follow that the administration of a similar dose at longer intervals would so result.

Very great practical importance would be attached to the discovery of a successful routine treatment which would necessitate the handling of bovines, constantly exposed to infection, only at long intervals and which would involve a comparatively small outlay on drugs during a year.

It is of interest to note here that Professor Browning, of the University of Glasgow forwarded to the Director of this Institution, under dover of a letter dated 2nd September, 1929, a reprint of an article by himself and co-workers (1929) in which the following passage occurred:- "While resistance can be acquired by trypanosomes subjected to increasing doses of the drug (a styryl compound), it is of interest that when in a particular animal each of successive relapses is treated by the same moderate dose, drug resistance does not tend to develop. In fact it has been shown that cure may ultimately be effected by this procedure."

PRELIMINARY EXPERIMENT.

The details of the experiment are tabulated in Table XIV. The arrangement of this experiment was to give 3 gm. doses of antimosan at intervals of four weeks to bovines infected with T.congolense. To control the results, temperatures were taken twice daily and blood smear examinations were carried out three times weekly. By the period of relapse is understood the number of days that elapsed after the last injection of antimosan before trypanosomes reappeared in the blood.

Table XIV;

T.congolense. Long Interval Treatment (28 days).

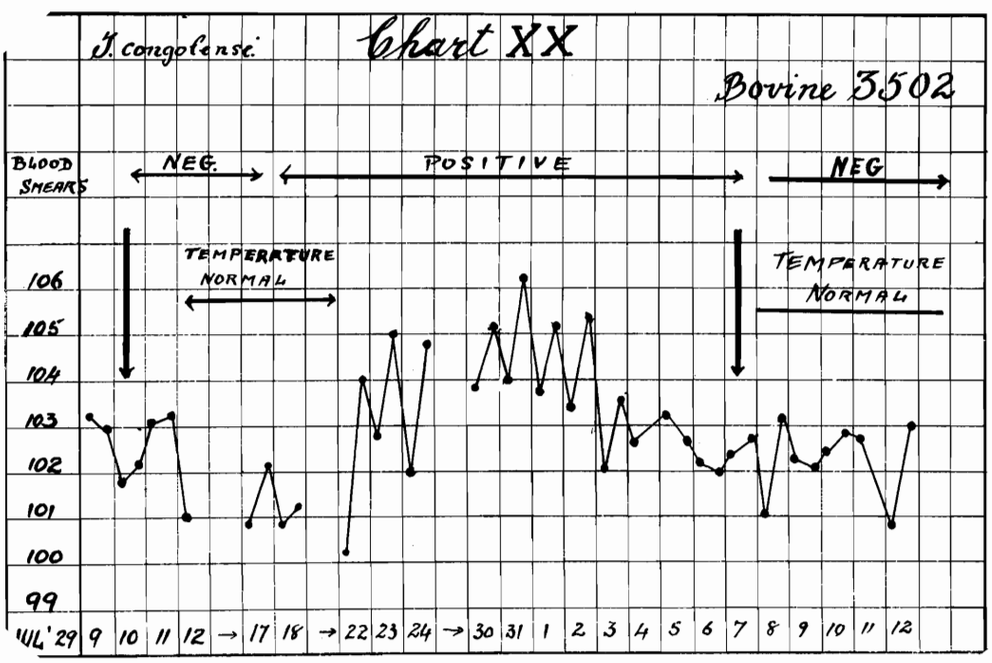
Bovine	Wgt. in Kg.	Date of infection.	Treatment commenced	Each dose in gm.	Dose per Kg.	Periods of Relapse.					Remarks.
2471	354	20/4/29	26/6/29	3	0.008	16	26	0	0	0	Sterilization
2473	483	20/4/29	26/6/29	3	0.006	16	0	0	0	0	"
3502	146	26/6/29	10/7/29	3	0.021	8	0	0	0	0	"
3520	191	26/6/29	10/7/29	3	0.016	12	14	26	0	0	"

Chart XX illustrates the sterilization of bovine 3502 by two doses of antimosan at a four-week interval.

Discussion.- The excellent results obtained in the above experiment were not anticipated. The ultimate results were, however, indicated by the remarkable fact of the lengthened period of relapse in those cases in which the trypanosomes did not disappear after the second injection. The uniformly successful outcome was obtained in spite of the fact that the bovines used varied considerably in weight. Bovine 2473 was two and a half times the weight of Bovine 3520, yet a similar dose of antimosan brought about a trypanosome-free interval sooner in the former than in the latter. It would appear that there is some factor present which results in quicker response to treatment by certain animals. It is the writer's opinion that the period of time that elapses after infection before treatment is instituted has a bearing on this matter. This aspect will be dealt with during the course of this chapter.

CONTINUATION AND MODIFICATION OF PRELIMINARY EXPERIMENT.

To support the results obtained in the previous experiment, further bovines were submitted to the four weekly administration of antimosan. In addition, a further modification of the treatment was carried out by reducing the amount of antimosan injected from 3 gm. to 1.5 gm. By this means it was hoped to obtain results which, although not comparable to those obtained by the use of 3 gm. dose, yet would bring about an


J. comp. Path.
Chart XX
Bovine 3502
BLOOD SMEARS
NEG.
POSITIVE
NEG
TEMPERATURE NORMAL
TEMPERATURE NORMAL
106
105
104
103
102
101
100
99
JUL. '29 9 10 11 12 → 17 18 → 22 23 24 → 30 31 1 2 3 4 5 6 7 8 9 10 11 12

appreciable reduction in the expenditure on the drug, perhaps with not too great a decrease of efficacy. The results are tabulated in Table XV.

Table XV.

T.congolense. Long Interval Treatment (28 days).

Bovine	Wgt. in Kg.	Date of infection	Treatment commenced	Each dose in gm.	Dose per Kg. Gm.	Periods of relapse.			
416	413	20/4/29	16/10/29	3	0.007	0	0	0	-
3524	166	26/6/29	16/10/29	3	0.018	0	0	0	0
2639	257	26/6/29	16/10/29	3	0.012	0	0	0	0
2464x	425	17/10/29	20/11/29	3	0.007	12	12	0	0
2634x	300	17/10/29	20/11/29	3	0.01	7	0	0	0
2468x	381	17/10/29	20/11/29	1.5	0.004	7	9	0	19
2994	218	17/10/29	20/11/29	1.5	0.007	9	9	0	0
3527	227	13/9/29	20/11/29	1.5	0.007	12	21	0	0
3627	318	13/9/29	20/11/29	1.5	0.005	12	9	9	0

x These three bovines were premune to one strain when re-infected with another strain on 17/10/29.

Discussion.- The 3 gm. dose of antimosan given at four-week intervals showed great efficacy in the three first tabulated bovines, the first administration in each case producing a trypanosome-free interval. In the remaining two such a period was not obtained until the third and second administration.

The results obtained by the administration of 1.5 gm. antimosan at four-week intervals would appear to be quite promising. If in conjunction with the table, the temperature charts, of which Charts XXI and XXII are included below as typical examples, are considered, it would appear that a comparatively small dose has a quite decided effect in controlling the disease. In another as yet uncompleted experiment, it has been shown that a bovine submitted to the Long Interval Treatment, using a dose of 3 gm. antimosan, can be re-infected during the interval by the injection of the homologous strain of T.congolense. Consequently, a bovine constantly exposed to infection would most likely, as far as the homologous strain is concerned, alternate between a state of sterility and a state of premunition. The use of a dose of 1.5 gm. antimosan in the circumstances would probably be indicated, as by its means a somewhat similar alternation of

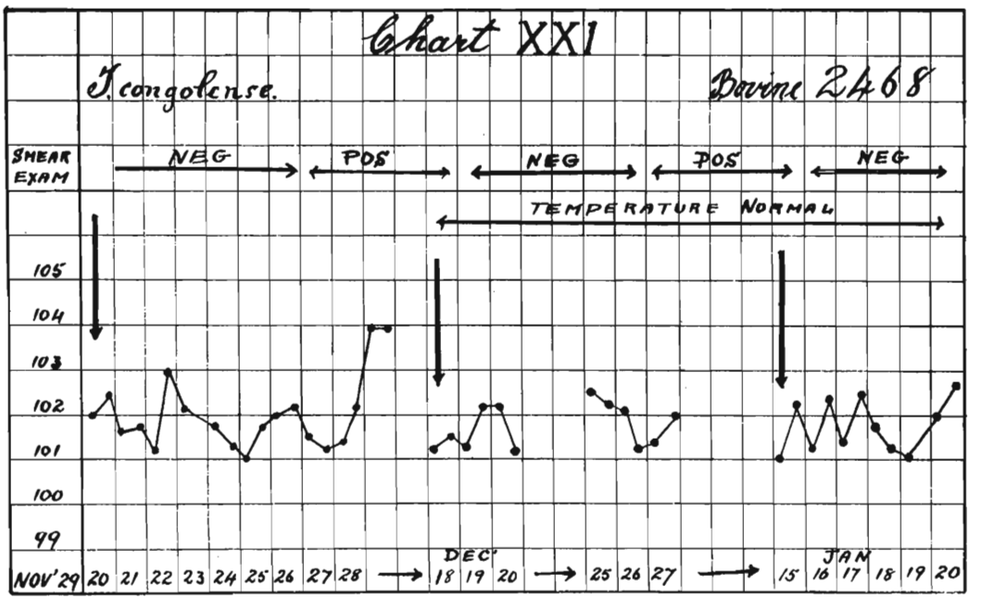


Chart XXI
T. congolense.
Bovine 2468
SMEAR EXAM
NEG
POS
NEG
POS
NEG
TEMPERATURE NORMAL
105
104
103
102
101
100
99
NOV'29 20 21 22 23 24 25 26 27 28
DEC' 18 19 20
25 26 27
JAN 15 16 17 18 19 20

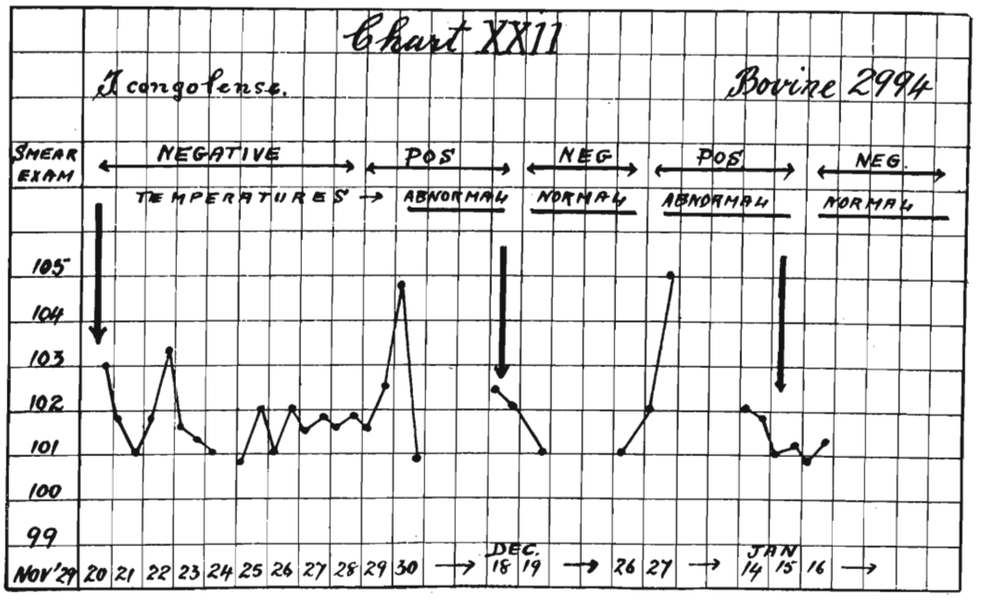


Chart XXII
T. congolense.
Bovine 2994
SMEAR EXAM
NEGATIVE
POS
NEG
POS
NEG
TEMPERATURES →
ABNORMAL
NORMAL
ABNORMAL
NORMAL
105
104
103
102
101
100
99
NOV'29 20 21 22 23 24 25 26 27 28 29 30
DEC. 18 19
26 27
JAN 14 15 16

sterility and premunition would be arrived at with a lessened expenditure on drugs. The practical value of the decrease in expenditure so brought about does not need emphasis.

Conclusions.- (1) The results with the 3 gm. dose of antimosan support those obtained in previous experiments.

(2) A dose of 1.5 gm. antimosan at similar intervals appears to be decidedly promising for practical application under field conditions.

SUMMARY AND CONCLUSIONS OF LONG INTERVAL TREATMENT.

Sterilization of bovines infected with T.congolense is obtained by the subcutaneous injection of 3 gm. antimosan when given at four-weekly intervals. In some cases this object is attained after the first injection. If not there is a gradual lengthening of the time before reappearance of the trypanosomes is noted until ultimately a trypanosome-free period of four weeks is obtained. This sterile condition is lost on re-infection of the bovine with a homologous strain. Consequently, under field conditions, one would expect bovines at times to alternate between sterilization and premunition. The latter state results when a bovine shortly after the Long Interval Treatmentis infected with a homologous strain. Cessation of a treatment which has produced either premunition or sterilization results consequently in an area where the bovine is constantly exposed to re-infection in premunition against the strains of T.congolense which have previously infected it. The writer is not at present in a position to say that such a premunition would be, under unfavourable conditions, sufficiently effective to enable the animal to survive.

The use of a smaller dose of antimosan may possibly be as efficacious in its ultimate results as far as the control of bovine trypanosomiasis is concerned as a larger dose. There was noticed, in connexion with the Long Interval Treatment and to a less extent, with the Short Interval Treatment, a certain amount of variation in the response to the injection of antimosan. This

variation could not be entirely ascribed to the weight factor. Tabulation as in Table XVI seems to indicate that the variation is correlated to a certain extent with the varying length of time which was allowed to elapse after infection before treatment was instituted. The date of infection of bovines infected with one strain and then infected with a different strain is entered in the table as under the date of infection with the latter.

Table XVI.

T.congolense. Variation in Response to Treatment.

Bovine	Infection to treatment in days.	Wgt. in Kg.	Treatment	Period of Relapse.			
3502	14	146	x L.I.T. 3 gm. doses	8	0	0	0
3520	14	191	" "	12	14	26	0
2464	34	425	" "	12	12	0	0
2634	34	300	" "	7	0	0	0
2471	67	354	" "	16	26	0	0
2473	67	486	" "	16	0	0	0
3524	112	166	" "	0	0	0	
2639	112	257	" "	0	0	0	
416	179	413	" "	0	0	0	
2468	34	381	" 1.5 gm. doses	7	9	0	19
2994	34	218	" "	9	9	0	0
3527	68	227	" "	12	21	0	0
3627	68	318	" "	12	9	9	0

x L.I.T. = Long Interval Treatment.

The response to treatment is judged by the number of injections of antimosan necessary to bring about a trypanosome-free interval. Those animals which were infected for a long time before treatment was commenced gave such an interval early, notwithstanding that the weights of these were well up to the average. Where the smaller dose of 1.5 gm. was used, this variation in response was not as well brought out on account of the variation in the time interval not being so great as in the case of the treatment with the 3 gm. dose.

Somewhat similar conclusions were arrived at in connexion with the application of the Short Interval Treatment. Table XVII illustrates the influence of late treatment when 3 gm. and 6 gm. doses were used. These two groups represent animals which have received identical treatment.

Table XVII.

Bovine	Infection to treatment in days.	Wgt. in Kg.	Treatment.	Result.
2702	29	220	x S.I.T. 3 gm. 2 doses	Sterilization.
2464	67	220	" "	Unsuccessful.
2468	67	220	" "	"
2743	82	181	" "	"
2634	168	238	" "	Sterilization.
2714	168	231	" "	"

S.I.T. = Short Interval Treatment.

Table XVIII.

Bovine	Infection to treatment in days.	Wgt. in Kg.	Treatment	Result
3627	33	318	x S.I.T. 6 gm. 1 dose	Unsuccessful
3542	41	204	" "	"
2743	194	236	" "	Sterilization.

x S.I.T. = Short Interval Treatment.

In Tables XVII and XVIII there appears to be an influence other than that of weight of the bovine. Greater success was obtained when the period before treatment was long.

There is scarcely sufficient evidence of the bovines grouped in Tables XVI, XVII, XVIII, to permit one to dogmatise but it undoubtedly appears that the response to treatment is influenced considerably by the time that elapses between infection and treatment. This point should prove to be of great value and importance in the treatment of bovine trypanosomiasis in the field. The success obtained in these experiments with late treatment will enable an easier sterilization to be attained in bovines which have been suffering from the disease for a long time, a state very likely to be encountered in undeveloped areas. A possible disadvantage of the Long Interval Treatment is that an infected animal might be exposed to adverse conditions of such a severity that the first dose given might be insufficient, under

such conditions, to carry it over to the end of the four-weekly interval when its second dose becomes due. This factor is not likely to be of influence if the bovine has received a number of doses as it would have, by then, undoubtedly developed a certain degree of resistance. However, if the conditions were such that there was danger that the first dose would not carry the bovine over to the second, a shorter interval between first and second dose could be employed. A few short interval doses before the institution of the Long Interval Treatment is not likely to interfere with the results obtained. Such a procedure is at present under trial in the field. Reports so far indicate that this modification is proving satisfactory.

Cheapening of the treatment of bovine trypanosomiasis will, as noted above, probably be brought about by decreasing the dose. It may even be possible to further decrease expenditure by increasing after the first few doses the length of the intervals. If one were dealing with only one strain of T.congolense no danger in introducing such an increase would be anticipated. However, one should be careful of increasing the length of the interval to too great an extent, especially if conditions are unfavourable, on account of the danger of the bovines concerned becoming infected with a heterologous strain of T.congolense and succumbing to this strain before the time of injection of the next dose comes around, because resistance to re-infection is only developed in a bovine under treatment to the homologous strain. This danger however, should not be of great moment if the animals be kept under close observation by an intelligent person. Any clinical manifestations of bovine trypanosomiasis would call for the administration of a dose before its pre-arranged time is due.

As far as our present knowledge of the strains of T.congolense goes, it is indicated that the administration of antimosan should be carried out in doses of 1.5 gm. to 3 gm. at regular not too long intervals to protect the bovines against the heterologous strains. It should also be remembered that, if a

bovine is sterilized of a particular strain of T.congolense, it again, in the temporary absence of this strain, becomes liable to an acute attack of bovine trypanosomiasis on the return of this strain.

No indication of the production of antimony-fast trypanosomes by the use of a 3 gm. of antimosan have been met with. The experiments in connexion with the use of the 1.5 gm. dose of antimosan although just completed, also indicate that no such antimony-fast trypanosomes are produced. Photograph 7(a) is of bovine 2727 while suffering from acute T.congolense infection and photograph 8(a) of bovine 3636 suffering from chronic T.congolense infection. Photographs 7(b) and 8(b) are of these two bovines after treatment. Photograph 9 is of bovine 2471 which is in a premunized state. It illustrates well the good condition notwithstanding the presence of infection.

(b_1) ANTIMOSAN IN EQUINE TRYPANOSOMIASIS.

Equines can be infected with all the three pathogenic trypanosomes but the one which is of most importance in equines is T.brucei, consequently this infection alone is dealt with in the following studies of the use of antimosan.

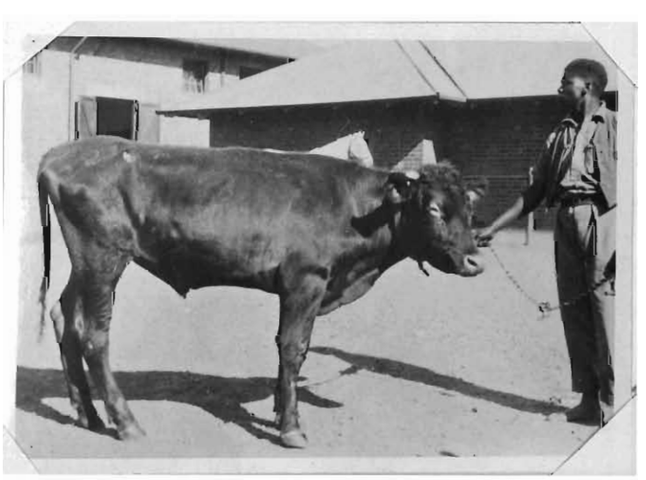
The literature available does not provide any information which might lead one to expect good results from the use of antimony compounds in T.brucei infection. Antimony potassium tartrate has been given numerous trials in T.brucei infection of equines and bovines. Its use was discontinued in both of these animals on account of the poor results obtained. Naganol is, at present, the drug usually selected for the treatment of this disease in horses. But on account of the excellent results obtained by the writer with antimosan in the treatment of bovine trypanosomiasis caused by Trypanosoma congolense, it was decided to test out this antimony compound against equine trypanosomiasis caused by Trypanosoma brucei. No striking results were anticipated from the employment of antimosan. The results were, however, surprisingly good. A great advantage in its use is the ease of

administration.

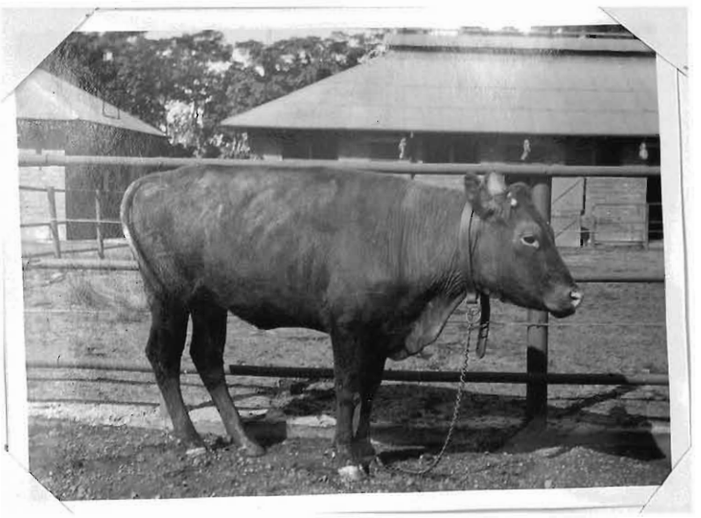
The antimosan utilized was the potassium salt in a 12 per cent. solution. This solution is hypertonic and it was found that it, in most cases, produced abscessation when given subsutaneously. It was avoided to a certain extent by injecting the solution slowly with massage. Later on a 7 per cent. solution, which is practically isotonic, was used and in no case did abscesses result. No particular precautions, as far as speed of injection and massage were concerned, were taken. In one case a horse was given subcutaneously, in the same spot, 40 c.c. of a 7 per cent. solution weekly for five weeks without showing any abscess formation. The site selected for the subcutaneous injections was always on the side of the neck. The sodium salt was used towards the end of the experiment. Similar good results were obtained. In the recording of the experiments no distinction is made between the potassium and the sodium salts.

The first experiment was so arranged that information could be obtained in connexion with the dose, the number of doses and to a less extent the intervals of administration. The particular strain of T.brucei utilized was obtained from a natural case in a donkey and proved to be extremely virulent to horses. Every horse which did not receive treatment died. The information obtained from this experiment is presented in Table XIX.

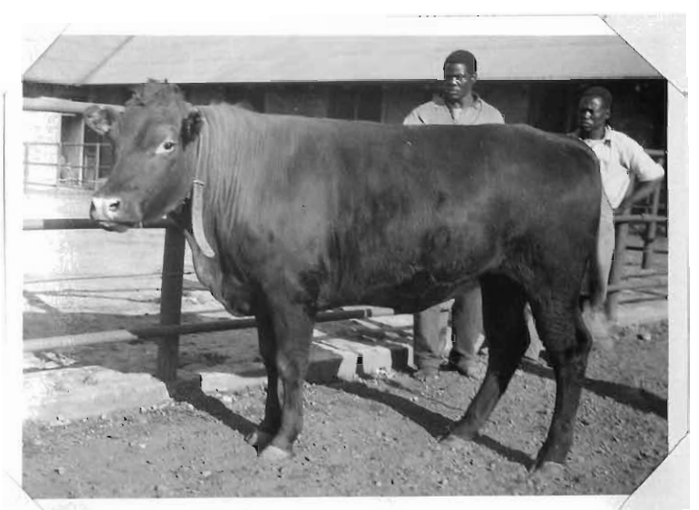
Photograph 7(a) B.2727. T.congolense infection. Before treatment for "close-up" see photograph 2.



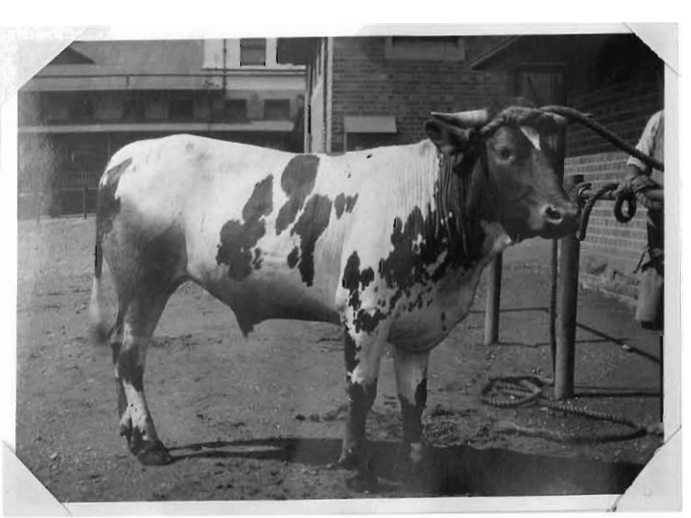
Photograph 7(b) B.2727. T.congolense infection. After treatment.



Photograph 8(a) B.3636. T.congolense infection. Before treatment. for "close-up" see photograph I.



Photograph 8(b) B.3636. T.congolense infection. After treatment. sterilization.



Photograph 9. B.2471. T.congolense infection. Premunition.

Table XIX.

T.brucei. Summary of Treatment with antimosan.

Equines	Infected on.	Appearance of Trypanosomes.	Treatment commenced	No. of doses and dosage.	Intervals	Reappearance of Trypanosomes.	Remarks.
19079	20.3.29	24.3.29	17.5.29	2 doses 3 gm.	weekly	21.6.29	Subsequently horse had a severe relapse.
19079	20.3.29	-	5.7.29	5 doses 3 gm.	"	25.9.29	Doing well up to 11.1.30.
19431	11.6.29	19.6.29	17.7.29	"	"	Nil	Sterilized.
19472	22.5.29	1.6.29	12.6.29	2 doses 6 gm.	23 days	-	Died 9.7.29.
18095	11.6.29	19.6.29	5.7.29	5 doses 3 gm.	weekly	13.8.29	Doing well up to 11.1.30.
18238	11.6.29	19.6.29	5.7.29	2 doses 6 gm.	"	3.8.29	Doing well up to 11.1.30.
17974	26.9.29	2.10.29	-	-	-	-	Control;died 27.11.29.
19377	16.7.29	22.7.29	-	-	-	-	Control; shot *in extremis*, 27.8.29.
18328[x]	12.4.29	23.4.29	-	-	-	-	Control;died 22.8.29.

x Equine 19328 was a donkey.

As will be seen from the above table certain of the horses were given more than one course of treatment. If horses were showing marked clinical indications of the disease the antimosan produced an almost immediate improvement in the case even though they had received a previous course of treatment.

Horses 19079 and 19431 were permitted to reach an advanced stage of the disease before treatment was instituted. The results obtained in these two animals were remarkably good. Horse 19431 became sterilized of the trypanosomes as was shown by negative smear examination for 13 weeks, by sub-inoculation into two horses with negative results and by a negative Complement Fixation Test, while horse 19079 attained a state comparable to that of premunition obtained in *T.congolense* infection of bovines by somewhat similar methods of treatment.

All of the three controls which received no treatment died. The donkey died in 19 weeks and the two horses in 8 and 7 weeks. Only one of the treated horses died and this one received a single dose on the 21st day after infection and another

44th day.

The progression of T.brucei infection in horses is considerably more rapid than that of T.congolense infection in bovines when the animals are kept under stable conditions. Consequently the treatment of equines by long interval methods, as has been found to be successful in T.congolense infection of bovines, probably would not be indicated in T.brucei infection of horses.

It was found that the treatment of this disease with antimosan in doses of 3 to 6 gm. in every case produced an improvement as could be judged by the temperature curve, the decrease of oedema when present, the decrease of the anaemia and the disappearance of the trypanosomes. The photographs10(a) and 10(b) are of horse 19431 before and soon after the cessation of treatment. Chart XXIII of the period before and during treatment illustrating the occurrence of the trypanosomes and the temperature curve of this horse is also included.

The beneficial effect of this drug, even when it does not produce sterilization, is well illustrated by horses 19079, 18238 and 18095. Notwithstanding the persistence of infection in these horses the temperature curves after treatment never showed the marked irregularity associated with T.brucei infection in horses. Although only one of the five horses treated was sterilized, it was decided to continue the experimentation to determine more exactly the probably requirements as far as the size of the dose and the intervals between administration are concerned. The number of horses used for this purpose was small, but the information obtained by varying the doses and the interval justifies the publication of the results.

ARRANGEMENTS OF THE EXPERIMENT.

In all five horses were introduced into the experiment. The intervals of administration were one week, two weeks and three weeks. The doses were 3.75 gm. and 5.25 gm., repeated a number of times, and ascending doses of 3 gm., 6 gm., and 9 gm. Control of

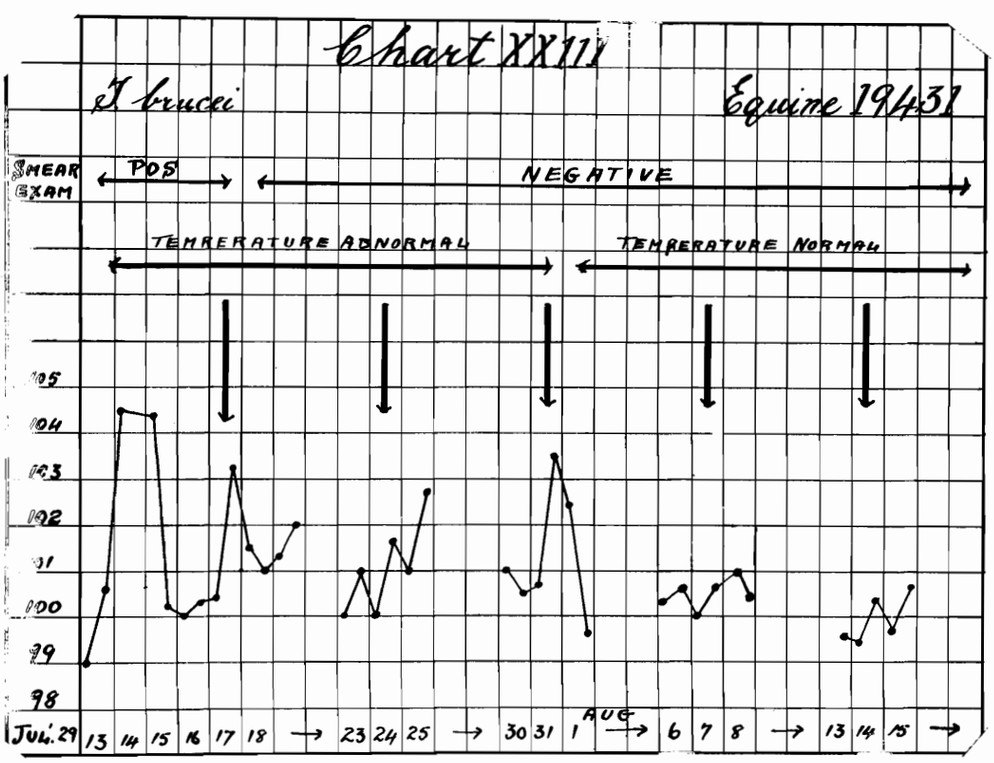
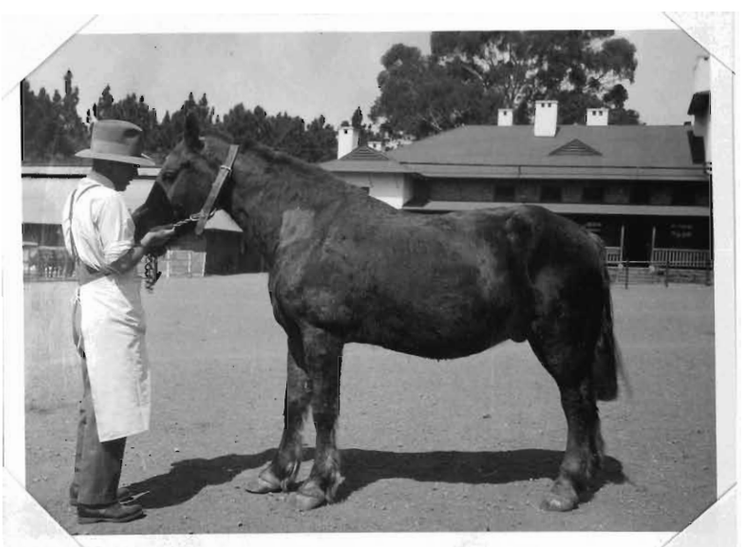


Chart XXIII
T. brucei
Equine 19431
SMEAR EXAM
POS
NEGATIVE
TEMPERATURE ABNORMAL
TEMPERATURE NORMAL
105
104
103
102
101
100
99
98
Jul. 29
13 14 15 16 17 18 → 23 24 25 → 30 31 1 AUG → 6 7 8 → 13 14 15 →

Photograph 10(a) H.19431. See photograph 5.



Photograph 10(b) H. 19431. T.brucei infection. After treatment.

the results of the treatment was based on the result of the examination of stained blood smears, on the appearance of the temperature curve and on the complement fixation test. In a few cases it was possible to test the sterility further by subinoculation into horses and reinoculation of the presumed sterile.

The table is arranged to give the various details in connexion with the treatment of each horse.

Table XX.

Antimosan Therapy.

Equines	Wgt. in Kg.	No. of doses and dosage.	Intervals	Total used	Result.
18095	382	6 doses of 5.25 gm.	3 weeks	31.5 gm.	Sterilization
17989	431	6 doses of 3.75 gm.	3 "	22.5 gm.	Failure
17990	423	8 doses of 3.75 gm.	2 "	30.0 gm.	"
19431	374	8 doses of 5.25 gm.	2 "	42.0 gm.	Sterilization
19079	308	3 doses of 3,6 and 9 gm.	1 "	18.0 gm.	"

The weights of the horses were obtained after the completion of the experiment, consequently the weights of the two ~~heifers~~ FAILURES were probably slightly higher, comparatively, than is indicated by the table, for, as a result of a successful treatment, there occurred an improvement in condition. However, even though the horses 17989 and 17990 were failures as far as sterilization was concerned, yet they also showed a definite improvement in condition. Thus, notwithstanding the slight disparity of weights, the results are regarded as comparable.

It can thus be seen that the dose of 3.75 gm., even though given repeatedly and at intervals of two and three weeks, would appear to be too small to produce sterilization in the two horses 17989 and 17990. In these two cases failure was determined by the detection of T.brucei in blood smears in the 9th week and 13th week respectively, by a positive complement fixation test in

both, and by re-appearance of the T.brucei temperature curves in the 11th week in both cases. Blood of horse 17989 was furthermore subinoculated into a number of other horses, it being used as a reservoir of T.brucei always with positive results. This horse is at present, 10 months after cessation of treatment, in good condition, although occasional irregularities of temperature are noted.

Of the three horses that were sterilized, one was treated at one-weekly intervals, one at two weeks and one at three weeks. Longer intervals were not feasible, because on account of the severity of T.brucei infection in horses the re-appearance of trypanosomes in the blood is associated with a rapid loss of condition and the development of marked symptoms. Trypanosomes were not found in these three horses during the intervals between treatment, but were found during the intervals in the two failures in one case as early as the 11th day. This finding might be regarded as additional evidence that the dose used in the failures was on the small side.

A difficulty in the study of the chemotherapy of trypanosomiasis is that of determining when an animal is sterilize For example, in the case of horse 19431 and also of horse 18095 it is quite likely that sterilization had been obtained long before the cessation of treatment. Of great interest in the matter of the dosage is the sterilization obtained in horse 19079. From the information obtained from the two failures, the first dose used, namely, 3 gm., may be regarded as too small to produce sterilization. Consequently sterilization must have been produced by either the 6 gm. or the 9 gm. dose. There would not, therefore have been any interference with the efficacy of the subsequent doses by the administration of the first dose. The same probably holds good in the cases of the other two sterilized animals, for in the previous experiment the use of two doses each of 6 gm. at an interval of a week did not produce sterilization.

The ascending doses which totalled 18 gm. were the most economical in this experiment, but it is quite likely that a

reduction in the total amount used in the other two cases of sterilization might be brought about by decreasing the number of doses. From the point of handling necessary, which is always of importance in dealing with horses which are not as a routine handled daily, the use of large doses is indicated, whether of the same size or ascending cannot now be stated.

The most important and, as has already been pointed out, difficult point in the efficient control of the results obtained in the treatment of trypanosomiases is the determination of sterility. After treatment with antimosan T.brucei becomes extremely rare in the blood. Consequently negative blood smear examinations is not of any great significance unless it be carried on over a long period and receives the support of other evidence, e.g. the temperature curve. But better still is the subinoculation of blood into horses which are then examined. The complement fixation test and re-infection have also been utilized for the purpose. The former gives good results but, unfortunately a horse does not become negative for some little time after sterilization. The latter was used in all three cases. It naturally results in the re-infection of the animal if it is sterilized, a condition which might not always be desirable even under laboratory conditions and never can be under field conditions. It is proposed to deal with each of these methods of determination of sterility in the three sterilized horses in turn.

Blood smear examination showed that each of the three horses was positive before treatment was commenced. Smear examinations were carried out three times weekly. To illustrate the difficulty of detecting T.brucei which one meets with at times, one might mention horse 19079 which only showed T.brucei once in blood smears over a period of 13 weeks. During the treatment of horse 19079 no trypanosomes were found. Complete smear examination subsequent to treatment was not carried out, for there were horses available for subinoculation. In horse 19431 smear examination was carried out during and for 12 weeks after cessation

of treatment with negative results. In horse 18095 similarly for a period of 11 weeks negative results were obtained.

The temperature curve was available for horse 19079 for six weeks when re-infection was carried out, and for horses 19431 and 18095 for 17 weeks. The indications were that none of the three were infected. The temperatures were somewhat irregular in horses 19431 and 18095 during the last interval, i.e. before the administration of the last dose.

The complement fixation test was carried out on the horses in the experiment. They all gave positive results either before or during treatment. The ultimate test, of course, in horses 18989 and 17990 was positive. In horse 19079 the test was positive two days before the last dose of antimosan, but was negative on the 28th day after cessation of treatment. This means that a negative result was obtained on the 42nd day after commencement of the treatment. In horse 19431 the test was positive after four doses had been given and became negative 11 weeks after the last dose, being doubtful during the 7th week. In horse 18095 the test was negative after the third dose and also during the 6th week after the last dose. The former of the two results in horse 18095 is rather surprising, for it seems to indicate that sterilization must have been produced by either the first or the second dose, a possibility which is not supported by the temperature curve. At any rate, it can be said that the complement fixation test becomes negative within a short period after sterilization.

As further cases of T.brucei infection were required for another experiment the opportunity was taken to test the sterility of horse 19079 by the injection of 50 c.c. of blood of this horse into horses 18095 and 19431. These two were then kept under observation for 15 days with negative results as far as the blood smears and temperatures were concerned. Their susceptibility was proved by the injection of 50 c.c. of blood of horse 17989, one of the failures. In both cases trypanosomes were found in the

blood smears on the 4th day, temperatures of 106 and 106.2 respectively being recorded on that day.

The observations in connexion with the susceptibility of horses 18096 and 19431 and subsequently of horse 19079 which when injected with blood of horse 17989 also became infected, are regarded as a good method of determining sterility, because the same strain of T.brucei was utilized.

The results obtained in these two experiments in the treatment of T.brucei infection of horses are definitely promising. It would seem that no difficulty would be experienced in obtaining sterilization if the dose be sufficiently larger. The ease of administration and furthermore the cost of the course of treatment are both in favour of the use of antimosan for this trypanosomiasis of horses. It might possibly be, however, that the drug styryl, which has been reported on, or a derivative of this drug, will be the drug of choice.

(c_1) ANTIMOSAN IN OVINE TRYPANOSOMIASES;

It has already been pointed out that the sheep is often the last of the domestic animals to come into contact with infected country. Consequently the same attention has not been devoted to the study of the chemotherapy of the disease in this animal. Graf produced T.brucei and T.congolense infection in sheep and utilised various drugs in the treatment. The work now to be reported is the results obtained by the writer in the treatment with antimosan of T.congolense infection of sheep. Cases of T.brucei infection were also produced but no chemotherapeutical trials have as yet been carried out.

No difficulty was experienced in the production of the infection in sheep by the intravenous injection of the blood of bovines or sheep infected with T.congolense. The sheep before treatment was instituted were kept for varying times and consequently the results obtained with the same doses are probably not strictly comparable, for it has been observed in bovines that those

which had been infected for varying periods before treatment was instituted gave different responses to treatment.

A number of the infected sheep died before treatment was commenced, but the mortality was undoubtedly influenced, in some cases, by concurrent heartwater. In general the infection produced in the sheep was of a subacute type, but in many cases the ultimate condition was of a chronic nature characterised by a loss of condition, an anaemia and a temperature curve showing persistent regular marked remissions and exacerbations.

For the experiments sixty-three sheep were utilized. This number does not include those sheep which died before treatment was instituted, but does include two sheep (S.25499 and S.28935), which died two and three days respectively after the injection of doses of antimosan. Death in both cases was due to trypanosomiasis, the institution of treatment having been delayed too long.

The antimosan used in all the experiments was the sodium salt in 12 per cent. solution. The doses in all the tables are in cubic centimetres of this solution. The antimosan was administered, with a hypodermic syringe and needle, subcutaneously, at the medial aspect of the right thigh. Notwithstanding that no disinfection or washing of the site was carried out at the time of the injection of the drug, yet in only one case did an abscess, which was small, result. The same site was always used, even when a series of weekly injections was given.

The majority of the sheep weighed approximately 35 Kg., the lightest being about 30 Kg. and the heaviest about 42 Kg.

The results of the treatment, i.e. whether sterilization had been obtained or not, were determined from the examination of the temperature curve, temperatures being taken twice daily, and of stained blood smears prepared three times weekly. When both the temperature curve and the blood smears did not indicate clearly that the sheep were free from infection, blood smears were continued for a period which in some cases extend to more than two months after cessation of treatment. It is considered

that this method of determination of sterility gives reliable results, although it would have removed all doubt if sub-inoculations from the sheep had been caried out. Charts XXIV and XXV illustrate successful and unsuccessful treatments of two sheep.

In the tables the sheep are grouped according to similarity of doses of antimosan. In some cases, when sheep were given doses which later were increased, the recording of the results may be included in more than one table, first in the tables recording the smaller doses and then in the tables of the larger doses. This procedure, however, is only carried out in those cases where it was possible to determine, before the administration of the larger doses, the failure of the smaller doses and where the interval between the smaller doses and the larger ones was at least six weeks.

The percentage of sterilization for the various groups is recorded in the last table.

EXPERIMENT 1.

Table XXI.

D.O.B. No.	Dosage in c.c.				Interval in days	Blood smear	Result
25499	3	-	-	-	-	-	Died on second day
26125	3	5	5	5	7	Positive	Unsuccessful.
27471	3	5	5	5	7	"	"

Discussion.- The blood smears obtained during the periods between the injections were negative, but trypanosomes were detected in the smears on the 34th and 11th days respectively after the last injections. The increase in dosage from 3 to 5 c.c. was instituted on account of the very slight influence the 3 c.c. dose had on the temperature.

EXPERIMENT 2.

In this series the doses were similar to those of Experiment 1, but an additional dose of 5 c.c. was given.

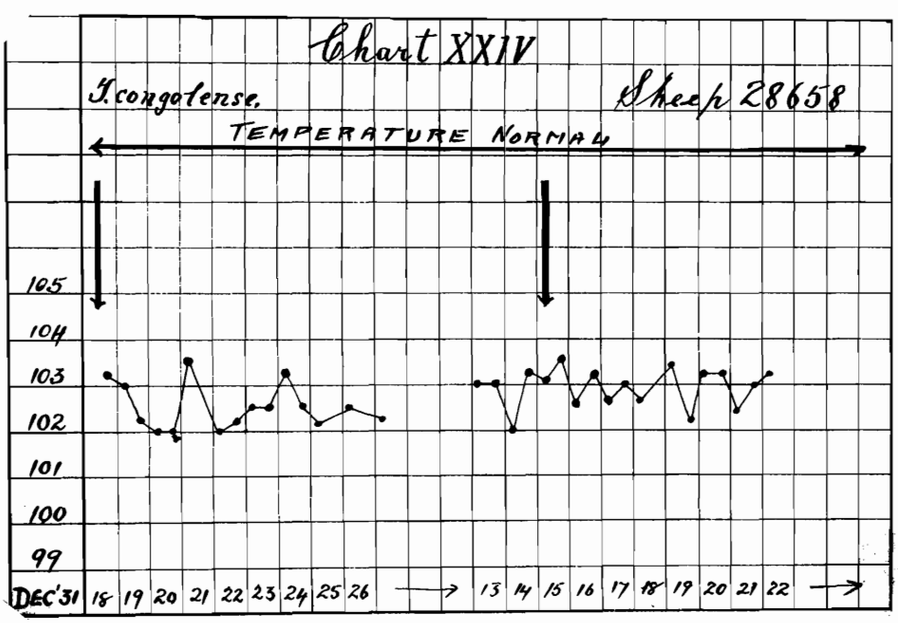


Chart XXIV
T. congolense,
Sheep 28658
TEMPERATURE NORMAL
105
104
103
102
101
100
99
DEC'31 18 19 20 21 22 23 24 25 26 13 14 15 16 17 18 19 20 21 22

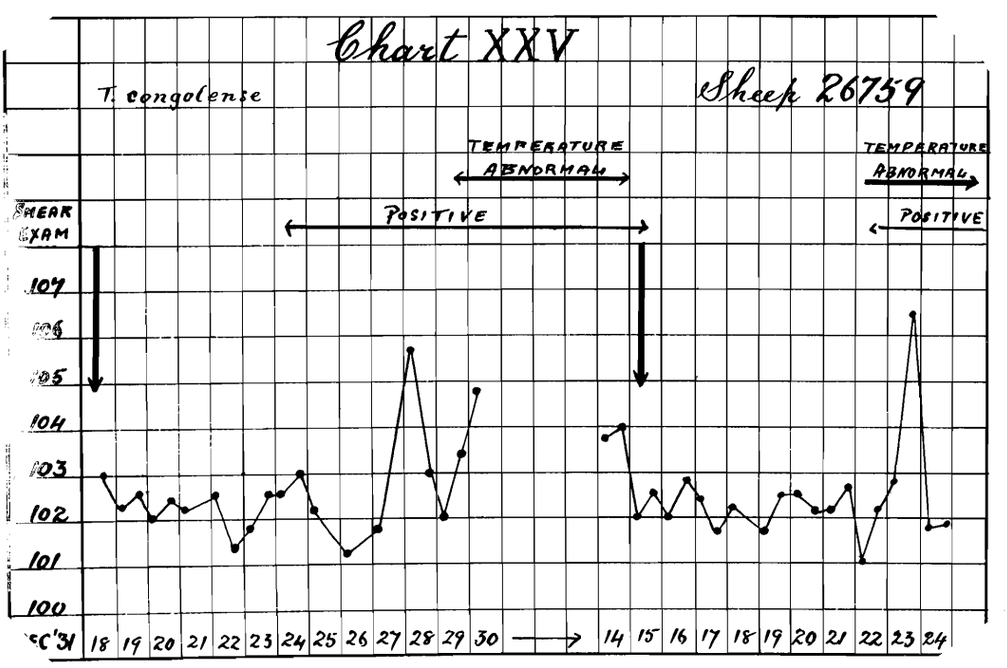


Chart XXV
T. congolense
Sheep 26759
TEMPERATURE ABNORMAL
TEMPERATURE ABNORMAL
SMEAR EXAM
POSITIVE
POSITIVE
107
106
105
104
103
102
101
100
DEC'31 18 19 20 21 22 23 24 25 26 27 28 29 30 14 15 16 17 18 19 20 21 22 23 24

Table XXII.

T.congolense. Antimosan therapy.

D.O.B. Nos.	Dosage in c.c.					Intervals in days	Blood smear	Result
24784	3	5	5	5	5	7	Positive	Unsuccessful.
25649	3	5	5	5	5	7	Negative	Sterilization.
26054	3	5	5	5	5	7	Positive	Unsuccessful.
28551	3	5	5	5	5	7	-	"
28807	3	5	5	5	5	7	Negative	Sterilization.
28834	3	5	5	5	5	7	Positive	Unsuccessful.
28957	3	5	5	5	5	7	Negative	Sterilization.

Discussion.- The introduction of an additional 5 c.c. dose resulted in the sterilization of three of the sheep. Probably further success would have been obtained if the treatment had been persisted in.

EXPERIMENT 3.

As some success had resulted from the employment of four 5 c.c. doses after one 3 c.c. dose, this series was given four 5 c.c. doses.

Table XXIII.

T.congolense. Antimosan therapy.

D.O.B. Nos.	Dosage in c.c.				Intervals in days	Blood smear	Result
25503	5	5	5	5	7	Positive	Unsuccessful.
26323	5	5	5	5	7	"	"
26519	5	5	5	5	7	"	"

Discussion.- If the result obtained from this experiment be compared with those of Experiment 2 it would appear that the administration of the preliminary 3 c.c. dose had some favourable influence.

EXPERIMENT 4.

This is a repetition of the previous experiment with, however, an additional 5 c.c. dose.

Table XXIV.

T.congolense. Antimosan therapy.

D.O.B. Nos.	Dosage in c.c.					Intervals in days	Blood smear	Result.
27660	5	5	5	5	5	7	Negative	Sterilizatior
28065	5	5	5	5	5	7	Positive	Unsuccessful
28317	5	5	5	5	5	7	"	"
28386	5	5	5	5	5	7	"	"
28524	5	5	5	5	5	7	Negative	Sterilizatior
28926	5	5	5	5	5	7	Positive	Unsuccessful
25539	5	5	5	5	5	7	"	"
26629	5	5	5	5	5	7	"	"
27547	5	5	5	5	5	7	"	"

Discussion.- The results obtained are no better than Experiment 2. The continuation of the use of a 5 c.c. dose does not present much hope of success. If further doses of 5 c.c. were to be given, the treatment for practical purposes would be too drawn out and too expensive.

EXPERIMENT 5.

The next series was arranged to give an ascending dosage with a maximum of 10 c.c. This served also to determine the tolerance of the sheep to fairly large doses.

Table XXV.

T.Congolense. Antimosan therapy.

D.O.B. Nos.	Dosage in c.c.				Intervals in days.	Blood smear	Result
25751	3	5	10	10	7	-	Sterilization.
25770	3	5	10	10	7	-	"
19151	5	5	10	10	7	-	"
25502	5	5	10	10	7	-	"
27526	5	5	10	10	7	-	"

Discussion.- No ill effects were observed as the result of the use of 10 c.c. doses. Trypanosomes were detected in smears before the third dose in all the sheep, but not subsequently. Probably the good results are, in this experiment, not so much dependent on the presence of the two first doses as due to the use of 10 c.c. as end doses.

EXPERIMENT 6.

For this series a slight increase was made in the

dosage to 7 c.c. Two sheep were given single doses, while the remainder received two doses at an interval of 28 days.

Table XXVI.

T.congolense. Antimosan therapy.

D.O.B. No.	Dosage in c.c.	Intervals in days		Blood smear	Result
20949	7	-	-	Positive	Unsuccessf.
21065	7	-	-	Negative	Sterilization
23487	7	7	28	Positive	Unsuccessf.
25734	7	7	28	Negative	Sterilization
25793	7	7	28	"	"
26759	7	7	28	Positive	Unsuccessf.
27438	7	7	28	"	"
28275	7	7	28	Negative	Sterilization
28658	7	7	28	"	"
28799	7	7	28	"	"
28871	7	7	28	"	"
28881	7	7	28	"	"
28919	7	7	28	"	"
28979	7	7	28	"	"

Discussion.- The increase of the dose to 7 c.c. and the interval to 28 days gave much better results. There was thus obtained increased efficiency with a decrease in the total c.c. of antimosan used.

EXPERIMENT 7.

The next series was devised for the purpose of determining whether a single dose of 10 c.c. would give the required result. In this experiment, in addition, were included those cases which were failures in previous experiments.

Table XXVII.

T.congolense. Antimosan therapy.

D.O.B. Nos.	Dose in c.c.	Failure from	Blood smear	Result.
24694	10	-	Negative	Sterilization.
28063	10	-	"	"
28641	10	-	"	"
28999	10	-	"	" "
28834	10	Experiment 2	"	"
26054	10	"	"	"
24784	10	"	"	"
25539	10	Experiment 4	"	"
26323	10	Experiment 3	Positive	Unsuccessful.
26629	10	Experiment 4	Negative	Sterilization.
27547	10	"	"	"
28386	10	"	"	"
28996	10	"	"	"
28317	10	"	"	"
28551	10	Experiment 2	"	Unsuccessful
26519	10	Experiment 3	"	"
28935	10	"	"	Died on third day

Discussion.- Rather disappointing was the failure of the three sheep which had failed in previous experiments. The four sheep which had not been subjected previously to treatment were all sterilized.

EXPERIMENT 8.

As fairly good results had been obtained by the long interval administration of 7 c.c., this series was arranged also at long intervals with a commencing small dose, a commencing large dose, and two large doses.

Table XXVIII.

D.O.B. Nos.	Dosage in c.c.		Intervals in days	Failure from	Blood smear	Result
28551	10	10	28	Exper. 7	Positive	Unsuccessful.
26519	10	10	28	"	Negative	Sterilized.
23556	10	10	28	-	"	"
23821	5	10	28	-	Positive	Unsuccessful.
24114	5	10	28	-	Negative	Sterilized.
25661	5	10	28	-	"	"
25808	5	10	28	-	"	"
28341	5	10	28	-	"	"
28497	5	10	28	-	"	"
28796	5	10	28	-	"	"
28943	5	10	28	-	"	"
28977	5	10	28	-	"	"
25739	10	5	28	-	"	"
27398	10	5	28	-	Positive	Unsuccessful.
27422	10	5	28	-	"	"
28795	10	5	28	-	"	"
28828	10	5	28	-	Negative	Sterilized.
28923	10	5	28	-	"	Sterilized.

Discussion.- The first two sheep were treated after failure to a series of small doses which had been given at weekly intervals and one 10 c.c. dose. In both cases there was an interval of 21 days before the two 10 c.c. doses were given. Then 5 c.c.-10 c.c. doses at 28 days interval were very much more successful than the arrangement 10 c.c.-5 c.c.

Summary.- Table XIX is compiled for the purpose of illustrating the comparison of the various arrangement of doses, intervals at which they were given and the percentage sterilized.

Table XXIX.

T.congdense. Summary of treatment.

Doses in c.c. of a 12% solution of antimosan.					Interval in days.	Reference	No.of sheep	No. sterilized.	% sterilized.
3	5	5	5	-	7	Experiment 1	2	0	0
3	-	-	-	-	-	"	1	0	0
3	5	5	5	5	7	Experiment 2	7	3	43
5	5	5	5	-	7	Experiment 3	3	0	0
5	5	5	5	5	7	Experiment 4	9	2	22
3	5	10	10	-	7	Experiment 5	2	2	100
5	5	10	10	-	7	"	3	3	100
7	-	-	-	-	-	Experiment 6	2	1	50
7	7	-	-	-	28	Experiment 6	12	9	75
10	-	-	-	-	-	Experiment 7	17	13	76
10	10	-	-	-	28	Experiment 8	2	1	50
5	10	-	-	-	28	"	10	9	90
10	5	-	-	-	28	"	6	3	50

Two doses or two end doses of 10 c.c. of antimosan whether given at 7 or 28 days interval, sterilized the sheep of trypanosomes in all cases except one. The percentage sterilized by the single dose of 10 c.c. was good, especially when it is taken into consideration that one of the failures was due to the too late institution of the treatment. The smaller doses gave disappointing and unsatisfactory results. The 7 c.c. dose would probably in practice be a suitable average one, but if sterilization were desired it would be necessary to give a third one. This dose was not tried out at the 7-day interval.

CONCLUSION.

1. For sterilization of sheep infected with Trypanosoma congolense the dose of the 12 per cent. solution of the sodium salt of antimosan necessary would probably be 10 c.c., i.e. 1.2 gm., repeated once or twice at an interval of 28 days.
2. A similar dose would probably produce sterilization if given at 7-day intervals.
3. The 7 c.c. dose of antimosan gave good results, whereas the 5 c.c. dose gave unsatisfactory results.
4. The practical and economical dosage for the production of sterilization is the 10 c.c. repeated.
5. The approximate dosage per Kg. live weight corresponding to a 10 c.c. dose of the 12 per cent. antimosan used is 0.03 gm. of antimosan.

ANTIMOSAN IN CANINE TRYPANOSOMIASIS.

The trypanosomiasis of dogs treated with antimosan was that produced by T.congolense. Quite remarkable were the results obtained not only in the production of sterilization but also in rapid alleviation and entire disappearance of the often advanced and acute symptoms. The sterilization was proved in every case not only by the disappearance of the trypanosomes and the return to normality of the temperatures, but also by re-infection and sub-inoculation into other dogs. In these tests the dogs were re-infected to prove sterilization and again treated. No decrease in efficiency of treatment was noted as a result of this procedure. On account of this repetition it is proposed to tabulate the details of each dog on a separate table. Table XXX gives the details of dog 985

Table XXX.

T.congolense Dog. 985 Weight 24 Kg.

Date of infection	Date of 1st treatment.	date of 2nd infection	Date of 2nd treatment	Date of 3rd infection	Date of 3rd treatment	Date of 4th infection	Date of 4th treatment.
24/8/31	5/9/31	2/10/21	31/10/31	21/12/31	31/12/31	18/2/32	9/4/32.

This dog died after the last treatment which was applied when it was in extremis. After each of the above treatments no trypanosomes were found in blood smears which were made daily and the temperatures were normal. The disease showed a definite tendency to become more chronic and consequently the period between infection and treatment could be lengthened during the progress of the experiment. The symptoms of the disease as the peracuteness decrease became much more evident. Consequently the results of treatment were more striking as the experiment progressed. Photographs 11(a) and 11(b) of this dog taken before and after treatment illustrates the effects of treatment

at a time when the symptoms were definitely established.

Table XXXI gives the details of dog 989.

Table XXXI.

T.congolense. Dog 989. Weight 21 Kg.

Date of 1st infection	Date of 1st treatment	Date of 2nd treatment	Date of 2nd infection	Date of 3rd treatment	Date of 3rd infection.
5/9/31	21/9/31	21/10/31	2/12/31	29/12/31	19/2/32.

This dog died 10 weeks after the last infection, no treatment having been given. The course in T.congolense infection in the dogs which did not receive the above type of infection-treatment manipulation was always short. There was thus a great lengthening of the course in Dog 989. The 1st treatment failed; in subsequent treatments the dose of antimosan was increased.

In the experiments on these two dogs the antimosan failed to sterilize only once out of six treatments given with one treatment doubtful in the case of dog 985 which died before it could be determined whether it was sterilized or not. The dose of antimosan in all the treatments for dog 985 was 0.025 gm. per Kg. of live weight and for dog 989 it was 0.017, 0.029 and 0.046 gm. per Kg. The above experiments are interesting also for the fact that there appears to be no sustained prophylactic action of antimosan, re-infection being possible as early as 27 days after treatment.

CHAPTER VII.

Antimosan-fastness of T.congolense.

Of paramount importance, before the institution of a chemotherapeutical campaign against trypanosomiases with a drug, is the determination whether the trypanosomes will or will not develop a resistance to the repeated use of the particular drug. If there be required for the treatment, whether the object be control or sterilization, a number of injections there is always the danger of non-sterilizing doses producing drug-fast parasites which would then interfere considerably with the campaign or even necessitate the use of some other trypanocidal drug. Especially useful would be a drug which did not produce drug-fastness in a campaign, the object of which was to produce not sterilization but premunition. Thus it was decided to carry out an experiment in guinea pigs, the object of which was to determine whether drug-fast T.congolense trypanosomes would or would not be produced in these animals by the use of antimosan. Antimosan was chosen as the drug for experimentation, because, if any extensive treatment of a trypanosomiasis were to be undertaken it would be in T.congolense infection of bovines with antimosan.

The experiment was arranged to be carried out in two distinct sections. In the first section a regular procedure was followed of treating the infected guinea pig with a non-sterilising dose of antimosan, and when the parasites re-appeared in the blood, a sub-inoculation was made into other guinea pigs to produce the second generation in guinea pigs which were then submitted to the same process of treatment and sub-inoculation to produce the third generation. The dose in c.c. tabulated is the average obtained in three guinea pigs of the same generation and represents the number of c.c. of a 1% solution of antimosan per Kg. of live body weight. Relapse indicates the average number of days after treatment to the reappearance of the trypanosomes.

Generations	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Dose	1.75	1.0	1.0	1.0	1.1	1.25	1.25	1.25	1.25	1.25	1.25
Relapse	6.6	1	4.3	2.0	2.0	4.5	0	1.5	3.0	2.5	4.0
Generations	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	22nd
Dose	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Relapse	0.3	2.6	3.75	0	3.0	2.6	3.0	3.0	2.0	3.5	2.0
Generations	23rd	24th	25th	26th	27th	28th	29th	30th	31st	32nd	
Dose	1.25	1.25	1.25	1.5	1.5	1.5	1.25	1.25	1.25	1.25	
Relapse	3.0	2.0	0.6	1.0	3.0	2.6	2.0	4.0	3.0	2.0	

In the second section of the experiment an endeavour was made to keep the number of sub-inoculations into fresh guinea pigs down to the minimum. An infected guinea pig was treated and all subsequent relapses in this guinea pig were similarly treated. Only when it appeared that the guinea pig would die was a sub-inoculation carried out into guinea pigs which, in turn, were submitted to the same procedure. The consequence was that the trypanosomes were exposed to action of the drug in each guinea pig a number of times. This arrangement was made for the reason that it approximated more closely to what would likely result if treatment in the field were to be carried out on an extensive scale. From the details submitted below the trypanosome of guinea pigs A can be traced through to the termination of the experiment in guinea pig J. After each guinea pig is recorded the number of doses and the quantity in c.c. of the 1% solution of antimosan used to each Kg. of live weight.

A (14 doses of 1.4 c.c.) - B (11 doses of 1.25 c.c.) -
C (1 dose of 1.25 c.c.) - D (11 doses of 2 c.c.) -
E (3 doses of 2 c.c.) - F (1 dose of 1.25 c.c.) -
G (3 doses of 1.25 c.c.) - H (1 dose of 3 c.c.) -
I (2 doses of 3 c.c.) - J (7 doses of 3 c.c.).

There were thus 54 doses varying from 1.25 c.c. to 3c.c. distributed over 10 guinea pigs.

Conclusions: There were no indications of the production of drug-fastness in the use of antimosan for the treatment of

T.congolense infection in guinea pigs either when each generation was treated once or when the trypanosome was submitted to the largest possible number of treatments in each guinea pig.

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