that appetite may be linked with microbial fermentation activity [9, 33, 34], and these findings are supported by studies in sheep which received peroral doses of representative antibiotic compounds and which subsequently showed depression of ruminal digestion with varying degrees of inappetance [35-38]. The anorexia observed in previous investigations on the effects of sulpha-compounds on rumen function [8, 9] and the degrees of diminished appetite recorded in this study probably also reflect an impairment of microbial activity. The depression of cellulolytic activity observed could well produce a reduced feed intake especially if the hypothesis is accepted that the voluntary intake of roughage is related to its rate of disappearanec from the reticulorumen [31]. The degrees of inappetance recorded in the present study were a funtcion of the sulphadimidine concentrations within the rumen and at higher concentrations the effect was more pronounced and even permanent in the one case.

Fairly marked variations in daily water intake were observed in the majority of the sheep studied but as noted previously gross diminution of feed intake almost invariable corresponded with a markedly reduced water consumption. This response was particularly evident in the Group A sheep following overdosage with sulphadimidine. This interference with normal fluid intake is of special significance with respect to a most important toxic effect of sulphonamide therapy, viz., crystalluria and renal damage, and although carnivores and omnivores are more prone to renal toxicity, sulphonamide-induced renal damage has been reported in the bovine [39]. In the ruminant sulphadimidine is only moderately soluble in urine and it has a relatively slow rate of excretion [40]. Urine

examination during the above studies demonstrated renal involvement in both sheep in both nutritional groups following overdosage. Crystalluria, haematuria and cellular elements indicating tubular irritation were evident although in no animal was an elevation of blood urea nitrogen observed. It would thus seem that the fundamental principles of sulphonamide therapy are applicable to the ovine and furthermore note must be taken of the possibility of a reduction of normal water intake of a sheep following oral dosing with a sulphonamide.

Reduction of rumen motility, both in rate and relative force of contraction of the primary mixing movements, occurred in the majority of sheep studied. Although this inhibition often, but not always, correspond to a period of appetite depression, it would appear that the redution of rumen motility was rather drastic compared with the degree of inappetance. A medorate interference was observed in both groups under standard dosage conditions and following induction of ruminal stasis. In the control studies when hyoscine alone was administered normal rumen motility returned immediately after administration of the parasympatholytic agent ceased. Gross overdosage of the sulpha-compound produced very severe and prolonged depressive effects in the Group A pair and in sheep no 11 this ruminal paresis was evident until corrective therapy was applied. The effect on Group B sheep was less marked but significantly lower rumen concentrations of sulphadimidine occurred in these sheep. Clark [41] noted that there is minimal alteration in the frequency or strength of the mixing cycles following fasting for a period of 24 hours and furthermore with prolonged fasting only a gradual decrease in ruminal motility occurs. However, it was emphasized that roughage did appear to play a most important part in general ruminal motility and the decreased movement recorded in these investigations is probably related to the reduced feed and water intake. However, since rumen wall activity is dependent upon central and peripheral control, other factors could be involved.

The glucose fermentation rate was affected in every study in which it was possible to record significant values. The higher concentrations of sulphadimidine produced noticably more drastic and prolonged reductions of glycolytic activity. Recovery of the ability of the rumen micro-organisms to ferment glucose did occur within a variable time period and usually only when the rumen fluid concentration of the sulpha-compound was low. When rumen stasis was artificially produced abolition of glucose fermentation occurred as rapidly as in the sulpha dosage trial and recovery required approximately the same time period. It thus seems that the glycolytic activity of rumen flora is particularly sensitive to any alteration of normal rumen function. These findings are consistent with those of Oyaert *et al* [8].

An immediate and dramatic cessation of cellulolytic activity, as reflected by the cellulose digestion indices, occurred in every experiment. However, recovery or a return to predosage values took place very readily and in fact was even observed during the dosage period in a few cases. It seems that adaptation of the micro-flora to the sulpha-compound occurred within several days. The induction of ruminal stasis without the administration of sulphadimidine did not influence the cellulose digestion index in the Group B sheep and produced a transient depression in one (no. 11) of Group A sheep. The inhibition of microbial cellulolytic activity by sulpha-compounds has been previously recorded by Oyaert *et al* [8], who also observed that the degree of suppression of cellulose digestion by sulphanilamide was dependent on concentration. A similar correlation for sulphadimidine was not evident although somewhat longer periods of time were required for the "recovery" of the cellulolytic rumen flora following gross overdosage. Physiological factors [42] which influence the rate of breakdown of cellulose are of considerable importance in studies which may alter the composition of the rumen liquor. The question of high dry-matter content being rather unfavourable to cellulolytic activity may well be a complicating factor whenever reduction of water intake occurs.

The total volatile fatty acid concentration within the rumen fluid underwent somewhat divergent changes under each experimental procedure. Decreases in v.f.a. levels were observed in Group B but not in Group A, sheep which had received the standard dose of sulphadimidine under normal conditions. However, in the case of induced ruminal stasis the Group A sheep showed an apparent accumulation of v.f.a. in the ruminal fluid, which was probably the result of decreased absorption, and which was then followed by a significant reduction in v.f.a. levels once

normal rumen motility was re-established. The control studies demonstrated a similar tendency. The Group A sheep once again showed mild decreases in the total v.f.a. concentrations which were also evident in the control studies. The overdosage of sulphadimidine to the Group A sheep produced an apparent accumulation followed by a depression of vf.a. levels. This may have been the reseult of the reduction of ruminal motility but this effect was absent in the Group B pair in which once again a less severe depression of v.f.a. levels was observed. The decrease in v.f.a. concentrations probably resulted from the inhibition of cellulolytic bacteria rather than any facilitated or increased absorption. This deviation following sulphadimidine dosage is discussed by Gilchrist & Clask [9] who recorded not only a decrease in total fatty acid levels but also a sharp fall of the propionic/butric acid ratio.

Protein utilization in the ruminant involves in part the bacterial conversion of both the protein and nonprotein nitrogen in the feedstuff into free ammonia in the rumen [43, 44]. This, as would be expected from the difference in dietary protein intake, the ammonia nitrogen levels obtained in the "good ration" Group A sheep were significantly higher than in the "poor ration" Group B sheep. However, in the majority of the series recorded the daily concentrations of the ruminal fluid ammonia nitrogen fluctuated rather markedly and no obvious tendency was evident except when rumen motility was reduced. It has been demonstrated that ammonia is absorbed directly from the rumen [45, 46] and the increased levels observed with decreased ruminal motility would seem to be explained on this basis. Fairly low levels of ammonia nitrogen persisted for several days following recovery in these cases.

The intraruminal dosage of sulphadimidine and the imposed experimental conditions lead to patterns of lactic acid concentration which were difficult to evaluate. Rather wide daily fluctuations and inconsistent levels were recorded and no obvious trend was apparent in most studies. The induction of ruminal stasis did produce high levels of lactate on the first day in both Group A sheep and this probably once again resulted from a decreased absorption. The fact that lactic acid concentrations within the rumen fluid were not significantly affected by the presence of graded sulphadimidine levels was regarded as an interesting feature as some interference with either lactate production or utilization by the rumen micro-flora had been anticipated.

The capacity of the rumen buffering systems was well demonstrated by the remarkably minor deviations of the pH values recorded under virtually all the experimental conditions imposed. The only significant alteration was observed in the rumen stasis studies in the Group A sheep in which a reduction of pH occurred probably as a result of volatile fatty acid and lactic acid accumulation [47, 48, 49]. Somewhat higher values were recorded in the case of overdosage of sulphadimidine to Group A sheep and with ruminal stasis studies in the Group B sheep. This tendency may have been the result of the reduction of feed intake.

The rumen fluid Eh values obtained produced interesting features in many instances. Broberg [50, 51] demonstrated a postprandial fall in redox potential and an elevation of levels following starvation. However, the redox potential in sick animals was stable while high levels were noted in cases of prolonged inappetance and acute ruminal acidosis. The significant point established, however, was that rH levels fell with increased bacterial activity in both *in vivo* and *in vitro* situations. The investigations on the effects of sulphadimidine produced frequent rather temporary positive deviations which often returned to predosage levels within 24 hours. Even more notable, however, was an apparent "over-compensation" with very low Eh values being recorded within the immediately succeeding time period. Generally there were marked overall daily fluctuations observed which stabilized as the rumen fluid sulphadimidine levels fell. This pattern would perhaps be expected from a continuous mixed culture with an ecology governed by competition. Baldwin & Emery [52] suggest that reductive characteristics of rumen fluid can be used as an index of fermentation rate in the same manner as pH.

Throughout these experiments no evidence of a disturbance of the acid : base status of any sheep was observed.

Inscipient ketonaemia appeared only with gross overdosage of sulphadimidine to Group A sheep but this did not progress to clinical ketosis and recovery occurred rapidly. Gilchrist & Clark [9] describe a case where sulphadimidine dosed to a sheep did indeed precipitate ketonaemia but there was some sign of recovery even before dosing was stopped.

Procos & Gilchrist [10] found that the dosing of sulphadimbidine alone without alteration of the original diet was ineffective in producing experimental ketosis.

The blood glucose and blood urea nitrogen levels remained within accepted normal limits in every case. Notwithstanding the renal irritation evidenced with overdosage of sulphadimidine the involvement was insufficient to lead to uraemia.

The sheep with normal rumen function which received the standard dose of sulphadimidine demonstrated a difference between the two nutritional groups. Whereas the concentrations within the rumen fluid were in the same broad range in both groups, viz. approximately 60—80 mg per 100 ml, the blood concentration curves differed. In the Group A pair there was an initial peak of about 20 mg per 100 ml and three distinct succeeding peaks which progressively decreased to about 10—12 mg per 100 ml (Figure 1). However in the Group A pair more of a plateau pattern resulted with less obvious peaks and blood levels of 16—20 mg were maintained throughout the dosage period (Figure 5). Allowing for differences in dosage regimens and presentations of sulphadimidine blood levels, the above results are in accord with previously published findings [53, 4, 5].

The induction of ruminal stasis produced rather bizarre rumen fluid sulphadimidine concentration curves. The levels attained in Group B sheep were not much higher than in the basic study but accumulation was apparent as the peaks which resulted from rapid absorption were absent during the period of stasis (Figure 6). The concentrations in Group A sheep were much higher, up to 280 mg per 100 ml, and again a pattern of accumulation was apparent (Figure 2). The hyoscine effects produced a profound influence on the sulphadimidine blood levels. The Group A sheep showed markedly reduced blood levels (2—6 mg per 100 ml) during the period of stasis and a dramatic elevation to about 30 mg per 100 ml following the cessation of hyoscine injections. A similar, although less striking situation was observed in Group B sheep. The sulphadimidine blood levels obtained following atropine induced ruminal paralysis (Figure 3) revealed that absorption of the compound did occur in the absence of any primary rumen movements but was drastically reduced to 10—13% of the values obtained with normal rumen function present.

Overdosage of the drug produced absorption patterns (Figures 4 and 7) at much higher concentrations but which were similar, although less regular, than those seen with standard dosage. One again the rumen concentrations in Group A sheep were much higher (up to about 600 mg per 100 ml). Furthermore the blood concentration curves in the Group B sheep were again plateau forms while peaks were evident in the Group A pair. The blood levels during the dosing period were in the same range in both groups viz. 50—65 mg per 100 ml.

Sulphadimidine is known to be rapidly absorbed following oral administration to sheep [5] and to cattle [3, 54] and the fundamental principles governing the distribution of sulphonamides across the rumen mucosa have been discussed by Austin [17].

The effect of the reduction of ruminal motility by the belladonna alkaloids on the absorption of sulphadimidine simply supported the observation by Oyaert *et al* [8] that absorption of sulphanilamide was greatly retarded when the rumen was paralyzed and furthermore these findings emphasized the warning of Nesic & Ibrovic [12] about dosing sulphonamides indiscriminately to ruminants when rumen stasis is present as a danger of accumulation seems to be very real.

The marked effect of different rations on the blood levels achieved and maintained has been described previously [15]. In addition starvation produces very prolonged and elevated blood sulphanilamide concentration and Dobson [14] suggests that this effect of starvation could be related to longer retention of the sulpha-compound in the rumen since, when a sheep is starved, the turnover of rumen contents has been observed to decline by a factor of three while rumen volume is not necessarily altered. The conlusion of Clark and Wessel [15] that, "In the ruminant, no pharmacological or toxilogical findings can be correctly interpreted without due regard being given to the basic diet and the feeding regime" would appear to be a tremendously important principle in ruminant therapeutics.

CONCLUSION

The general conclusion which may be reached from this study would seem to be that sulphadimidine may be administered orally to sheep for therapeutic purposes pro-

vided the following facts are borne in mind:

a) Temporary interference with normal rumen function and microfloral activity will probably occur although spontaneous recovery is **likely.**

b) Ruminal stasis will lead to a diminshed absorption of the compound and a consequent reduction of blood levels. Furthermore repeated doses may well accumulate within the rumen.

c) Sufficiently high concentrations of sulphadimidine are highly detrimental to normal rumen function.

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BOOK REVIEW

WOOL GROWTH

M. L. Ryder and S. K. Stephenson

London: Academic Press Inc. First Edition 1968. pp. xi, 806. Price 168s nett.

Although written for teachers and students of wool biology, the wool producer who endeavours to improve the quality of wool and production of his flock will find much valuable information in this book which, to the knowledge of the reviewer, is not available in any single volume in the English language.

The apparent lack of comprehensive information on wool production throughout the world is remedied in the first part with a survey of the main breeds and sheep rearing systems. This introduction, comprising 205 pages, serves as an excellent basis for the scientist unfamiliar with farming practice.

Parts two and three, the core of the book, are devoted to wool biology. These contain the newest information on the anatomy of the skin, the histology of the follicle and wool fibre structure. It is however the theoretical discussion on the components influencing wool growth and fleece, and the genetic and envirenmtal factors influencing fleece variation which will ensure for this publication a wide circle of readers among wool scientists as well as wool producers.

Part four starts with a discussion of fleece characteristics, wool faults and their measurement. The rest of the book is devoted to a technical discussion of the physical properties of wool and wool textile processes.

The book contains many outstanding photographs and illustrations and is well printed on good quality paper.

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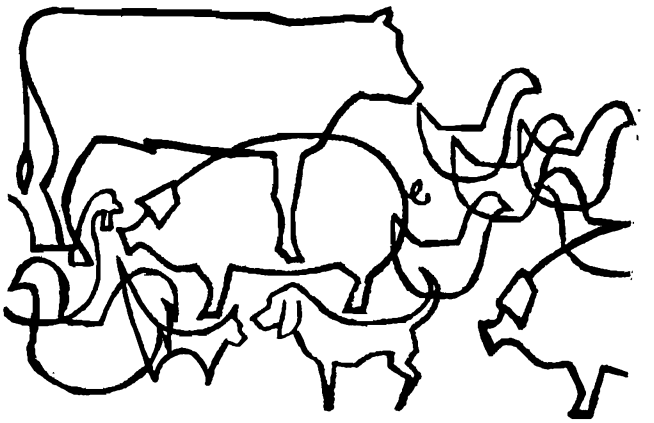

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Jl S. Afr. vet. med. Ass. 40 (2): 179-182 1969

THE INFLUENCE OF PROGESTERONE AND STILBOESTROL ON THE THYROCALCITONIN CONTENT OF SHEEP THYROIDS

P. C. BELONJE*, C. H. VAN NIEKERK*, I. M. R. VAN AARDE** AND G. L. SMIT*

SUMMARY

It is demonstrated that progesterone increases the amount of thyrocalcitonin in the thyroids, possibly by decreasing its secretion. Stilboestrol does not influence this effect of progesterone, nor does it have an effect *per se* upon thyrocalcintonin content of the thyroid..

The possible relevance of these findings to bovine parturient paresis is discussed.

INTRODUCTION

The ability of thyrocalcitonin to lower blood calcium by inhibiting bone resorption, even in the presence of parathyroid hormone [1], suggests that it may play an important role in the aetiology of certain hypocalcaemic conditions. For example, in the cow suffering from milk fever, there is a five to six fold increase in circulating parathyroid hormone [2], but, as there is a concomitant depletion in the thyrocalcitonin content of the thyroid gland [3], it is assumed that the thyrocalcitonin has entered the circulatory system and inhibited the action of the parathyroid hormone. This may explain why the administration of parathyroid hormone is ineffective in the treatment of parturient paresis [4].

As many hypocalcaemic conditions are associated with pregnancy and parturition, it was considered necessary to investigate the effects of progesterone and oestrogen (as stilboestrol) on the thyrocalcitonin content of the thyroid. For this purpose young Merino ewes were employed, while rats were used to assay the thyrocalcintonin content of their thyroids.

MATERIALS AND METHODS

Animals and treatments

Twenty four ewe lambs (6 months old) were employed in the experiment. They were all oöphorectomized on 24 October, 1968 to exclude any interference from endogenous ovarian hormones. The experiment commenced on 4th November, 1968 and the following treatments were applied to four groups of six animals.

1. Control group—seven daily injections of peanut oil.
2. Progesterone group—seven daily injections of 10 mg progesterone (in peanut oil).
3. Stilboestrol group—seven daily injections of 1 mg stilboestrol (in peanut oil).
4. Progesterone+Stilboestrol group — seven daily injections of 2+3 above.

For convenience sake, the experiment was staggered over a period of 24 days. All treatments, however, were administered concurrently and each sheep received its appropriate intramuscular injections for seven consecutive days. On the eighth day they were reweighed and, prior to being killed, a blood sample was taken from each animal for plasma calcium analysis by the naphthalhydroxamic acid precipitation method [5].

Experimental design

The treatments described above were arranged in a 2×2 factorial design in completely randomized blocks. Blocking was done on initial body weight and at the termination of the experiment the mean body weight for all groups showed minimum variation, namely 50.7±0.6 lb. The four treatment combinations may be denoted as:

(1), p, s, and ps,

in which (1) denotes the control group, p the progesterone group, s the stilboestrol group and ps the progesterone+stilboestrol group.

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The statistical methods employed are described by Snedecor and Cochran [6].

Preparation of the thyrocalcitonin extract [7]

Immediately after weighing and slaughtering, the thyroid glands of each sheep were dissected free, accurately weighed, folded in aluminium foil and placed in ice. With the minimum of delay, the glands of each sheep were homogenized, together with 5 ml 0.1 N HCl per gram of tissue, in a stainless steel homogenizer, surrounded by an ice bath. The homogenate was then centrifuged at 10,000 g for 90 minutes at 2°C. The supernatent was removed, brought to 70°C in a boiling water bath, cooled and then once again centrifuged as before. The final supernatent was then stored overnight at −10°C.

Bioassay [8]

A random sample of three week old Wistar rats of both sexes was employed (mean body weight 42.60±0.97 g). The rats were starved for 24 hours prior to the assay, which consisted of an intraperitoneal injection of 1 ml of the extract and subsequent collection of aortic blood (60 minutes after injection), for plasma calcium analysis [5].

Four rats were used for the estimation of the thyrocalcitonin content of each extract, but in 10 of the 24 samples only three rat values were obtained due to insufficient blood for analysis.

A further 20 rats received 1 ml of the 0.1 N HCl solution as an outside control.

RESULTS

The mean plasma calcium values for the four sheep groups and five rat groups are given in Table 1.

Table 1: THE MEAN PLASMA CALCIUM VALUES, WITH STANDARD ERRORS, OF THE FOUR SHEEP GROUPS AND FIVE RAT GROUPS, IN mg Ca/100 ml PLASMA

Groups	Sheep values	Rat values
Control	11.92±0.34	9.63±0.22
Progesterone	12.22±0.34	8.57±0.22
Stilboestrol	11.42±0.34	9.05±0.22
Progesterone + stilboestrol	12.30±0.34	8.40±0.22
Outside control	—	11.08±0.20

Plasma calcium values of the sheep (See Table 1)

The appropriate analysis of variance on the plasma calcium values of the 24 sheep indicated no treatment differences. The calculated F value to test for these differences was 1.38 which falls far below the values required for significance, namely 3.29 and 5.42 for 5% and 1% risk respectively.

Plasma calcium values of the rats (See Table 1)

The analysis of variance applicable to a randomized block design was carried out on the mean rat calcium values obtained for the 24 extract samples. By comparing the within sheep variation with the error variance, as obtained from this analysis, it was verified that the slight imbalance in numbers or rats used per extract did not result in any appreciable heterogeneity of error. The F value for treatment differences obtained from this analysis was 6.347, which is highly significant (the level of significance is approximately ½ of 1%).

Using the symbolism (1), p, s and ps to denote treatment means, the results of the assay can be summarized as follows:

The differences p-(1) and ps-s provide two estimates of the progesterone effect. The average of these two estimates, namely:

$$P=\tfrac{1}{2}[(p\text{-}1)+(ps\text{-}s)]$$

is the so-called "main effect" of progesterone, and the difference of the two estimates, namely:

$$PS=\tfrac{1}{2}[(p\text{-}1)-(ps\text{-}s)]$$

in which the division by 2 places the measurement on a single rat, single sheep basis, is the so-called "interaction" of the progesterone and stilboestrol.

The "main effect" of stilboestrol is measured in a similar manner and the interaction is a symmetric expression i.e. SP=PS.

Point estimates and 95% interval estimates of these effects are given in Table 2.

Table 2: THE POINT ESTIMATES AND 95% INTERVAL ESTIMATES OF THE TREATMENT EFFECTS ON RAT PLASMA CALCIUM LEVELS

Effect	Point estimate	95% Interval estimate
P	−0.85±0.22	$-1.32<P<-0.39$
S	−0.37±0.22	$-0.84<S<+0.09$
PS	−0.20±0.22	$-0.67<PS<+0.26$

As can be seen from Table 2, the progesterone treatment of the sheep resulted in a significant lowering of the plasma calcium content in the rats. The level of significance of this effect is approximately $^1/_{10}$th of 1%, leaving no doubt that this effect is real. The interaction shows that the effect of progesterone does not depend on the presence or absence of stilboestrol. Although the main effect of stilboestrol was not significant, there is a slight indication that stilboestrol treatment of the sheep may result in a slight lowering of the blood calcium levels in rats. It would, however, require a more precise experiment to demonstrate whether this is so or not (level of significance of the S effect is approximately 10%).

The correlation between sheep and rat plasma calcium values.

By performing an analysis of covariance on the sheep and rat calcium values and calculating a coefficient of correlation from the error line of the analysis, one obtains a measurement of the relationship between the plasma calcium values of the sheep and the rats, quite apart from any treatment effects. A calculated correlation of -0.465 was obtained, which is almost significant at the 5% level (the value required for significance is -0.482).

The efficiency of blocking

The efficiency of the blocks design relative to a completely randomised design on the sheep was estimated at 125% from the sheep values and 135% from the rat values. While one would expect the rat value to be somewhat lower, it is quite possible for chance variation to cause this anomaly. It is clear, however, that an appreciable gain in precision resulted from the blocking done on the sheep and that this gain carried over to the second phase of the experiment.

Comparison between the two rat control groups

The mean of the rat plasma calcium values of the factorial control group, (1), which received thyroid extracts from sheep not treated with any hormone, was compared with the mean values of the outside control group of rats, $\overline{X}$, which received only HCl.

The estimated difference was:

$\overline{X}-(1)=1.46\pm0.296$ (mg Ca/100 ml plasma).

Since the difference is almost five times as large as its standard error, there can be no doubt that the plasma values of the rats receiving thyroid extract were much lower than those receiving only the HCl vehicle.

DISCUSSION

It is clear from the preceding evidence that the thyroid of the sheep contains demonstrable quantities of thyrocalcitonin. Moreover, progesterone increases the content per gram very significantly. However, stilboestrol does not influence this effect of progresterone, nor does it have an effect *per se* upon the thyrocalcitonin content of the thyroid.

Although the negative correlation between the plasma calcium values of the sheep and the rats did not quite attain significance at the 5% level, it does suggest that progesterone causes an increase in the thyrocalcitonin content per gram of thyroid tissue, mainly by inhibiting its secretion.

In attempting to relate the above results to the possible role of progresterone in parturient paresis, the following arguments could be considered. The increased amounts of progesterone, secreted during pregnancy, may increase the thyrocalcitonin content of the bovine thyroid. This apparently is not affected by the rise in oestrogen which occurs prior to parturition. Some mechanism must, however, be responsible for the depletion of thyrocalcitonin in the cow suffering from parturient paresis [4]. Moreover, the apparent action of progesterone to cause a retention of thyrocalcitonin may also explain the beneficial effect of progesterone administration in the prevention of milk fever [9].

ACKNOWLEDGEMENTS

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BOOK REVIEW

SHEEP HUSBANDRY AND DISEASES

ALLAN FRASER AND JOHN T. STAMP

London: Crosby Lockwood & Son. Fifth edition (1968). pp. V, 406. Price 50s nett.

In the preface to the first edition in 1949 Dr. Allan Fraser, D.Sc., M.D. stated clearly his fears for the dogmatism of modern science which threatens to reduce the sheep to a metabolic machine converting calories to mutton and wool and calling the unexplained miracle of the birth of a lamb reproduction. In the preface to the present edition he again refers to this "slightly unfashionable approach"—an approach which has been welcomed by many readers who appreciate Dr. Fraser's conciliation between the traditional knowledge and experience gained in his youth of Scottish sheep-farming and of Scottish sheep and his profound scientific knowledge of sheep husbandry.

The first and greater part of this book deals with sheep husbandry. It starts with a comprehensive survey of the history, distribution and description of the sheep breeds with emphasis on British sheep. (The Republic is still referred to as the "Union" of South Africa). The chapters on reproductive physiology of the ram and ewe are lucid and written for the farmer as well as the student and scientist. It is however the chapters on production, nutrition and husbandry which bear testimony of the wide practical knowledge of the author and contain many personal observations and remarks which will be heartily supported by practical stud and flock farmers. A special chapter dealing with the intensive rearing of sheep has brought this fifth edition in line with modern trends in sheep farming and production in the U.K.

The second part on sheep diseases is contributed by Dr. John Stamp. His approach is equally practical and refreshing. The introductory chapter explains the various causes of disease, the application of chemotherapy and an approach to the diagnosis of disease. In subsequent chapters diseaes are classified according to characteristics of sudden death, a longer course, chronic debilitating diseases and diseases with localized symptoms. Only diseases occurring in the British Isles are discussed in a style which makes it understandable for both flock master and scientist.

Although written essentially for the U.K. the contents cover many aspects not found in comparable publications and this book can be recommended to all persons interested in sheep farming.

The type is clear and the many photographic illustrations add to the excellence of this well produced book.

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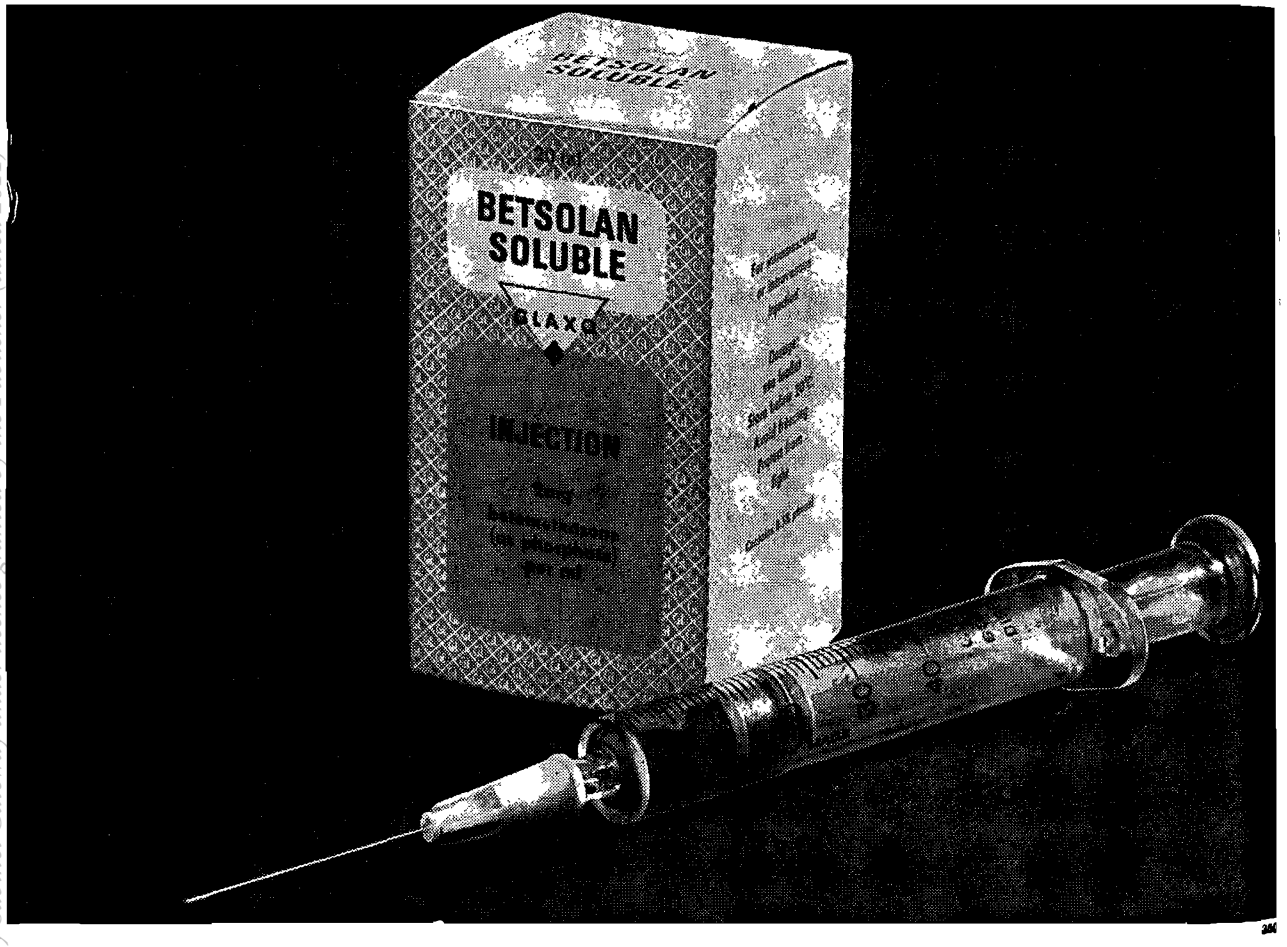

Jl S. Afr. vet. med. Ass. 40 (2): 185-190 1969

THE BRUCELLA MILK RING TEST AND THE ONDERSTEPOORT MRT ANTIGEN

R. W. WORTHINGTON*

SUMMARY

The sensitivity of the Onderstepoort milk ring test (MRT) antigen has been increased and standardized according to FAO/WHO recommendations. The reasons for and the implications of this change are discussed. The MRT on can milk samples is a valuable test for the detection of infected herds and a simple, reliable test to check the brucella free status of clean herds. The test is probably not suitable for use on bulk milk. The test is a valuable method for the rapid detection of individual animals excreting brucella in the milk and has limited application for differentiating S19 vaccinated animals from infected cattle. Common sources of error in the milk ring test are discussed.

INTRODUCTON

The composition of the milk ring test (MRT) antigen issued by Onderstepoort has recently been altered so as to conform with the recommendations of the FAO/WHO Expert Committee on Brucellosis [1]. It was also decided that in future the issue of milk ring test antigen would be restricted to registered veterinarians. The purpose of this paper is therefore to review briefly some of the literature on the use and significance of the MRT and to discuss the changes made in the South African antigen and the effect that these changes will have on the interpretation of test results.

THE MILK RING TEST ANTIGEN

Milk ring test antigen consists of a suspension of killed *Brucella abortus* organisms which have been stained with either haematoxylin (purple blue colour) or with tetrazolium (red). Methods of preparation and staining antigens have been adequately described elsewhere [2]. What is of particular significance in the present context is that the sensitivity of the test is indirectly proportional to the density of the cell suspension used, i.e. the more dense the bacterial suspension used the less sensitive is the test. The Onderstepoort antigen issued before 1967 was considerably denser than that recommended by the FAO/WHO expert committee with the result that the test was less sensitive than the standard test used in most countries. As long as this was the position it meant that we were unable to take advantage of the extensive experience gained in other countries during their brucellosis eradication campaigns and that we would have to determine our own interpretation standards. The sensitivity of the Onderstepoort antigen was therefore increased by reducing the cell density.

USES OF THE MILK RING TEST

a) Herd test

The main use of the MRT is as a herd test for the recognition of infected herds in the initial stage of eradication campaigns and as a simple, cheap and reliable check of the brucella free status of herds once an area has been established as a brucella free area [3, 4]. Can milk samples may be used when doing herd tests as the dilution of antibody in milk cans is not usually so high as to prevent recognition of positive titres. It is generally those cows that have infected udders that give high MRT titres, so that in theory infected animals without infected udders could easily be missed. In practice however, infected herds usually contain sufficient cases with infected udders for the test to be reliable in recognizing infected herds. The efficacy of the method is shown by the fact that it has been used successfully in Holland [5], Ireland [6], Belgium [7], the United States [8] and many other countries during eradication campaigns.

The value of the test in bulk milk samples is still open to some doubt. Many dairy farmers are changing to a system of storage

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of milk in bulk tanks and the question of reliability of the test in bulk milk has again arisen. Roepke *et al* [9] state that "a study of the milk ring test titres of individual milk samples from 303 reactor cows showed 80 per cent of such samples could be diluted 1 : 25 with mixed negative milk and still obtain 2+ or stronger ring test reaction. On this basis the probability of obtaining a positive ring test reaction on mixed milk from 25 cows when two are reactors is 96 per cent and 99 per cent, when milk of three reactors is included." On the other hand Hill & Cremers [10] conclude from their studies that a titre of 1/16 or more was only found in a small percentage of cases. The findings of Frank [11] and Jaartsveld [12] appear to support this view. These findings indicate that a test on bulk milk would not be sufficiently reliable. Christie *et al* [6] also believe that the test cannot be used on bulk milk. On the other hand according to Sjollema [13] "monthly ring tests of mixed samples by a skilled investigator are hardly less useful than is quarterly testing of can samples for contagious abortion."

There is little doubt that the MRT is the simplest and most economical way of locating infected herds. Janney *et al* [14] found that "of 3,615 herds in two counties the ring test missed only one herd which contained a lone reacting animal which proved to be a shedder, and this animal was negative to the whey test.

In Calumet county with 1,521 herds it cost $1,014-00 to locate by means of the county-wide blood test each herd which contained reactors. Using the milk ring test as a screening test it cost $104-28 to locate each herd which contained reactors.

The cost of locating a herd containing reactors in Ocinto county was $536-92 in the area blood test and only $102-60 with the ring test."

The ring test is also the ideal method of conducting surveys to establish the incidence of the disease in an area and has frequenty been used for this purpose [15, 16, 17]. The use of the MRT as a herd test is dependant on the use of an antigen of sufficient sensitivity and the value of the changes made to the Onderstepoort antigen are therefore obvious.

b) Individual animal test

To understand the value of the MRT as a test for individual animals it is necessary to appreciate the fact that the bulk of the antibodies found in milk are produced in the udder [18, 19]. The presence of antibody in milk is therefore usually an indication of the presence of antigen in the udder. It is thus easy to understand that the MRT is reliable only when udder infection is present.

In cases where the infection is confined to other organs positive blood titres and negative ring tests may be found. Bryan, *et al* [20] found an overall agreement of 78.7% between the MRT and the serum agglutination test in 955 lactating cows tested. Only 38.1% of 244 blood test positive cows reacted positively to the ring test and 93% of MRT positive cows were postive to the agglutination test. It can therefore be seen that the MRT is not an accurate indicator of infection in individual cows.

The test has, however, been found to be a simple and accurate method of recognizing cows with udder infection [21, 22] and it is therefore a valuable method for tracing cows excreting organisms in their milk. Ferguson and Robertson [21] found that 85% of cows with ++ or higher ring test reactions on milk diluted 1/10 also gave positive biological tests on milk, while none of the 300 MRT negative cows were biologically positive. According to Alton & Jones [2] a MRT titre of 1/32 can often be correlated with the presence of brucella organisms in the milk.

The MRT has also been used to distinguish S19-vaccinated animals from infected animals [23, 24]. Ferguson & Robertson [21] found that after S19 vaccination cows developed MRT titres 8—10 days after vaccination and these titres persisted for over a year and even into a second gestation but when the milk was diluted 1/10 postive titres were not seen beyond 30 days after vaccination. Hill [25] considered MRT titres of 1/10 or over to be probably due to infection. Van Drimmelen [23, 24] considered a reaction at a dilution of 1/4 to be positive while lower titres were probably vaccine titres. Van Drimmelen's work was done with the less sensitive antigen so that a titre of 1/10 as recommended by other workers will probably be more reliable using the new Onderstepoort antigen. The differentiation of vaccine titres from titres due to infection with virulent *Br. abortus* is now more generally done on serum using a series of tests of which the complement fixation test is the most important. Other tests used for this purpose are the heat inactivation test, Coomb's test, mercapto-ethanol test, rivanol test and milk whey agglutination tests. The differentiation

of vaccine titres from titres due to infection should not therefore be considered an important application of the MRT.

SOURCES OF ERROR IN THE MRT

False positive results may occur when milk from a cow freshly in milk, from a cow which is drying up or from a cow suffering from mastitis is tested. These findings are explained by Kerr *et al*[18] who believe that serum agglutinins do not appear in the milk except in the milk of early lactation, late lactation and in some forms of mastitis.

Pietz *et al*[26] found that storage at 35°F was the most suitable method of preserving milk for the MRT. Storage at 74°F and addition of mercuric chloride as a preservative caused a rapid drop in titre. Soured milk is totally unsuitable for testing.

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*Gibbons, W.J. (1951). Vet. Med., *46*:397.

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HEREDITARY EFFECTS AND THEIR ELIMINATION**

IVAR JOHANSSON*

Various defects appear from time to time in all species and breeds of farm animals. Morphological defects observable in the newborn are usually called congenital defects. Other defects appear at a later stage, for example genital malformations or functional sterility which are difficult to diagnose before puberty. Sometimes hereditary defects are spoken of in contrast to environmentally induced defects but there is no clear borderline between them. A certain phenotypic defect may in one case be due to the genetic constitution of the animal and in another case to environmental agencies. One classification that possibly can be made is into morphological and biochemical defects, but even here there may be transitional cases.

With regard to hereditary defects, Hadorn [1] proposed that the responsible genes should be classified into lethal, semi-lethal and sub-vital. When a gene in effective dose always causes the death of the carrier before attainment of puberty it is called lethal, when the percent survivors is above zero but less than 50, the gene is said to be semi-lethal, and when more than 50 but less than 100% of the defective genotypes survive, compared to animals with the corresponding normal alleles, the gene is said to be sub-vital.

Hereditary defects may be due to chromosomal aberrations (losses or duplications of a certain chromosomes, translocations of chromosomal parts, deletions or inversions of parts of the same chromosome) or to gene mutations. However, there is no distinct borderline between, for example deletions and gene mutations.

Recent research has shown that numerous congenital defects in man and animals can be induced by nutritional deficiencies or by administration of certain chemicals to the pregnant mother. The branch of science dealing with congenital defects is called teratology, and substances inducing malformations are said to have teratogenic effects. Just a few examples will be given. By injecting such drugs as insulin, cortisone, sulfanilamid, trypan blue etc. into pregnant females of laboratory rodents it has been possible to produce in the young practically the whole array of congenital malformations which are known to appear spontaneously in the same species and in farm animals. The sensitivity of the foetuses for such teratogenic agents shows a great variation between different stages of pregnancy. As a rule, the sensitivity is greatest when an organ is in the most rapid progress of differentiation during the early stages of pregnancy.

Some defects may be due to dietary deficiencies, for example, in rats and pigs lack of vitamin A in the diet may cause anophthalmia or microphthalmia (lack of eyes or reduced size of eyes), cleft palate, harelip, cryptorchism, etc. Lack of riboflavin, folic acid or pantothenic acid may also cause malformations. When certain defects, which otherwise are hereditary, are induced by environmental causes, the induced defects are said to be phenocopies of the hereditary defects.

The effect of a drug, or a dietary deficiency on foetal development may be different at different stages of pregnancy. Landauer [2], working on chickens, found that insulin injected into the eggs 72 hours after the beginning of incubation produced rumplessness, similar to the hereditary type of this defect, in about 5% of the embryos surviving to the 17th day of incubation, whereas injections 120—135 hours after the initiation of incubation caused a pronounced increase in the frequency of beak abnormalities, short legs and abnormal eyes but no cases of rumplessness. By the injection of nicotine sulphate at the level of 2.5 mg per egg several defects were produced in more than 50% of the embryos;

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the most characteristic defect being shortening and twisting of the neck. When the dose was doubled, practically all the embryos were defective in several different ways. Another interesting finding in Landauer's experiments was that the severity of the induced defects seemed to differ between breeds. That there are individual differences within the same breed is clearly established.

Table 1: EXAMPLES OF CONGENITAL DEFECTS, INDUCED AND HEREDITARY

(Partly from Kalter and Warkany [4])

Organ and type of anomaly	Rodents: induced by		Hereditary in farm mammals		
	Drugs	Diet def.	Cattle	Sheep	Pigs
Dermal					
Epithelial defects			X l (r)		X (r)
Hairless: lethal	X		X l (r)		
semilethal			X sl (r)		X (r)
Skeletal					
Lack of mandible (Agnathia)	X	X	X l (r)	X l	
Short jaw (Brachygnathia)	X	X		X	
Leg "amputations," 1–4 legs	X	X	X l (r)	X l (r)	X l (r)
Achondroplasia: Partially dominant (bulldog)	X		X l (r)		
Recessive (bulldog)			X l (r)	X l (r)	
Short spine (Parocormia)		X	X l (r)	X l (r)	X l (r)
Stiff joints (Anchylosis)			X l (r)	X l (r)	X l (r)
Cleft palate and hare-lip	X	X	X l	X l	X l
Polydactyly and syndactyly	X	X	X (d)	(X)	X (d)
Nervous and sense organs					
Waterhead (Hydrocephalus)	X	X	X l (r)	X l (r)	X l (r)
Anophthalmia (lack of eyes)	X	X	(X)	(X)	X
Michophthalmia (small eyes)	X	X			X
Ataxia (lameness)			X l (r)	X l (r)	X l (r)
Visceral and circulatory organs					
Atresia ani			(X)	(X)	X sl
Cryptorchidism	X		(X)	(X)	X (r)
Hydrops			X l (r)	(X)	X l (r)
Metabolism (Biochemical defects)					
Dwarfism			X sl (r)	(X)	(X)
Porphyria			X (r)		X (pd)
Albinism			X (r)		

X = cases analysed, common, (X) = rare or uncertain cases.

l = Lethal, sl = sublethal, r = recessive, d = dominant gene.

The examples of congenital defects listed in Table 1 show that:

1. The same or similar types of defects appear in several species of farm mammals. They appear also in poultry.

2. Many morphological defects have been produced as phenocopies in rodents by drugs or dietary deficiencies. This has been shown experimentally, not only with rodents but also with poultry.

It is highly probable that many of the malformations are phenocopies due to drugs, dietary deficiencies or virus infections of the mother during pregnancy. Vitamin A deficiency in pregnant sows may cause anophthalmia or microphthalmia in newborn pigs, and lack of iodine may cause goiter and hairlessness.

Most morphological malformations show a wide spectrum of manifestation, or expressivity, and in some cases the presence of a mutant gene in otherwise active dose may not be expressed at all in the phenotype, i.e. the penetrance of the gene is incomplete. Varying expressivity and incomplete penetrance may have a strictly genetic basis, but in some cases environmental influences may be partly or wholly responsible. The residual inheritance, sometimes called "the genetic background," is probably the most common cause. When the assumed penetrance is low, say 50% or less, it is preferable to state that the mode of inheritance is unknown rather than to give a false impression of exactitude in the genetic analysis by estimating a figure for penetrance. The hereditary defects in farm animals do not always follow the simple Mendelian scheme of inheritance. Some examples of varying expressivity and complicated genetic situations may be given.

Several different types of achondroplasia (disproportionate dwarfism) occur in cattle. The head and the legs are markedly shortened. It has been known for a long time that Dexter cattle are heterozygous for a partially dominant gene which in single dose is responsible for the shortlegged Dexter type and in double dose causes extreme achondroplasia (bulldog calves which are non-viable). When Dexters are mated *inter se* they produce normal animals, Dexter and bulldogs according to the ratio 1 : 2 : 1. Completely recessive achondroplasia has been reported in several breeds of cattle and in sheep. The bulldog calves are usually born alive but non-viable. Hereditary partial achondroplasia is also known where the calves are viable although with reduced viability. This type is caused by a partially dominant gene.

As a case of varying expressivity the "amputated" calves in the Friesian breed may be mentioned. The legs may be completely lacking in the newborn calves (in such cases the head is extremely short), or they may be only partly amputated in varying degree. Calves which are very deformed are usually dead at birth, whereas the less deformed calves may live for several days or weeks.

Other cases of congenital malformations may have a hereditary basis but the mode of inheritance is unknown. As an example the defect *atresia ani* in pigs may be mentioned. The expression of *atresia ani* varies between as well as within sexes. The blind end of the rectum is closer to the skin in the female piglet and the rectum usually opens into the vagina, forming a cloaca which makes it possible for the pigs to defecate. In the male pigs the defect is lethal, if surgery is not resorted to. Operated male pigs have been mated to defective sows. In such experimental matings 13 litters with a total of 115 pigs were produced; 51.3% of those were afflicted with *atresia ani*.

Superficially, the hypothesis that *atresia ani* is caused by a recessive gene with somewhat less than 50% penetrance might seem to fit the data fairly well, but the situation is probably even more complicated. For example, when a defective boar was mated to unrelated defective sows, 48% of the offspring were defective but when the same boar was mated to his own defective daughters 67% of the offspring were defective. Similar observations have been made with regard to other defects in pigs, for example "kinky tail" [3].

Inbreeding has a depressive effect on the viability and growth rate of the progeny, and when the mother is inbred she provides a less favourable intra-uterine environment for the foetuses than when she is not inbred. It is possible, therefore, that inbreeding increases the frequency of congenital defects, not only because it increases the frequency of segregation of lethal and semi-lethal genes,

but also because of its general depressing effect on metabolism and growth rate.

Some congenital defects show a tendency to appear together in the same animal, for example cleft palate and hare-lip, especially when these defects are strongly manifested. Some congenital malformations can not be diagnosed by exterior examination of the newborn animal, for example *atresia coli* or kidney atrophy, but an autopsy is needed. Hypoplasia of the internal genital organs, for example the ovaries and testicles, is usually discovered first after attainment of puberty.

Errors in metabolism

Biochemical defects form an interesting group of congenital defects which have been studied particularly in man, but they are also found in domestic animals. Albinism may be mentioned as an example. The amino acid, tyrosine is a building stone in the synthesis of protein, and it is also the raw material for melanin in skin, hair and feathers. The formation of melanin involves a long series of oxidation and polymerization processes. The early stages of oxidation require the presence of the enzyme, tyrosinase. If tyrosinase is not present, the reactions are blocked and no melanin is formed. The result is albinism as known to occur in poultry, rodents and cattle, for example. Albinism is always due to a recessive gene in double dose.

One of the best examples of the detailed genetic control of protein synthesis, and the importance of even slight changes in the amino acid sequence of a protein, is obtained from investigations on haemoglobin variants in man. The haemoglobin molecule is constructed from about 600 amino acid groups bound together in two α- and two β-chains, each with a porphyrin ring and an atom of iron. Individuals homozygous for the so called "sickle-cell" type of haemoglobin suffer from a severe form of anaemia and their death rate is high compared to normal individuals. However, the heterozygotes have a greater resistance to malaria than non-carriers for the "sickle-cell" gene, which is important in regions where malaria is prevalent. Under other conditions they have approximately normal viability. The "sickle-cell" anaemia is due to the fact that in both β-chains of the haemoglobin the animo acid glutamine (in normal haemoglobin) is replaced by valine. The presence or absence of glutamine at this site is determined by a single gene. Several other inherited biochemical defects are known in man. In farm animals we know only a few cases, probably because of less intensive investigations. However, biochemical genetics covers the whole field of metabolic and immunity reactions in the body, and the importance of this branch of genetics will probably increase in the near future. A great deal of work has been done on the inheritance of blood groups, different types of serum and milk proteins, etc. in farm animals, but this work does not fall under the heading "congenital defects."

Numerous papers and several reviews and books have been published on hereditary defects in farm animals [5, 6]. Last year, Lauvergne [7] published a catalogue comprising 226 anomalies in cattle with a proven or postulated monofactorial type of inheritance. A short description is given of each abnormality and a special index shows in which breeds they have been found. However, as yet no estimates have been made of the frequency of mutations into various defective genes, or the frequency of such genes in the various breeds.

"Accidents" in development

A number of defects appear with very low frequency in all species of farm animals and have never been reported to aggregate in certain lines or strains, for example *duplications* (conjoined twins, duplication of hind- or forelegs, etc.), *acrania* (no or incomplete skull), *exencephaly* (exposure of the brain), *spina bifida* (clefts of the vertebral column with exposure of the spinal cord) etc. It is likely that such defects are caused by "accidents" in development. The alternative would be dominant mutations which eradicate themselves as soon as their effect is manifested.

How to decide whether or not a congenital defect is likely to be hereditary.

The first thing is to find the answers to the following three questions:

1. Is the defect known to be hereditary in the same species of animals?
2. Does it repeat itself after matings between certain animals? Is it a certain bull that throws defective calves when he is mated to cows which have one or more common ancestors with the bull?

3. Has the mother been sick and received medical treatment, or are there any reasons to suppose a dietary deficiency during pregnancy?

The elimination of unfavourable recessive genes from the breeding populations

Dominant lethal genes eliminate themselves in the first generation when they appear, but recessive genes can pass through many generations hidden in the heterozygotes, when the frequency of the gene is low, as is usually the case. At the early stages of AI cattle breeding the dangers were pointed out of spreading defective genes to a large number of offspring from heterozygous bulls. In the individual case this danger exists, but, considering the population as a whole, AI is no more risky than natural breeding. There are no reasons to expect that carrier bulls should be chosen more often for breeding in A.I. than in natural breeding. However, the possibility of reducing the frequency of undesirable genes is much greater in AI than in natural breeding. The private breeders often try to conceal the fact that some of their animals throw malformed calves, but a member of an AI association usually reports it because he does not want to have it repeated. Owing to their greater use, the carriers are usually discovered earlier in AI than in natural breeding.

Natural selection tends to reduce the frequency of recessive lethal and semi-lethal genes, but the process is rather slow, especially when the frequency of the gene is low (Table 2). In order to accelerate the process of elimination it may be necessary to test suspected male carriers by planned matings, especially in natural breeding.

Tests for heterozygosity of males

Usually the frequency of heterozygosity in the breeding population is not known, and therefore, it can not be considered. The question to be answered is: How many progeny, not showing the recessive trait, are needed in order to reduce to a certain level the probability that a heterozygous male passes the test without being unveiled as a heterozygote? This probability will be denoted P(WrC)=probability for wrong conclusion. First we will discuss cases where the male produces only one offspring with each female.

1. Mating the male to recessive females (when these are viable and fertile). If the male is a carrier of the same recessive gene as the females possess in homozygous condition, the probability for homozygous recessive offspring at each mating is 0.5, and for n fertile matings the probability is 0.5^n. The value of n for a certain level of probability, for example 0.05, can be obtained from equation $0.05=0.5^n$, or in this case n=4.32, i.e. 5 offspring of the dominant type are needed, with no recessives appearing. In order to

Table 2: THE REDUCTION IN THE FREQUENCY OF THE RECESSIVE GENE WHEN NO HOMOZYGOTES (aa) ARE USED FOR BREEDING.

$q_n = \frac{q_o}{1+nq_o}$ where q_o = initial frequency of a and q_n = frequency in the nth generation

Gene frequencies P_A	q_a	Frequency of heterozygotes: Aa $2pq$	Frequenzy of homozygotes: (aa) q^2	Ratio $2pq/q^2$	Generations required to decrease the frequency of q^2 one step
0.8	0.2	0.32	0.04	8	5
0.9	0.1	0.18	0.01	18	10
0.95	0.05	0.095	0.0025	138	80
0.99	0.01	0.0198	0.0001	198	900
0.999	0.001	0.001998	0.000001	1998	

Table 3: NUMBER OF OFFSPRING REQUIRED (*all of the dominant type*) IN ORDER TO REDUCE THE PROBABILITY TO 0.05, 0.01 AND 0.001 RESPECTIVELY THAT THE MALE IS CARRIER FOR A CERTAIN RECESSIVE GENE (*one offspring per female*)

The male is mated to	Probablity P(WrC) that a carrier male produces offspring of the dominant type only in n matings	Number of offspring (n) needed to reduce P(WrC) to the levels stated		
		0.05	0.01	0.001
1. Homozygous recessives (aa)	0.5^n	4.3	6.6	10.0
2. Known heterozygotes (Aa)	0.75^n	10.4	16.0	24.0
3. Daughters of known heterozygotes (50% AA+50% Aa) 4. Own daughters (50% AA+50% Aa)	0.875^n	22.5	34.5	51.7

reduce the probability to 0.01 the number of dominant offspring needed would be 7 (Table 3).

2. Mating the male to known heterozygous females (*Aa*). *If the male is heterozygous* for the recessive gene a, the probability of dominant offspring is at each mating 0.75, and the probability of obtaining offspring of the dominant type only in n matings is $P=0.75^n$.

3. Mating the male to daughters of known heterozygous females, assuming that the sires of the daughters are AA. The probability is that 50% of these daughters are heterozygous (Aa) and 50% homozygous (AA). The probability that a heterozygous male does not produce any recessive offspring in n matings to this group of females is 0.87^n.

4. The male is mated to his own daughters. If the male is heterozygous for a certain recessive gene, 50% of his daughters will also be heterozygous for the same gene, on the assumption that the dams were all homozygous dominants (AA).

The probability that the heterozygous male does not produce any recessive offspring in n matings to his own daughters is 0.875^n. This test which is often called *the inbreeding test*, has the advantage that it is a test for any recessive gene which the male carries, irrespective whether this gene is previously known or not. The disadvantage is that it involves intense inbreeding which may cause decreased viability and fertility of the offspring.

Further Discussion of Test No. 4

In Table 3 the situation was simplified in order to facilitate the practical applications. The fact that recessive traits segregate out in one or several herds indicates that the genes for these traits have reached a certain level of frequency in the population. *It is* difficult to estimate this frequency, especially when it is low. However, an example will be given showing the influence of the frequency (q) of the recessive gene *a* when bulls are tested by matings to their own daughters. Assuming that no recessive can be used for breeding, the frequency of heterozygotes (Aa) in the population would be $\frac{2pq}{p^2+2pq}=\frac{2q}{1+q}$ since $p+q=1$. The frequency of the recessive gene would be $\frac{q}{1+q}$ and the frequency of the dominant gene $\frac{1}{1+q}$. From the gamete frequency in the population and among the daughters of the bull it can be calculated that the probability of obtaining the dominant type only when the bull is mated to his daughters is $\frac{(7+3q)}{(8+4q)}$ instead of simply $\frac{7}{8}$ and the probability that no segregation occurs in matings to n daughters is $\left[\frac{7+3q}{8+4q}\right]^n$. Taking the frequency of *a* in the general population into consideration changes the $P_{(WrC)}$ very little as shown in Table 4. The accuracy of the test will be slightly increased when the same number of offspring is produced as stated in Table 3.

There are no reasons for carrying out expensive inbreeding tests if the male is not suspected to be carrier for an unfavourable recessive gene. If one of his parents is a carrier, the probability that a son (or a daughter) is a carrier, is 0.5, and the probability that a grandson is a carrier would be 0.25.

Table 4: PROBABILITY OF (AA + Aa) IN ANY SIRE × DAUGHTER MATING and the number of offspring required to decrease $R_{(WrC)}$ to 0.01 when the frequency (q) of a in the cow population is considered.

q	0	0.05	0.1	0.2
P(AA + Aa)	0.875	0.872	0.869	0.864
No. of progeny for $P_{(WrC)} = 0.01$	35	34	33	32

$$\left[\frac{7+3q}{8+4q}\right]^n = 0.01$$

"Automatic" tests

In an AI association where each bull produces a large number of offspring on a random sample of cows (random with regard to unfavourable recessive genes), no special tests are needed. If the frequency of the gene in the general population has reached the level of 0.02—0.05 the bulls will be tested automatically at an early stage. The segregation of recessives *(aa)* when a heterozygous bull *(Aa)* is used on the cow population would be $0.5\frac{q}{1+q} = \frac{q}{2(1+q)}$ and the probability of obtaining the dominant type of offspring only in n matings would be $\left[\frac{2+q}{2(1+q)}\right]^n$. Table 5 shows the number of offspring needed in order to reduce $P_{(WrC)}$ to 0.05, 0.01 and 0.001 respectively for stated gene frequencies in the cow population.

Table 5: "AUTOMATIC" TESTING OF BULLS FOR RECESSIVE GENES

Gene frequency (q) in the cow population ⟹	0.02	0.05	0.1	0.2	0.3
Frequency of aa segregation after each mating when an Aa bull is used ⟹	0.01	0.025	0.05	0.1	0.15
Probability P(WrC)	Number of offspring required in order to reduce the probability that the bull might be a carrier (Aa) to stated levels.				
0.05	304	124	64	34	24
0.01	467	191	99	53	38
0.001	701	287	148	79	56

$$P_{(aa)} = 0.5\left[\frac{q}{1+q}\right] = \left[\frac{q}{2(1+q)}\right] \text{ for each mating}$$

$$P_{(AA+a)} = \left[1 - \frac{q}{2(1+q)}\right]^n = \left[\frac{2+q}{2(1+q)}\right]^n$$

for n matings

For this kind of test to function properly, it is necessary that all abnormal calves are reported to the AI center. This is practiced in several coutries in Northwestern Europe. In Holland, calf births are reported within 3 days after the occurrence, and if a calf is abnormal, the abnormality is described. When possible the malformed calves are sent to the veterinary health service for examination. It is required that the first 750 births after each AI bull are reported. This system of reporting seems to have worked quite well.

Testing males of polytocous species, for example pigs or poultry

The simplest way of testing a boar for undesirable recessive genes is to mate him to known recessive sows. Only two litters are needed in order to reduce the probability that the boar passes the test without throwing any defective piglets. (Table 6).

Table 6: NUMBER OF DAUGHTERS NEEDED WHEN A BOAR IS TESTED FOR RECESSIVE GENES WITH REGARD TO 5 DIFFERENT LITTER SIZES AND 3 LEVELS OF PROBABILITY

No. of pigs per litter ⟹	8	10	12	14	16
Probability that an Aa boar may pass the test	Number of daughters needed with one litter each				
0.05	5.0	4.7	4.5	4.4	4.4
0.01	7.7	7.3	6.9	6.8	6.7
0.001	11.6	10.9	10.4	10.2	10.1

$$P_{(AA+Aa)} = [0.5 + 0.5(0.75)^m]^n$$

m = number of piglets per sow.

n = number of sows.

If the boar is tested in sire × daughter matings, it does not suffice for an effective test that the boar produces a certain number of offspring, but he must be tested on several daughters since a daughter may be either homo- or heterozygous. If the boar is heterozygous, the probability is that 50% of the daughters are AA and 50% Aa. As shown before, it is not necessary to consider the frequency of the recessive gene (or genes) in the population. When the boar is mated to heterozygous daughters, 25% homozygous recessive piglets segregate out, and this is true for any recessive gene which the boar might carry. On an average 75% of the pig-

lets represent the dominant type with regard to any recessive gene. The probability that a randomly chosen daughter produces m piglets of the dominant type only is $0.5+0.5\ (0.75)^m$ and the probability that n daughters show the same result is $[0.5+0.5\ (0.75)^m]^n$. The number of daughters necessary to reduce $P_{(WrC)}$ to 0.05, if each daughter produces one litter of 10 piglets, can be obtained from the equation $0.05=[0.5+0.5(0.75)^{10}]^n$. The answer is 5 daughters. Table 6 shows the number of daughters required for different probability levels and different litter size. The litter size may, of course, vary between daughters, but it can be seen from the table that variations in the litter size is much less important than variation in the number of daughters. The same principles apply to families of full sibs in poultry, m=number of birds per full sib group and n=full sib groups from the same male.

SUMMARY AND CONCLUSIONS

1. Congenital defects are not always hereditary. Sometimes they may be due to environmental influences through the mother during pregnancy, for example dietary deficiencies or infections. In other cases, the foetus may carry a hereditary disposition for arrestment in differentiation and growth, which is manifested only under certain environmental conditions. Such defects as harelip, cleft palate and *atresia ani* seem to belong to this group. Many otherwise hereditary congenital malformations may be induced as phenocopies by the intra-uterine environment.

2. Hereditary as well as environmentally induced malformations show usually a great variation in their phenotypic manifestation. Hereditary morphological and physiological defects are usually caused by recessive autosomal genes. In some cases the dominance of the normal allele may be incomplete, thus making it possible to identify the heterozygotic carriers, e.g. achondroplasia in Dexter cattle. Only a few hereditary biochemical defects are known as yet in farm animals but many more will probably be revealed when the search for such defects is intensified.

3. The efficiency of mass selection against recessive deleterious genes is a very slow process when the frequency of the genes in the population is low, and mating tests of suspected male carriers may therefore be indicated. The most efficient test is to mate to known heterozygotes. With artificial insemination no special tests are necessary, because all males will be "automatically" tested as soon as they have produced a few hundred offspring in matings to a random sample of the female population, provided that the recessive gene has reached a frequency of 2 percent, or more. (Table 5).

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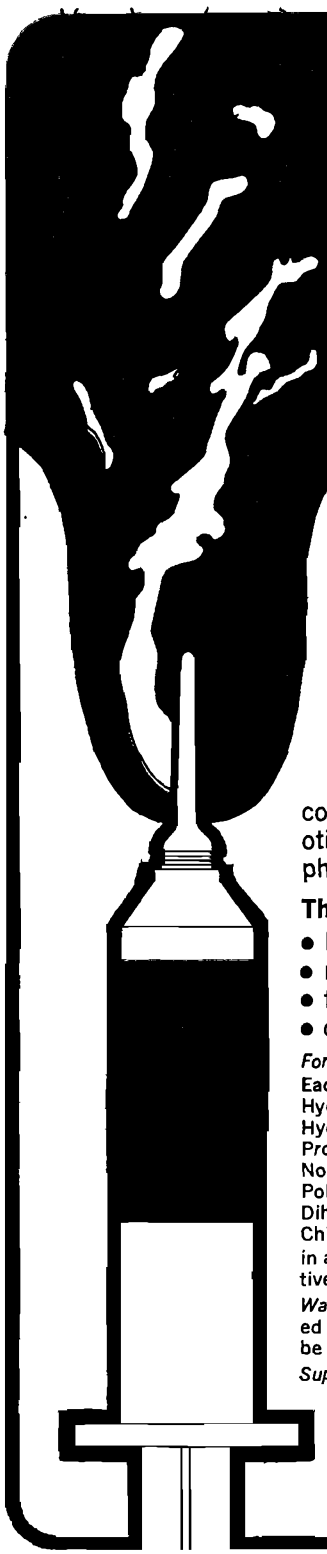

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Formula

Each 10 cc. of the suspension contains:

Hydrocortisone acetate	20 mg.
Hydrocortisone sodium succinate	12.5 mg.
Procaine penicillin G	100,000 I.U.
Nobobiocin (as sodium nobobiocin)	150 mg.
Polymyxin B sulphate	50,000 units
Dihydrostreptomycin base (from the sulphate)	100 mg.
Chlorobutanol anhydrous (chloral deriv.)	50 mg.

in a special bland vehicle that permits maximum dispersion of the active ingredients.

Warning: When used in the treatment of mastitis, milk taken from treated animals within 72 hours (6 milkings) after latest treatment must not be used for food or marketed for cheese making.

Supplied: In 10 cc. single-dose plastet with mastitis tip

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Jl S. Afr. vet. med. Ass. 40 (2): 201-206 1969

THE INCIDENCE OF SALMONELLA IN THE ABATTOIR AND SOME BUTCHER SHOPS OF PRETORIA

V. RISLAKKI*

SUMMARY

The incidence of salmonellas in meat and edible offal in the municipal abattoir and some retail butcher's shops situated within the Pretoria city area was studied.

The results revealed that: 1) Only a few of the samples collected during the process of slaugtering contained salmonellas. 2) In samples taken after slaughtering from offals and floor washings, salmonellas were found relatively frequently (14.8%). 3) In butcher's shops salmonellas were most frequently isolated from blocks, minced meat and offals. 4) Salmonellas belonging to 21 serogroups were isolated from 34 samples out of 458 taken (7.4%).

INTRODUCTION

Species of Salmonella of animal origin are responsible for cases of salmonellosis in humans, occasionally developing into very serious and extensive outbreaks. In Sweden, for example, an outbreak involving 8845 persons occurred in 1953[1]. These infections nowadays involve an ever increasing number of consumers due to the increased output of slaughter-houses, food processing plants and butcheries. Meat products and other foodstuffs can be contaminated from the sick or latently infected animal acting as the principal reservoir or by personnel who are chronic carriers.

Exhaustive information on the infections caused by salmonellas is not usually easy to obtain. Routine laboratory examinations and periodic special investigations, however, are providing details of the situation and making it possible to take effective preventive measures.

Statistical information from both medical and veterinary sources is available regarding the salmonella situation in South Africa. From this it is clear that the infection is still very common and the number of the serotypes isolated is rather numerous[2-7].

Unpublished data at the Veterinary Research Institute, Onderstepoort, reveal that 412 isolations of 38 different Salmonella species were made from cattle and pigs between 1961 and 1967. Of these nearly half (202) were *S. dublin*. Other common serotypes were *S. typhimurium* (45), *S. isangi* (18), *S. chester* (14), *S. caledon* (11) and *S. infantis* (10).

The present study concerns the occurrence of salmonellas in the municipal slaughterhouse and retail butcher shops in Pretoria.

In this report, the term "offal" refers to portions of the gastro-intestinal tract of ruminants.

MATERIAL AND METHODS

The bacteriological examinations were performed in the Section Bacteriology, Veterinary Research Institute, Onderstepoort.

The investigation was divided into three parts.

Part I: Specimens taken in the Pretoria municipal slaughterhouse during the process of slaughtering (Table 1).

Part II: Specimens taken in the slaughterhouse after slaughtering. These samples were mostly from offals intended to be sold as food (Table 2).

Part III: Specimens taken from butcher shops situated in areas occupied by Bantu, Coloureds, Indians and whites (Table 3).

Samples were taken twice a week in sterile 50 ml glass flasks with ground-in stoppers in the forenoon and delivered to the laboratory, where the examination usually proceeded immediately.

All samples from the slaughterhouse were of porcine or bovine origin. The species of Salmonella isolated and the number of samples can be seen from Tables 1 and 2.

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This investigation was carried out during 1967-68 by the author at the Veterinary Research Institute, Onderstepoort, Republic of South Africa. The investigation was supported by a grant from the "Nordiska Afrika Institutet".

Samples from Pretoria retail butcheries were obtained from 15 butcher shops in areas for nonwhites (=33% of the total number of 45 shops), and from 28 butcher shops in districts occupied by whites (=14% of the total of 205 shops).

The samples were inoculated (a) directly onto MacConkey agar and (b) into brilliant green selenite F enrichment broth. After incubation for 20 hours at 37°C the MacConkey agar plates were inspected and suspect colonies subcultured onto bromthymol blue lactose sucrose agar. Loopfuls of the enrichment broth were streaked onto SS agar, from which suspect colonies were eventually subcultured onto bromthymol blue lactose sucrose agar. The subcultures were then used for biochemical tests and serological typing.

The isolates were tested biochemically in the following substrates: Nitrate-agar Difco (4 days at 37°C), glucose, lactose, dulcitol, sucrose, maltose, inositol, indol, phenyl alanine, urea, malonate, MR, KCN and TSI.

Serological typing was performed on object glass slides with sera from the Veterinary Research Institute, Onderstepoort.

RESULTS

The results are presented in Tables 1—5.

Table 1 PART I: SAMPLES TAKEN AT SLAUGHTER

Specimen	Number	Positive for *Salmonella*	Salmonella isolated
1. Bovine bile	15	—	—
2. *Bovine mesenteric* L. nodes	15	3	**S. chester** (d+s) **S. dublin** (s) **S. kingston** (s)
3. Bovine carcass* washing water	15	—	—
4. Bovine muscle**	15	—	—
5. Calf bile	15	—	—
6. Pig bile	15	—	—
7. Pig mesenteric L. nodes	15	—	—
8. Pig carcass* washing water	15	—	—
9. Pig muscle**	15	1	**S. newport** (d+s)
	Total 135	4=3,0%	

(d) = results obtained directly from MacConkey agar.
(s) = results obtained after enrichment in brilliant green selenite F.
* = direct from hose nozzle.
** = from **M. triceps brachii** (exposed by routine incision for cysticecosis examination) after carcass washing.

Table 2 PART II: SAMPLES TAKEN AFTER SLAUGHTERING INSIDE THE SLAUGTERHOUSE

	Specimen	Number	Positive for Salmonella	Salmonella isolated
1.	Floor water-offal washing room, near drain	18	2	**S. humber** (s) **S. gambaga** (s)
2.	Floor water—beef dressing hall	18	1	**S. loanda** (d)
3.	Floor water—pig dressing hall	18	2	**S. chester** (s) **S. gwaai** (s)
4.	Cattle offal with contents of intestine	18	2	**S. newlands** (s) **S. derby** (s)
5.	Calf offal with contents† of intestine	18	2	**S. saint-paul** (s) **S. dublin** (s)
6.	Pig offal with contents of intestine	18	7	**S. chester** (s) **S. chester** (s) **S. typhimurium** (s) **S. gwaai** (d+s) **S. saint-paul** (s) **S. saint-paul** (d+s) **S. newlands** (s)
		Total 108	16=14.8%	

(d) and (s) = Table 1.

† = some specimens were from condemned intestines showing evidence of enteritis.

Table 3 PART III: SAMPLES TAKEN FROM PRETORIA BUTCHER SHOPS

Specimen	Number	Positive for Salmonella	Salmonella isolated	
Group A				
1. Minced meat	15	2	**S. kimuenza**	(s)
			S. langenhorn	(s)
2. Offal	15	—	—	
3. Blocks	15	2	**S. mim**	(s)
			S. chagoua	(s)
4. Axes, saws, knives	15	1	**S. cerro**	(s)
5. Floor	15	—	—	
Total	75		5=6.7%	
Group B				
1. Minced meat	28	2	**S. chagoona**	(s)
			S. gwaai	(d)
2. Offal	28	3	**S. typhimurium**	(s)
			S. wandsworth	(d)
			S. anatum	(d)
3. Blocks	28	3	**S. umbilo**	(s)
			S. typhimurium	(s)
			S. paratyphi A, var. Durazzo	(d)
4. Axes, saws, knives	28	1	**S. anatum**	(d)
5. Floor	28	—	—	
Total	140		9=6.4%	
Grand Total	215		14=6.5%	

A: 14 butcher shops in areas for non-whites.
B: 28 butcher shops in areas for whites.
(d) & (s)=Table1.

(1) Only a few (3%) of the samples taken during the process of slaughtering contained salmonellas. (*Part I*, Table 1).

On the other hand, samples taken after slaughtering from offals, and from bovine, pig and offal washwater rather frequently contained salmonellas (14.8%). (*Part II*, Table 2).

(2) The separately recorded results from the butcher shops situated in areas for non-whites and whites, however, indicate that there was no appreciable difference between the frequency of salmonella isolations (6.7% and 6.4% respectively) (*Part III*, Table 3).

(3) The butcher shop blocks most frequently yielded salmonellas. Minced meat and offals were also relatively often contaminated. (Table 3).

(4) The isolation of *S. parathyphi* A leads one to suspect that one or more of the employees might have been a chronic carrier in the butcher shop.

(5) The number of Salmonella species isolated and the distribution of the various serogroups is shown in Table 4.

Table 4—SEROGROUPING OF SALMONELLA ISOLATED FROM 458 SAMPLES FROM THE ABATTOIR AND SOME BUTCHER SHOPS IN THE PRETORIA AREA

Serogroup	Type	Number
A	**S. parathyphi A,** var. Durazzo 2, 12 : a : —	1
B	**S. saint-paul** 1, 4, 5, 12 : e, h : 1, 2	3
	S. chester 4, 5, 12 : e, h: e, n, x	4
	S. derby 1, 4, 5, 12 : f, g : —	1
	S. kingston 4, 12, 27 : g, s, t : —	1
	S. typhimurium 1, 4, 5, 12 : i : 1, 2	3
	S. kimuenza 1, 4, 12, 27 : 1, v : 1, 5	1
C_2	**S. newport** 6, 8 : e, h : 1, 2	1
	S. loanda 6, 8 : 1, v : 1, 5	1
D_1	**S. dublin** 1, 9, 12 : g, p : —	2
E_1	**S. anatum** 3, 10 : e, h : 1, 6	2
	S. newlands 3, 10 : e, h : 1, 7	2
G_1	**S. mim** 13, 22 : a : 1, 6	1
G_2	**S. chagoua** 1, 13, 23 : a : 1, 5	2
K	**S. langenhorn** 18 : m, t : —	1
	S. cerro 18 : Z_4, Z_{23}	1
L	**S. gwaai** 21 : Z_4, Z_{24}	3
	S. gambaga 21 : Z_{35} : e, n, Z_{15}	1
M	**S. umbilo** 28 : Z_{10} : e, n, x	1
Q	**S. wandsworth** 39 : b : 1, 2	1
53	**S. humber** 53 : Z_4, Z_{24}	1
		34=7.4%

DISCUSSION

Investigation and statistical data [2-7], show clearly that salmonelloses in South Africa still constitute a serious public health problem as well as being a frequently occurring zoonoses.

Brede has stated that it can almost be compared to tuberculosis (*"les salmonelloses présentent un importance très grand. En tant que problème sanitaire, elles suivent immédiament la tuberculose"*). In approaching this problem it would seem sensible to examine the frequency of salmonellas in slaughter animals, slaughterhouses and butcher's shops more closely. Important information may thus be obtained concerning the latent infections in domestic animals, and the isolated serotypes could then be compared with those isolated from man in order to elucidate the epidemiology of the disease. In addition, some insight could be obtained regarding the role of persons employed in slaughterhouses and butcher's shops.

ACKNOWLEDGEMENTS

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Finally, I am indebted to Dr. L. W. van den Heever, Faculty of Veterinary Science, University of Pretoria for suggestions and for revision of the manuscript.

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BOOK REVIEW

TIERÄRZTLICHE OPERATIONSLEHRE

E. Berge and M. Westhues

Verlag Paul Parey. Berlin and Hamburg: 29th Ed. 1969. pp. xvi+411, Figs. 337. Price not stated.

This very well known work on operative surgery has reached its 29th edition—an adaquate testimony to its general acceptance. The 27th and 28th edition as well as the English translation of the 28th edition have been reviewed in this Journal. It is therefore not proposed to repeat the process in detail. The present edition follows the pattern of its predecessor. Operations which have been superseded have been deleted or referred to very briefly while newer operations and techniques are included. Regarding the latter no attempt has been made to include "all" techniques but only those tried out extensively. The book is thus keeping pace with established surgical practice.

The format has been enlarged slightly. The quality of paper, printing and illustrations are as good as previously.

C. F. B. H.

Jl S. Afr. vet. med. Ass. 40 (2): 207-226 1969

THE SIR ARNOLD THEILER MEMORIAL LECTURE, 1968

HUGH. MCL. GORDON*

INTRODUCTION

May I first express my very great appreciation of the great honour conferred on me by the invitation to give this lecture in memory of Sir Arnold Theiler. This is the greatest honour of my career as a veterinarian.

Arnold Theiler was born on 26th March, 1867 at Frick, a peaceful and beautiful village in Aargau in Switzerland. He became a doyen among veterinarians and farmers. His memorial at Onderstepoort is a reminder of his work and his love. He looks outward still for there is much yet to be done.

Let me begin with an invocation and as you read it think of the man and his works as we again pay homage to his memory — "to our common enterprise. To the insatiate taskmaster who is ever exacting the best, last ounce of effort or the longest hour of drudgery, yet whose wage is of the highest, though he pays neither in riches or contentment, only a craving that can never be satisfied, an unrest that will not be stilled ... To the goal of our endeavour, though the mists obscure it, though we know not even if that we follow be the call of truth itself or but the yearning of our own restless quest... To the god of things hidden, of questions unanswered, of mysteries unrevealed" (Clark, 1915).

"Let us now praise famous men, and our fathers that begat us". Let us recall Sir Arnold Theiler, a famous man and deserving of praise, a father among veterinarians — his offspring the seeds of inspiration and encouragement sown not only here in this well-loved country of his adoption, but throughout the world of veterinary science. My first meeting with him was on 21st June, 1928 when I was in my second year in Veterinary Science. These were difficult times and Australia, with a great part of the world, was entering the "Depression" — dark days when one wondered about the future of the profession one hoped to attain. Sir Arnold talked to us of his work in South Africa. He gave us inspiration and encouragement, he made us feel that we were part of the greatest profession in the world. He gave us faith in ourselves and our profession. In him we saw the "goal of our endeavour", in us he created "an unrest that will not be stilled".

He talked to us again in 1934, with the same energy and stimulation as in 1928. By 1934 I had tasted a little of the bittersweet of research with its disappointments and triumphs. Sir Arnold had words of comfort and encouragement for us all. At that time there was parrallel work on the oesophageal groove reflex in progress here at Onderstepoort and at the McMaster Laboratory — we did not know that Clunies Ross and Mönnig were racing neck and towards a common goal. Clunies Ross is alas no longer with us — how good it is to have his rival, Dr. Mönnig with us to-day.

Sir Arnold Theiler's visit to Australia and New Zealand in 1934 was recorded in the Australian Veterinary Journal for December of that year. Perhaps you would like to hear what is written there — "Veterinarians in New Zealand and New South Wales were delighted recently to welcome Sir Arnold Theiler who, accompanied by Lady Theiler, was on his way to South Africa from the United States, where he had attended the International Veterinary Congress.

"In New Zealand Sir Arnold had been particularly interested to gain personal knowledge of certain of the deficiency diseases of those islands, and particularly Bush Sickness. In company with the Chief of the Live Stock Division, Mr. Barry, he travelled extensively.

"Before leaving New Zealand Sir Arnold was informed that he had been elected an Honorary Life Member of the Zealand Veterinary Association.

"Sir Arnold's stay in Sydney was very

*McMaster Laboratory, C.S.I.R.O., Australia.

brief, but he found the opportunity to spend a considerable amount of time at the Glenfield Veterinary Research Station and at the McMaster Animal Health Laboratory. As in New Zealand, his Australian colleagues were delighted to find Sir Arnold's keenness of analytical observation in no way dimmed and profited greatly from an association, unfortunately brief, with a veterinarian who will always rank as one of the most distinguished scientists of a generation.

"Sir Arnold was the guest of the Veterinary Association of New South Wales at their annual dinner on the night before his departure from Sydney. His health was proposed by Professor J. D. Stewart, and the evening was further enlivened by Major C. J. Sanderson's Rabelaisian reminiscences of his association with Sir Arnold Theiler and South Africa in the early years of the century.

"Sir Arnold will reside for some time at Onderstepoort, where he will again take up active research, thus adding to those distinguished contributions to knowledge which he has continued to make since he relinquished his position as Director of that great institution."

Thus time runs on and it has become my privilege to present this lecture in honour of a great man who gave me encouragement and "restless quest" so many years ago.

In thinking about an appropriate subject for this lecture many conflicting aspects arose. Firstly, it was necessary to select something to which I have given a good deal of thought and to which a considerable amount of research has been devoted. Secondly, it was necessary that the subject should be of reasonable general interest. The fascination of and the necessity for specialization is one of the great problems of modern science; and there is the even greater problem of attempting to offset the fragmenting influence of specialization; the necessity for synthesis.

This is the day of the specialist and expert — but the need for the "synoptic vision" is pressing in its urgency; the more so when one takes the point of view of the protagonist of preventive veterinary medicine. This view must dominate our thinking when the health and productivity of flocks and herds are at stake; for it is upon these attributes of husbandry that the prosperity of the grazing industries must depend.

As an apologia one may quote Geertz "... scientific explanation oes not consist as we have been led to imagine, in the reduction of the complex to the simple. Rather it consists in a substitution of a more intelligible complexity for one which is less so. One may go even further and argue that explanation often consists of the substitution of complex pictures for simple ones, while striving to retain persuasive clarity."

Ecology has always fascinated me. A great many years ago I walked in my imagination with Jan Christian Smuts as he strode about his beloved country while the examples of ecology crystallised into the principles of his "holism". I spent 1935, not walking, but riding over the countryside on thirty sheep "stations" in N.S.W. — our sheep man disdains to be called a farmer and his land is not a farm, it is a station! I had to look at pastures and soil and sheep and disease as a whole — and while the call of straight ecology was strong, it was necessary to go beyond this pure concept of the organism and its environment, for here were abnormal changes of all kinds, from invasion by weeds and inferior grasses, to soil erosion and an increasing burden of disease, especially parasitic disease. So ecology became epidemiology — let us not debate whether it should be epidemiology or epizootiology; Wedman (1965) has done this for us. Might we call epidemiology pathological ecology?

We preached learnedly the doctrine that prevention was better than cure but alas we could not practice our precept because our epidemiological knowledge was deficient. Clearly the synthesis of management was essential in the application of preventive veterinary medicine, which so often fails because animal husbandry is poor. One recalled the visit of Sir Arnold Theiler to Australia in 1928 and his introduction in his report (1929) to the Commonwealth of Australia — "Investigations into the problems of research in animal health in Australia have been approached by me in a somewhat wider sense than is usually given in the interpretation of the term health. I consider health to be that condition of an animal which is the most suitable for maximum economic exploitation. This definition has accordingly a purely utilitarian aspect, but, as the principal object of research work from the point of view of the Council for Scientific and Industrial Research is to assist the primary industries, it served as my guide. A non-productive animal is apparently an unhealthy, or better, a diseased

animal. The main object in studying diseases is to find their causes. Once they are found and can be explained, the question of prevention or cure is frequently solved at the same time, which means either removal of the cause or rendering the animal unsuitable for the development of the disease-causing agency".

Theiler's investigations and research reflect this view; he had a "synoptic vision".

From my earliest associations with parasitic diseases in sheep the great variations in incidence and severity from season to season and flock to flock were alike confusing and challenging.

Again Jan Smuts came to the rescue in "Holism and Evolution" with the phrase "transformation of causality". The multiplicity of factors, biological, parasitological, economic, husbandry, meteorological, which are concerned with the epidemiology of helminthosis in sheep may somehow be sublimed in the "synoptic vision" (a term attributed originally to Plato, I believe) inherent in the implications of the "transformation of causality".

This was the genesis of the second reason for the selection of the subject of this lecture. I trust that it will be an overall view, with some interest for most veterinarians.

THE PARASITIC WAY OF LIFE

The helminth parasites of sheep are mostly highly specialised, and have probably had a very long association with their host during evolutionary time. Adjustment to parasitism — which is a biological state, not a disease — was inherent in this association, but a need for adjustment to pathogenic effects probably did not arise because, in general, pathogenic effects depend on numbers, and in the normal ecological relationship the numbers of parasites were rarely excessive. The tremendous biotic potential of the parasite, expressed in manifold ways, indicates that the parasitic way of life was hazardous. There are remarkable adaptations in parasites — concerned chiefly with reproduction. There is the hermaphroditism of most trematodes, and the pudendal propinquity of those in which the sexes are separate (Schistosomes), the enormous egg production by ascarids and *Haemonchus contortus*, the alternatives in the life-cycle of Strongyloides, the multiplication of the trematodes in their intermediate hosts, the "togetherness" of Syngamus, perhaps the "indecency" of Trichosomoides.

There have been many definitions of a parasite (Van Beneden, 1889, Elton, 1927, Hall, 1936, Taylor, 1947). Boughton (1955) says that by definition a parasite is parasitic, i.e. it is taking something from its host — if there are too many parasites we may have parasitic disease. Noble and Noble (1964) come close to an inclusive definition — "invasion with compromise", for this implies the whole range of host-parasite relationship —

the ideal of adaptation

the exquisite compromise with environment

the incompatibility which necessitates epidemiology

the perversion which provokes pathogenicity

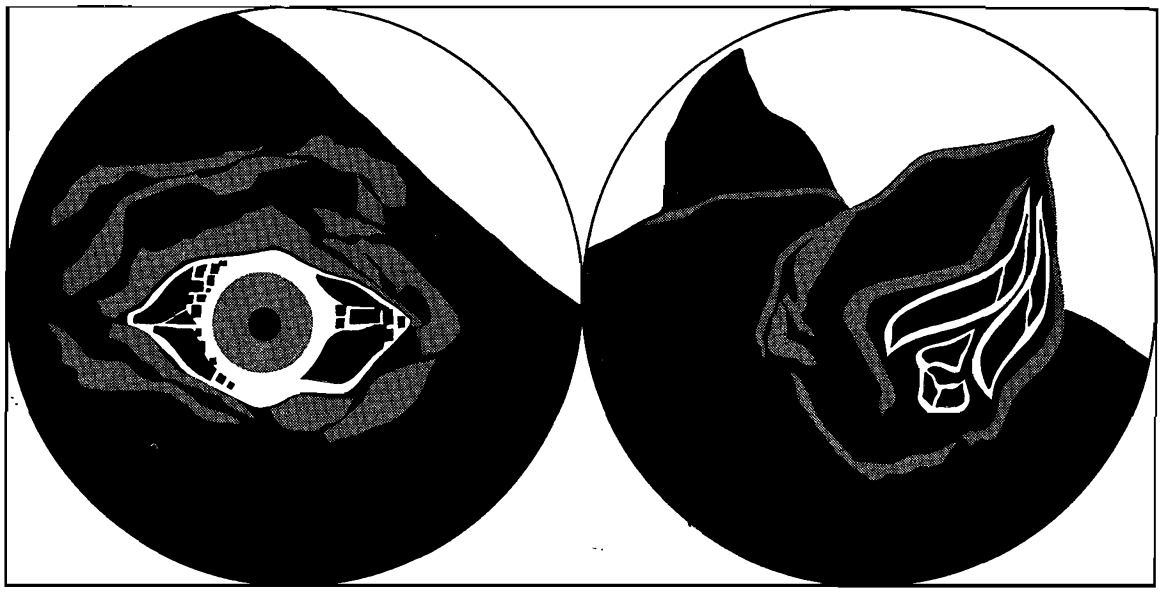
the challenge of control.

From broad definitions one must focus down to special attributes, especially in the consideration of parasitic disease. Despite the tremendous amount of research which has been carried out there is still a persisting misunderstanding about certain aspects of the helminthoses. One might contrast helminths and bacteria as causes of disease. The worm parasites are mostly large and easily recognized and man knew them as inhabitants of his animals and himself from earliest times, but he did not always recognize them as causes of disease — a case of familiarity breeding contempt! There was no mystery about the helminths, but the bacteria clearly were a much more serious menace — fear of the unseen! Further, the concept of disease needed change to accommodate the helminths. Chronic disease, sub-clinical disease, illthrift — these are characteristic of the helminthoses, in contrast to the acute, the fulminating disease due to many microorganisms. Further again, it was hardly possible to measure the economic effects of helminthosis until we had highly effective, non-toxic anthelmintics which enabled us to apply "diagnosis through control" (Gordon, 1967).

EPIDEMIOLOGICAL EXCURSION

The sheer complexicity of the epidemiological background of parasitic disease is almost enough to deter an overall study. Individual components, e.g. the bionomics of the free-living stages or the host/parasite relationship, permit a clear experimental approach with a reasonable restriction of para-

In superficial eye and ear conditions of domestic animals...



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meters. The precise form of the disease may be simple enough, e.g. the anaemia of haemonchosis, but the genesis of an outbreak is usually very complex. J. H. Whitlock and his colleagues (1966) refer to haemonchosis as an "orderly disease", and employ fairly complicated mathematics to prove it so! I am old fashioned enough to think of the epidemiology of the helminthoses as a refined version of natural history — which enables, indeed, compels a very wide view of the interplay of climate, immunology and husbandry.

"These NATURE's works, the curious mind employ,

Inspire a soothing melancholy joy;

As fancy warms, a pleasing kind of pain

Steals o'er the cheek, and thrills the creeping vein!

Each rural sight, each sound, each smell combine;

The tinkling sheep-bell, or the breath of kine;

The new-mown hay that scents the swelling breeze,

Or cottage-chimney smoking through the trees".

(Natural History of Selbourne, Gilbert White).

Always behind my thinking about epidemiology was the need to control disease. This dominating thought goes back to early conditioning as a child growing up among the sheep in New England in northern N.S.W. closely familiar with the ravages of outbreaks of haemonchosis, very conscious of ineffective anthelmintics and the drudgery of drenching. Our training in veterinary science had a very strong emphasis on preventive veterinary medicine — we learnt, as Maurice Hall put it "that parasites must be controlled, rather than that we must accept and adjust to parasitism, and that parasites in general are harmful, and are not either harmless or beneficial". Finally, as a member of a Government research organisation, supported by funds from the sheep and wool industries, there was a clear obligation to the economy of these industries.

So to seek a guide or model, to simplify, to explain, and eventually to direct the attack. The Reed-Frost model, described by Abbey (1952) was based on the following assumptions:

"The infection is spread directly from infected individuals to others by a certain kind of contact (adequate contact) and in no other way.

"Any non-immune individual in the group, after such contact... will develop the infection and will be infectious to others only within the following time period, after which he is immune.

"Each individual has a fixed probability of coming into adequate contact with any other specified individual in the group within one time interval, and this probability is the same for every member of the group.

"The individuals are wholly segregated from others outside the groups.

"These conditions remain constant during the epidemic".

This appeared rather too restrictive, perhaps too precise for the helminthoses. Instead, the Epidemiological Model which emerges from the paper on Generalization of Epidemic Theory: An Application to the Transmission of Ideas, by Goffman and Newill (1964) has been used. This has been modified to the following form.

The necessary elements involved in the process of the spread of infectious disease are those of:—

1. a specified population

2. an exposure to infectious material.

The members of the specified population include:—

a. infectives

b. susceptibles

c. removals.

Exposure to infectious material directs us to the great variety of ways in which infection occurs, e.g. prolonged exposure, sudden, massive exposure.

After exposure (infection) there are several consequences. In general one thinks of:—

1. Transmission and spread of the disease

2. Prediction of the epidemic course

3. Discovery of the threshold densities of

the population that might be passed before an epidemic can develop ("An epidemic occurs when the process of susceptibles being transformed to infectives crosses a certain threshold").

EPIDEMIOLOGICAL MODEL

The epidemiological excursion led to the need for an epidemiological model to provide signposts, landmarks, highway guides and, to stretch the analogy, perhaps highway patrols to keep the traffic in order.

Let us examine the model in more detail and orientate its precepts towards the helminthoses of sheep.

1. Specified Population

What animals are at hazard? Are they young, adult, raised on the farm, newly introduced? If newly introduced, where have they come from, what parasitic diseases might they bring with them, are they highly susceptible to local diseases?

A. Infectives — sources of infection

a. *All sheep* — from the principles of the epidemiology of the helminthic diseases of grazing animals; that every animal is infected and that contamination of the environment, by egg laying, is continuous.

b. *Specific Sheep*

i. Ewes — commonly lightly infected because they have acquired some degree of resistance, but usually continue to pass sufficient worm eggs to ensure infection of their lambs ("susceptibles").

ii. Young sheep — lambs and weaners which have little resistance and acquire early infections which may result in heavy infections later on by the process of "self-augmentation" (Tetley, 1959).

B. Susceptibles

a. *Highly Susceptible*

i. *To all parasites* — lambs and weaners which have not yet developed resistance. These sheep are likely to suffer malnutrition which may delay their acquisition of resistance.

ii. *To certain species* — most sheep remain susceptible to *Haemonchus contortus* and *Fasciola hepatica,* and to a somewhat less extent, *Oesophagostomum columbianum* and *Ostertagia* spp.

b. *Moderately Susceptible*

i. *Young sheep* from one to two years old in which the development of resistance is still in progress.

ii. *Pregnant ewes* in which resistance may decline or even be lost. Further, pregnancy and lactation may cause a latent infection with retarded worms (hypobiosis) to resume development, with serious consequences for the ewes themselves, but especially for their lambs. The ewes may become anaemic (haemonchosis, fasciolosis, hookworm infections), their milk production will then decline and the growth of their lambs will be reduced. The lambs will have to graze sooner and longer and they will then be more likely to acquire heavy infections. They may experience immunological tolerance as part of a chain reaction — susceptible ewe, susceptible lamb, susceptible weaner, and there may be life-time consequences in relation to susceptibility and productivity.

iii. *Introduced sheep* which have come from regions of low rainfall where their experience of helminth infections has not been sufficient to stimulate resistance must be classified among the susceptibles. Even adult sheep may be in this category and from the point of view of control they may require considerable care for their first year in the new environment. Again the introduction of sheep from a region of predominantly summer rainfall to one of predominantly winter rainfall, and vice versa, may expose them to new experiences of infection, e.g. oesophagostomosis, chabertiosis, haemonchosis, ostertagiosis. Movement of sheep during drought is a particular example.

C. Resistant or Immune

Resistant sheep are usually those which have been exposed to experience of infection for a year or more. Older sheep are usually experienced sheep and while age *per se* does not necessarily confer resistance, the older sheep tend to suffer less from the effects of parasitic disease, and develop resistance more rapidly than young sheep. It must be noted that even adult sheep may have little resistance to haemonchosis and fasciolosis. Well fed sheep develop resistance more rapidly than those suffering malnutrition and their resistance is maintained better — but note that a high plane of nutrition does not neces-

sarily prevent sheep becoming heavily infected.

Definitions of immunity and resistance do not always give a clear indication of the precise role of the immune sheep in the flock — "The function of immunity is not the measuring emergency reactions to infection but an understanding and implementation of the mechanisms which result in the preservation of the norm; ... immunology is concerned with the basic function of protection from a wide variety of injurious influences — a physiological problem rather than a priestly cant to ward off satanic visitations" (Francis, 1950).

From the applied view it is essential to differentiate clearly between resistance to the establishment of an infection and resistance to the effects of an infection once is has become established.

Immunity sterilans is rare in helminthology — the immune or resistant sheep commonly continues to harbour a few worms which continue to lay eggs and thus the sheep may be still an "infective".

The immune response by the sheep varies greatly for different species of worms, e.g. resistance to *Trichostrongylus* spp. is usually strong and persistent, but resistance to *Haemonchus contortus* is weak and often breaks down. Immunity is a relative condition; it may break down if the host suffers malnutrition or if it is exposed to a very heavy dose of larvae.

These manifold conditions show that the precise role of the "immune" host as part of the specified population in the epidemiological model is not readily defined. From the control point of view we may be grateful that with the acquisition of resistance the role of the sheep as a susceptible has been reduced — but let us not neglect its persisting role as an infective.

D. Removals

Host animals may be removed, actually, or effectively in an epidemiological sense, from the specified population in several ways:—

a. *Death* — as in a severe outbreak with heavy mortality. Before death the sheep will have been an important infective and was also a susceptible. In a series of outbreaks in Australia in the early 1960's mortalities ranged from 10 to 100 *per cent.*

b. *Disposal* — as when infected animals are sold or slaughtered.

c. *Treatment* — when it is highly effective and results in a tremendous reduction in the contamination rate of pastures represents a removal factor in the population. Thus in considering a recently treated flock as the "specified population" it might be considered as removed temporarily from the epidemiological scene.

If the principle of "the safe residual worm burden" is applied, by appropriate treatments and management, in control, it might be regarded as an example of "removal" in the epidemiological model.

It should be noted, so great is the biotic potential of many parasites, that anything less than about 90—95% reduction is not likely to have other than a transitory effect in an outbreak.

In one trial the regular monthly treatment of 60% of a flock was insufficient to prevent a 100% mortality in the 40% which represented the controls. In another trial in which 75% of the flock was treated monthly, the remaining 15% of the controls, although there was no mortality, suffered severe illthrift and their productivity was greatly reduced (Gordon, 1963b).

d. *Immunity* — here again it is easy to court confusion if precise definition distracts attention from the realities of health and husbandry. If an immunity sterilans is present, clearly the animal is removed from the epidemiological exhibition — it becomes a bystander. If there is a parasitological premunition, the persisting infection may be benign as far as the health of the host is concerned, but the host is usually still an infective. If we think of the "immune" host as one which, while continuing to harbour a few parasites, will not accept any more, or one in which there is a slow turnover of parasites at a low level, it is a near-removal from the population inasmuch as it is playing little part in the population dynamics of the parasite and is doing nothing to increase the parasite population. Indeed it can be argued that such an individual is playing a useful role — the so-called vacuum cleaner role — in helping to keep the parasite population at a low level by

destroying a high proportion of the infective larvae it may ingest (Taylor, 1957, 1961). From the point of view of control the grazing together of young (susceptible) and older (immune) animals may have merit — as opposed to the common husbandry procedure of grazing the young sheep, e.g. weaners, as a separate flock, thereby concentrating the susceptibles.

If one considers the development or existence of resistance, in the sense of resistance to the effects of the disease, there are several epidemiological consequences which must be kept in mind. Firstly, the host is infected, it is tolerating a worm burden, it is not suffering severely (economically or physically) by virtue of being mature or big enough or sufficiently well-fed to offset the day to day needs of its parasites which perhaps are behaving more like "messmates" than parasites! Secondly, there is a state of balance — but not necessarily a clear equilibrium — between a series of environmental states including the nutritional status of the host and the immunological nexus in that particular host-parasite relationship. Such a state of balance should imply an aphorism with implications for prognostic diagnosis — "stability is but balance, and wisdom lies in masterful administration of the unforeseen" (Bridges). We may then regard the resistant host as outside the epidemiological arena — but only for the time being — we must be prepared for the "unforeseen"; but with the knowledge, born of the experience of epidemics, that host-parasite relations are usually unstable.

We might take these particular aspects of the host-parasite relationship further into the realms of experimental parasitology with Sprent (1959) and his discussion on immunological tolerance, or with Dineen (1963) with his discussions on the threshold levels and how they may be influenced by antigenic disparity between host and parasite and the flow of antigence information, with the implications for the several aspects of the host-parasite relationship, including pathogenesis which may follow a subliminal tolerance of an increased worm burden, and the progressive "resistance" which may develop with successive infections as the threshold level decreases.

Finally, in thinking of immunity as a factor affecting the "removals" in the epidemiological model it is essential to keep in mind that the occurrence and intensity of immunity in a flock is just one more variable in the whole ecological picture; that it is a statistic which, while it may be predictable, can seldom be absolute.

2. Exposureto Infection

Having set out the necessity for specifying the population which may be on exhibition in the ecological arena, it is then necessary to consider the exposure of the population to infection. For the common nematode parasites of sheep exposure is by ingestion of infective larve. The exceptions are Strongyloides and the hookworms which may infect by skin penetration, and Trichuris where the infective egg must be ingested.

The extent of the infection may determine the nature of the subsequent disease depending on whether light, medium, heavy, overwhelming — and there are more subtle variations in the helminthoses. A massive intake of larvae may produce an acute outbreak if all of the parasites mature quickly as in haemonchosis, or if tissue damage is severe as in oesophagostomosis. With other species, e.g. *Ostertagia* spp. intake of a large number of larvae often results in an infection consisting chiefly of retarded worms which may resume development at some, often unpredictable, time many weeks or months in the future.

One thinks of the overwhelming infections with *Nematodirus* spp. which cause disastrous losses in lambs in Great Britain. Here an accumulation of eggs develop *en masse* in the spring. In Australia we see occasionally somewhat similar outbreaks of nematodirosis when the eggs have accumulated during some months of dry weather and mass hatch after good rain. Many of these outbreaks occur in regions of relatively low rainfall, and may affect sheep up to a year old because it may be their first experience of infection. These outbreaks commonly take the stockowner by surprise because they occur in low rainfall regions where helminthosis is not a common disease and because they may occur in older sheep usually regarded as having developed resistance. Some of the original observations on nematodirosis in low rainfall regions were made in the Great Karroo by Ryksen (1939).

Again the form of the exposure to infection may affect the character of the disease.

Acquisition of infective larvae may take place in a short period as in the nematodirosis outbreaks discussed above, or may be an extended process countinuing for many weeks or months. A great many of the helminthoses have an insidious onset and develop so slowly over so many weeks that a diagnosis of helminthosis may never be made and the resulting unthriftiness is attributed to malnutrition or a poor season or other unkind activities of Mother Nature!

Associated with the exposure to infection one thinks of the epidemiological characteristics of the parasitic diseases. There may be a simple infection with a single species, but more often there are mixed infections with a multiplicity of symptoms and difficulties in diagnosis. The nature of the parasitic damage must be envisaged because it may determine the course and consequences of the infection. Recovery from haemonchosis is usually rapid and complete, but the effects of oesophagostomosis may be life-long and cannot be readily alleviated.

The economic consequences of the form of the infection are a special aspect of the epidemiological form of the disease. The actual economic form of the enterprise may influence the epidemiology of parasitic disease, e.g. the introduction of sheep from low to high rainfall regions. In Australia it is common practice to breed first-cross ewes (Border Leicester-Merino) in regions of light rainfall, and take them when ready to be mated at about 12 months old to high rainfall regions to produce prime lambs after mating with Dorset Horn rams. These young ewes have little resistance because they have not had sufficient experience of infection in the low rainfall region where they were bred. They are exposed to infection in their new surroundings, they are subjected to the stresses of pregnancy and lactation, they are expected to produce a rapidly growing lamb. The husbandry of this enterprise predisposes to parasitic disease by dictating the nature of the exposure to infection. Further, in this form of enterprise any setback to the health, and especially the lactation of the ewe and growth and fattening of the lambs is very serious. Merino sheep raised for wool production can withstand a good deal of setback due to malnutrition and helminthosis, without seriously interfering with the economics of the enterprise.

3. After Exposure to Infection

During the past 20 to 30 years there must have been many thousands of experimental infections of sheep with the common worm parasites. The infections have ranged from the daily dose or "trickle" infection, to the massive single dose system, sometimes with millions of larvae. It is little wonder that there is a great variety of opinion about the nature, form and progress of helminthosis in sheep and the development of resistance. Under field conditions the infections acquired by grazing sheep probably partake of all of these variations depending on the epidemiological circumstances, and such variations no doubt account for the differences observed in field outbreaks.

If the administration of larvae is spaced appropriately it is now possible to predict within reasonable limits what will happen — acute infection, chronic infection, immune tolerance, immunological exhaustion, self-cure, hypobiosis, resistance, immunity. In the field all sorts of combinations must occur with tremendous differences between sheep and between occasions. While these variations are largely unpredictable, it must still be a guiding principle in epidemiology — where this is related to the development of control measures — to attempt to forecast what may happen from time to time and to then prescribe preventive action.

While the outcome of the epidemiological situation may not be precisely predictable at any given time, at least it is possible to set out several categories of consequences when a flock has been exposed to infection.

A. An Outbreak of Clinical Disease

This is the simplest situation. It is usually readly diagnosed by faecal examination, symptoms or autopsy. Once diagnosed, treatment is urgent — and the anthelmintic must be highly effective in order to resolve the outbreak. Selection of the anthelmintic depends on precise diagnosis — may it be a selective anthelmintic, e.g. an organic phosphorus drug in haemonchosis, or must it be a wide-spectrum drug in a mixed infection including large bowel parasites. Or — in view of some studies made here in South Africa should one think in terms of an anthelmintic which, while reducing the worm burden so that the clinical disease has been cured, yet leaves

enough worms to retard reinfection? There is indeed much to be thought about in this observation — more studies during outbreaks are essential (Stampa *et al.*, 1968).

Having discussed the simple situation, it is necessary to go back a little to think about the genesis of the outbreak. What are the factors concerned? Time and the form and degree of exposure are the chief components.

a. *Time*

There are a number of important "times" in the flow of an outbreak. The very concept of "outbreak potential" has time factors — how long before an outbreak can develop, given the appropriate situation on day 1? How long will an outbreak continue?

There are times related to the stages and phases of the life-cycle and to the duration of the interval between generations. The interactions between time and climatic conditions are very important components of the epidemiological model, firstly, in determining the developmental periods of the free-living stage and then the survival periods of the infective phase. One thinks of the work of the Dinniks (1958) in Kenya where the developmental period from egg to infective larvae of *Haemonchus contortus* was prolonged well beyond the commonly accepted 7-10 days, due to lower temperatures which while not low enough to kill, were sufficient to retard development.

A knowledge of timing is essential to the understanding of epidemiology and in the application of epidemiological information in control. *Fasciola hepatica* wanders in the liver tissue for many weeks before it reaches the bile ducts and begins to produce eggs to contaminate the environment. The occurrence of chronic fasciolosis is usually dated some time beyond 10 to 12 weeks after exposure to infection. Meanwhile, if the infection has been heavy, one may expect acute fasciolosis. The pre-patent period is about 12 weeks and if infected animals are treated effectively during this period, or removed from pastures which have snail habitats, the epidemiological cycle will be broken. With a knowledge of the location of snail habitats and of the general husbandry of the flock it may be possible to time certain events in order to achieve control of fasciolosis.

A plan may be briefly stated in stages—

i. Treat the flock.

ii. Graze over pastures which have snail habitats.

iii. Remove the flock from these pastures to pastures without snail habitats within 10 weeks. (This precludes contamination of snail habitats from parasites which may have been acquired since treatment.)

iv. Wait a few weeks to allow recently acquired flukes to develop to a stage where they will be readily vulnerable to anthelmintics.

v. Treat the flock and after a week to allow fluke eggs to be cleared from the bile ducts return to the pastures where snail habitats are present.

With such a plan the pastures which harbour snails may be used with reasonable safety and there will not be an increase in the fluke population. Indeed with a rigid plan it may be possible to eliminate the parasite. The timing of events is all important.

b. *Form and Degree of Exposure*

It may be necessary to differentiate between the initial exposure, e.g. the lambs ("susceptibles") when they first begin to graze a pasture which has been contaminated by the ewes ("infectives") and a later exposure due to an increase in the amount of infective material (larvae) which results from the increased rate of contamination by the infected lambs ("self-augmentation" of Tetley, 1959).

The degree of exposure may determine the form of the outbreak — massive infection followed by acute disease; prolonged intake of smaller doses of larvae followed by chronic disease.

The stability of epidemic processes is discussed by Goffman (1966). We need not be carried away by his mathematical treatment, either to a safe haven if we have the necessary mathematical comprehension and perhaps even complacency, or lacking these, to drift in the sea of biological variation which is the reality behind statistics. However, we may use his general concepts. The epidemic process may be in any one of a set of three states at a point in time —

i. *Increasing state:* the change in the rate at which the number of infectives accrues with respect to time is positive,

ii. *Decreasing state:* the change in the rate at which the number of infectives accrues with respect to time is negative, and

iii. *Stable state:* the change in the rate at which the number of infectives accrues with respect to time is zero.

Here we might look back for a moment to remind ourselves of Robert Bridges' comment, "our stability is but balance, and wisdom lies in masterful administration of the unforeseen". Although Bridges was the Poet Laureate most helminth epidemiologists and indeed veterinarians, will "get the message"!

Have we then come "full circle", from the apparent simplicity of a tangible outbreak, as a consequence of exposure to infection, which we can see, diagnose and treat, through the possible variations, biological and mathematical, which after all only reflect the infinite capacity and cunning of our parasite enemy, back again to one of the aspects of the "freedom of necessity" — to find some pattern of behaviour. The pattern may be artificial for we must "go along" with Crofton (1963) — "It is apparent that the relationships between sheep and their parasites are complex. Many interacting forces are at play. Some of these we may not even recognize and others have influences which are difficult to measure and discern. They all contribute to a dynamic system and they cannot be isolated as single factors without changing the complex relationships and destroying the system to which they belong. This is reflected in the account which has been given, for the act of writing imposes a false linearity on a multidimensional complex".

Perhaps we should be duly grateful when our epidemiological model yields a clear-cut outbreak!

B. Unthriftiness

Precise definition of unthriftiness (illthrift) is difficult. Sub-clinical disease is not quite correct. My attempt is that "an unthrifty is one which is producing less than the maximum which is genetically possible". Unthriftiness usually presents difficulties in diagnosis, especially differential diagnosis to isolate and identify the several possible causes which may be operating singly or in combination. "Diagnosis through control" may be the simplest means, expecially when it is necessary to measure the economic effects of the diseases which are present.

A fairly simple example may suffice. In a field trial groups of Merino weaners grazed together; one was treated monthly with a highly effective anthelmintic, one was treated with the same anthelmintic each third month. During the period from 6 to 18 months of age there was little difference in the total weight gain of the two groups, but in the group treated monthly 80% of the sheep weighed 70 lb or more at the end of the trial, while in the group treated only each third month, the figure was 64%. Precisely what these differences might have made in the life-time performance of the sheep remains to be measured, but in the short term if the farmer had offered these sheep for sale the group treated monthly would have commanded a better price, and this, with a smaller "rejection" of the lighter sheep would emphasise the role of unthriftiness as one consequence of exposure to infection.

C. Resistance

The influence of immunity and resistance as factors affecting the "Removals" from the population in the epidemiological model has been discussed earlier. It is necessary to reiterate certain aspects because these phenomena are manifest as one of the results of exposure to infection.

The form and duration of the infection influence the development of resistance. The manifestations of resistance are manifold, probably with a continuous series of changes which we break up artificially in order to discern whether patterns exist. Delayed development, slower growth, smaller worms, variations in morphology, reduction and finally inhibition of egg laying, death of the parasite, resistance to reinfection, premunition, self-cure — all may be included in the widest definition of resistance. Among the more specialised manifestations — they might perhaps be termed negative aspects — are immune tolerance and immunological exhaustion. It is not necessary to venture too deeply into the precise experiences of infection which are necessary to evoke these phenomena, but be content to accept them as immunological intrusions into epidemiology which must be recognized as factors in the epidemiological model.

It is necessary to stress again that a clear differentiation is required between resistance to the establishment of an infection (immu-

nity?) and resistance to the persistence and effects of an infection which has already become established (resistance?).

The complex role of nutrition as it may affect the acquisition of infection indirectly, e.g. by regulating grazing behaviour, or may influence the development and persistence of the resistant state, must be recognized and included in the model.

D. Hypobiosis

There are many examples of hypobiosis — temporary cessation of vital physiological processes — among the helminths. Witenberg (1961) points out that "true diapause, which is characterised by a state of obligatory rest for a certain prolonged period, apparently does not occur in parasitic worms. Cases of curtailment or cessation of activity must be regarded as phenomena of adaptation which allow the egg or larva to perform the stage transformation without disturbance, or the adult to survive unfavourable environmental conditions. This kind of cryptobiosis has usually no predetermined limit and may end as soon as conditions become suitable". Witenberg discusses hypobiosis in relation to eggs, free larvae, encysted larvae, resting of nematode larvae in the unborn foetus, histotropic phase of nematode larvae and hypobiosis of worms due to hypobiosis of the host (e.g. trematodes in hibernating snails).

When exposure to infection is followed by hypobiosis a new chain of reactions is initiated which may end many weeks or even months later in an outbreak of disease. There is much to be learnt about hypobiosis in helminthosis.

There is some evidence that the conditions under which the eggs and larvae developed may influence the nature of the infections which may result. The work of the Glasgow team on bovine ostertagiosis is the "classic" case. In the field in south-western Scotland larvae taken in during the spring and summer usually produce the common Type I disease as a heavy infection with mature parasites which have developed in the regular pre-patent period of about three weeks. Larvae ingested from the same pastures in the autumn tend to develop to the late 4th stage and may then remain in this retarded or inhibited (hypobiotic) stage for weeks or even months (Pre-Type II disease), finally resuming development to produce a fulminating Type II ostertagiosis. It has been suggested that exposure of the freeliving stages to cold and to prolonged sunlight may pre-condition the larvae to undergo hypobiosis in the 4th stage in the host (for recent discussions on bovine ostertagiosis see Hotson, 1967).

What the factor or factors may be which trigger the resumption of development of retarded worms present a fascinating series of problems. It appears likely that there will not be one, but many causes and that in due time after much experiment and argument the phenomenon will be neatly incorporated as one of the magnificent manifestations of adaptation in the host-parasite relationship. At present the problems may perhaps be best conveyed in the words of Omar Khayyam —

> "Myself when young did eagerly frequent
> Doctor and Saint, and heard great argument
> About it and about; but evermore
> Came out by the same Door as in I went."

However, there is some experimental work which is beginning to illuminate the scene.

When sheep harbouring infections with *Ostertagia* spp. consisting of adult and retarded worms were treated with an anthelmintic Dunsmore (1963) found that the adults were killed, and that the retarded forms then resumed development. This work was analogous with Gibson's (1953) findings with *Trichonema* spp. in horses. There is a good deal of circumstantial evidence from the field that in ostertagiosis in sheep and cattle removal of adult worms is comomnly a trigger to the resumption of development of hypobiotic forms. In some instances the use of an anthelmintic has appeared to initiate an outbreak of disease of the Type II form described by the Glasgow workers. It should be noted that while most of the modern wide-spectrum anthelmintics are effective against immature worms, they are not necessarily effective against the retarded forms.

Taylor and Michel (1953) have set the phenomenon in perspective when they conclude "that a tendency to become dormant during the larval stage, which is so characteristic a feature of the freeliving larvae of parasitic nematodes and is an essential requirement for their use of intermediate hosts,

is not an uncommon occurrence during their life in the final host. The purpose in each instance is essentially the same, i.e. to carry it through a period in which the environment is unsuited to development, on the ground for instance, when it waits for a suitable host; as a parasite of an intermediate host, where it waits for a suitable final host, or in a resistant final host where inhibited development serves the parasite in enabling in to wait until some depression in the host's state of resistance allows it to grow to maturity".

Later observations suggest strongly that it is not only in a resistant host that inhibition may occur, e.g. Sommerville's (1954) work on *Ostertagia* spp. in sheep. In some instances there well be combinations of circumstances concerned in what eventually appears as a most admirable adaptation.

Perhaps the work of Gibbs (1968) in Canada presents one of the clearest cases. Here the ewes are housed during the winter and there is no reinfection with *Haemonchus contortus*, but the sheep continue to harbour infections with retarded worms. The worms resume development when the ewe begins to lactate after lambing in the spring. Gibbs has been able to stimulate the worms to resume development earlier on in the winter by treating the ewes with prolactin, and he has been able to repeat this even in non-pregnant ewes. Here it is clear that lactation is a signal to the retarded worms. If one looks at these findings epidemiologically, even "logically", it is clear that they provide a fine example of adaptation in its widest sense. There is no advantage for the parasite to be present in the adult egg-laying stage in the ewes during the winter when the eggs will not survive — either because the sheep are housed, or because climatic conditions are fatal. Further the presence of adult worms may have a stimulating effect on the development of resistance. What better than to remain in a retarded, hypobiotic state until the conditions are appropriate for a continuation of the life-cycle, that is, in the spring when climatic conditions are suitable for the development of the eggs and larvae, and moreover, when a new generation of "susceptibles", the lambs, has appeared. What are the "conditioning" factors which determine that the larvae taken in by the ewe in the late summer and autumn will become hypobiotic and virtually hibernate through the winter?

It is probable that there will be found a number of "conditioning" factors, probably each appropriate to the epidemiological circumstances which prevail, but we must beware of expecting to find perfection. If we think for a moment of two opposing situations, one natural, one man-made, it will be clear why we must expect conflicting evidence. Firstly, host and parasite have been associated in adapting during long periods of evolutionary time — perhaps even as Sprent (1959) says, "accordingly it is probably that as the host becomes tolerant to the parasite, so the parasites, while having the host, so to speak at their mercy, have been compelled, in the evolutionary sense, to modify their behaviour so as to ensure, for their own survival, the host's survival." Sprent goes on to say that "the perpetuation of host-parasite association over evolutionary time would seem to require that, just as there has been progressive development in the potential immunological response to a new parasite, there has also occurred a progressive adaptation of the host to the habitual parasites". Here is the situation which as the late J. A. Gilruth used to say concerned "Happy worms in happy hosts".

Secondly, I think of the effects of domestication and husbandry and the movement across the earth of hosts and parasites into new and unforeseen environments. Is it any wonder then that the epidemiology of helminthosis, even the individual helminthoses, is a complex? Such a complex may so exasperate the ecologist that he turns from the host-parasite situation in domestic animals to the wildlife scene as presenting something simpler, not yet wholly muddled up by man! To the epidemiologist the situation presented by the husbandry of domestic animals should be pure stimulus and challenge. If he succumbs to the demands for simplicity he is likely to be caught up in the vast web of immunology. Logically of course this should be the first thought, for here are many abnormalities of association, with husbandery cutting across so many of the old evolutionary patterns. One might even extend this speculation to include the hypothesis by Shad (1966) which proposed "... non-reciprocal cross immunity as one of several mechanisms evolved in the adaptation of a parasite to its environment and limiting the populations of competing parasitic species. Non-reciprocal cross immunity may, therefore, be viewed as a special kind of competitive interaction between two species, which is mediated by a third species,

the host. The hypothesis provides a theoretical framework which may make some cases of non-reciprocal acquired resistance to parasites more compatible with immunological concepts. Furthermore, it provides a basis for understanding the function of antibodies that appear in parasitic infection, but are ineffective against the species eliciting this response."

Where there is some abnormality of association one may discern some weakness of which advantage might be taken to achieve control. When control of disease becomes the primary consideration epidemiological involvement again may turn towards the simple approach with the basic thought being the suppression of the biotic potential of the parasite by whatever means are available. Make use of the knowledge of the life-cycle and immunity and the general principles of epidemiology, but beware of the seductions of acceptance and adjustment to parasitism, which while biologically attractive may be epidemiologically dangerous.

This has been a diversion down a byway — a side-track in the epidemiological excursion — but hypobiosis may well be one of the central facts of certain helminthoses and so must be given a good deal of thought, speculative though some of it may be.

Hypobiosis is listed here as a fourth consequence of exposure to infection. The simple outbreak was formerly the common result but modern management and the newer anthelmintics have largely eliminated it. The insidious erosion of productive potential in unthriftiness is one of the challenges of the pastoral revolution. The use of a modern anthelmintic is often required in order to effect a differential diagnosis. The development of resistance and immunity while a "consummation devoutly to be wished", is yet elusive and defies easy application. Hypobiosis, however mediated in its onset and whatever the release mechanism may be, is an epidemiological complication which makes diagnosis difficult and control confused.

SOME GENERAL PRINCIPLES

The epidemiological model has been discussed as a summary or guide and on the theoretical aspect of epidemiology one might continue to find examples and speculate on their place in helminthosis. With the control of helminthosis as the aim and object of research in epidemiology one must examine some of the more practical and applied aspects.

1. Biological Principles

A. Every sheep is infected

This bears a relationship to diagnosis, for the demonstration of the presence of worms or worms eggs in simply confirming what we know already! Predictive or prognostic diagnosis is required — to tell us what an existing worm burden means in relation to the development of heavier infections on the one hand, or what its significance may be as a current cause of impaired productivity, on the other.

B. Contamination of the environment is continuous — but the intake of larvae is intermittent

This is the general situation for most species, but one thinks of the special examples of *Nematodirus* spp. and *Trichuris* spp. There may be an accumulation of *Nematodirus* spp. eggs over many weeks, followed by mass hatching after rain. In Great Britain there is a parallel case, especially with *N. battus*, but the stimulus to hatch is complex and appears to be related to cold and moisture, then warmth.

Trichuris spp. eggs do not hatch until swallowed by the sheep and it is probable that grazing habits, especially close grazing, determine when infections will be acquired.

The free-living infective larvae must have moisture to enable their movement in the vegetation. Commonly rain is required before heavy infections are acquired.

C. Life-cycle

There is a pattern with variations, each having specific epidemiological significance, e.g. the tremendous egg production by *Haemonchus contortus* which offsets the high efficiency of anthelmintics, and the relative vulnerability of the free-living stages to climatic conditions.

The life-cycle may be divided into stages and phases in order to discern the pattern, which may then be differentiated according to species. The parasitic stage has many variations — duration, migration and hypobiosis all influence the epidemiology of the disease. The intervention of an intermediate host adds further stages and phases to the life cycle. The free-living stage is equally various if one thinks of the vulnerability of *Haemonchus contortus* and *Oesophagostomum*

columbianum, and of the resistance of *Nematodirus* spp. and *Trichuris* spp.

One may regard the parasitic and free-living stages as static, against which the attack may be static (strategic), while the contamination phase and the infection phase are dynamic and against them an attack must also be dynamic (tactical).

Keep in mind the "natural" controls — resistance and immunity against the parasitic stage, climatic conditions against the free-living stage, and contrast them with the managerial, man-made controls.

It is always tempting at this point to sheer away from epidemiology and plunge into the practicalities of control measures — the excuse being evident that the planning of control must be derived from the knowledge of epidemiology.

2. Economic Principles

(with acknowledgements to Boughton, 1957)

A. Parasites are parasitic

By definition this is so and we must conclude that, for the domestic animals and man, parasites are not harmless or beneficial.

B. Parasitic disease is related to the number of parasites, and damage produced. Are there any non-pathogenic parasites? It may be necessary to "weigh and measure" in order to detect economic loss, or to apply "diagnosis through control".

For an example of a parasite sometimes dismissed as of little consequence consider *Trichuris* spp. A heavy infection with 500 worms in a young sheep produced severe lesions in the caecum, reminiscent of Johne's Disease, and prolonged the diarrhoea and delayed the recovery in a flock treated with an anthelmintic which was very effective against other species.

C. Parasitic disease affects the whole flock

Every animal is infected, all are exposed to infection, overall productivity is impaired, and treatment and control must be applied to every sheep. One consequence of this principle is the necessity for safe, non-toxic anthelmintics which must often be used to treat "healthy" sheep, and for reasonably cheap anthelmintics

3. Ecological Principles

These are principles which may influence the stages and phases of the life-cycle in the very broadest sense.

A. Meteorological conditions

The development, survival and accessibility of the infective larvae are related to moisture, temperature and shelter. The climograph (bioclimatograph) for a region and the known optimum conditions for the development of the free-living stage may enable an approximate prediction of the geographical and seasonal occurrence of certain helminthoses (Gordon, 1963a). A sheepman, Loneragan (1964, 1966), has applied this knowledge, combined with an estimate of the significance of worm egg counts made at a given time as an indication of the possible rate of increase of the parasite population, in order to determine whether preventive anthelmintic treatment is required.

It must be remembered that while there are optimum conditions for the development and survival of the free-living stages in faeces, soil and herbage, development may proceed slowly under sub-optimal conditions, and provided these conditions are not lethal, the life-cycle can be completed. This accounts for the presence of certain species in sheep far from the regions where the disease produced by the species is endemic. It also explains the occasional outbreak in regions not commonly regarded as climatologically suitable for the parasite.

Recent studies on the bionomics of the free-living stages (Donald, 1968) indicate that the persistence of infective larvae in pastures may be a good deal longer than is usually accepted, and that the maximum larval population in the herbage may not be attained for many weeks after the deposition of the eggs. The precise effects of climate and shelter (depending on the nature of the sward), and the amount of herbage, on the number of larvae which persist and become accessible to grazing sheep are being studied anew under the conditions which have developed as a consequence of the pastoral revolution — dense sward with a heavy "mat", and intensive grazing.

The results of the earlier observations cast doubts on the value of resting pastures and rotational grazing as a control measure,

unless the resting period is at least two months. However, it must be stressed that management in the control of parasitic disease is a compromise. Flock and pasture management must have as the primary objectives the optimum productive growth of the herbage and the best nutritional conditions for the sheep, only incidentally contributing to the control of parasitic disease. Modern anthelmintics permit such a compromise.

B. Pastures

The sward provides a microclimate for the free-living stages which may be very different from the climate measured by conventional means. A dense sward of sown pasture provides much more protection against desiccation than a sparse growth of native pasture. Further, there may be a tremendous dilution of larvae as the herbage grows. If a rapid growth of herbage is achieved by a system of resting pastures the dilution of larvae may well be a useful contribution towards reducing the intake of larvae — providing of course that the sheep do not graze the pasture down to a low level very quickly.

C. Host-parasite relations

Cross infections with certain species may occur between sheep and cattle, particularly with *Fasciola hepatica, Haemonchus* spp., *Trichostrongylus axei,* and probably *Nematodirus* spp. One consequence of the pastoral revolution has been the grazing together of sheep and cattle to a greater extent than was formerly possible. The precise epidemiological consequences of cross-infections have not been studied in the field.

There are interactions between species in the same host, often manifested by self-cure (Stewart, 1955, Gordon, 1968). Does the repeated intake of larvae of *Haemonchus contortus* in the field interfere with the development of infections with *Ostertagia* spp. and *Trichostrongylus spp.*? There is an interesting field for epidemiological study here. The use of 2,6-diiodo-4-nitrophenol to prevent the development of *H. contortus* may be a useful technique in such studies.

D. Special significance of the dung-pat of cattle

Eggs and larvae develop and survive in the protection of the dung pat and there may be mass releases after rain. Whether there is a similar situation in the dung-pat of sheep remains to be determined. This form of sheep faeces in becoming common when sheep graze sown pastures — a great contrast to the usual pellet form.

E. Nutritional conditions

There may be multiple effects on the epidemiology of helminthosis, from the direct effect of adequate nutrition supporting resistance, to the indirect effects which may regulate grazing habits.

4. Emergent Principles

These principles are more closely concerned with the control of disease. They are derived from the basic lines of flow in the epidemiological model, they bear a close relationship with the stages and phases of the life-cycle, perhaps they bring us back to reality!

A. Seasonal patterns of disease

The pattern may be imposed basically by climatic influences, modified by husbandry practices. Thus lambing in the spring or early autumn may coincide with climate conditions favourable for the free-living stages of *Haemonchus contortus.* Again the winter housing of ewes in Canada adds a premium to the hypobiotic state of *Haemonchus contortus,* persisting, not provoking reaction, but reactivated by lactation in the spring.

B. Application in control

A regular pattern in the occurrence of disease enables a strategic approach, an irregular pattern requires a tactical approach. The strategic approach may obviate the necessity for precise diagnosis. A tactical approach may demand a diagnosis — but epidemiological information which indicates and indentifies the predisposing circumstances may reduce the needs for a "clinical" diagnosis. A periodical preparation of "An Appreciation of the Situation" may be adequate guidance for the application of control measures.

C. Control through management is compromise

Animal and pasture management must be coordinated to achieve optimum productivity, but this may not necessarily provide control of parasitic disease. Modern anthelmintics, applied epidemiologically, enable a compromise.

D. Seven uses of anthelmintics

Curative use ("economic salvage in emergencies") is necessitated by failure of control, either through human frailty or due to the tremendous biotic potential of the parasites. Strategic and tactical use prevent parasitic infection becoming parasitic disease. Diagnosnostic use ("diagnosis through control") may be applied to measure the economic importance of parasitic disease and to isolate and identify the causes of unthriftiness. Immunological use may enable a regulation of the host's experience of infection to avoid immune tolerance and immunological exhaustion, and even to stimulate the immune mechanism. Ecological use is experimental to modify and regulate the general picture of the host-parasite relationship. Special or exceptional use applies the special qualities of an anthelmintic, e.g. the effects of small daily doses of phenothiazine on the eggs and larvae in the faeces.

CONCLUSION

Epidemiology is a complex — the epidemiology of helminthosis of sheep is very complex. An epidemic develops when "the process of susceptibles being transformed to infectives crosses a certain threshold". The interactions of climate, husbandry and the biology of the parasites and their host provide a fine challenge to all who are concerned with sheep, from the breeder who must pursue his ideal economically, the shepherd who must apply control effectively, the extension officer who must prepare a continuous "appreciation of the situation", the veterinarian who must devise the means for the control of the disease, to the helminthologist who must go back to basic principles every now and then to uncover and define more clearly the peculiarities of the parasitic way of life.

The epidemiological model — an "idealization of the real situation in which the complex process is reduced to its essential properties" — was introduced as a guide.

The broad approach to the problem, an example of the application of the "holism" of Smuts, especially the "transformation of causality" and of the concept by Theiler of the non-productive animal being an unhealthy or diseased animal, was aimed to generalise in the hope that most would gain something of interest, that some would perhaps glean in the content of the words of Szent-Györgyi, "discovery consists of seeing what everybody has seen and thinking what nobody has thought."

It has been a very great honour and privilege to have been able to give this lecture as a tribute to the life and work of Sir Arnold Theiler, lovingly described by his daughter as "a simple veterinarian with friends the world over."

NOTES

In seeking inspiration and instruction to enable me to prepare this lecture I read many things, including Ecclesiasticus in the Apocrypha; Jan Christian Smuts (written by his son); Smuts: Study for a Portrait (Smuts Memorial Lecture by W. K. Hancock); Smuts' Presidential Address to the British Association in 1931, The Scientific World Picture of To-day (Nature 128 : 521-529); Commando (Deneys Reitz); Holism and Evolution (Smuts, 1926); The Natural History of Selbourne (Gilbert White); Life and Work of Sir Arnold Theiler (P. J. du Toit and Cecil Jackson, 1936, in Jl. Sth. Afr. vet. med. Ass. 7 : 135-177); Dr. Gertrud Theiler's manuscript of her monograph on her father's life, and the account of the unveiling of the Sir Arnold Theiler Memorial by General J. C. Smuts (Jl. Sth Afr. vet. med. Ass. 11 : 1-2, 1940).

Information on the use of 2,6-diiodo-4-nitrophenol for the prevention of reinfection of sheep by *Haemonchus contortus* came from a personal communication from Dr. Claudia Alves da Rocha. A paper was presented at the Brazilian Veterinary Congress in 1967.

SLIDES

During the lecture a series of slides was shown to illustrate, perhaps illuminate, some aspects of the discussion. These included colour slides of the village of Frick in Aargau (kindly provided by Hans Suter of CIBA), of representative country scenes in Australia to show regions visited by Theiler during his visit in 1928, and a "Peaceful Scene" of sheep and pastures to indicate the need for the "synoptic vision" and for thinking of the "transformation of causality". Other slides were concerned with population dynamics and parasitic disease, a tabulation of the special attributes of parasitic diseases due to helminths, the "epidemiological model", the safe residual worm burden, an outbreak of haemonchosis, measurement of the effects of helminthosis as a contributing cause of unthriftiness, stages and phases of the life-cycle and climographs.

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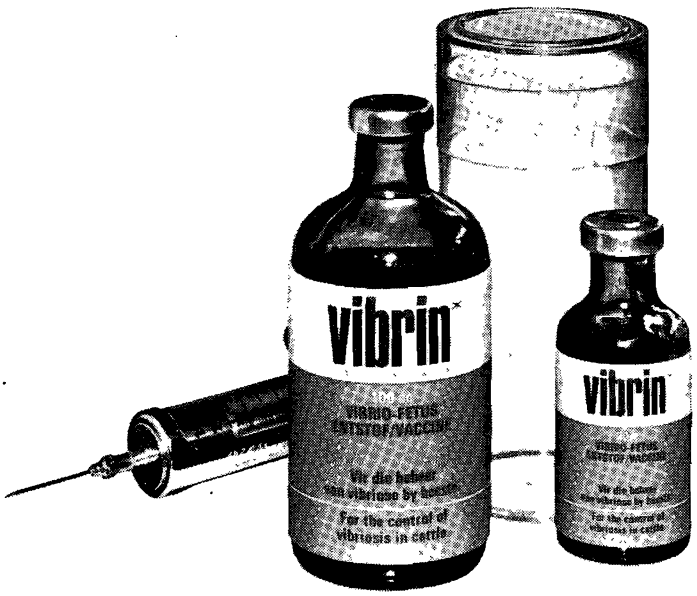

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BOOK REVIEW

VETERINARY MEDICINE

D. C. BLOOD AND J. A. HENDERSON

London: Bailiére, Tindall & Cassell. Third Edition ,1968. pp. xi, 899; Tabs. 22. Price 110s. nett.

The authors of Veterinary Medicine are to be congratulated on the appearance of a third revised edition only eight years after the first publication. This alone is an indication of the immense popularity of this text book which is regarded as the most comprehensive publication on diseases of the large domestic animals in the Western world.

The book has been redesigned and reset in a new format which, despite an increase of ten per cent in the amount of material presented, has made possible the production of a less cumbersome volume than the previous editions.

The plan of the book remains the same; the first part deals with the general principles of diseases and the second part discusses specific diseases and conditions. The thorough revision is particularly evident in the second part where pertinent new information has been added to the descriptions of most of the disease conditions with selected recent references appearing in the reference lists. New sections appear on Rift Valley fever, inclusion body rhinitis, mucosal disease, colitis-x of horses and oesophagogastric ulceration of pigs.

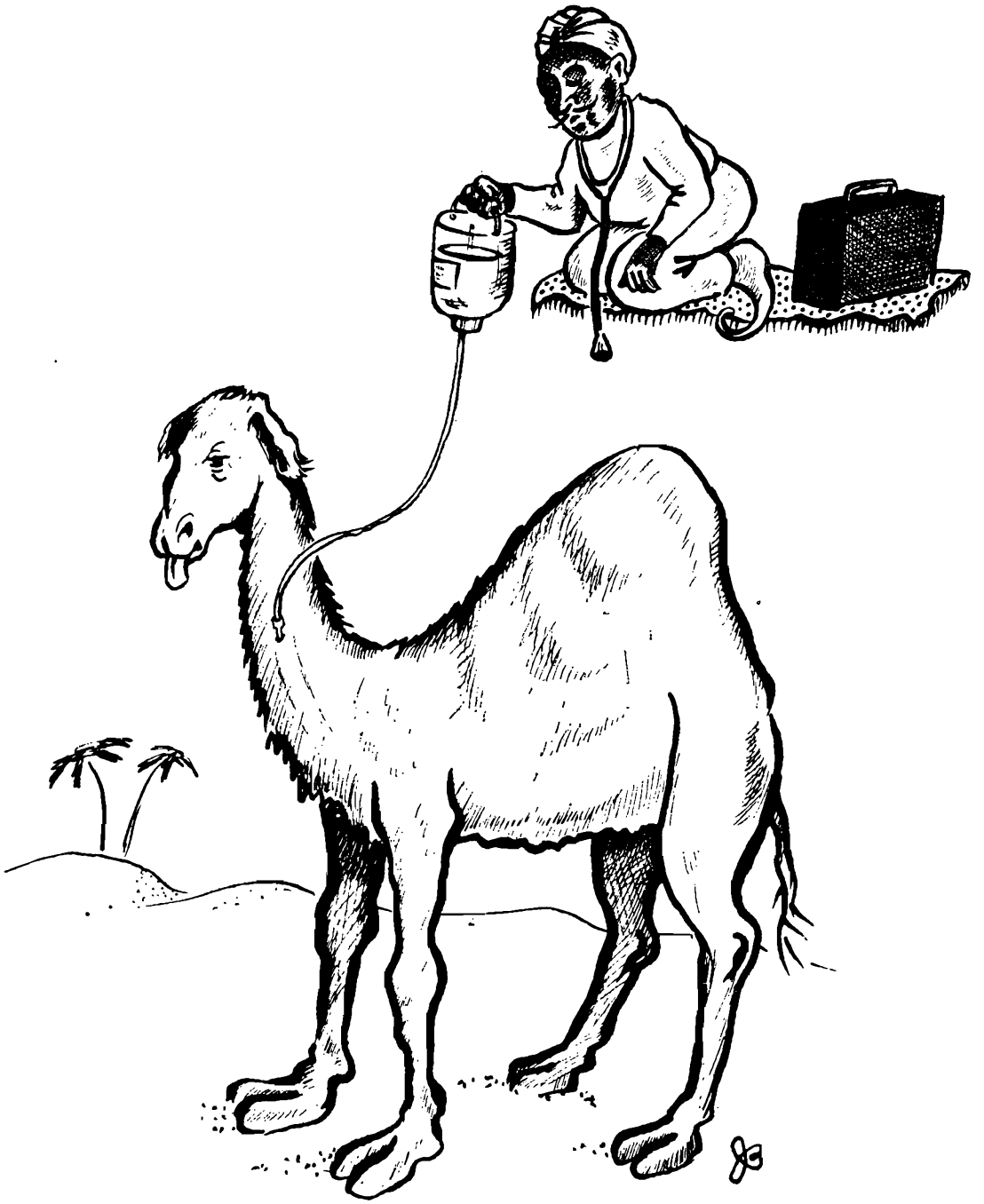
The best recommendation for a well written text book is usually that it should find a place in the personal libraries of students as well as practitioners. This is certainly true of *Blood & Henderson* but its value as a reference volume for teachers of veterinary medicine is equally important. The tables appearing in the text summarise information on aetiological agents, differential diagnoses, levels of minerals, toxic oral doses, etc.—all valuable material which facilitates teaching.

The text covers the whole subject of veterinary medicine with appropriate information on rare and obscure conditions, many of unknown or uncertain aetiology—a great consolation and help to the consultant who often has difficult cases referred to him.

As always the printers have produced a well printed and bound book at a very reasonable price.

K. v. d. W.

Veterinarians around the World 3



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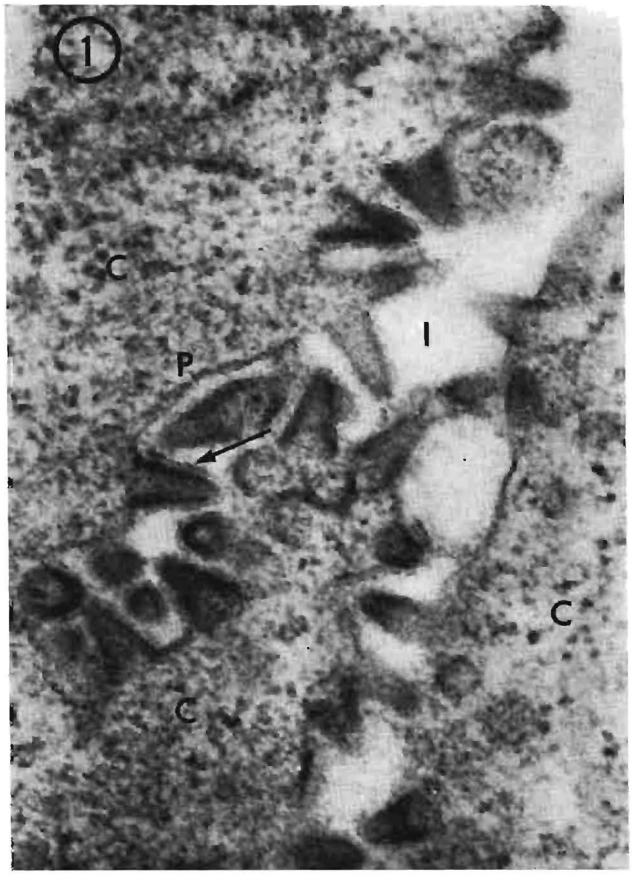

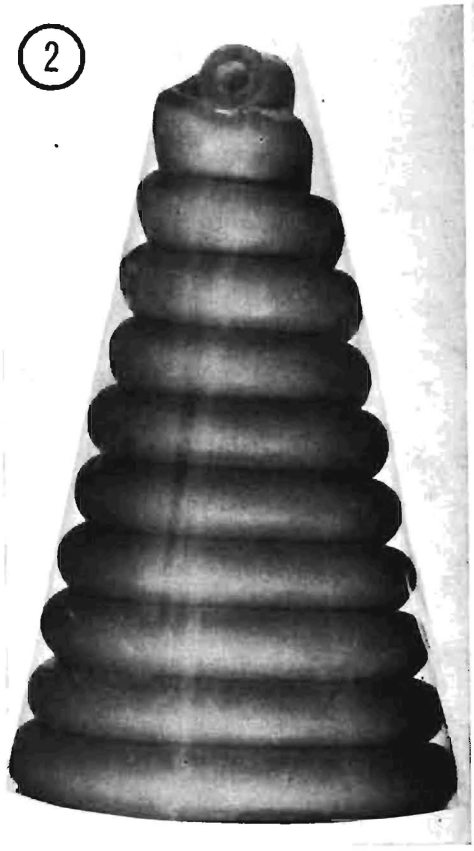

THE STRUCTURE OF BOVINE EPHEMERAL FEVER VIRUS

G. Lecatsas

Molecular Biology Section, Veterinary Research Institute, P.O. Onderstepoort.

Electron microscopic studies have shown that this virus is cone-shaped having a height of 176 $m\mu$ and a basal diameter of 22$m\mu$. Morphologically it bears some resemblance to the Rhabdovirus group (rabies, vesicular stomatitis) of bullet-shaped viruses.

Fig. 1. Cone-shaped viruses apparently emerging from BHK 21 cells grown in tissue culture. Membranous covering of virus particle (arrow) is derived from the cell membrane P. C = Cytoplasm. I = Intercellular space. Magnification: ×80,000.

Fig. 2. Proposed model of bovine ephemeral fever virus: A nucleoprotein spiral with approximately ten turns enclosed in an envelope derived from the host cell membrane.

DIE STRUKTUUR VAN DRIE-DAE-STYWESIEKTE VIRUS

G. Lecatsas

Seksie Molekulêre Biologie, Navorsingsinstituut vir Veaartsenykunde, Pk. Onderstepoort.

Elektromikrospokiese studies toon aan 'n kegelvormige virus met hoogte van 176 $m\mu$ en 'n basis van 88 $m\mu$ in deursnit. Morfologies is daar 'n mate van verwantskap tussen hierdie virus en die koeëlvormige Rabdovirus-groep (hondsdolheid en vesikulêre stomatitis).

Fig. 1. Kegelvormige virusse in weefselkultuur van BHK 21-selle wat blykbaar die sel verlaat. Die virusomhulsel (pyltjie) is afkomstig van die selmembraan P. C = Sitoplasma. I = Intersellulêre spasie. Vergroting: ×80,000.

Fig. 2. Voorgestelde model van kegelvormige drie-daestywesiekte virus: 'n Nukleoproteinspiraal met ongeveer tien draaie, bedek met 'n omhulsel afkomstig van die selmembraan.