

### C; ANTIMONIALS.

The important discovery of Plimmer and Thomson (1907), acting on the suggestion of Cushny, of the trypanocidal effect of potassium antimony tartrate on trypanosomes in experiments on rats gave a marked stimulus to investigations on the use of salts of antimony. Previous to this the chemotherapeutical work was to a great extent concentrated on various dyes and salts of arsenic especially atoxyl. Plimmer and Thomson also tried out sodium antimony tartrate on account of the toxic and irritant effects of the potassium salt. Within a short period a considerable amount of data had been collected which, on the whole, <sup>WAS</sup> were distinctly indicative of ultimately successful results with the use of antimony salts in the treatment of some, at any rate, of the trypanosomiases.

Manson (1908) was the first to use an antimony salt for the treatment of human trypanosomiasis. He administered sodium antimony tartrate subcutaneously, intramuscularly and orally, but as the administration produced considerable local damage and nausea the use was not persisted in.

Broden and Rodhain (1908), in order to avoid the objectionable effects of pain and irritation resulting from hypodermic injections, gave the solution of potassium antimony tartrate intravenously with only occasionally mild symptoms of intoxication.

On account of the superiority of atoxyl over other arsenicals used at the time, e.g. arsenious acid, sodium arsenite it naturally followed that research would be directed along the lines of producing some antimony salts which would not have the disadvantages of potassium antimony tartrate, salts which would correspond to the more advanced arsenicals. Brein<sup>e</sup> and Nierenstein (1908) succeeded in preparing the p., m., and o. aminophenyl-stibinic acids. The o. compound was early discarded. The p. compound gave most promise and could be given hypoder-

mically. From this time numerous other antimonials were introduced. These were both the pentavalent and trivalent compounds. The former were found to be inefficient. Consequently for the trypanosomiasis the trivalent antimony compounds were the ones which were ultimately selected for trial.

Rowntree and Abel (1910) prepared and tested out further antimonials namely antimony sodium, thioglycollate and the triamide of antimony thioglycollic acid.

The complex organic antimony compounds now available amount to a considerable number. In rapid succession were produced Sb 212, stibenyl, stibosan, urea stibamine, neostam, neostibosan, antimosan, fouadin. Some of these were pentavalent compounds and more applicable to other tropical diseases than the trypanosomiasis. The introduction of the trivalent organic antimony compounds lead to a definite advance in the chemotherapy of the trypanosomiasis.

#### C (a) OTHER THAN ANTIMOSAN.

Notwithstanding the enormous amount of experimental work carried out on the use of antimonials in the treatment of the trypanosomiasis since the publication of Plimmer and Thomson on the use of potassium antimony tartrate, it is surprising that, until the last few years, we find that the chief reliance practically throughout the African continent for the treatment of some of the trypanosomiasis of domestic animals has been placed on potassium antimony tartrate. This drug was introduced to Southern Africa for treatment of trypanosomiasis by Bevan (1910) who used it in cattle. Andrews (1913) and later Curson (1928) carried out a considerable amount of field and laboratory work and recommended it as the drug of choice. An influencing factor for its use is its cost which is low. As this drug has been and is still used extensively in Zululand, it is necessary to write shortly on its advantages and disadvantages before continuing to the consideration of antimosan. Graf (unpublished) used in his experiments antimony sodium thioglycollate, the triamide of

antimony thioglycollic acid, Sb 212 and stibosan at times in combination with naganol. His results are tabulated together with the results obtained by other workers in South Africa with antimonials in Table IX.

Potassium antimony tartrate is always administered by the intravenous route and is recommended for use in T.congolense and T.vivax infections in cattle. In West Africa it is also used for the latter infection in horses. The number of injections in a course of treatment, the dosage and the intervals at which the injections are given vary somewhat in the different areas. In Zululand a single injection consists of 1 to 1.5 gm. in 20 to 30 c.c. water, and, as a routine measure, five injections are given on consecutive days. The Tanganyika authorities employ eight consecutive weekly injections of 20 c.c., each of a 4 per cent. solution. Other countries utilize one or other of these two, which represent the shortest and the longest courses of treatment, or slight modifications of them.

The vein selected for the injection is usually the jugular. Consequently the animal in most cases has to be controlled by casting or some other complicated method. Bovines living in or near tsetse fly areas are not usually of much value and are left to range practically uncontrolled. Any treatment instituted therefore must take into consideration that the animals are often troublesome to collect and difficult to handle and control. If the drug utilized in the treatment is to be injected, the administration by the subcutaneous or intramuscular routes instead of by the intravenous, would simplify matters considerably.

The operation of intrajugular injection is by no means a simple one. Skill is especially necessary when the material to be injected is as irritant as the solution of potassium antimony tartrate is. The local effects of faulty administration might be extremely serious, interfering often with the subsequent administration of the drug. A trypanocidal drug which could be administered by the subcutaneous or intramuscular routes would be a great

advance in the chemotherapy of bovine trypanosomiasis.

Potassium antimony tartrate has been found to be of little or no value in treatment of bovine trypanosomiasis caused by T.brucei. Even though this parasite is comparatively rare in bovines and does not produce marked ill-effects in the animals, yet it would certainly add to the value of any treatment if it could be shown that it was also effective against T.brucei type of bovine trypanosomiasis. The efficacy of potassium antimony tartrate in bovine trypanosomiasis caused by T.congolense and T.vivax does not need to be commented on here as the references in literature on this point are numerous.

A decided advantage in favour of the use of potassium antimony tartrate is its cheapness. In Africa, especially in those areas where it becomes necessary to institute treatment for trypanosomiasis, the bovines are of small value. Consequently any treatment employed must be cheap enough to justify its use on an economical basis. Potassium antimony tartrate fulfils this condition ideally, the cost of an average dose being approximately one-fifth of a penny. The cost of the drug is thus of practically no hindrance to its wide application in bovines of even small value. Even though the first essential in the use of a treatment for such bovines is cheapness yet it should naturally be an ideal to provide the most effective method of treatment possible and then to endeavour to cover any additional expense connected with the drug by the improvement of the type and class of animals kept. The introduction of improved breeds in any area is often dependent on the introduction of better methods of treatment of diseases; the native stock, usually more resistant, are replaced only when improved methods of disease control enable the more susceptible but greater revenue producing improved cattle to survive.

TABLE IX.

Antimonials other than Antimonial.

Name of drug	Species of animal.	No. of animals.	Maximal single dose.	Maximal total per animal.	Controlled by:	Remarks.
Potassium antimony tartrate.	Sheep & goats.	35	0.69 gm.	2.3 gm.	Graf & D.V.S.	In some cases with arsenicals, Naganol and other antimonials.
"	Bovines	4	1.2	" 7.5	D.V.S.	-
"	"	9	1.5	" 7.0	Robinson	In some cases with Neosalvarsan and Tryparsamide.
"	Equines	2	2.6	" 8.0	D.V.S.	-
Sb-Na-thioglycollate.	Sheep & goats.	4	3.4	" 17.0	Graf &	In some cases with Sb-thioglycollamide.
Sb-thiocollamide.	"	5	4.8	" 24.0	"	In some cases with Sb-Na-thioglycollate
Sb 212	"	6	1.5	" 7.1	Graf	In some cases with Stibenyl and Stibosan.
"	Bovines	2	1.5	" 7.0	D.V.S.	-
Stibenyl	Sheep	1	1.8	" 9.0	"	With Sb.212 and Stibosan.
"	Bovines	2	3.0	" 11.0	"	-
"	Equines	2	2.5	" 17.2	"	-
Stibosan	Sheep & goats.	3	1.2	" 9.0	Graf	With stibenyl and Sb. 212.
"	Equines	3	3.5	" 25.0	D.V.S.	-

(b) ANTIMOSAN.

On account of the known efficacy of various antimonials against certain of the trypanosomiases affecting the domestic animals it was decided to choose from the antimony compounds for further investigations. This work was commenced by the writer in January 1929 and is still being continued. The drug antimosan now known as "old antimosan" was chosen at that time.

Antimosan or "old antimosan" is a product of the I.G. Farben-Industrie - Aktiengesellschaft, Elberfeld, and is described as a complex antimony salt of pyrocatechin. In this compound the oxide of antimony is so firmly combined that the salt remains unaltered in the  $P_H$  conditions of the body fluids. The drug can be given intravenously, subcutaneously or intramuscularly. It is a white powder easily soluble in water and contains 12.5 per cent. of metallic antimony. It was supplied (a) in ampoules of 25 c.c. in a 12 per cent. solution, (b) in ampoules of 40 c.c. in a 7 per cent. solution and (c) in tablet form each tablet representing 1 gm. This antimosan was the potassium salt.

The following information in connection with its toxicity is obtained from a note by Hans Schmidt (1931).

For mice 0.27 to 0.37 mg Sb. when in form of potassium antimony tartrate was lethal; 0.14 was badly borne. Whereas 0.25 mg. Sb. in form of antimosan was well borne, 1.0 mg. Sb. being required to produce lethal effects. In rabbits the dosis tolerata for potassium antimony tartrate in Sb. was 2.7 mg. per Kg. and for antimosan 10 mg. per Kg.

Khašil (1931) found that for dogs potassium antimony tartrate was six times more toxic than antimosan.

Uhlenhuth, Kuhn and Schmidt in 1924 reported on the action of antimosan in cases of trypanosomiasis. Curson (1926) studied its effects on T.congolense and T.brucei infection of mice and came to the conclusion that, in these animals, it was a good curative agent for the T.congolense infection but not a promising one for the T.brucei infection.

Trials of antimosan in trypanosomiasis of domestic animals were subsequently made in a few parts of Africa. Some success was attained in its use at Onderstepoort in 1926 by Graf who for various reasons did not follow up this work. He did not publish his results. In Tanganyika Territory (1927, 1928) a few bovines were treated with antimosan by itself and in combination with potassium antimony tartrate, the former drug being given subcutaneously and intravenously. The outcome of this work was the suggestion that antimosan be given intravenously in T. congolense infection of bovines. The use of the intravenous method of administration, <sup>in</sup> the writer's opinion, immediately destroys one of the chief advantages of the drug.

As far as the ease of administration is concerned, antimosan is immeasurably superior to potassium antimony tartrate. There is when the subcutaneous route is employed no necessity for elaborate methods of control, there is elimination of the often severe complications associated with the escape of the irritant potassium antimony tartrate solution into the perivascular tissue and further, as will be later shown, there is a great reduction of the number of administrations necessary to bring about sterilization or to maintain the bovines in health in areas where they are constantly exposed to re-infection. For the subcutaneous administration the use of a crush, such as can be easily constructed locally and such as is <sup>s</sup> often required for other operations of a like nature, e.g. anthrax immunization, provides a simple and speedy method of control for the injection. The handling of the individual animals is almost entirely eliminated.

No complications or objectionable sequelae of any importance have been noted in connection with the subcutaneous administration of antimosan in bovines. In horses the 12 per cent. solution produced at times considerable damage to the tissues at the site of inoculation. In bovines, however, no damage of any moment was observed from the use of this hypertonic solution. At times swellings appeared as a result of the injection which was carried out without any special precautions,

but in only one case was an abscess observed and at no time was there interference with subsequent injections. For this reason the treatments hereafter described were all carried out with the 12 per cent. solution given subcutaneously. The advantage of the use of this solution is that there is a considerable reduction of the bulk of the solution as compared with the 7 per cent. solution, but this advantage possibly is not great enough to justify the continuation of its use. Its replacement under field conditions by the 7 per cent. solution is indicated. Recently the potassium salt has been replaced by the sodium salt and the strength reduced to a 6.3 per cent. solution.

The cost of treatment with antimosan taking into consideration the value of the individual bovines which are usually found in areas exposed to trypanosomiases is a factor which may interfere considerably with the wide use which is justified by its efficacy. However, it may be anticipated that the improvement in the stock which might be expected to follow as the result of improvements in chemotherapy of the disease will produce a more extended use of the drug.

#### (a<sub>1</sub>) ANTIMOSAN IN BOVINE TRYPANOSOMIASES:

The consideration of the chemotherapy of antimosan in the trypanosomiases of the various domestic animals is undertaken separately for the different animals. In bovines the trypanosome which is of special importance is T.congolense but bovines become infected also with T.vivax and T.brucei. Consequently the effects of the drug on the two last named parasites should also be considered. No opportunity, however, has presented itself to test out the efficacy of antimosan in T.brucei infection of bovines. It is not anticipated that antimosan treatment would prove to be a failure in T.brucei infection of bovines for the REASON That T.brucei infection of horses, which is much more severe in its effects in horses than in bovines has shown, as will be noted later in this report, a good response to treatment



with antimosan.

BOVINE TRYPANOSOMIASIS DUE TO T.VIVAX.

For this experiment five bovines were utilized. These were infected by sub-inoculation from an infected bovine on the 10th January, 1929, by the injection of 10 c.c. blood subcutaneously. In no case was there are failure in the transmission. Diagnosis was chiefly centred on the examination of lymphatic gland smears. Unfortunately there was transmitted over at the same time piroplasmosis and anaplasmosis with the result that the animals lost condition rapidly and had to receive treatment for these diseases. One of the experimental animals died from the anaplasmosis.

Table X gives the details in connexion with this experiment. The antimosan in every case was given subcutaneously in a 12 per cent. solution.

Table X.

Bovine	Date of infection.	Complications.	Treatment commenced.	Wgt. of each dose. gm.	No. of doses	Intervals	Remarks.
2715	10/1/29	Piroplasmosis Anaplasmosis	31/1/29	3 gm.	1	-	5/2/29 died Anaplasmosis.
2765	"	"	12/3/29	1.8	3	8 days	Sterilization. No tryps. found to 27/5/29.
2766	"	"	4/3/29	1.8 1.2 1.8	2 2 1	8 days	Sterilization. No tryps. found up to 27/5/29.
B.2743 <sup>x</sup>	"	"	-	1.2 1.3 3.0	1 1 1	8 days	Sterilization. No <u>T.vivax</u> found up to 27/5/29.
B.2727	"	"	Control	-	-	-	Tryps. were found up to 28/8/29.

x this bovine was found to have become infected with T.congolense just before treatment, which did not sterilize it of T.congolense

DISCUSSION.

Notwithstanding that the bovines in this experiment

lost condition rapidly on account of the trypanosomiasis<sup>and</sup> inter-current affections, yet they showed no constitutional disturbance as a result of the injection of the antimosan. It was not expected that the mortality among these animals would be high for our experience has been that, if bovines suffering from T.vivax disease are kept under good conditions, the disease rarely proves fatal. The one animal that died in this experiment had an extremely severe attack of anaplasmosis.

On account of the comparatively minor importance of T.vivax infection and the success which was obtained in the above preliminary trial of antimosan in the treatment of T.vivax disease, it was decided not to institute further trials but rather to concentrate on the more important and urgent problem of T.congolense infection.

#### CONCLUSION.

(1) Of the four bovines treated with antimosan, three were apparently sterilized and one died of anaplasmosis.

(2) The control showed T.vivax for a period of 230 days.

#### BOVINE TRYPANOSOMIASIS DUE TO T.CONGOLENSE.

The antimosan therapy of bovine trypanosomiasis caused by T.congolense is dealt with below under two headings namely (1) Short Interval Treatment, and (2) Long Interval Treatment. By the former is understood treatment by the administration of antimosan at intervals of 8 days or less and by the latter at intervals of 28 days.

#### SHORT INTERVAL ANTIMOSAN THERAPY.

The treatment of trypanosomiasis in general has been almost entirely on the lines of the administration of the selected drug on consecutive days, or at intervals of a few days at the most. Consequently such short interval methods of administration were followed in the first trials of the efficacy of antimosan in T.congolense infection of bovines.

PRELIMINARY EXPERIMENT.

For the first experiment five bovines were utilized. These bovines were infected by the subcutaneous injection of 5 c.c. citrated guinea pig blood rich in T. congolense. In no case<sup>was</sup> there a failure in the transmission. Diagnosis was chiefly centred on the examination of blood smears. Table XI. gives the details in connexion with this preliminary trial. The antimosan in every case was given subcutaneously as a 12 per cent. solution.

Table XI.

T. congolense. Short interval treatment.

Bovine	Date of infection	Complications.	Treat-ment com-menced.	Wgt. of each dose. gm.	No. of doses.	Inter-vals.	Remarks.
2634	10/1/29	-	3/2/29	1.2	5	8 days	Unsuccessful
2639	"	anaplasmosis	23/1/29	3	5	8 days	Sterilization.
2702	"	-	8/2/29	3	2	8 days	"
2709	"	anaplasmosis	16/2/29	1.2	5	4 days	Unsuccessful.
2714	"	-	Control	-	-	-	Smears + after 5 months.

Discussion: As a result of the treatment bovine 2634 showed a marked decrease of the severity of the disease for a period of 10 weeks when the trypanosomiasis again commenced to show acute exacerbations. During treatment the trypanosomes were found during all the intervals between injections. Bovine 2709 showed results very similar to those of 2634 but acute exacerbations did not become evident. The ultimate result of treatment in this animal even though it was a failure as far as sterilization was concerned was excellent. Its anaemia disappeared entirely, the red cell count returning to normal, its condition became good and trypanosomes became difficult to find. Bovines 2639 and 2702 were both sterilized. This was proved by negative blood smear examinations over a period of 9 and 10 weeks respectively, by sub-inoculation each into two susceptible bovines and by the test for determination of sterility to be described later.

Conclusions: (1) Subcutaneous injections of 3 gm. antimosan repeated twice and five times at intervals of 8 days resulted in sterilization.

(2) Subcutaneous injections of 1.2 gm. antimosan repeated five times at intervals of 8 and 4 days did not produce sterilization.

ANTIMOSAN AT INTERVALS OF 7 DAYS;

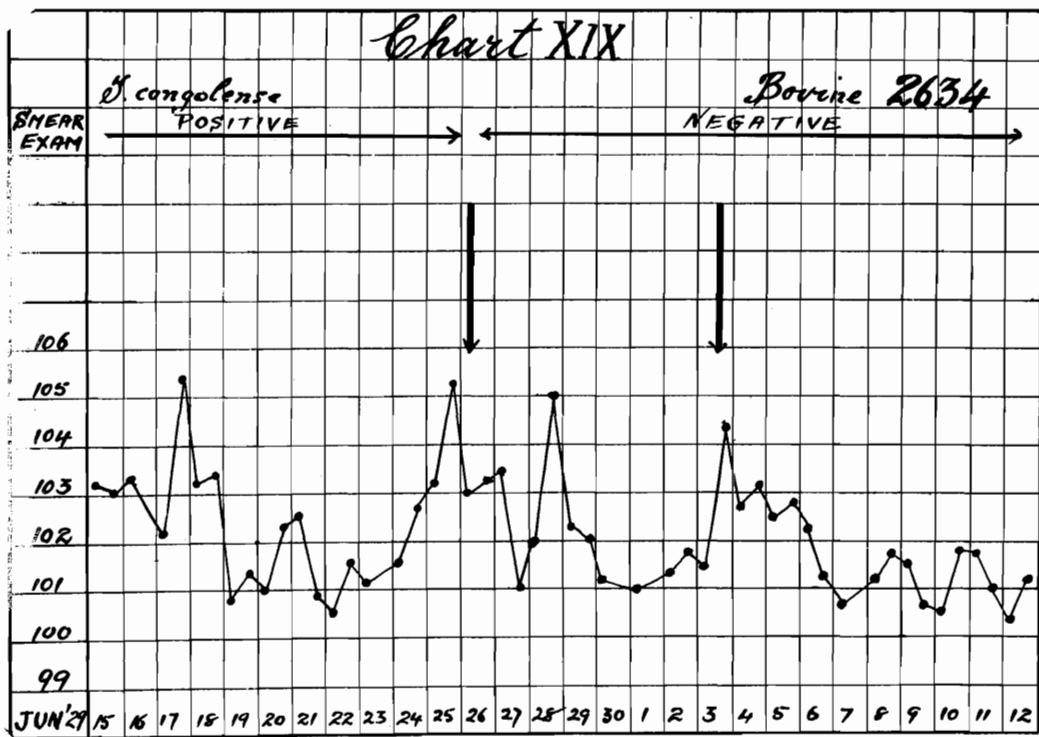
As a result of the above experience, further bovines were submitted to treatment. Details are tabulated on Table XII. Of these five bovines two, namely 2634 and 2714, were one failure and the control respectively of the previous experiment and one, 2743, was brought forward from the T.vivax experiment. This latter animal had become stable infected with T.congolense just prior to the treatment which was successful against the T.vivax infection, but unsuccessful against the T.congolense infection. The time of infection in each case is the date of the original infection with the exception of 2743, where the date indicates the first detection of T.congolense in the blood smears.

Table XII.

T.congolense. Short interval treatment(7 days).

Bovines	Wgt. in Kg.	Date of infection.	Date of Treatment commenced	Each dose in gm.	No. of doses	Dose per Kg.	Remarks.
2634	238	10/1/29	26/6/29	3	2	GM. 0.013	Sterilization. Negative smears for 11 weeks.
2714	231	10/1/29	26/6/29	3	2	0.013	-do-
2464	396	20/4/29	26/6/29	3	2	0.008	Unsuccessful. Tryps found 7th week.
2468	375	20/4/29	26/6/29	3	2	0.008	Unsuccessful. Tryps. found 8th week.
2743	181	5/4/29	20/4/29	3	2	0.017	Unsuccessful. Tryps. found 3rd week.

Chart XIX illustrates the sterilization of bovine 2634 by two doses of antimosan at a seven-day interval.



Discussion.- It is of interest to note that bovine 2634, which had not been sterilized by the previous treatment of 1.2 gm. repeated 5 times yet became sterilized by the administration of the same total quantity divided into two doses, whereas bovine 2743 which was of less weight and which failed (see Table X) to an administration of 6 gm. given as 1.2 gm., 1.8 gm., and 3 gm. again failed to a total of 6 gm. divided into two doses. Yet it is remarkable that a single dose of 6 gm. resulted in sterilization of this animal (see Table XIII). The two bovines 2464 and 2468 considerably heavier than the others did not become sterilized. However, there was a marked depression of the virulency of the trypanosomes, the anaemia practically disappeared and the condition of the animals improved. The greater weight of these two probably was a factor in the failure of the treatment.

Conclusions.- Antimosan in a 3 gm. dose repeated at an interval of one week produced sterilization in two out of five bovines. Of the failures two were decidedly heavier than the successful cases and one had been submitted to increasing doses previously.

#### ANTIMOSAN AT INTERVALS OF LESS THAN SEVEN DAYS.

To enable a saving of time to be brought about in the treatment when the object is to obtain rapid sterilization of bovines, for example, when it is desired to remove bovines from an infected to a non-infected area, it was decided to test out somewhat larger doses than had previously been employed, or moderate doses repeated on consecutive days. The results obtained are tabulated in Table XIII. As previously, the date of infection of bovine 2743 indicates the date on which T. congolense was first found in blood smears, whereas in the other three bovines it indicates the date of inoculation.

Table XIII.

T. congolense. Short Interval Treatment.

Bovine.	Wgt. in Kg.	Date of infection.	Date Treatment commenced.	Each dose in gm.	No. of doses	Dose per Kg.	Remarks.
2743	236	5/4/29	16/10/29	6	1	0.025	Sterilization. Negative smears to 51st day.
3627	318	13/9/29	16/10/29	6	1	0.019	Unsuccessful.
3542	204	13/9/29	24/10/29	6	1	0.029	Unsuccessful.
3527	218	13/9/29	24/10/29	3	2	0.014	Sterilization. Negative smears to 71st day.

Discussion.- The results were not sufficiently promising to justify the continuation of experimentation along these lines especially as constitutional disturbances were noted in bovine 3542 and 3627. Alternative methods of treatment with a greater expenditure of time have proved to be so successful that it is not considered that the above procedure would develop into a method of treatment which could be recommended for practical application. It is worth while again to note that sterilization of bovine 2743 resulted with a dose of 6 gm. whereas two previous failures had occurred with a similar dosage administered as three ascending and two equal doses. This animal was intermediate in weight of 3627 and 3542, the two failures. It, however, had been infected for approximately 150 days longer. The variation in response to treatment of bovines infected for varying periods will be referred to again in this article.

Conclusions.- (1) The administration of more massive single doses of antimosan to obtain immediate sterilization was not of sufficient promise to justify, at this stage, the continuation of the experimentation.

(2) Similar conclusions were arrived at in connexion with the administration of antimosan on consecutive days.

## SUMMARY AND CONCLUSIONS OF SHORT INTERVAL TREATMENT.

The lines of experimentation in the foregoing treatments of bovine trypanosomiasis caused by T. congolense have followed more or less the lines generally advocated in the treatment of trypanosomiasis in general. By the time these experiments on Short Interval Treatment had reached this stage, considerable advance had been made in the experiments in connexion with the Long Interval Treatment. As this latter held out such great promise in the surmounting of the problem of bovine trypanosomiasis all facilities available were devoted to its study. The experimentation on the Short Interval Treatment consequently was discontinued.

As far as the experiments went, however, it would appear that a dose of 3 gm. of antimosan given twice at weekly intervals would be insufficient to bring about sterilization in bovines above 250 Kg. in weight. The indication is that the dose should be increased or perhaps preferably repeated more often. Even for bovines lighter than 250 Kg. this treatment might prove successful.

## LONG INTERVAL ANTIMOSAN THERAPY.

Until such time that methods are developed and instituted to destroy the tsetse fly, the sterilization of bovines of trypanosomes which have to continue to live near or in tsetse areas is of problematic advantage. Such sterilized bovines become susceptible again and would consequently have to resist further attacks of the disease. The sterilization of bovines has not proved a difficult undertaking but no lasting benefit can be looked for unless such bovines are removed entirely from further contact with infection. If a true immunity were to result after recovery such sterilization would be of considerable benefit, but no evidence of such immunity has been obtained throughout this work.



Consequently, when bovines have to live in or near tsetse fly belts, some substitutional method of control must be devised. The treatment already described under Short Interval Treatment results in sterilization, but it is manifestly impossible on account of expense of drugs and of handling to be continually treating bovines by that method. It was conceived that this difficulty might be overcome by utilizing a long interval routine method, a method which would entail a minimal amount of handling and a small annual expenditure on drugs.

It is a well known fact that the treatment of trypanosomiasis with arsenic compounds has already<sup>WAYS</sup> been on the principle of the administration of large doses with the object of destroying the trypanosomes as quickly as possible. The danger otherwise is that drug-fast trypanosomes may be developed. Somewhat similar conditions are held to govern the treatment of trypanosomiasis with antimony compounds.

Bearing in mind these points, it can be seen that the institution of experiments for the object of observing the effect of doses of an antimony compound given at long intervals appeared to be scarcely justified, especially after the failures recorded in the treatment of Bovine 2743 already referred to,

Notwithstanding this, such experiments were decided on, not with the idea that sterilization would be obtained, but for the purpose of determining whether by such means bovines could be enabled to survive in tsetse fly areas. It was fully expected that such a procedure would result in the production of drug-fast trypanosomes, but it was hoped that a method might be obtained whereby the trypanosomes are caused to disappear for a period in between the administration of the drug. Such disappearance or partial control of the disease would then free the animal for part of the time at any rate from the detrimental effects of the parasite and enable<sup>E</sup> it thus to survive in infected areas. Furthermore, it was argued that even though non-sterilizing doses at short intervals might result in the production of drug-fast parasites, yet it did not necessarily