

Some Experiments on the Drug Treatment of Trypanosomiasis.

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These experiments were commenced in the year 1910 by Dr. H. Sieber, and were continued in 1911 by W. H. Andrews.

During 1912 further experiments in "combined treatment" have been undertaken by W. H. Andrews in collaboration with Dr. Wölfel, of German East Africa, and Mr. van Raes, of the Belgian Congo.

Species of the Trypanosome concerned.—In all cases the trypanosomes concerned were of the *T. congolense* type (or *T. pecorum* of Bruce), but in the earlier experiments a number of different strains were used, including one obtained from Hartley, S. Rhodesia, and several strains from different localities in Portuguese East Africa.

The later experiments all refer to the "Guara-Guara" strain sent from Portuguese East Africa in May, 1910, by Mr. W. Jowett, and described by him in the *Journal of Comparative Pathology and Therapeutics* for September, 1910.

With regard to Jowett's description, it may be remarked that the strain in the Onderstepoort laboratory was obtained from an inoculated guinea pig, and that only the small form, without a free flagellum, was observed.

In August, 1911, the original strain was lost, and had to be recovered from a heifer when dying from a relapse after treatment with Arsenophenylglycin.

In subsequent experiments the strain used was, therefore, one which had been resistant to arsenophenylglycin in one passage.

It may be mentioned that in recent outbreaks of trypanosomiasis in Zululand and N'gamiland (Bechuanaland Protectorate) the trypanosomes have also been of the *T. congolense* type, and indistinguishable morphologically one from the other, and from the strains above-mentioned.

Virulence of the Trypanosomes.—No significant differences with regard to the lethality for the various species of domesticated animals, and to the duration of infection and incubative period, were noted in the several strains of trypanosomes utilised.

Horses and mules were largely used in the earlier experiments, and in them the trypanosomes were generally first seen in the peripheral blood about 21 days after inoculation, the period varying between 15 and 30 days; in one case they were not recorded until the forty-first day.

A small number of these horses and mules, which were subjected to treatment varying in the different cases, died from 2 to 5 months after inoculation, and in a considerable number of cases the animals were killed, after the lapse of several months, on account of emaciation and debility, but this debilitated condition could not in all cases be attributed entirely to the trypanosome infection.

On the other hand, some animals appeared to be surviving for very long periods without showing any signs of serious ill-health, and in this connection the case of one animal (Mule 5,338) may be quoted

The mule was injected with the Guara-Guara strain (from a sheep) on the 25th January, 1911, and trypanosomes were found in the peripheral blood on the 9th February, the 15th day.

The mule was not treated, but was kept as a control animal.

On the 12th August, nearly 7 months after infection, the animal was in rather poor condition, although otherwise appearing to be in good health, but inoculation of two sheep, each with 20 c.c. of blood, gave positive evidence of trypanosome infection; the mule was killed three weeks later on account of limitations of space, all infected animals being kept in fly-proof stables.

It would thus appear that the infection tends to run a very slow course in equines, although the majority of infected animals would ultimately succumb from anaemia. For this reason the later experiments were made with sheep.

Only two experiments were made with *cattle*, and in these the trypanosomes appeared in the blood 11 and 12 days respectively after inoculation. Both animals were treated unsuccessfully with arsenophenylglycin, and death occurred in 43 and 95 days respectively.

The strain (Guara-Guara) is apparently considerably more virulent for cattle than for horses and mules.

A very considerable number of *sheep* were under observation, as, in addition to those used in experiments on specific methods of treatment, there were those used to keep up the strains, the sheep being generally used by us for this purpose.

In sheep, the trypanosomes appeared in the peripheral blood between 4 and 18 days (in one case 34 days) after inoculation, the average period being 5—6 days.

The duration of the disease varied within very wide limits; rather less than 50 % of infected sheep died within one month of inoculation, the period exceptionally being as short as 7 days.

In the other cases, the sheep survived for months, and, in about 10 % of cases, even for a year.

A number of *dogs* were injected with the blood of infected animals, with the object, in most cases, of observing the effect of some previous treatment on the latter.

In those dogs which became infected, the trypanosomes appeared in the peripheral blood in 9—17 days, and the animal died from 15 to 36 days after inoculation.

In one case, however, a dog, injected with the blood of a mule which had undergone drug treatment, showed trypanosomes only after a lapse of 31 days, and it survived for 51 days, when it was killed by other dogs in the same kennel.

The results of inoculations from Mule 5,338 (a control animal, infected with the Guara-Guara strain) are curious. A dog injected on the 139th day of the infection with 100 c.c. of the mule's blood, did not contract the infection, whereas two sheep injected 60 days later with 20 c.c. and 40 c.c. respectively of the blood, died from trypanosomiasis in 36 and 50 days respectively (comp. Mule 5,145, treated with arsenophenylglycin, and Mule 5,174, treated with Novoflavin).

The problem of the drug treatment of trypanosomiasis in domesticated animals differs from that of the treatment of Sleeping Sickness in man, in that the attention which may practically be paid to individual cases is necessarily much more limited in the former case.

A method of treatment, if it is to be of any practical importance in such a country as Rhodesia or Moçambique, must involve the performance of only very few and simple operations, in order that it may be applied to a large number of patients within a reasonably short period, and that the assistance and equipment required may be cut down to a minimum.

We were, therefore, concerned chiefly with the effects of only a small number of injections, and treatment was sometimes abandoned in cases which might possibly have recovered, had the treatment been continued for longer periods.

On the other hand, it was recognised that the desired results could not be expected from any one trypanocidal agent at present known, and that a combination of two or more drugs was essential.

In practically all cases the agents were injected, in dilute solution, into the jugular vein.

Before giving the results of experiments with several combinations of drugs, the following observations on the trypanocidal actions of certain individual agents may be recorded.

TABLE I.—TREATMENT WITH ARSENOPHENYLGLYCIN.

Animal.	Strain of Trypanosomes.	Time after Trypanosomes first seen in blood.	Dose of drug employed.	Time of disappearance of Trypanosomes from blood.	Interval before re-appearance in blood.	Inoculations of Blood after Treatment.				Result.	Remarks.
						Animal.	Vol. of blood.	Time after treatment	Result.		
Horse 4316	Hartley	21 days	0·06 gm.	Same day	—	1 dog	20 c.c.	30 days	†	Relapse	Died (debility) after 4½ months. Subjected to further treatment.
Mule 3050	"	Same day	0·057 "	"	—	"	"	8 days	—		
Horse 4018	"	1 day	0·06 "	"	54 days	"	10 c.c.	35 days	†	"	"
Mule 3900	"	Same day	0·058 "	"	—	"	20 c.c.	30 days	†	"	"
Horse 3883	"	"	0·058 "	"	—	"	10 c.c.	8 days	—	"	"
						"	20 c.c.	35 days	†	"	"
						"	10 c.c.	8 days	—	"	"
Mule 4969	"	"	0·06 "	"	—	"	20 c.c.	35 days	†	" }	Killed less than 5 months after inoculation.
						"	10 c.c.	8 days	—		
						"	20 c.c.	35 days	†	"	"
						"	10 c.c.	8 days	—	"	"
						"	20 c.c.	35 days	†	"	"
						"	10 c.c.	8 days	—	"	"
Horse 5016	"	25 days	0·06 "	"	—	"	40 c.c.	107 days	—	—	Died (Trypanosomiasis) 2 days after treatment.
Mule 5145	Guara-Guara	34 days	0·059 "	"	—	1 dog	40 c.c.	50 days	—	Relapse	Killed (in fairly good health) after 8 months.
						2 sheep	100 c.c.	90 days	—		
Horse 5973	"	9 days	0·06 "	"	44 days	"	20 & 40 c.c.	169 days	†	"	Subjected to further treatment.
Heifer 1371	"	8 days	0·074 "	"	71 days	2 sheep	20 c.c.	65 days	†	"	Killed in extremis, after 95 days.
Heifer 1372	"	3 days	0·059 "	After 2 days	6 days	"	"	"	—	"	Died after 43 days.
Sheep 2641	Massonzane	7 days	0·06 "	Same day	17 days	"	"	"	—	"	Subjected to further treatment.
Sheep 2137	Hartley	3 days	0·07 "	"	21 days	"	"	"	—	"	"
Sheep 2262	"	3 days	0·057 "	"	"	"	"	"	—	"	"
Sheep 2621	Massonzane	7 days	0·075 "	1 day later	16 days	"	"	"	—	"	"
Sheep 2623	"	7 days	0·075 "	"	27 days	"	"	"	—	"	"
Sheep 2777	Zendoga	5 days	0·09 "	Same day	—	1 sheep	40 c.c.	3 months	—	"	Died (accidental) after 15 months.
Sheep 2136	Hartley	2 days	0·067 "	"	21 days	"	"	"	—	Relapse	Subjected to further treatment.
Sheep 2146	"	3 days	0·0616 "	"	"	"	"	"	—	"	"
Sheep 2260	"	36 days	0·0617 "	"	"	1 dog	10 c.c.	22 days	—	"	Died (debility) after 19 months.
Sheep 2133	"	2 days	0·06 "	"	21 days	"	"	"	—	Relapse	Subjected to further treatment.
Sheep 2257	"	4 days	0·06 "	"	"	"	"	"	—	"	"
Sheep 2640	Massonzane	8 days	0·062 "	"	22 days	"	"	"	—	"	"
Sheep 2643	Zendoga	3 days	0·06 "	"	15 days	"	"	"	—	"	"
Sheep 1295	Hartley	6 days	0·06 "	"	—	"	"	"	—	"	Further treatment, 15 days later.

The experiments performed, together with the results obtained, are given in tabular form (table I).

I. ARSENOPHENYLGLYCIN.

It will be seen that 9 infected horses and mules were injected with doses of arsenophenylglycin varying from 0.057 to 0.06 gm. per kilogramme of body weight, and that relapse followed in every case, but that of a horse which died two days after the injection was made. Trypanosomes were actually seen in the peripheral blood, after treatment, only in two instances, but in the other cases their presence was demonstrated by sub-inoculations into sheep or dogs, such inoculations usually giving positive results 30—35 days after the treatment.

It must also be mentioned that in Mule 5,338, kept as a control animal, microscopic examination of the blood frequently gave negative results (and, on two occasions, inoculation of dogs).

Ehrlich and his collaborators have fixed the maximal therapeutic dose of arsenophenylglycin for the horse at 0.05 gramme per kilogramme of body weight. (Bulletin de l'Institut Pasteur, April, 1909.)

It is, therefore, evident that the above doses, all of which proved to be insufficient, could not with safety be increased.

Two cases deserve special attention; in the first place it should be noted that Mule 4,969 gave a positive result with a subinoculation of blood taken from the mule 35 days after treatment, but that similar inoculations, on the 68th and 107th days respectively, proved negative.

Secondly, Mule 5145 gave negative results with sub-inoculations into dogs performed on the 50th and 90th days after treatment, although in the latter case 100 c.c. of blood was injected; yet 79 days later 20 c.c. of blood was infective to sheep.

In both cattle relapse followed treatment; the dose of 0.059 gm. per kilogramme, given in one case, was obviously insufficient, as the trypanosomes disappeared but slowly from the peripheral blood, while in the other case a dose of 0.074 gm. was well supported, and gave a more promising result. We were not able to make further experiments with cattle, although such are perhaps very desirable, in that the losses in Moçambique are apparently most serious in bovines.

The maximal therapeutic dose of arsenophenylglycin for cattle should be determined more exactly.

Of the sheep, eleven animals were treated with a single dose of arsenophenylglycin, varying in different cases from 0.057 to 0.075 gm. per kilogramme of body weight; in each case there was a relapse, the trypanosomes in all cases reappearing in the peripheral blood, and generally about 21 days after treatment (limits 15-27 days).

One sheep (S. 1295) was given a dose of 0.06 gm. per kilo., and no relapse had occurred up to the 15th day, on which day further treatment was instituted.

Two other sheep, which received doses of 0.09 and 0.0617 gms. respectively, appear to have been cured thereby; trypanosomes were never recorded in the peripheral blood, subsequent to treatment, and in each case a sub-inoculation gave a negative result.

The favourable result obtained with Sheep 2777 might be ascribed to the size of the dose (0.09 gm.) of arsenophenylglycin employed, but this will not explain the case of Sheep 2260, in which a dose of only 0.0617 gm. was given.

In this connection it is of interest to note that in the case of Sheep 2621, in which the results are the least favourable of those obtained with sheep, a dose of 0.075 gm. was employed.

With regard to the stage of the infection at the time treatment was undertaken, it will be seen that in different cases the injection was given from a few hours to 36 days after trypanosomes were first seen in the peripheral circulation, but that the results were not clearly affected by this factor.

2. *NOVOFLAVIN*.—See Table II.

Six equines received each a dose of 0.01 gramme, and one mule was given 0.0098 gramme per kilogramme body weight.

In three cases the animal treated was suffering from a relapse after treatment with arsenophenylglycin, and in these cases the trypanosomes disappeared from the peripheral blood and were not found again, the animals being killed on account of general debility about two months after the injection of Novoflavin was given.

In one of the four cases in which Novoflavin was given to animals otherwise not treated, the animal was shown to be infected by sub-inoculation of blood into sheep, on the 169th day after treatment.

This animal (the mule which received only 0.0098 gm.) was killed six and a half months after treatment, although the general health was fairly good.

In the other three cases the animals had to be killed on account of general debility; this was done 11 days after the treatment in one case, and after 25 days in the other cases, and no relapse was observed.

Eleven sheep were injected with a dose of from 0.009 to 0.013 gramme of Novoflavin per kilogramme, and in three cases the drug produced a fatal intoxication; in two other instances the sheep died, 12 and 15 days respectively after treatment, from an accidental complication, pneumonia.

In the remaining six cases, all of which had previously been treated unsuccessfully with arsenophylglycin, or with salvarsan, relapses occurred, trypanosomes reappearing in the blood 13 to 29 days after treatment.

In view of the fact that on several occasions a dose of 0.01 gm. per kilo. proved fatal, and that on two occasions a fatal intoxication was produced by a dose of 0.013 gm., it is evident that in the sheep the doses given approximated closely to the maximal therapeutic dose, and that they could not with safety be increased.

With equines also a dose of 0.01 per kilo. on two occasions (out of 10) proved fatal, although a larger dose was given in two cases, without producing any serious effects. The dose of 0.01 gm. is therefore considered as the maximum dose for equines.

Novoflavin is, therefore, to be classed, with several similar drugs, as a useful trypanocidal agent, which, however, in the majority of cases, will fail completely to sterilise.

TABLE II.—TREATMENT WITH NOVOFLAVIN.

Animal.	Strain of Trypanosomes.	Time after Trypanosomes first seen in the blood.	Dose of the drug employed.	Time of disappearance from blood.	Interval before re-appearance in blood.	Inoculations of Blood after Treatment.				Result of treatment.	Remarks.
						Animal.	Vol. of blood.	Time after treatment.	Result.		
Horse 4398	Guara-Guara	30 days ..	0.01 gm.	Same day	—	1 dog	10 c.c.	11 days	—	No Relapse	Killed (poor condition) 11 days after treatment.
Mule 4924	N'hongoon...	31 days ...	" "	" "	—	"	40 c.c.	10 days	—	"	" " 25th day
Mule 4698	Massonzane	" "	" "	" "	—	"	" "	" "	—	"	" " "
Mule 5174	Guara-Guara	33 days ...	0.0098 "	" "	—	"	" "	50 days	—	Relapse	" " "
Mule 3050	Hartley ...	*52 days ...	0.01 "	" "	—	2 Sheep	100 c.c.	90 days	—	No Relapse	Killed (in fairly good condition) 6½ months after treatment.
Mule 3900	"	* " "	" "	" "	—	1 dog	20 & 40 c.c.	169 days	†		
Mule 3883	"	" "	" "	" "	—	"	10 c.c.	16 days	—	"	Killed (debility) within 2 months.
										"	Killed (debility) in about 2 months.
										"	Killed (debility) 2 months after treatment.
Sheep 2620	Guara-Guara	About 7 days	0.01 gm.	Same day	—	—	—	—	—	Intoxic't'n	Died (intoxication) after 1 day.
Sheep 2637	N'hongoon...	" "	" "	" "	—	—	—	—	—	"	Died (pneumonia) after 15 days.
Sheep 2641	Massonzane	*About 29 days	" "	" "	29 days	—	—	—	—	Relapse	Subjected to further treatment.
Sheep 2137	Hartley ...	*About 24 days	" "	" "	" "	—	—	—	—	"	" " "
Sheep 2262	"	* " "	0.009 "	" "	13 days	—	—	—	—	"	" " "
Sheep 2621	Massonzane	*About 35 days	0.013 "	" "	—	—	—	—	—	—	Died (pneumonia) after 12 days.
Sheep 2623	"	*About 37 days	" "	" "	—	—	—	—	—	Intoxic't'n	Died (intoxication) 1 day later.
Sheep 2767	"	†About 19 days	" "	" "	—	—	—	—	—	"	Died (intoxication) 2 days later.
Sheep 2768	"	†About 19 days	" "	" "	—	—	—	—	—	Relapse	Died Trypanosomiasis after 18 days.
Sheep 2136	Hartley ...	*About 23 days	0.01 "	" "	29 days	—	—	—	—	"	Subjected to further treatment.
Sheep 2146	"	*About 24 days	" "	" "	13 days	—	—	—	—	"	" " "

* Signifies that the animal was first injected with arsenophenylglycin, and had shown signs of a relapse.

† Signifies that the animal was first treated unsuccessfully with Salvarsan.

3. SALVARSAN, or "606."

In the accompanying table (Table III.) it will be seen that this drug was administered to 15 sheep infected with Trypanosomiasis.

In the first three cases the animals received Salvarsan after unsuccessful treatment with arsenophenylglycin and novoflavin, and the drug was injected into the jugular vein as a 1% solution in normal saline.

In the remaining cases Salvarsan was injected at an early stage of the infection, these results being therefore free from any sources of error connected with the treatment with other agents; the strain used, however, had been recovered from an animal dying after unsuccessful treatment with arsenophenylglycin, and had subsequently been passed through 5 sheep, untreated. In these later cases, the drug was given, intravenously, as a 1-500 neutral solution.

In order to determine the maximum therapeutic dose, ten normal sheep were injected intravenously with varying doses of Salvarsan (in 1-500 neutral solution) with the following results:—

- 2 sheep received 0.02 gm. of "606" per kilo—no effect.
- 2 sheep received 0.03 gm. of "606" per kilo—some salivation.
- 2 sheep received 0.04 gm. of "606" per kilo—some salivation.
- 2 sheep received 0.05 gm. of "606" per kilo—both sheep died.
- 2 sheep received 0.06 gm. of "606" per kilo—both sheep died.

The maximum dose was therefore fixed at 0.04 gm. per kilo.

The results of treatment were not at all encouraging, although in five cases the dose given was actually that determined as the maximum.

In practically all cases, after the injection of large doses of Salvarsan, the trypanosomes in the peripheral circulation diminished in number for from two to five days, but they never actually disappeared, and a few days later they increased very rapidly.

It is therefore apparent that, at least as far as concerns sheep infected with this particular strain of trypanosome, Salvarsan has only a very feeble trypanocidal action.

COMBINATIONS OF TRYPANOCIDAL AGENTS.

1. ARSENOPHENYLGLYCIN AND NOVOFLAVIN.

- (a) One injection of each agent given in succession. See Table IV.

Of the four equines subjected to this treatment, three received the injection of Novoflavin after an interval of 52 days, when a relapse after Arsenophenylglycin had already been demonstrated; in the fourth case the two injections were separated by an interval of only 9 days.

TABLE III.—TREATMENT WITH SALVARSAN.

Animal.	Strain of trypanosomes.	Time which had elapsed since trypanosomes were first seen in the blood.	Dose of Salvarsan per kilo.	Effect on the trypanosomes in the peripheral blood.	Remarks.
Sheep :					
*†2641	Massonzane.	59 days.	0·008 gm.	No effect	Subjected later to other treatment.
*†2136	Hartley.	53 days.	0·01 gm.	"	" "
*†2137	"	55 days	0·014 gm.	"	" "
3773	Guara-Guara.	4 days.	0·02 gm.	"	" "
3766	"	3 days.	0·02 gm.	"	" "
3757	"	3 days.	0·02 gm.	Fewer in number on second day, numerous on third day.	Died (trypanosomiasis and pericarditis) in 12 days.
3756	"	4 days.	0·03 gm.	Fewer in number for 3 days ...	Died (trypanosomiasis) in 76 days.
3769	"	3 days.	0·03 gm.	No effect	Died (trypanosomiasis) in 12 days.
3768	"	4 days.	0·03 gm.	Fewer in number for 5 days ...	Subjected later to other treatment.
3810	"	2 days.	0·04 gm.	" "	" "
3813	"	"	0·04 gm.	" " " "	" "
3765	"	3 days.	0·04 gm.	Fewer in number for 3 days ...	" "
3811	"	1 day.	0·04 gm.	" " " "	Died (trypanosomiasis) in 21 days.
3764	"	1 day.	0·04 gm.	No effect	Died (trypanosomiasis and heartwater (?)) in 30 days.
3775	"	2 days.	0·04 gm.	Fewer in number for 3 days ...	Subjected later to other treatment.
3812	"	Control.	—	—	Died (trypanosomiasis) after 77 days.

* Previously treated unsuccessfully with arsenophenylglycin.
 † Previously treated unsuccessfully with novoflavin.

In no case were trypanosomes again seen in the blood, and inoculations into dogs on the 16th day (and in one case also on the 55th day) gave negative results, but all 4 animals were destroyed, about two months after the second injection, on account of general debility and emaciation.

It would appear that in these cases the debility was due, in large measure, to causes other than the trypanosome infection, and the animals were old and in poor condition at the commencement of the experiment.

Of the fourteen sheep, two died within a day from intoxication with Novoflavin, and two succumbed to accidental complications, and one sheep died from the effects of the previous infection (and treatment possibly, in part), before a relapse could be expected.

In two cases, where the injections were made on two successive days, no signs of relapse were detected, and the sheep died from accidental causes, nine and fourteen months respectively after the treatment was concluded.

All the remaining seven sheep showed relapses, the trypanosomes reappearing in the blood from 13 to 29 days after the second injection.

The treatment may therefore be considered to be worthy of more extended trial, but the debilitating effects of both infection and treatment should be counteracted, as far as possible, by commencing treatment early, and by giving the two injections at a reasonably short interval, before any relapse has occurred.

TABLE IV.—COMBINED TREATMENT WITH ARSENOPHENYLGLYCIN AND NOVOFLAVIN—TWO SUCCESSIVE DOSES.

Animal.	Interval between appearance of trypanosomes in blood and treatment.	Dose of Arsenophenyl-glycin per kilo. body weight.	Interval before relapse was detected.	Interval between the two injections.	Dose of Novoflavin per kilo.	Interval before trypanosomes re-appeared in the blood.	Inoculations made subsequent to the second injection.				Remarks.
							Animal.	Vol. blood.	Time after treatment.	Result.	
Horse 3883	21 days	0·058 gm.	35 days	52 days	0·01 gm.	—	1 dog	10 c.c.	16 days	—	Killed (debility) 2 months after treatment.
Mule 3050	0 day	0·057 gm.	35 days	52 days	0·01 gm.	—	1 d g	10 c.c.	16 days	—	Killed (debility) within 2 months after treatment.
Mule 3900	0 day	0·058 gm.	35 days	52 days	0·01 gm.	—	1 dog	10 c.c.	16 days	—	Killed (debility) about 2 months after treatment.
Mule 3509	40 days	0·06 gm.	—	9 days	0·01 gm.	—	2 dogs	{ 10 and 40 c.c.	{ 16 and 55 days	—	Killed (debility) about 70 days after treatment.
Sheep 2621	7 days	0·075 gm.	16 days	28 days	0·013 gm.	—	—	—	—	—	Died (Pneumonia) 13 days after treatment.
Sheep 2623	7 days	0·075 gm.	27 days	30 days	0·013 gm.	—	—	—	—	—	Died (Intoxication) 1 day after treatment.
Sheep 2775	5 days	0·075 gm.	—	1 day	0·005 gm.	—	1 sheep	40 c.c.	2½ mths.	—	Died (Gastro-Enteritis) 9 months after treatment.
Sheep 2776	11 days	0·075 gm.	—	1 day	0·005 gm.	—	—	—	—	—	Died (Toxaemia) 14 months after treatment.
Sheep 1295	6 days	0·06 gm.	—	15 days	0·01 gm.	—	—	—	—	—	Died (Intoxication) 1 day after treatment.
Sheep 2133	2 days	0·06 gm.	21 days	21 days	0·01 gm.	20 days	1 sheep	20 c.c.	20 days	†	Died (Gastro-enteritis) 21 days after treatment.
Sheep 2257	4 days	0·06 gm.	21 days	21 days	0·01 gm.	—	—	—	—	—	Died (Trypanosomiasis) 12 days after treatment.
Sheep 2640	8 days	0·062 gm.	22 days	22 days	0·01 gm.	—	—	—	—	—	Died (Gastro-enteritis) 15 days after treatment.
Sheep 2643	3 days	0·06 gm.	15 days	15 days	0·01 gm.	20 days	—	—	—	—	Died (Trypanosomiasis) 20 days after treatment.
Sheep 2146	3 days	0·0616 gm.	21 days	21 days	0·01 gm.	13 days	—	—	—	—	Subjected to further treatment.
Sheep 2262	3 days	0·057 gm.	21 days	21 days	0·009 gm.	13 days	—	—	—	—	do.
Sheep 2136	2 days	0·067 gm.	21 days	21 days	0·01 gm.	29 days	—	—	—	—	do.
Sheep 2137	3 days	0·07 gm.	21 days	21 days	0·01 gm.	29 days	—	—	—	—	do.
Sheep 2641	7 days	0·06 gm.	17 days	22 days	0·01 gm.	29 days	—	—	—	—	do.

(b) One injection of Arsenophenylglycin, followed by successive doses of Novoflavin.

(See Table V.)

Five of the sheep given in Table IV. were given a second dose of Novoflavin, and in one case a third injection of this drug was given.

In two cases the animal died shortly after the second injection, and the effect on the trypanosomes could not be determined; in two cases a relapse was recorded, the trypanosomes re-appearing in the peripheral circulation, one month and three and a half months respectively, after the injection of Novoflavin.

In one of the latter two cases, a third injection of Novoflavin was given, but the animal died from general debility 21 days later, and the result could not be observed.

Only in one case was a favourable result recorded; the sheep survived for a year after the second dose of Novoflavin, and no signs of a relapse were detected.

Unfortunately the experiment as a whole is not at all conclusive.

TABLE V.—ARSENOPHENYLGLYCIN, AND SUCCESSIVE DOSES OF NOVOFLAVIN.

Animal.	First dose of Novoflavin (per Kilo).	Interval before Trypanosomes reappeared.	Interval between the two injections.	Second dose of Novoflavin (per Ki.o).	Interval before Trypanosomes reappeared in the blood.	Remarks.
Sheep 2262	0.009 gm.	13 days.	30 days.	0.013 gm.	—	Died (Enteritis) 12 days after the injection.
„ 2146	0.01 gm.	13 „	30 „	0.01 gm.	3½ months.	Died (Gastro-enteritis) 1 year after the injection.
„ 2136*	0.01 gm.	29 „	39 „	0.0125 gm.	—	Died 1 year after the injection.
„ 2137*	0.01 gm.	29 „	39 „	0.012 gm.	—	Died (Intoxication) a few hours after the injection
„ 2641* }	0.01 gm.	29 „	39 „	0.013 gm.	34 days.	Treated again as given below.
	As shown above		34 „	0.0134	—	Died (Debility) 21 days later.

* These animals received one injection of Salvarsan, in the interval between the first and second doses of Novoflavin.

(c) *Arsenophenylglycin and Novoflavin*—simultaneously.

Only three animals were treated by this method, and the results are inconclusive.

The details of the cases are as follows:—

Horse 4,018. (See Table I.)

Relapse after treatment with Arsenophenylglycin. Injected intravenously, 7 days after the trypanosomes reappeared, with 0.05 gm. Arsenophenylglycin + 0.005 gm. Novoflavin, per kilo. The trypanosomes disappeared from the blood, and were not again recorded, but the horse died 52 days after injection, from general debility and emaciation.

Sheep 2,650.

No preliminary treatment.

The sheep received, 17 days after trypanosomes appeared in the peripheral blood, 0.05 gm. arsenophenylglycin + 0.005 gm. Novoflavin per kilo.

The trypanosomes disappeared, and were not again recorded.

Death occurred 12 days later from pneumonia.

Sheep 2,622.

No preliminary treatment.

The sheep received, 23 days after trypanosomes appeared in the blood, 0.05 gm. arsenophenylglycin + 0.005 gm. Novoflavin per kilo.

The trypanosomes disappeared, and were not again recorded.

Death occurred after 4 days from Peritonitis (Rupture of the Abomasum).

2. ARSENOPHENYLGLYCIN, TRYPANBLUE AND SODIUM ARSENITE.

Three sheep were injected with the three drugs, given simultaneously, 15 days after trypanosomes were first seen in the peripheral blood.

The drugs were injected, as 1% solutions, in the following doses:

Arsenophenylglycin, 0.07 gm. per kilo, body weight.

Trypanblue, 0.025 gm. per kilo, body weight.

Sodium Arsenite, 0.001 gm. per kilo, body weight.

Unfortunately the doses proved to be excessive, and the animals succumbed in 1 day, 4 days, and 11 days, respectively.

3. ARSENOPHENYLGLYCIN, TRYPANBLUE, AND TARTAR EMETIC.

A. Sheep were injected (intrajugular) with:—

0.06 gm. arsenophenylglycin per kilo—in 1-100 solution;

0.02 gm. trypanblue per kilo—in 1-200 solution; and

0.01 gm. tartar emetic per kilo—in 1-500 solution.

At first the three injections were given on successive days, but such treatment proved to be too severe, and intervals of 2 days were observed later.

After 8 days, the treatment was recommenced, the doses being:—

0.05 gm. arsenophenylglycin per kilo—in 1-100 solution.

0.02 gm. trypanblue per kilo—in 1-200 solution.

0.01 gm. tartar emetic per kilo—in 1-500 solution.

Sheep 3,771.

Treatment started 4 days after the trypanosomes first appeared in the blood.

Trypanosomes disappeared after the first injection (arsenophenylglycin) and did not re-appear.

Sheep died—*intoxication*—3 days after the second dose of arsenophenylglycin.

Sheep 3,760.

Treatment started 4 days after trypanosomes appeared in the blood.

Trypanosomes disappeared after first injection of tartar emetic, and did not re-appear.

Sheep died—*Intoxication*—the day the second dose of trypanblue was given.

Sheep 3,774.

Treatment started 6 days after trypanosomes appeared in the blood.

Trypanosomes disappeared on the first day of the treatment, re-appeared on the fourth day, and then disappeared, under the influence of the tartar emetic; no relapse was seen. Death occurred 5 days after the first course of treatment concluded, from *Intoxication*.

Sheep 3,754.

Control—died from Trypanosomiasis after 6 weeks.

Result.—The above doses are evidently excessive.

N.B.—The maximal therapeutic dose of Tartar Emetic for sheep was found by experiment to be 0.01 gm. per kilogramme of body weight.

B.—Sheep were injected as follows:—

1st treatment

0.06 gm. arsenophenylglycin	(1-100) per kilo	} With intervals of two days.
0.02 gm. trypanblue	(1-200) per kilo	
0.01 gm. emetic	(1-500) per kilo	

and 12 days later

0.04 gm. arsenophenylglycin	(1-100) per kilo	} With intervals of two days.
0.01 gm. trypanblue	(1-200) per kilo	
0.008 gm. emetic	(1-500) per kilo	

In the three animals mentioned below Trypanosomes appeared in the blood three or four days previous to the commencement of treatment.

Sheep 3,772.

The trypanosomes disappeared from the blood after the first injection, and no relapse has been found up to the present date.

The animal is still living (4th August, 1912), 5 months after the treatment.

Sheep 3,761.

The trypanosomes disappeared after the first injection; no relapse until death occurred, 6 days after the second course of treatment, from *Intoxication*.

Sheep 3,770.

The trypanosomes disappeared after the first injection, and did not re-appear.

The sheep died, 3 days after the second course, from *Enteritis*.

Sheep 3,763.

Control—died from Trypanosomiasis after 5 weeks.

The result in one out of three sheep is apparently very satisfactory, but the above doses were obviously proving very severe.

C.—Sheep received the following intrajugular injections:—

0.06 gm. arsenophenylglycin	(1-100) per kilo	} With intervals of two days.
0.02 gm. trypanblue	(1-200) per kilo	
0.01 gm. emetic	(1-500) per kilo	

and 8 days later

0.05 gm. arsenophenylglycin	(1-100) per kilo	} With intervals of two days.
0.02 gm. trypanblue	(1-200) per kilo	
0.007 gm. emetic	(1-500) per kilo.	

Sheep 3,762.

Treatment commenced 3 days after trypanosomes were first seen in the blood.

The trypanosomes disappeared after the first injection, and were not again seen, although after death, which occurred 70 days after the conclusion of the second course of treatment, the lesions presented were those of *Trypanosomiasis*.

Sheep 3,758.

Treated three days after trypanosomes were first seen in the blood. The trypanosomes disappeared after the first injection, and re-appeared for one day only, six days after the second treatment concluded. Death occurred ten days after the second treatment, from *Chronic Intoxication*.

Remarks.—The treatment was still too severe, although in one (out of two cases) a relapse appears to have occurred.

D.—Sheep were injected with the following:—

0.05 gm. arsenophenylglycin	(1-100) per kilo	} With intervals of two days.
0.02 gm. trypanblue	(1-200) per kilo	
0.01 gm. emetic	(1-500) per kilo	

and 12 days later,

0.01 gm. trypanblue	(1-200) per kilo	} With intervals of two days.
0.04 gm. arsenophenylglycin	(1-100) per kilo	
0.008 gm. emetic	(1-500) per kilo	

N.B.—The undermentioned sheep had previously been treated unsuccessfully with Salvarsan.

Sheep 3,766.

Treated 13 days after trypanosomes were first seen in the blood.

The trypanosomes disappeared after the first injection, and reappeared, for one day only, on the ninth day after the injection of emetic. The animal was too weak to permit the carrying out of the second course of injections.

Death occurred, 15 days after the first course, from *Intoxication*.

Sheep 3,773.

Treated 15 days after trypanosomes were first seen in the blood. The trypanosomes disappeared after the first injection, and reappeared 8 days after the second course of treatment concluded.

Death occurred, 46 days after the second course, from *Trypanosomiasis*.

Sheep 3,756.

Control (also relapsed after Salvarsan treatment).

Died from Trypanosomiasis, 76 days after inoculation.

Remarks.—The treatment was too severe, but insufficient to cure completely those animals which did not succumb to the effects of the trypanocidal agents injected.

E.—The doses were reduced to the following:—

0.04 gm. arsenophenylglycin	(1-100) per kilo	} Intrajugular, with intervals of two days.
0.01 gm. trypanblue	(1-200) per kilo	
0.008 gm. emetic	(1-500) per kilo	

and, after 11 days,

0.05 gm. arsenophenylglycin	(1-100) per kilo	} Half intrajugular and half given subcutaneously, with an interval of three days.
0.008 gm. emetic	(1-500) per kilo	

*These two sheep had previously been treated unsuccessfully with Salvarsan.

**Sheep 3,810.*

Treated 14 days after trypanosomes were first seen in the blood. The trypanosomes disappeared from the peripheral blood after the first injection, and re-appeared (in small numbers) 3 days later.

They disappeared after the injection of emetic, re-appearing on the third day, for one day only. Five days later they were again recorded, and persisted for three days, until the second course of injections was commenced.

No relapse was observed.

Death occurred, 35 days after the second treatment, from *Peritonitis*.

**Sheep 3,813.*

Treated 14 days after the trypanosomes were first seen in the blood. The trypanosomes disappeared after the first injection, re-appeared ten days after the emetic injection, and on the following day disappeared again, under the influence of the second injection of arsenophenylglycin.

The animal is still alive (4th August, 1912) and no relapse has been noted to date (128 days after the second course concluded).

Sheep 3,819.

Treated 22 days after trypanosomes were first seen in the blood. No second course of injections was given.

The trypanosomes disappeared after the first injection, and no relapse has been observed up to the present date.

The animal is apparently in excellent health at the present date (4th August, 1912), 133 days after the conclusion of the treatment.

Sheep 3,759.

Treated 38 days after the appearance of trypanosomes in the blood.

Only one course of three injections was given.

The trypanosomes disappeared from the peripheral blood after the first injection of arsenophenylglycin, and were not again recorded. The animal is still alive (4th August, 1912), 132 days after the suspension of treatment.

Sheep 3,765.

Treated 37 days after the appearance of trypanosomes in the blood. Only the first course of injections was given.

The trypanosomes disappeared after the first injection, and were not again recorded.

The sheep is still alive, 132 days after the suspension of treatment.

Remarks.—In three cases the first course of injections has apparently proved successful, no relapses being recorded up to the present time, more than four months after the termination of the treatment.

In two cases this first course of injections proved insufficient, and a second course of the injections was given; this appears to have succeeded in both cases, although in one case death occurred 35 days later, from an accidental complication.

The later history of the four sheep, which have survived until the present date, will be of considerable interest, and the experiment cannot yet be considered to have terminated.

4. ARSENOPHENYLGLYCIN AND TARTAR EMETIC.

A.—Sheep were injected intravenously with:—

One does of 0.05 gm. Arsenophenylglycin (1-100) per kilo, and 3 successive doses of 0.005 gm. Emetic (1-500) per kilo, with intervals of two days between the injections.

Four sheep were subjected to the above treatment, and in each case the treatment was too severe, the animal succumbing 1-3 days after the third injection of emetic was given.

B.—Alternate injections of

0.05 gm. arsenophenylglycin (1-100) per kilo and
0.01 gm. emetic (1-500) per kilo

At first the injections were made at intervals of 2 or 3 days (*a*), but later the interval was increased to four days (*b*).

In all the cases mentioned below, treatment was commenced 3-5 days after trypanosomes were first seen in the blood.

Sheep 3,871.

Four injections (*a*). Trypanosomes re-appeared in the blood 14 days after the last injection. Other treatment was then instituted.

Sheep 3,873.

Two injections (*a*). Trypanosomes re-appeared in the blood 10 days after the last injection. The sheep died from *Trypanosomiasis*, 12 days after the second (emetic) injection.

Sheep 3,879.

Four injections (*a*) followed in 9 days by two injections (*b*). Trypanosomes were seen in the blood, for one day, 3 days after the third injection of arsenophenylglycin, and they re-appeared 8 days after the third dose of emetic. Other treatment was then instituted.

Sheep 3,877.

Two injections (*a*), followed in 13 days by two injections (*b*). Relapses occurred 10 days after the first dose, and 8 days after the second dose, of emetic. Further treatment was then commenced.

Sheep 3,872.

Two injections (*a*), followed in 13 days by two injections (*b*). Relapses occurred 10 days after the first injection of emetic, and 8 days after the second dose. Other treatment was then commenced.

Sheep 3,866.

Four injections (*a*), followed in 9 days by two injections (*b*). Trypanosomes re-appeared in the blood, for one day, 7 days after the second injection of emetic, and for one day on the 8th day after the third dose of emetic.

Other treatment was then instituted.

Thus six sheep were treated by this method, the number of injections made varying in different cases from two to six.

Relapses occurred in every case, the trypanosomes re-appearing in the peripheral blood from 7 to 14 days after the injection of the emetic. It is evident that this treatment is not at all effective, and that the interval between the injection and the relapse was becoming distinctly shorter, when several injections were given.

C.—Treatment consisted in injecting into the jugular vein:—

0.04 gm. Arsenophenylglycin (1-100) per kilo	}	Given simultaneously.
0.005 gm. emetic (1-500) per kilo		

The injection (of the same quantities) was repeated at intervals varying in the different cases.

The following sheep were treated by this method, treatment being commenced 3 to 5 days after trypanosomes were first seen in the peripheral blood.

Sheep 3,801.

1st injection.—Relapse not seen.

2nd injection, 5 days later—Relapse in 12 days.

3rd injection, 15 days later—Relapse in 10 days.

4th injection—15 days later—Relapse in 10 days.

Died (*Trypanosomiasis*)—15 days after the 4th injection.

Sheep 3,802.

1st injection—Relapse not seen.

2nd injection—5 days later—Relapse after 8 days.

3rd injection—11 days later—Relapse after 8 days.

4th injection—19 days later—Relapse after 10 days

Other treatment was then instituted.

Sheep 3,803.

1st injection—Relapse after 9 days.

2nd injection—9 days later—Relapse after 6 days.

3rd injection—7 days later—Relapse after 1 day, 3 days, etc.

Died (*Trypanosomiasis*)—18 days after the third injection.

Sheep 3,804.

1st injection—Relapse after 8 days.

2nd injection—9 days later—Relapse after 15 days.

3rd injection—26 days later—Relapse after 10 days.

Other treatment was then instituted.

Sheep 3,805.

- 1st injection—Relapse after 8 days.
 2nd injection—after 9 days—Relapse after 6 days.
 3rd injection—after 7 days—Relapse after 10 days.
 4th injection—after 19 days—Relapse after 10 days.
 Other treatment was then instituted.

Sheep 3,782.

- 1st injection—No relapse seen.
 2nd injection—after 5 days—Relapse after 9 days.
 3rd injection—after 11 days—Relapse on 1st and 5th days, etc.
 4th injection—after 19 days—Relapse after 10 days.
 Died (*Trypanosomiasis*)—18 days after the fourth injection.

Three sheep, which had previously been treated unsuccessfully with alternate doses of these two agents (treatment 4B), were given one simultaneous injection of the doses mentioned above.

In each case trypanosomes reappeared in the peripheral blood ten days after the injection.

The method is evidently not effective, or the doses given are insufficient, for in every case injection was followed by relapse, usually in 8 or 9 days.

It is significant that on two occasions the relapse occurred only *one* day after the third injection.

It is doubtful whether the doses could be increased by more than a very small amount.

5. COMBINED TREATMENT WITH QUININE AND ATOXYL.

This experiment was suggested by the publication of Dr. Helm "Heilung von Trypanosomiasis in zwei Fällen" (Archiv für Schiffs- und Tropen-Hygiene, 1911).

Six sheep, infected with the "Guara-Guara" strain of trypanosome, were treated as follows:—

Two doses of 2 grammes of Quinine Hydrochloride were administered per os. with an interval of three days, 3 grammes of atoxyl (in a 1-50 solution) was injected into the jugular vein, on the day following that on which the second dose of quinine was given.

In one case this treatment was recommenced, ten days after the injection of atoxyl.

Each of the sheep used in this experiment weighed approximately 34 kilogrammes.

It will be seen, by reference to Table VI., that we did not by any means get the satisfactory results obtained in two cases by Dr. Helm.

In two of our cases the animal died shortly after the injection of atoxyl, and the dose (approximately 0.088 gm. per kilo.) could not, therefore, be increased with safety.

In two sheep the trypanosomes in the peripheral blood diminished in number, but never actually disappeared.

In the remaining two animals, both of which had previously been treated with Salvarsan, the trypanosomes disappeared for a short period (two and four days respectively), and then increased rapidly in numbers.

TABLE VI.—TREATMENT WITH QUININE AND ATOXYL.

Animal.	Interval between the appearance of trypanosomes in the blood and treatment.	Effect of the treatment of the trypanosomes in the peripheral blood.	Remarks.
Sheep 3817	4 days.	Trypanosomes diminished for 5 days after the injection of atoxyl.	Died (trypanosomiasis) 7 days after the atoxyl was given.
Sheep 3816	4 days.	Trypanosomes diminished for 4 days after the injection of atoxyl.	Died (trypanosomiasis) 7 days after the atoxyl was given.
Sheep 3815	4 days.	Trypanosomes disappeared after the injection of atoxyl.	Died (intoxication) 1 day after the atoxyl was given.
Sheep 3818	3 days.	" "	" "
*Sheep 3768	13 days	Trypanosomes disappeared for 2 days after atoxyl was given.	Died (trypanosomiasis and enteritis) 10 days after the atoxyl was given.
*Sheep 3775	13 days.	Trypanosomes disappeared for 4 days after atoxyl was given.	Died (trypanosomiasis and enteritis) 11 days after the atoxyl was given and 1 day after the first dose of quinine in the second course.
Sheep 3755	Control.	—	Died (trypanosomiasis) in 9 days.

* Previously treated unsuccessfully with salvarsan.