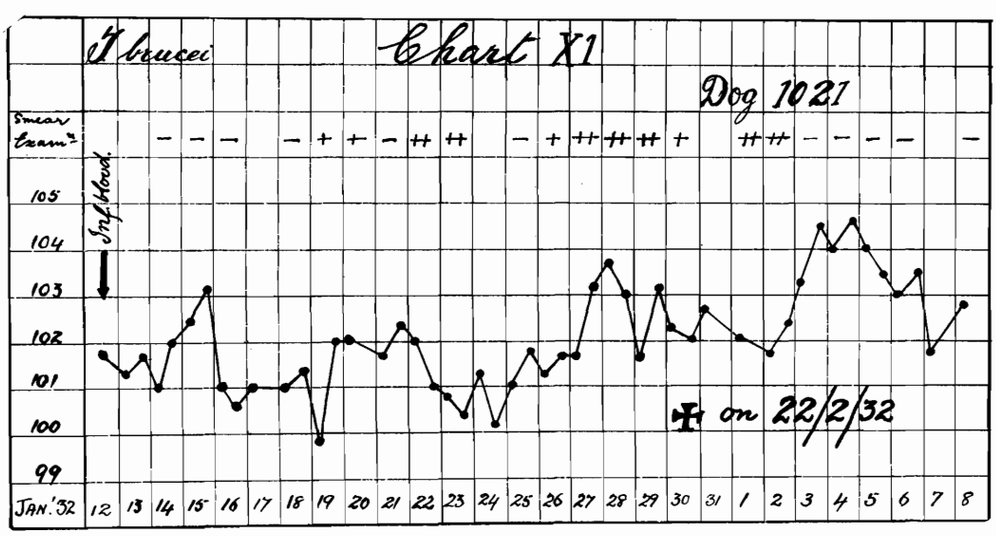
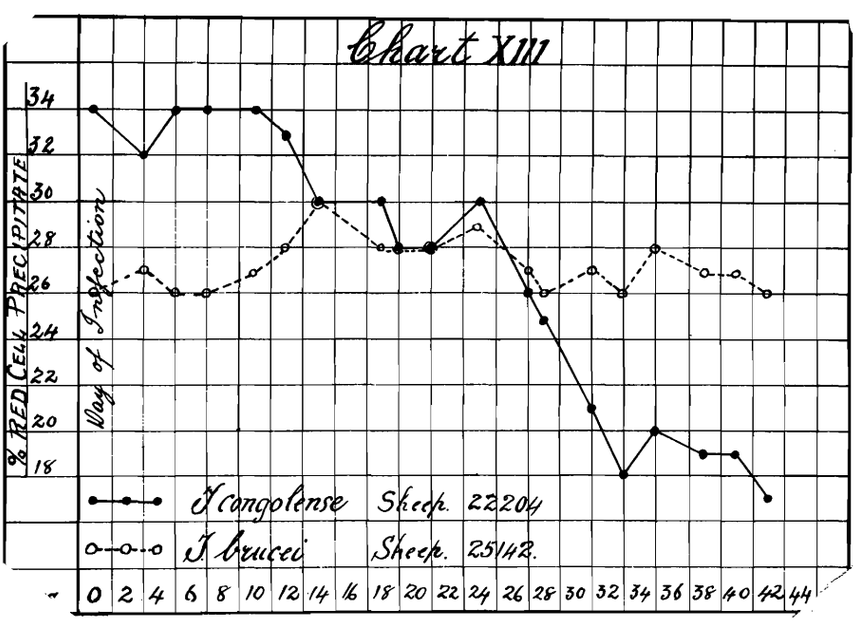
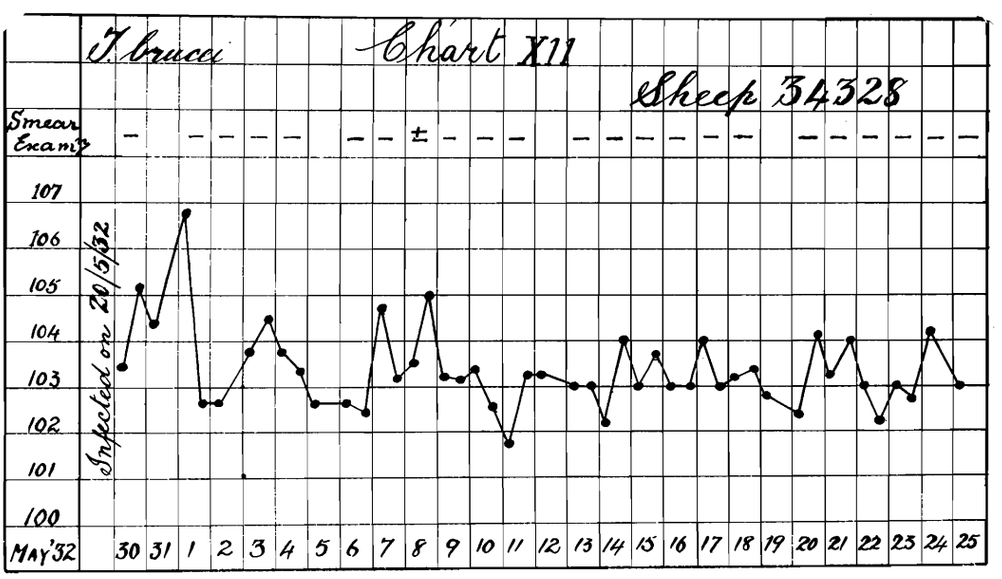
produced by the subcutaneous injection of blood of a horse infected with T.brucei. The shortest period of incubation in these dogs was, when judged from the first appearance of the parasite in blood smears, seven days and from first elevation of temperature sixteen days. If further sub-inoculation had been carried out possibly shorter periods would have been obtained, for the strain of T.brucei had been maintained in horses for two and a half years. The trypanosomes were in most cases difficult to find in the blood smears. The course compared with T.congolense infection in the same animal was long and even though death resulted ultimately in all the cases there was not the same acuteness associated with the infection as with that of T.congolense. Other than the fever symptoms there were no symptoms of note. The absence of oedema and of eye-lesions as compared with the definite development of these in T.congolense infection is of interest.

(iii) Symptomatology in sheep. Of all the trypanosomiases observed T.brucei infection of sheep gave the most meagre symptoms. Other than mild fever symptoms, no abnormality was determined. The temperatures at the commencement of the infection are somewhat irregular but they soon settle down to a comparatively non-oscillating type. The parasites were extremely difficult to find in the blood smears. As an illustration of the difficulty of diagnosing the infection by this means may be instanced the blood smear examination of three sheep the first, second and third generation of the trypanosome after leaving the reservoir, a horse. In the first generation one trypanosome was found in 22 blood smear examinations, and in the second and third no trypanosomes were found in 60 examinations. The complement fixation test was positive. In addition gland smears were examined with negative results. There are no indications of anaemia when judged from the determination of red cell precipitate Chart XIII gives the determinations in a sheep infected with



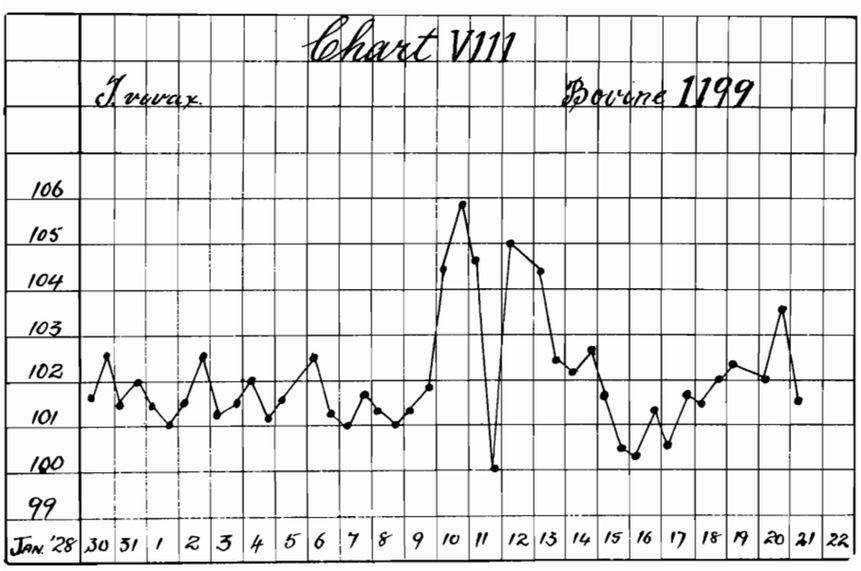




T.*brucei* and in one infected with *T.congolense*. In another sheep the red cell precipitate showed practically no variation during the period of observation which extended from the 24th June to 26th August 1932.

(c.) SYMTOMATOLOGY OF T.vivax INFECTION.

Symtomatology in bovines. This infection was produced in all cases except one by the injection of the blood of an infected bovine. As these carriers of the infection had at some previous time been infected with Piroplasma bigeminum and, or, Anaplasma marginale the T.vivax infection was complicated with these diseases. Consequently the temperature curve did not give a true index of the T.vivax infection. In the exception noted above the T.vivax was transmitted to a bovine by the bite of a tsetse fly. This temperature curve on Chart VIII is thus the only true curve of T.vivax infection available. The temperatures subsequent to the initial stage do not show marked exacerbations and remissions. Constitutional disturbances are not conspicuous. There is at first some inappetence and a loss of condition, the latter of which might not be fully recovered for a few months. During this period of bad condition the coat is dry and poor in appearance. The superficial lymphatic glands are enlarged. The parasites are difficult to find in the blood smears. In one case, bovine 2727, the animal was not treated and regular examination of both blood and gland smears was carried out over a period of 44 weeks. During the 33rd week T.vivax was found in a gland smear, but no trypanosomes were found either in blood and gland smears for 15 weeks before and 11 weeks after this finding, representing in all an examination of 108 smears. T.vivax infectio in bovines under the conditions prevailing at Onderstepoort thus is a disease which does not produce effects of any great severity. The majority of animals recover, i.e. they regain their normal condition and appearance without the institution of treatment. The possibility of a spontaneous sterilization must also be considered. As an example of this may be instanced the case of bovine 2611. This bovine was infected by the injection of the blood of an infected bovine on 14th July 1928. T.vivax was found in stained smears on the 8th day after infection. The



last finding of T.vivax was in a smear made on the 9th December 1928. It is quite possible that a more frequent examination of smears would have resulted in the finding of T.vivax at a later date than this. On the 4th July 1929 the animal was splenectomised. The smear examination was more intensive shortly before and for some years after the splenectomy nearly 900 blood and gland smears being examined, but no trypanosomes were found. Furthermore, inoculations of blood of bovine 2611 into three susceptible bovines were carried out with negative results in August 1929, April 1930 and May 1931. (I wish to thank the Director for permission to quote this example from his experiments).

CHAPTER V.

Diagnosis of Trypanosomiases.

The diagnosis of the trypanosomiases dealt with was, in the majority of cases, most conveniently arrived at by the examination of stained smears made from blood or gland tissue. In some cases more especially during the course of experimental chemotherapeusis the use of this method did not suffice for it often happened that smear examination did not determine the presence of the parasites. In such circumstances other methods had to be utilised.

Before dealing with the various methods employed for arriving at the diagnosis the following comparisons made between T. congolense and T. vivax infection of the bovine from the point of view of smear examinations and the notes on the smear examination of these infections in the other animals are of interest.

To obtain a comparison of the relative frequency of the trypanosomes in blood and gland smears in T. congolense and T. vivax infections of bovines counts of a large number of trypanosomes were made. In the case of blood smears the number of trypanosomes in 50 fields and in the case of gland smears the number in 25 fields were made with the exception which is noted, of a case where an estimate was arrived at on account of the large number of trypanosomes present. It is realized that the figures do not represent a high degree of accuracy on account of the varying thickness of the smears from day to day, but as the counts were made over a large number of fields the ultimate results do give a fair indication of the relative frequency of the two species in gland and blood smears. The smears were all made by the same assistant. A cypher represents a negative examination after a prolonged search. Tables III and IV summarize the results obtained before the institution of treatment.

TABLE III.

T. Congolense - Smear Examination.

Bovine.	Blood Smears				Gland Smears				Highest count in	
	No.of Fields	No.of ~~types~~ TRYPS.	No.of +smears	No.of -smears	No.of fields	No.of ~~types~~ TRYPS	No.of +smears	No.of -smears	50 blood fields	25 Gland field
2634	900	1,730	18	0	450	31	6	12	414	11
2639	350	779	7	0	175	14	3	4	216	9
2702	850	558	17	0	425	8	7	10	78	2
2709	1,050	1,326	21	0	525	17	9	12	221	7
2714	3,750	3,241	68	7	1,500	38	22	38	542	8
Total	16,900	7,634	131	7	3,075	108	47	76	542	11

Discussion:

The above figures demonstrate clearly that the diagnosis of T. congolense infection should be based on blood smear and not gland smear examination, for in the case of blood smear examination one trypanosome is found on an average in slightly less than one field whereas in case of gland smears 1 trypanosome is found only after examination of approximately 28 fields. Furthermore, the proportion of positive smears to negative smears in the examination of blood smears is approximmately 19 to 1, where-as in examination of gland smears it is about 1 to 1.6. Several high counts were obtained in examination of some blood smears, but in case of the gland smears, the counts were always low.

TABLE IV.

T.vivax - Smear Examination.

Bovine.	Blood Smears.				Gland Smears				Highest count in	
	No.of Fields	No.of Tryps.	No.of +smears	No.of -smears.	No.of Fields	No.of Tryps.	No.of +smears	No.of -smears	50 blood Fields	25 Gland fields
2715	500	5	5	5	250	50	7	3	1	19
2727	1,050	12	3	10	500	139	16	4	6	39
2743	800	1	1	15	375	111	10	5	1	77
2765	400	4	3	5	200	552	7	1	2	436
2766	800	12	5	13	450	155	13	5	8	46
Total	3,650	34	17	56	1,775	1,007	53	18	8	436

In the blood smear series it is seem that it is necessary to examine approximately 107 fields to detect one Trypanosome, whereas the examination of not quite two fields in the case of gland smears, is necessary for this purpose. Also there were, in the blood smear series, 56 negative smear examinations in every 73 examined, while in the case of the gland smear examination only 18 were negative of a total of 71. A remarkably high count of 436 trypanosomes in 25 fields was obtained in a gland smear. The highest obtained in a blood smear was only 8 trypanosomes in 50 fields.

When a relapse occurred after treatment in T. congolense infection, trypanosomes, were always more numerous in the blood smears than in the gland smears. Table V gives the details in connection with the examination of bovine 2634 in which animal treatment did not bring about sterilization.

Table V.

Bovine 2634. T. congolense - Smear examination during and after treatment.

Blood Smear.				Gland Smear.				Highest Count in:	
No.of fields	No.of Tryps.	No.of +smears	No.of -smears	No.of Fields	No.of Tryps.	No.of +smears	No.of -smears	50 blood fields	25 Gland fields
1,300	4,858	26	0	625	32	8	17	2,425[x]	12

[x]This figure was calculated from a count over 20 fields.

In this bovine the only one in which a count was continued for any length of time, the disparity previously noted between blood and gland smear examination is still more marked. Nearly four trypanosomes were found per field of the blood smear, whereas it was necessary to examine on an average nearly 20 fields to find one trypanosome in the gland smears. The highest count of trypanosomes found in 50 fields of a blood smear was over 2,400. In the gland smear made at the same time a careful search failed to reveal a single trypanosome.

From the foregoing it may be concluded that in T. congolense infection of bovines the smear diagnosis

is centered on blood smear examination whereas in T. vivax infection it is centered on gland smear examination, the gland and blood smear examination being, respectively, of little practical assistance. Montgomery and Kinghorn (1908) make mention of gland puncture as a means of detecting the parasite regarding it as secondary in importance to the examination of blood smears. They were dealing with T. congolense and their results, consequently, are understandable. Dutton and Todd (1907) also used the gland puncture method in the Congo. They regarded as a most successful method of diagnosis. With gland examination they obtained 28 positives out of thirty cases whereas the blood examinations gave them only 13. There is little doubt that many of these cases must have been due to T. vivax. Recently du Toit (1929) has recommended the examination of stained gland smears as the method of choice in the diagnosis of T. vivax infection of bovines. The results obtained by the writer and shewn in Table IV support his contention in a striking manner.

So difficult is the diagnosis at times in the trypanosomiases especially after the animal has been treated that the deductions made after testing drugs might be entirely wrong unless great care be exercised. The smear examinations particularly are apt to lead one into errors and it would be advisable to regard only positive smears as giving full value. In natural cases similar precautions in connection with the value of negative smears should be taken for under specially favourable conditions of food and housing the parasites may be extremely rare. Even in a virulent infection of T. brucei of horses it often happens that blood smear examination gives negative results, although the case is obviously passing to a fatal issue. These precautions are particularly necessary when dealing with T. brucei. It therefore became necessary to devise other methods of diagnosis or what was even of greater importance of determining sterilization of an animal after treatment. The following

methods thus even though in some cases of no practical use in other than experimental work proved of assistance and worth for the purpose.

(1) Blood smear and gland smear examination in stained and wet smears. These are the absolute and direct methods for diagnosis. As already mentioned value in most cases should only be placed on a positive result or on a negative result when supported by an examination over a long period and by a temperature curve not indicative of a trypanosome infection.

(2) The subinoculation of blood into laboratory animals ~~or domestic animals~~ is unsatisfactory on account of the difficulty even in the most favourable cases of obtaining the infection in the small animals. The time infection may take to evince itself in these animals is an additional objection. The sub-inoculation of blood from one species into the same species has always proved satisfactory and it probably the method of choice. The cost of this method, however, is a definite objection and it was not employed in every case.

(3) Serological tests. The complement fixation test was of some service. It was utilized in T. brucei infection of horses and T. congolense infection of bovines. In the former as antigen T. equiperdum proved to be fairly satisfactory but to obtain the best results it was necessary to prepare T. brucei antigen. In the latter considerable difficulties were met with in the preparation of a suitable T. congolense antigen.
Robinson (1931) has recently reported on these tests.

(4) The method of determining whether trypanosomes were present or not which was of greatest use in these experiments was by means of re-infection with the same strain. This method was utilized in a number of different animals which had been or were infected with T.congolense and T. brucei and in most cases gave clear, readable results. This method was particularly useful in those

animals which, as a result of treatment, attained a high state of premunition. In such cases the smear examinations were often negative over a period of months, the animal was in good condition and health and blood examinations showed no anaemia yet one had little doubt that the animal still laboured trypanosomes. Bovine 2708 can be cited as a good example of this type. During a period of eleven weeks of blood smear examination carried out three times weekly, T. congolense was only found on four occasions, on three of these occasions only one being found after prolonged search.

This method of re-infection is naturally only of assistance under conditions of experimentation where it is desired to determine the effectiveness of treatment without the necessity of carrying out the laborious process of smear examination over a long period.

A disadvantage of this method is that it is necessary to permit a certain period of time to elapse after the completion of the treatment to enable the bovines to get rid of the resistance to re-infection which is always present in T. congolense infections of bovines after recovery from the disease. A bovine which has been sterilized by treatment reacts in one of two ways. Either it reacts as if it were a bovine which had not had an attack of the disease previously, i.e. after a short incubation period trypanosomes become numerous, there is a thermal reaction and anaemia develops, or it drops directly into a state of premunition. Whether it reacts as in the first or second case depends entirely on the period of time that is permitted to elapse after sterilization. The writer is not at present in a position to state definitely the length of the period of time necessary to bring the reaction under the first mentioned heading, but it is certainly less than 152 days and more than 52 days. Charts XIV and XV are introduced to illustrate this point. The method of re-infection as a test for sterility is represented in Table VI.

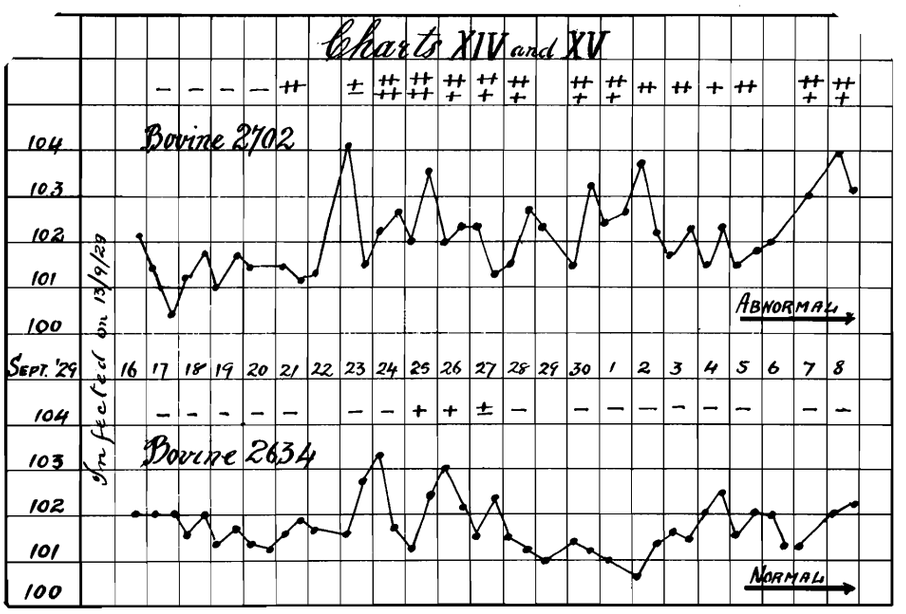


TABLE VI.

Test for Determination of Sterility.

Bovine.		Days after Inoculation.																
		4	5	6	7	8	10	11	12	13	14	15	17	18	19	20	21	21
2702	sterile	-	-	-	-	1+	1+	4+	5+	3+	3+	3+	3+	3+	2+	2+	1+	2+
2639	"	-	-	-	-	1+					3+	1+	2+	1+				1+
2634	presumed sterile	-	-	-	-	-	-	-	2+	2+	1+	-	-	-	-	-	-	-
2714	"	-	-	-	-	-	-	-	-	-	-	-	-	1+	-	-	-	-
2634	Premune	-	-	-	-	-	2+	1+	3+	2+	-	-	1+	2+	2+	-	1+	1+
2468	"	-	-	-	-	-	-	-	-	-	-	-	1+	-	-	-	-	-
2743	"	3+	-	-	-	1+	1+	2+	3+	2+	2+	-	-	-	-	3+	2+	-
2709	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3506	Presumed non-infected	-	-	-	1+	2+	4+	2+	2+	4+	2+	2+	3+	1+	2+	3+	3+	3+
3525	"	-	-	2+	2+	3+	1+	2+	4+	2+	1+	2+	3+	1+	3+	3+	3+	1+
3524	Newly infected	4+	3+	-	1+	3+	2+	2+	3+	3+	2+	2+	1+	2+	4+	4+	3+	4+
416	Chronic	-	2+	3+	-	-	-	-	-	-	1+	2+	-	-	-	-	-	-
3627	Control	1+	2+	2+	4+	4+	3+	4+	4+	2+	3+	2+	2+	3+	3+	3+	2+	3+

Trypanosomes absent (-), very rare (1-), rare (2-), frequent (3-), very frequent (4-)

Explanation of Table.

The two bovines 2702 and 2639 had been proved to be sterile by negative blood smear examinations over periods of 18 and 17 weeks respectively, and by sub-inoculating 100 c.c. blood from each into two bovines. These four bovines them in turn were submitted to blood smear examinations and clinical observations for 13 weeks, in addition to the test now under discussion, with negative results.

The two bovines 2634 and 2714 classified As "presumed sterile" are animals which were treated and which, for a period of 11 weeks, gave negative results to blood smear examinations.

The four "presumed non-infected" bovines 3506, 3525, 3527 and 3542 are bovines which had been used to test by sub-inoculation the sterility of bovines 2639 and 2702 referred to above.

For purposes of comparsion there are also introduced four "premune" bovines 2464m 2468, 2743 and 2709, one bovine 3524, recently infected, one chronic case, 416, and one control 3627.

By examination of the table and the charts it will be seen that the bovines can be classified according to the occurrence of the trypanosomes and the thermal reaction into three groups.

Group I. This one includes the two "steriles" all of the "presumed non-infected" and the "control". They all behaved as if they were receiving infection for the first time.

Group II. In this group are the two "presumed steriles" and the "premune". A variation among these is the very early appearance in one premune bovine of trypanosomes. The irregularity in connection with the re-infection of two "presumed steriles" as compared with the two "steriles" of Group I is due to their great resistance on account of this test having been carried out at a comparatively short time after their sterilization. By this test alone, therefore, it is not possible to determine sterility unless the bovines to be tested be retained for a period longer than these were namely 52 days, before submitting them to the test.

Group III. Includes the "newly infected" and the "chronic types. In neither of these could any change be detected. In adidition, bovines of this group showed more frequent trypanosomes and greater irregularity of temperature than those of Group II.

Conclusions:

(1) A test for determination of sterility is described which has proved to be of some assistance when it is necessary to determine whether a bovine has been sterilized or not as the result of treatment.

(2) A necessary essential to the proper working of the test is that sufficient time be allowed to elapse before carrying out the test to permit of the disappearance of the resistance to re-infection which is always present for some time after sterilization in T. Congolense infection of bovines.

(3) If such a period is not allowed, there arises the difficulty of differentiating between such a resistant sterile bovine, which after test may only show occasional

trypanosomes and a strongly premune one which, whether tested or not, will show trypanosomes often only at long intervals.

In T. congolense infection of dogs a similar resistance was determined. This resistance could be conveniently used to produce different lengths of the course of the disease which resulted in the appearance of additional symptoms.

In T. brucei infection of horses the method of determining sterility by re-infection is somewhat more definite and clear cut than in T. congolense infection of bovines - there does not appear to be a carry over in the sterile horse of a resistance comparable to that found after sterilization of a bovine of T. congolense. Consequently the test of sterility by re-infection may be carried out much earlier in T. brucei infection of horses. As examples of this lack of resistance to reinfection may be quoted the following:

Horse 19079 infected with T. brucei was treated over a period of 14 days. Indications were that the treatment had produced sterilization, the complement fixation test being already negative on the 28th day after cessation of treatment and sub-inoculations of 50 c.c. blood into two horses on the 27th day giving negative results. This horse 19079 on the 42nd day after cessation of treatment was given an inoculation of 50 c.c. infected horse blood. It reacted as if it had not previously had T. brucei infection giving an incubation period of 6 days. It would appear from the above that the period of 42 days could be considerably reduced. In T. congolense infection of bovines the period of carry over of resistance is apparently greater.

(5) Although the appearance of the temperature in the trypanosomiases was not utilized for the diagnosis, yet it was found to be of considerable assistance as an indicator of the presence or absence of the trypanosomes. It was

definitely of no assistance in those cases which were in a high state of premunition. In such cases it was quite common to determine by smear examination and subinoculation the presence of parasites in the absence of abnormalities of temperature.

CHAPTER VI.

CHEMOTHERAPY - GENERAL REMARKS.

Since David Livingstone (1858) first suggested, the use of arsenic in the treatment of trypanosomiases numerous drugs have been tested for this purpose. It is not proposed to deal here in detail with the various drugs used in the treatment of trypanosomiases throughout Africa in man and animals but rather to restrict the references to drugs used on domestic animals in the Union of South Africa.

In the forefront appeared the arsenicals. Later various dyes were given extensive trials. In both the arsenicals and the dyes it was found that the results obtained in the treatment of trypanosomiases of mice, rats and other laboratory animals were often very promising but that these drugs when submitted to trial on the domestic animals did not fulfil their promise.

The chemotherapy of the trypanosomiases is considered from two points of view. The first, the ideal in most circumstances, is to produce by treatment a complete sterilization of the animal body of all trypanosomes: the second is the control of the trypanosomes in the animal body to such an extent, by the use of appropriate drugs, that, even though the trypanosomes continue to live in the tissues, the animal itself does not and will not even under unfavourable conditions show any detrimental effects of this persistence of infection. The subsidiary points to be considered in the selection and use of a drug are the ease and facility of administration and the absence of any marked direct detrimental effect on the animal's tissues. Many drugs, if administered in effective doses, destroy the trypanosomes but produce at the same time tissue destruction or toxic results of such a nature that their use cannot be persisted in.

It is proposed now to consider under various headings the drugs, which have been utilised in the treatment of trypanosomes of the domestic animals in the Union.

A. DRUGS OTHER THAN ANTIMONIALS AND QUINOLINES.

It was decided to introduce this section for the purpose of providing as complete a history as possible of the chemotherapy in the Union. With one exception ~~of~~ none the drugs mentioned has been of any practical use, being discarded after trials for some reason or other chiefly on account of inefficacy. This section, however, serves not only as a historical record but also as an indication of the large number of drugs utilised and the large amount of work that was undertaken for the purpose of obtaining efficient methods of combating the trypanosomiases.

These drugs are, for convenience, grouped in Table VII. An endeavour has been made in this table to allocate as accurately as possible the investigators who were concerned with the work. I wish to acknowledge here my indebtedness to those of my colleagues who gave me permission to utilise their unpublished results.

Some of the earliest experimental investigations on the chemotherapy of trypanosomiases in the Union were carried out by Andrews (1912) in 1911 who continued the work commenced by Sieber in 1910. The following drugs or combinations of them were used by these workers in T.congolense infection of equines, bovines and sheep: potassium antimony tartrate, novoflavin, arsenophenylglycin, salvarsan, quinine, trypan blue, sodium arsenite and atoxyl.

Curson (1928) a few years later tested out the action of a large number of drugs. In addition to his very thorough tests of potassium antimony tartrate to which reference will be made later in this thesis, he gave trials to the following, arsenious oxide, sodium arsenite, atoxyl, neosalvarsan, hydroxy-amino-phenylarsenate of sodium; salicylarsenate of mercury; trisulphide of arsenic; triple peptonate of iodine, arsenic and mercury; colloidal silver; quinine sulphate; ethylhydrocupreine; trypan blue; potassium citrate.

The reference "D.V.S." in Table VIII indicates that the work was carried out under the supervision of the Director of Veterinary Services, the name of the actual investigator, if any, being unknown.

TABLE VII.

Drugs other than Antimonials and Quinolines.

Name of drug	Species	No. of animals	Maximal single dose	Maximal total per animal.	Controlled by:	Remarks.
Arsenophenyl-glycin	Sheep	50	2.9 gm.	5.9 gm.	D.V.S.	In some cases with T.B., Novoflavin etc
"	Bovines	2	22.0 "	22.0 "	"	-
"	Equines	16	22.0 "	32.5 "	"	In some cases
Naganol 5 parts (with Sb 212.3 parts).	Equines	7	5.0 " of mixture	35.3 " of mixture	Graf	with Novoflavin Coopers Dip.
"	Bovines	2	4.5 gm. of mixture.	63.0 gm. of mixture.	D.V.S.	-
"	Sheep and goats.	6	2.0 gm. of mixture.	11.0 gm. of mixture	Graf	In some cases with Stibosan.
Novoflavin	Sheep	20	0.6 gm.	1.3 gm.	D.V.S.	In some cases with Neosalvarsan, Arsenophenylglycin.
"	Equines	9	3.3 "	3.3 "	"	In some cases with Arsenophenylglycin.
Neosalvarsan	Sheep	13	1.4 "	1.4 "	"	" Novoflavin etc
"	Equines	3	10.0 "	10.0 "	"	-
"	"	1	3.0 "	6.0 "	Robinson	-
"	Bovines	3	2.0 "	4.0 "	"	-
"	"	1	2.0 "	4.0 "	"	With potassium antimony tartrate.
Trypan blue	Sheep	18	1.1 "	1.5 "	D.V.S.	With Arsenophenylglycin, Neosalvarsan, Nat.arsenicum.
Coopers dip (per os).	"	9	1.0 "	1.0 "	"	With Arsenophenylglycin.
"	Equines	3	18.0 "	18.0 "	"	"
Natrium Arsenicum.	Sheep	3	0.05 "	0.05 "	"	" , T.B.
Quinine hydrochor. (per os).	"	3	6.0 "	6.0 "	"	With atoxyl, Neosalvarsan.
Atoxyl	"	6	3.0 "	3.0 "	"	With quinine, neosalvarsan.
Tryparsamide	Bovines	7	20.0 "	80.0 "	"	In some cases with B.205.
"	Equines	1	15.6 "	125.0 "	"	-
"	"	1	10.0 "	10.0 "	Robinson	-
"	Bovines	4	20.0 "	40.0 "	"	-
"	"	1	10.0 "	20.0 "	"	With potassium antimony tartrate.
B.205	Bovines	2	10.0 "	10.0 "	D.V.S.	With T.B.
T.10	"	1	5.4 "	32.0 "	"	-
T.11	"	2	7.0 "	14.0 "	"	-
T.12	"	1	7.5 "	52.5 "	"	-
T.13	"	1	6.2 "	25.0 "	"	With B.205.
T.18	Equine	1	10.0 "	30.0 "	"	" Naganol
T.19	"	1	5.0 "	10.0 "	"	" "
M.201	Bovines	2	3.0 "	18.2 "	"	" B.805
M.203	"	1	3.0 "	3.0 "	"	-
M.204	"	1	3.0 "	9.0 "	"	With B.805.
M.207	"	3	4.5 "	7.6 "	"	-

Table VII (Contd.)

Name of	Species	No.of animals.	Maximal single dose.	Maximal total per animal.	Controlled by:	Remarks.
B.805	Bovines	2	6.6 gm.	20.0 gm.	D.V.S.	With M.201, M.204.
B.802	"	1	6.3 "	37.0 "	"	-
Copper Chlorophyllin.	"	1	4.7 "	4.7 "	"	-
Naganol	Equines	3	3.6 "	14.0 "	"	With T.18, T19
"	Bovines	5	10.0 "	30.0 "	Robinson	-
"	Equines	3	10.0 "	10.0 "	"	-
Oxal arsenicum + " sulphur.	Bovine	1	0.2 "	0.2 "	"	-
Brilliant phosphin.	"	1	0.5 "	0.5 "	"	-

B. QUINOLINES.

Published results by Browning, Cohen, Ellingworth, and Gulbransen (1929) on the trypanocidal properties of various derivatives of anil and styryl quinoline have shown these to be capable of producing sterilization in rabbits and mice infected with Trypanosoma brucei. The derivatives which gave most satisfactory results were Nos. 8 and 90. The strains of T.brucei used were Prowazek's and ferox.

The Director of this Institution arranged with Professor Browning that derivative No. 90, which was regarded as being slightly more suitable than No. 8, be further tested against T.brucei infection of horses and T.congolense infection of bovines A supply of compound No. 314 was forwarded for the purpose by the Medical Research Council. This compound is 2 (p-acetylamino-styryl) -dimethylanimo-quinoline methosulphate, and compound No. 90 being the corresponding methochloride.

Large doses of No. 90 (0.02-0.3 mgm. per Kg. of body weight) were effective but frequently produced toxic effects in the animal injected. Smaller doses (0.004-0.0075 per Kg. of body weight) if repeated several times were efficacious and well tolerated. The recommendation thus for No. 90 was that fractional doses be given at weekly intervals until six or more doses had been administered.

In the rabbits and mice No. 90 was given subcutaneously in a watery solution. The intravenous method was not utilized on account of the possibility of the production of fatal results. The subcutaneous injections did not produce any observable irritation of the subcutaneous tissue. In the human subject doses of 0.003 gm. per Kg. given intramuscularly have been well tolerated.

Compound No. 314, which was the one received for test, has a solubility of 3.5 per cent. vol. It was recommended that it be given in watery solution in doses similar to those used in No. 90

T.brucei INFECTION OF HORSES.

Five horses were utilised for the purpose of testing the efficacy of No. 314 in T.brucei infection of horses. No control was kept, as the virulency of the strain of T.brucei used had been well established in previous chemotherapeutical tests by the writer. Furthermore, the one horse which did not respond to the treatment died of trypanosomiasis. The strain of T.brucei used was obtained from a natural case of the disease in a donkey.

The dose of No. 314, namely, 0.004 gm. per Kg. of body weight, was the same, with one exception, for all the cases treated. The intervals between administration were, in every case, one week. But variations were made in the number of doses given and, in an endeavour to overcome the difficulty of irritation to the tissues, variations were also made in the strength of the solutions. No attempt was made in this experiment to determine whether a dose smaller than that recommended could be efficaciously employed.

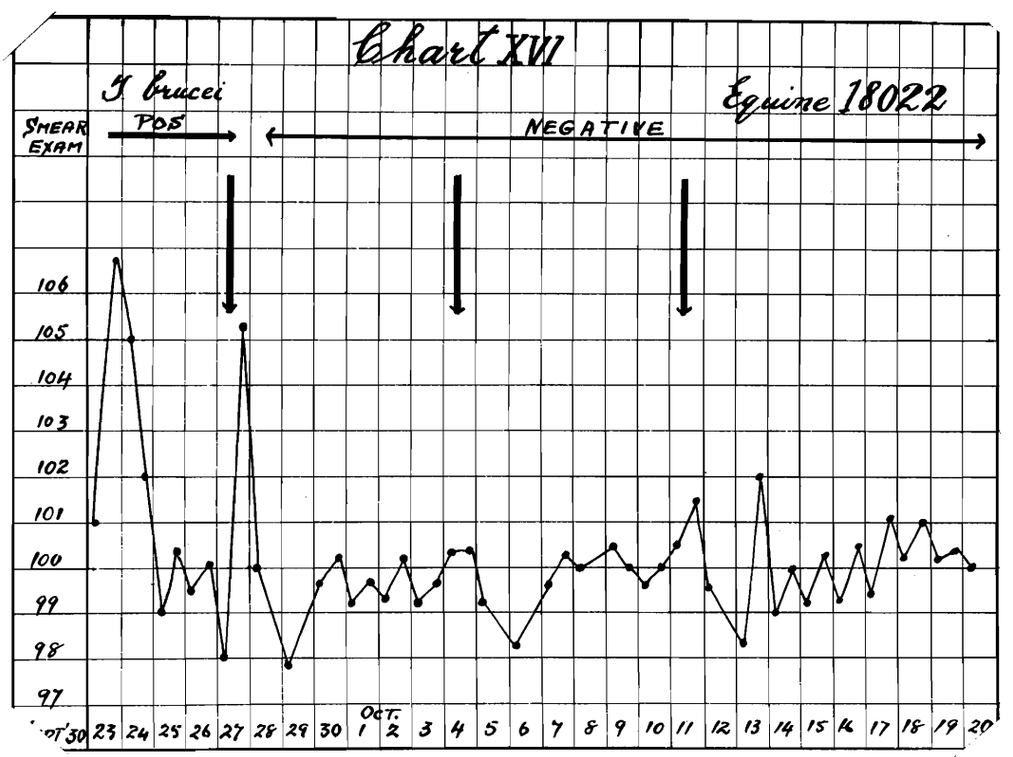
The table gives the details in connection with the experiment and the charts (XVI and XVII) illustrate two cases, one a sterilization and one a failure.

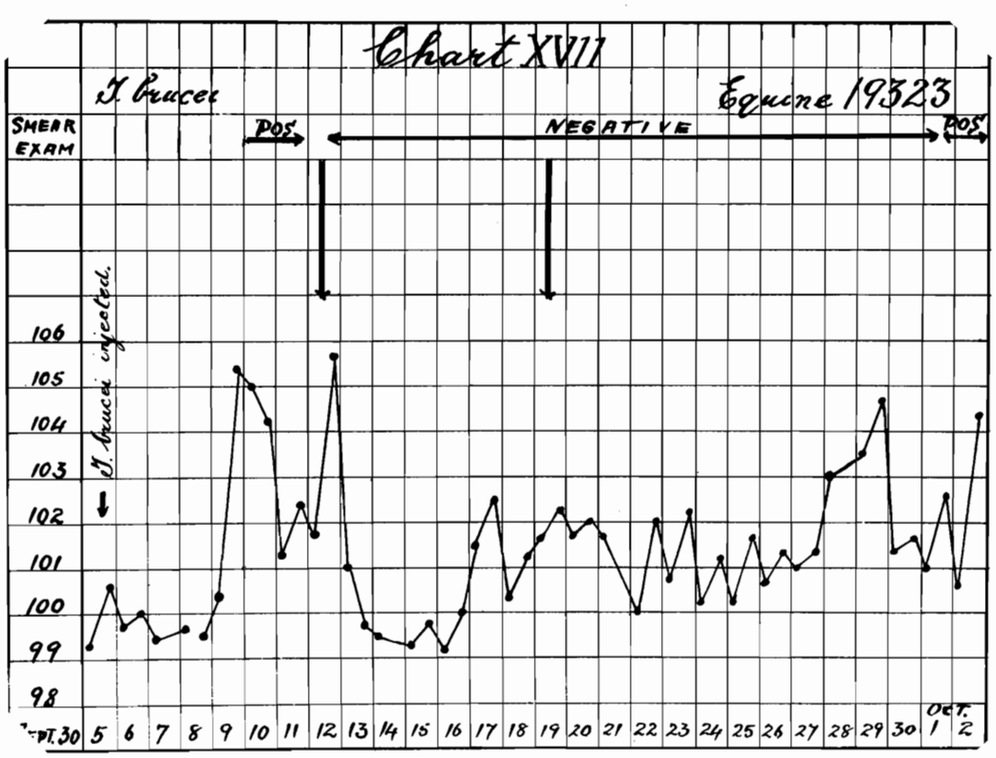
Table VIII.

T.brucei. Treatment with No. 314.

Equine	Wgt. in Kg.	Infection date.	Commencement of treatment.	No. of doses and dosage.	Intervals.	Remarks
18095	342	5/9/30	12/9/30	2 doses of 1.4 gm.	1 week	Sterilization
19323	454	5/9/30	12/9/30	2 doses of 1.8 gm.	"	Died 5/11/30, Tryp. seen.
18022	363	5/9/30	27/9/30	2 doses of 1.5 gm. and 1 dose of 0.8 gm.	"	Sterilization
19431	374	5/9/30	12/9/30	3 doses of 1.5 gm.	"	"
17990	423	9/12/2o	15/8/30	6 doses of 1.6 gm.	"	"

Discussion.- Horse 17990, the first one treated, was given in all six doses. The second dose was given intravenously and produced alarming symptoms. Immediately after the injection,





the horse dropped to the ground with symptoms of marked respiratory distress, frequent pulse and muscular twitchings. It did not recover sufficiently to stand up until after an hour. This was the only case when the drug was administered by any other route than the subcutaneous. After the treatment had commenced, T.brucei was not found in blood smears, and the temperature from then remained normal except for a short period of three days, due most likely to dermatitis gangrenosa produced by the injection. It is quite likely that sterilization was obtained prior to the cessation of treatment, for the complement fixation test was already negative by the 13th day, being doubtful on the 6th day.

Of the two horses which were given two doses one only was sterilized. This one gave a negative complement fixation test, negative blood smear examination for 10 weeks and normal temperatures for same period. The other horse, 19323, for which Chart XVII is submitted, was not sterilized, trypanosomes being found on the 13th day and subsequently. It is of interest to note that the latter horse had not previously been in a T.brucei experiment, whereas horse 18095 was one which had been sterilized and re-infected just prior to its introduction into this experiment. One is forced, therefore, to take into consideration the possibility of the previous infection having some influence on the treatment with No. 314. The two remaining horses, 18022 and 19431,both gave negative complement fixation tests and negative smears examinations and normal temperatures for seven and nine weeks respectively. Horse 18022 received for its third dose approximately half the other doses. As this horse had not been in any previous chemotherapeutical tests, it is an example of sterilization with two and a half doses uninfluenced by any former treatment. Chart XVI illustrates the treatment of this horse.

There is thus good evidence to claim for No. 314 the possibility of bringing about sterilization in T.brucei infection

of horses in a dose of 0.004 gm. per Kg. of body weight if more than two doses be given and, in some cases, although other factors might be influencing the result, with two doses.

Consequently it is much to be regretted that such excellent results lose some of their value by the fact that invariably the subcutaneous injection produces severe, often alarming, destruction of tissues below the site of injection. In every case there occurred swellings, most of which became abscesses, often deep-seated. In some also there was a dermatitis which became gangrenous. An endeavour was made to overcome this disability by varying the concentration, the vehicle and the mode of administration. The drug thus was given in percentages varying from 1 to 3.4, with and without massage during injection and in saline, distilled water and 10 per cent. glucose. The injection of the solution in small quantities at a number of sites resulted in only slight swellings. Also when the drug was given subcutaneously in sterile olive oil the swellings were only minor.

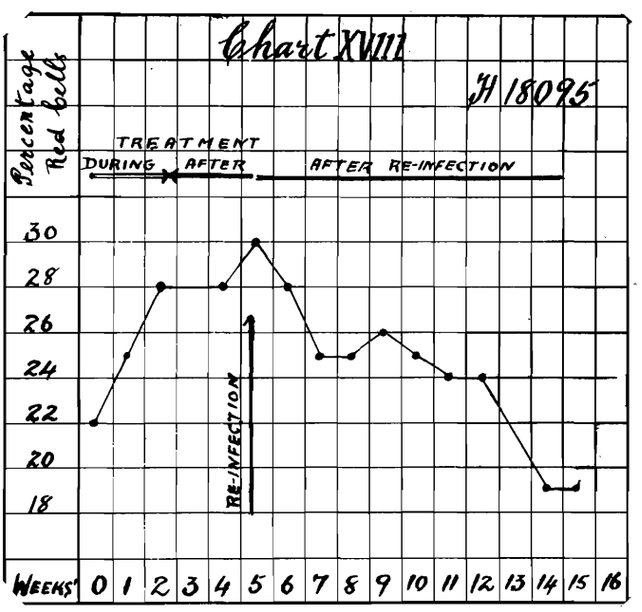
The swellings consequent on the injection of the drug often took two or more days to become evident. Prior to this the site of injection had a higher local temperature and a fairly well delineated slightly raised area.

The following experiment was devised to determine firstly whether doses smaller than .004 gm. per Kg. of body weight would produce toxic symptoms when administered intravenously and secondly, whether such smaller doses would prove to be efficacious

The horse used in this experiment was 18095, which had previously in these experiments been sterilized by two full doses, the last of which was administered on the 19th September 1930. Infection was produced in this horse by the inoculation of 50 c.c. blood of the T.brucei reservoir horse 17989. The parasites were found in blood smears and the temperature curve showed the exacerbations and remissions found in T.brucei infection of the horse. This reaction to the injection of T.brucei incidentally proved the sterility of horse 18095 and was additional proof of the efficacy

of the treatment to which this animal had previously been submitted. The dose decided on for treatment of this horse in this experiment was half that previously used. Calculated on the live body weight on the basis of .002 gm. per Kg., it amounted to .7 gm. and was administered intravenously at weekly intervals for five weeks. No signs of intoxication were observed. As a result of this course of treatment sterilization was obtained. The proof of this was the negative smear examination for a period of 27 days after the last dose, the absence of temperatures suggestive of T.brucei infection and its reaction when injected 28 days after the last dose, with the same strain of the parasite obtained from horse 17989. Chart XVIII of the red precipitate of this horse illustrates the changes of the anaemia during and after the treatment, and also after the re-infection, additional indications of the efficacy of the drug.

On account of the success obtained in the treatment in the previous experiment it was decided to introduce a further horse for the purpose of determining whether a dose of .002 gm. per Kg. of live body weight would produce, when given intravenously on five consecutive days, toxic symptoms or not and whether such a course would produce sterilization. Thus horse 20342, which had been infected by the subcutaneous injection of 100 c.c. of blood of horse 18095 was submitted for five days to the daily intravenous injection of .002 gm. per Kg. No toxic effects other than a slight uneasiness were noticed. Subsequent to treatment some slight elevations of temperatures appeared, but these did not resemble those of a T.brucei infection. Smear examination was negative for 8 weeks. The complement fixation test was negative. Sub-inoculation of a large quantity of blood of horse 20342 into a susceptible horse gave temperatures somewhat resembling those of a T.brucei infection, but no trypanosomes were found during 10 weeks daily examination. The sterility of horse 20342 was further more demonstrated by its reaction to the injection of T.brucei infected blood.



T.congolense INFECTION OF BOVINES.

Compound No. 90 had been tested out on mice infected with T.congolense. No therapeutic action was detected.

To test compound No. 314 two bovines were used, a third being kept as a control. The drug was given at the same rate as in the horses, namely 0.004 gm. per Kg. of body weight. The injections were carried out on the left side of the neck, the subcutaneous route being employed. The solution was a 1 per cent. one in distilled water.

The two bovines, 3637 and 3684, showed no apparent response to the injections. The temperature curves were not influenced, the temperatures remaining high, and T.congolense were in both cases found in smears made 24 hours after the administration. On the 3rd day they were recorded as frequent.

In both the injected animals swellings occurred commencing at the site of injection and passing backwards to the posterior abdominal region and across to the opposite side. These swellings persisted for some weeks but no sloughing took place.

The bovines were not given a second injection.

Styryl-quinoline No. 314 thus is an efficient sterilizing drug for use in T.brucei infection of horses. It was first shown that the dose for this purpose was 0.004 gm. per Kg. of body weight and that more than two doses at intervals of one week should be used. Later it was shown that half the above dose, if five doses be given, was efficacious and furthermore, that this half dose could be given intravenously without the production of symptoms of intoxication whereas the intravenous injection of a full dose did produce alarming symptoms. The half dose was eqqally efficacious whether given at weekly intervals or on consecutive days. The administration of the drug subcutaneously cannot be recommended for in both equines and bovines it produced marked irritation and destruction of the tissues. In T.congolense infection of bovines it had apparently no therapeutic action.