Further Results of the Experimental Treatment of Trypanosomiasis; being a Progress Report to a Committee of the Royal Society.

By H. G. Plimmer, F.R.S., W. B. Fry, Captain R.A.M.C., and H. S. Ranken, Lieutenant R.A.M.C.

(Received September 19,—Read November 3, 1910.)

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

These experiments have been carried out with the same strain of Surra as was used before, at the Brown Institution, and at the Elstree Farm of the Lister Institute.

Further Experiments with Antimony upon Dogs by New Methods.

In the last Report† we summarised the results obtained by treating dogs suffering from Surra with antimony. We found that in some dogs the subcutaneous or intramuscular administration of antimony or of its salts caused inflammatory swellings and often abscesses, with much constitutional disturbance, and therefore, although certain good results had been obtained, the method seemed impracticable for the treatment of animals upon a large scale. We then tried the effect of *intravenous* injections of the salts of antimony and even of the metal itself, and this would seem at present to be the most promising method of giving antimony, for, if the injection of the metal into the veins be carried out successfully no irritation is caused. If the salts be injected in solution the elimination is so rapid that no good result can be obtained in the acute form of trypanosomiasis with which we have been working, so the actual injection of the metal itself has been successfully carried out in now a large number of instances. In the last two Reports mentioned above experiments have been recorded with antimony (metal) in a state of extremely fine division, and it was shown that it was relatively much more powerful than the salts in its action upon trypanosomes, and that its effects lasted much longer than those of the salts. The particles of metal, which are very minute—they vary roughly from 0·5 μ to 2·5 μ—are taken up by the leucocytes, and some compound which is soluble in the *liquor sanguinis* is apparently formed by them. We are

* 1907, B, vol. 79, pp. 500—516; 1908, B, vol. 80, pp. 1—12, and 477—487; 1909, B, vol. 81, pp. 354—371.

† 'Roy. Soc. Proc.,' B, vol. 81, p. 367.

not certain what happens: but in rabbits stained films of blood, taken at various intervals after the intravenous administration of the metal, seem to show that the leucocytes go on taking up the particles until they can contain no more, and then disintegrate at varying periods afterwards, thus providing a constant, though small, supply of antimony. The disintegration would seem to be due to the destruction of the leucocytes by the antimony they have taken up, but on the other hand, many of the leucocytes which have not taken up too much appear to be quite healthy. We have found that both cerebro-spinal fluid and blood serum are, as far as a solution of any trypanocidal substances is concerned, by themselves quite without action on metallic antimony, even when kept in contact with it for periods up to three days. Experiments were undertaken with these fluids after their contact with the metal both on infected animals and upon trypanosomes *in vitro* with entirely negative results.

The time at which no more particles can be found in, or in which they disappear from, the leucocytes, varies in the different animals; in rabbits they can be found for three or four days; in horses we have not found them after three days; and in dogs not after two.

Sufficient soluble antimony is formed in all these animals after an appropriate dose of the metal to drive the trypanosomes out of the peripheral blood in about $2\frac{1}{2}$ hours; that is, more than double the time taken by the soluble salts to attain the same result.

We have not had in rabbits, dogs, or horses any plugging of capillaries with the metal, but the animals appear to be more sensitive to over-dosage with the metal than with the salts. Testing the dosage on healthy uninoculated animals is also fallacious, as a fatal dose to a healthy animal is apparently borne well by a similar animal when its blood is full of trypanosomes; we believe, also, that in the same animal a large dose is better borne when the blood contains trypanosomes than when it is free.

When antimony in this form is prepared in larger quantities, it becomes more difficult to remove impurities from it. We mention this as we have had deaths from doses which had previously been well borne, which we attribute to these impurities, but these difficulties are now overcome.

Of the 26 dogs treated with antimony, the details of which are given in the last Report, one (No. 1) lived for over 15 months in good condition, and was then killed. Inoculations from all the organs into rats proved negative, and the animal was regarded as cured; twelve others (Nos. 2, 5, 7, 9, 10, 11, 13, 14, 16, 17, 18, 19), which lived for from 48 to 94 days, if inoculation into rats from the organs after death can be accepted when negative as evidence of cure, may also be regarded as cured.

Average Duration of Untreated Disease, 14 Days.

No.	Weight in kilos.	No. of doses.	Substance used and quantity of dose.	Recurrences.	Remarks.	Gain or loss of weight.	Subinoculations from organs.
						kilos.	
27	9¼	18	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	*Living* 1 *year* 121 *days after inoculation*.....	+ ½	
28	10¼	9	8 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	Died on 75th day from pneumonia	+3	Negative.
29	9¾	10	7 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	Died on 84th day from pneumonia	+1	Negative.
30	9	12	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	3	Died on 168th day with nervous symptoms, "fits"	−1¼	Positive from C.S.F.
		3	50 mg. ant., intravenously				
31	8½	9	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	2	Died on 87th day: kidneys inflamed	−1	Negative.
		3	20 m. 5 per cent. lith. ant. tart. diluted, intravenously				
32	11	4	20 m. 5 per cent. lith. ant. tart. diluted, intravenously	1	Died on 154th day, 3 months after last dose. Spleen large	+1¼	Negative.
		2	80 mg. ant., intravenously				
33	9	2	20 m. 5 per cent. lith. ant. tart., intravenously	1	Died on 116th day. Trypanosomes in blood	− ½	Positive.
		4	60 mg. ant., intravenously				
34	8¼	2	20 m. 5 per cent. lith. ant. tart., intravenously	3	Died on 81st day from pneumonia.....	−1¼	Negative.
		4	80 mg. ant., intravenously				
35	9½	2	80 „ „	—	*Living* 347 *days after inoculation*.....	+1	
36	13	4	90 „ „	1	Died on 88th day. Trypanosomes in blood.....	− ½	Positive.
37	9	4	80 „ „	—	Died on 67th day with nervous symptoms	−1¼	Negative.
38	7	3	60 „ „	2	Died on 40th day. Probably poisoned with ant.	−1½	Negative.
39	9½	5	60 „ „	2	Died on 76th day. Probably poisoned with ant.	+1	Positive.

The preceding Table shows in outline the results with 13 other dogs since treated with antimony in the method indicated.

The last two dogs probably died from the effects of antimony, as other dogs at the same time died after the first dose of that particular sample.

Dogs are particularly susceptible to both Surra and antimony, but if we accept negative sub-inoculations made from the organs after death as fair evidence of cure, this table and the previous one will work out as follows:—

In the first Dog Table (in the last Report), on this assumption, out of 26 dogs, 13 may be regarded as cured, and in the one above, 2 are alive and 7 gave negative results, that is 22 out of 39 altogether. The indications are also that treatment with the metal intravenously has yielded better results than that with the salts, either intravenously or intramuscularly.

The technique in dogs is difficult, as only the saphenous veins in the hind legs are available without an operation, and the suspension of the metal in salt solution must be as dilute as possible.

Intraperitoneal Injection of Antimony (Metal).

A series of experiments on the effects of intraperitoneal injections of the metal has been made on rats and rabbits. Both bear the metal quite well in the peritoneum, and neither pain nor inflammation were caused. The metal rapidly disappears from the peritoneum, and after three or four days none can be seen. In rats, one dose of 15 milligrammes will keep the trypanosomes out of the blood for an average of 25 days. No los of weight or appetite occurred. In dogs this method is impracticable, as the injection causes acute peritonitis.

Rats Treated with Arsenophenylglycin and Antimony.

In the last Report is an account of some experiments with arsenophenylglycin. A rat treated with one dose of arsenophenylglycin had a dose of lithium antimonyl tartrate given to it the day *before* recurrence took place in other rats treated with arsenophenylglycin. The rat lived 222 days without recurrence; and, regarded as cured, it was reinoculated and treated intraperitoneally with one dose of antimony (metal): it died 32 days after, from a recurrence. Another rat treated with one dose of arsenophenylglycin and five doses of lithium antimony tartrate died of the disease: in this case the latter drug was not given until *after* recurrence took place.

These experiments show again that no protection is afforded by a previous attack, and also that the time at which treatment is begun is of importance.

New Arsenic and Antimony Compounds.

Five Surra rats were treated with a new arsenic-camphor compound kindly sent to us for trial by Dr. Morgan, of the Imperial College of Science. They died at about the same time as untreated rats, and the substance was found to be too irritating for use.

Eleven Surra rats were treated with a new organic antimony compound, also sent to us by Dr. Morgan. This caused considerable swelling, and did not effect the disappearance of the trypanosomes. The arsenic compound had no effect, the antimony a small effect. *In vitro*, the latter killed the organisms very slowly.

Treatment of Goats with Antimony.

According to Musgrave and Clegg* inoculated Surra in goats is always fatal, and they give 18 days as the time the untreated disease runs.

We have treated two goats with antimony. One has had three doses of about 80 milligrammes each of the metal suspended in egg-yolk and oil subcutaneously, and is living and well 15 to 16 months after inoculation. The other had 15 doses of 20 m. of 5 per cent. lithium antimonyl tartrate, and later, when it became ill with nervous symptoms, four doses of 150 milligrammes of the metal intravenously. It became paralysed and died 236 days after inoculation.

Inoculations into rats from organs and cerebro-spinal fluid were negative.

Treatment of Surra Rabbits with Antimony.

The results of treatment of rabbits with antimony have been striking. They were left until they were very ill with the disease, with sores on the face, swollen genitals, œdema, eyes and nose inflamed, and unable to stand; after treatment all these symptoms disappeared, and their deaths were, we believe, due to poisoning with an impure antimony.

The table on p. 145 sets forth the principal points.

Inoculations made from Nos, 2, 3, and 4 into rats after death were negative.

It will be noticed that after three doses of the salt there was a recurrence, and also after one dose of the metal, but not in those rabbits which had more than one dose of the metal.

* 'Trypanosoma and Trypanosomiasis,' 1903.

No.	No. of doses.	Material used, and quantity.	Recurrences.	Remarks.
1	3	5 m. 5 per cent. lith. ant. tart., intravenously	1	Died on 116th day. Recurrence occurred on the 115th day, when the impure metal was given.
	1	30 mg. ant., intravenously		
2	3	30 mg. ant., intravenously	—	Died on 80th day.
	1	30 mg. ant., intraperitoneally...		
3	2	60 mg. ant., intravenously	—	Died on 183rd day, probably killed with impure antimony. Had increased 300 gr. in weight.
4	2	30 mg. ant., intravenously	—	Died on 147th day, probably as No. 3. Had increased 180 gr. in weight.
5	1	30 mg. ant., intravenously	1	Died on 79th day. Lost 500 gr. in weight.

Experiments with Surra Rats after One Dose of Antimony.

We have had the following curious experience with infected rats which had been treated with *one* dose of lithium antimonyl tartrate. One dose of this drug causes the disappearance of all trypanosomes from the blood, and keeps the blood free from them for a variable period, the average time of recurrence being 18 days. We wished to find out how soon the blood of a rat thus treated became infective to other rats on sub-inoculation, and whether it would prove to be so before trypanosomes could be found in it by ordinary microscopic examination. Sub-inoculations were therefore made from the treated rat's blood at various intervals from the 2nd to the 16th day after the one dose of antimony. It was then found that the blood of the original rat was infective long before any trypanosomes could be found in the peripheral circulation; but also that this infectivity was not constant, an infection being produced on one day, and none occurring on one or two subsequent days. Moreover, as the table shows, the course of the disease (incubation period especially) was very prolonged. Ordinary untreated infective blood produces in rats a recognisable infection in from two to four days, and death in from six to seven days. In these cases either the trypanosomes could not be found in the peripheral blood at all, the only lesion being the very large spleen, or were not found until the 28th day after inoculation. These prolonged incubation periods appeared to have no relation to the time of the recurrence in the original rat, and the infection was brought about by blood which not only showed no trypanosomes, but appeared to be perfectly normal in structure. We have no explanation of these results, but as all the rats which died had very large spleens we can only suppose that the trypanosomes were present in either some form or place which we did not recognise or find. If this be so the

experiments are of interest as showing the influence on the trypanosomes, and the constant effects, of one dose of antimony.

Table giving Details of the above-mentioned Experiments.

Rats inoculated from treated Rat 1 on—	Death occurred on—	Spleen.	Trypanosomes present (+) or absent (0) from blood.	Remarks.
5th day after dose ...	—	—	—	Living 103 days.
7th " " ...	31st day	Very large	0	
10th " " ...	16th day	Large	0	
12th " " ...	—	—	—	Living 95 days.
14th " " ...	13th day	Very large	0	
Ditto from Rat 2 on—				
2nd day after dose...	—	—	—	Living 52 days.
4th " " ...	—	—	—	Living 50 days.
7th " " ...	—	—	—	Living 47 days.
10th " " ...	28th day	Very large	+ +	
16th " " ...	41st day	Enormous	0 in blood, spleen, liver, adrenals.	

It will be noticed that death was delayed up to as long as 41 days. Two other rats were given a relatively smaller dose of antimony, and rats inoculated from these on the 4th, 7th, 9th, and 11th days after the dose died with trypanosomes in their blood on the 24th, 19th, 19th, and 29th days respectively, the incubation period only being much prolonged.

Experiments upon Surra Horses with Antimony.

Inoculated Surra in horses runs a quicker course than that acquired naturally, and it is difficult to give a certain time for the disease, as a good deal depends on the condition of the horse, and experimental horses are usually old and worn-out and would succumb more quickly than young ones. Cases of untreated experimental Surra in horses have died as early as the 14th day, but the time probably depends very much upon the age and condition of the animal. The horses we have used were inoculated from rats, and the incubation period was practically six days. Musgrave and Clegg* give 6 to 13 days as the incubation period, so that our strain of Surra is a fairly virulent one for horses.

Both intramuscular and intravenous injection of antimony (metal) have been tried. Doses of 2 grammes have been given, intravenously, and if sufficiently diluted with warm salt solution no unpleasant symptoms

* *Op. cit.*

follow. Large doses are best borne when injected into a peripheral vein in one of the legs, and one injection of 1 gramme will keep the blood free from trypanosomes for from 2 to 3 weeks. If the injection be made into the jugular vein considerable depression, and even faintness, may be induced. The use of a peripheral vein involves throwing the horse, which is risky if repeated doses have to be given. We have not yet ascertained the maximum dose. All the horses died with nervous symptoms, and although life has been prolonged (in one case to 121 days) none of them has shown satisfactory results. We have persisted in our experiments with metallic antimony because we hoped that, as it is taken up by the leucocytes, it might possibly be carried by them to, and be deposited in, parts not accessible otherwise; also, because we know that in syphilis the protozoon causing the disease is killed by the injection of metallic mercury We know also that syphilis requires a continued treatment, and it may be that in trypanosomiasis a continued treatment also is necessary. The effects produced by metallic antimony are so striking that we are emboldened to think that if the methods could be improved a much better effect would be obtained.

The following Table summarises the experiments upon horses.

No.	No. of doses.	Material and quantity given.	Recurrences.	Remarks.
1		Intramuscular—		
	7	20 per cent. suspension of sod. ant. tart. in Lambkin's medium. Doses from 1 to 20 c.c.; in all 36 c.c.	—	No trypanosomes seen for 17 days before death. Died on 44th day with nervous symptoms and thoracic œdema. Spleen normal.
	1	5 c.c. 5 per cent. ant. in Lambkin's medium		
		Intravenous—		
2	8	Ant. in salt solution. Doses from 0·5 gr. to 1·25 gr., altogether 5·5 gr.	2	No trypanosomes seen for 19 days before death. Died on 52nd day with nervous symptoms.
3	8	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 9·75 gr.	3	No trypanosomes seen for 20 days before death. Died on 121st day with nervous symptoms.
4	6	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 4·5 gr.	—	No trypanosomes seen after first dose. Died on 56th day with nervous symptoms. Had persistent thoracic œdema.

With the exception of No. 1, these horses were old and worn-out.

It will be noticed that none of these horses had trypanosomes in the blood for some time before death; No. 3 appeared to be quite well up to the 90th day, and No. 4 up to the 40th day, and both could apparently have worked up to these times. In all cases death was preceded by a very sudden aggravation of symptoms referable to the nervous system.

We considered that treatment by injection into the cerebro-spinal space was impracticable in horses owing to the distance of the spinal canal from the surface, and to the fact that the spinal cord extends much further down the canal than in man.

Experiments with Silver Salts.

Three Surra rats were treated with protargol in doses varying from 0·5 c.c. of a 1-per-cent. solution to 1 c.c. of a 2-per-cent. solution.

Three Surra rats were treated with argyrol in similar doses.

Three Surra rats were treated with silver nitrate in doses varying from 1 c.c. of a 0·02-per-cent. solution to 1 c.c. of a 1-per-cent. solution.

All these rats died at the usual time: death was not delayed, nor were the trypanosomes reduced in number in the blood. *Post-mortem* examination showed that they had died with the usual signs of acute trypanosomiasis, with very large spleens, etc. At the sites of injection of protargol there was œdema and brown staining of the tissues in a circumscribed area: there was more marked œdema and swelling after injection of argyrol, and œdema and localised necrosis of the tissues after injection of silver nitrate. It would appear that the silver salts are not absorbed from the site of injection. Presumably they have a primary cauterising action on the tissues, and are quickly transformed into silver chloride, which is inert.

In vitro experiments were carried out with these three compounds, all of which are soluble in distilled water. Equal sized drops of blood containing trypanosomes and of the solutions were mixed, and the results observed under the microscope.

Protargol solutions from 1/200 to 1/10000 produced very little effect; in 1/200 dead trypanosomes were seen in 30 minutes, but many were alive in 24 hours. Silver nitrate in solutions from 1/200 to 1/10000 has much the same effect as protargol.

Some trypanosomes are killed in 30 minutes by the stronger solutions, but the remainder are as active as the controls up to 24 hours and longer.

Argyrol (which contains 30 per cent. silver) in solutions of 1/1000 to 1/10000. The stronger solutions are still less active than those of protargol and silver nitrate; and the more dilute have no effect up to three hours. Thus it appears that these bodies have no effect except the early effects of the stronger solutions; they probably combine with the salts in the blood-plasma and corpuscles, and become inert.

Citrate of silver in its strongest solution of 1/4000 is also inert.