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FURTHER RESULTS OF THE EXPERIMENTAL TREATMENT OF TRYPANOSOMIASIS; BEING A PROGRESS REPORT TO A COMMITTEE OF THE ROYAL SOCIETY.¹

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THE following results are a continuation of the work of which summaries have already appeared in the *Proceedings of the Royal Society*.²

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

A.—CONDITION OF THE ANIMALS LIVING AT THE DATE OF THE COMPLETION OF THE TABLES IN THE LAST PAPER.

Rats treated with Sodium Antimonyl Tartrate, 1 per cent. (p. 478).

No. 7 died 428 days after inoculation.

„ 32 „ 409 „ „

„ 35 „ 371 „ „

Rats treated with Sodium Antimonyl Tartrate, 5 per cent., in Colonel Lambkin's Medium (p. 482).

No. 13 died 216 days after inoculation.

¹ Reprinted from the *Proceedings of the Royal Society*, B, vol. lxxx.

² B, vol. lxxix., 1907, pp. 500-516; B, vol. lxxx., 1908, pp. 1-12 and 477-487.

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Rats treated with Antimony (metal), 5 per cent., in Colonel Lambkin's Medium (p. 483).

| | | | | | |
|--------|------|-----|------|-------|--------------|
| No. 10 | died | 205 | days | after | inoculation. |
| „ 17 | „ | 367 | „ | „ | „ |
| „ 25 | „ | 385 | „ | „ | „ |
| „ 27 | „ | 399 | „ | „ | „ |
| „ 29 | „ | 360 | „ | „ | „ |

Rats treated with Lithium Antimonyl Tartrate, 0.25 per cent. (p. 485).

| | | | | | |
|-------|------|-----|------|-------|--------------|
| No. 4 | died | 145 | days | after | inoculation. |
| „ 5 | „ | 229 | „ | „ | „ |
| „ 6 | „ | 257 | „ | „ | „ |
| „ 8 | „ | 241 | „ | „ | „ |
| „ 10 | „ | 209 | „ | „ | „ |

Most of the above rats died from cold; none of them died from the disease, and no trypanosomes were found in their blood or organs, and inoculations made therefrom were entirely negative.

B.—FURTHER EXPERIMENTS.

Rats treated with Lithium Antimonyl Tartrate, 1 per cent.

A further series of experiments has been carried out with this substance on a large number of rats, giving four doses of 4 to 5 minims (according to weight) of a 1-per-cent. solution subcutaneously, a dose being given every other day. Practically by this method every rat can be cured. They have lived for varying periods, up to 249 days, and in no case have trypanosomes been found after death in the blood or in the organs. No rat has died of the disease, and in no case thus treated has there been a recurrence. The results have therefore been more constant than those attained with sodium or potassium antimonyl tartrates. The treatment was begun on the third or fourth day after inoculation; it will be seen below that when it is left until the number of trypanosomes in the peripheral blood is very great, although they may be driven out of the blood, it does not cure; so that the time at which treatment is commenced is of considerable importance.

It has also been given intravenously in rabbits, but with far less effect than when given subcutaneously. The elimination in this case is very rapid, to which fact we attribute its comparatively feeble action.

Rats treated with Lithium Antimonyl Tartrate on the Fifth or Sixth Day of the Disease.

The blood at this period of the disease is swarming with trypanosomes, and experiments were made in order to see what effect this salt of antimony would have upon the disease at this period. If one dose of 5 minims of a 1-per-cent. solution be given the rats die on the seventh day, so that little or no effect is produced. If two such doses be given, one on the fifth and one on the sixth day, the average time of death in ten rats was nineteen and a half days, and living trypanosomes were found in the blood at death. When four doses were given, one on each day from the fifth to the eighth, the time in three rats was lengthened to eighty-one to eighty-six days; in one of these even living trypanosomes were found in the blood after death. By comparing these results with those mentioned in the former section it will be seen that the time at which the administration of the drug is begun is of importance, as well as the number of doses. The animals stand the best chance of cure when no recurrences take place, and this is best ensured by the method described in the previous section.

Further Experiments made with Rats treated with Antimony in Order to find out in what Organs the Trypanosomes are latent.

Following on the experiments made on rats treated with sodium antimonyl tartrate, with the view of finding out where the trypanosomes are latent, and recorded in the last paper,¹ a further series of experiments has been made on rats inoculated with Surra, which is more amenable to treatment with antimony than the Nagana used in the former series, and completely treated (that is, given a curative series of doses) with lithium antimonyl tartrate; this, as stated in the paper referred to, appears to be the most active of this variety of salt.

Seven rats were treated with four doses of 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate, and they were killed in succession, one on the sixth, seventh, tenth, fourteenth, sixteenth, twenty-second, and thirtieth days after the last dose. The livers and bone-marrow were made into an emulsion with the minimum quantity of 0.89 per cent. salt solution, and 1 cc. of the emulsions of these organs and 1 cc. of heart's blood was injected separately into other rats. The results were entirely negative. Microscopic preparations were made of the material injected and no organisms were seen, and none of the sub-inoculations gave a positive result.

¹ *Proc. Roy. Soc., B*, vol. lxxx., p. 487.

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Experiments made in Order to see if any Protection was afforded by Initial Treatment with Antimony.

A series of six rats was treated with four doses of 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate, one dose every other day in the same manner as when given for curative purposes. They were then inoculated with Surra, one on the first day after the completion of the treatment, and one on the second, fourth, fifth, ninth, and tenth days after. They all died on the fifth or sixth day after inoculation, just as untreated rats would have done, so that antimony in this very soluble form is of no protective use in rats, owing most probably to its rapid elimination.

The blood of an uninfected rat treated as above has also been used in the *in vitro* experiments recorded below.

Rats treated with Sodium Antimony Lactate and with Antimony Sodium Calcium Lactate.

Through the kindness of Messrs. von Heyden we have been enabled to make some experiments with the above compounds. The sodium antimony lactate contains 26 per cent. of antimony, and the antimony sodium calcium lactate 17 per cent., so they are both much weaker in antimony than the tartrates which we have used. By the addition of a small quantity of lactic acid we were able to get a 1-per-cent. solution of both salts, and in this strength the solutions were not very irritating, but neither with rats nor with larger animals are they as effective as the tartrates or the metal.

The following table shows the results obtained with sodium antimony lactate 1 per cent.

Average duration of untreated disease 6.9 days:—

| Rats of 150 to 200 grammes weight | Number of doses, and quantity | Recurrences | Lived | Remarks |
|-----------------------------------|-------------------------------|-------------|--------|---|
| 1 | 2 of 4 minims | 0 | 9 days | Died from enteritis. |
| 2 | 4 of 4 " | 0 | 20 " | Died from retained foetus. |
| 3 | 4 of 4 " | 1 | 37 " | — |
| 4 | 5 of 4 " | 1 | 46 " | — |
| 5 | 6 of 4 " | 2 | 100 " | } (No trypanosomes found in any of these rats after death.) |
| 6 | 4 of 5 " | 0 | 74 " | |
| 7 | 5 of 5 " | 1 | 48 " | |

The following table shows the results obtained with antimony sodium calcium lactate 1 per cent.

Average duration of untreated disease 6.9 days:—

| Rats of 150 to 200 grammes weight | Number of doses and quantity | Recur- rences | Lived | Remarks |
|-----------------------------------|------------------------------|------------------|---------|--|
| 1 | 3 of 4 minims | 1 | 83 days | No trypanosomes found <i>post mortem</i> . |
| 2 | 4 of 4 " | 0 | 25 " | Living trypanosomes found <i>post mortem</i> . |
| 3 | 5 of 4 " | 2 | 68 " | No trypanosomes found <i>post mortem</i> . |
| 4 | 6 of 4 " | 2 | 45 " | " " " |
| 5 | 5 of 5 " | 2 | 64 " | " " " |
| 6 | 6 of 5 " | 2 | 68 " | " " " |
| 7 | 8 of 5 " | 2 | 57 " | " " " |
| 8 | 4 of 7 " | 0 | 131 " | " " " |

On dogs the effect was very much less marked than on rats, and an effective dose became inconveniently large.

The following experiments show the relatively greater time taken for these salts to act as compared with the sodium or lithium antimonyl tartrates, which drive all the trypanosomes from the peripheral blood in about an hour after the dose.

A Surra rat was taken on the fourth day, when the trypanosomes are numerous in the blood, and 5 minims of a 1-per-cent. solution of sodium antimony lactate were injected.

Blood was taken and showed the following:—

Half hour after injection: Trypanosomes affected by the drug are extremely active, and show a tendency to swell.

One hour after injection: Very few normal trypanosomes to be seen; nearly all are swollen and spherical in shape (= "battledores"). Still large numbers.

One and a half hours after injection: Much smaller number of trypanosomes to be seen; a few "battledores"; a few motionless ones, and one or two normal forms.

Two hours after injection: "Battledores" have all disappeared; one or two slowly moving normal forms seen.

Two and a half hours after injection: Ditto.

Three and a half hours after injection: No trypanosomes found.

A similar experiment made with a rat treated with antimony sodium calcium lactate yielded practically the same result. Further experiments made with these drugs *in vitro* will be mentioned later.

Experiment made with Antimony (Metal in state of finest Division) suspended in various Oily Media.

Since the curative results following treatment with the metal antimony¹ suspended in Colonel Lambkin's medium seemed

¹ Roy. Soc. Proc., B., vol. lxxx, p. 483.

promising, many trials have been made with the metal suspended in other oily media, such as olive oil, cod-liver oil, lanolin, egg-yolk, &c., in order, if possible, to obviate, or at any rate reduce, the extremely irritating properties of the metal, which seriously interfere with its practical use.

In olive oil a 5-per-cent. suspension was used; with one dose of 3 minims Surra rats lived for fifteen days, and died with living trypanosomes in their blood. Seventeen Surra rats were given one dose of 5 minims on the fourth day of the disease, and they lived from 41 to 133 days; in these there were no recurrences, nor were trypanosomes found after death, and sub-inoculations were in every case negative. Six Surra rats were treated with the same dose in order to observe the time taken for the complete disappearance of the trypanosomes from the blood.

Blood was taken and showed the following:—

Half hour after injection: Trypanosomes very active.

One hour after injection: As numerous; show evidences of swelling.

One and a half hours after injection: Still numerous; nearly all swollen; some "battledores."

Two hours after injection: Very few forms found; all "battledores."

Two and a half hours after injection: No trypanosomes seen.

Two Surra rats were taken on the fifth day, when the blood was swarming with trypanosomes, and 6 minims were given. Two and a half hours after the rats were killed, and smears were made from the lungs, liver, spleen, kidney, bone marrow, heart's blood, and brain. In none of the specimens could a trypanosome be found after prolonged examination.

This oil was also given to several rats upon recurrences after treatment with small doses of the lactates mentioned above; in these cases the effect was much less marked, even although the number of trypanosomes in the blood was much less than in the rats treated for the first time. This accords with our general experience that recurrences are much more difficult to deal with than the initial infection, and this applies to all the drugs we have tried.

A suspension in cod-liver oil took four hours to drive the trypanosomes out of the peripheral blood.

The suspension in egg-yolk appeared to act in rats better than in any other. In dogs, however, the results were variable; sometimes strikingly good, at others no better than the other mixtures; sometimes causing great irritation and sloughing, sometimes not

causing any irritation at all. We have rats alive for more than 120 days after inoculation, with no recurrences, after one dose.

An experiment was made to see how long one dose took to drive the trypanosomes out of the blood. A Surra rat on the fourth day was treated with 5 minims of a 5-per-cent. suspension.

Blood was taken and showed the following :—

Three-quarters of an hour after injection : Trypanosomes much affected, but not decreased. Many “battledore” forms.

One and a quarter hours after injection : Trypanosomes reduced in numbers ; all swollen and “battledore” forms, very little movement.

Two hours and a half after injection : No trypanosomes found.

Experiments with Quassia.

Dr. Guillemard, of Cambridge, suggested that quassia, on account of its known poisonous effects on some of the lower forms of life, should be tested for its trypanocidal qualities. A series of experiments was therefore undertaken on rats.

Six Surra rats were treated on the third and following days of the disease with a 5-per-cent. solution of the pharmacopœial extract of quassia ; they were given three doses subcutaneously—5 minims on the third day, 10 minims on the fourth, and 10 minims on the fifth day. The trypanosomes were entirely unaffected, and the animals died on the sixth to seventh day. Another series of twelve Surra rats was treated with a two hours' decoction of quassia-wood made with the minimum amount of water. Of this three doses were given—5 minims on the third day, and 10 minims on the fourth and fifth days. The trypanosomes in these rats were also entirely unaffected, and the animals died on the sixth to seventh day. It was also tried intravenously in rabbits in doses of 30 minims of the decoction ; no effect was produced, and the rabbits died on or about the forty-second day.

Experiments made *in vitro* correspond with these results, and will be described later.

Experiments with Arsenophenylglycin.

Professor Ehrlich kindly sent some of this substance to Dr. Bagshawe, the Director of the Sleeping Sickness Bureau, with which we have made some initial experiments upon rats. Ehrlich found that Nagana mice could be cured, in practically every case, with this substance. But the effects on larger animals, so far as we have gone, are not quite so satisfactory, and it compares in this

undesirable manner very well with the antimony tartrates, with which we can cure practically every case of Surra in rats, but which do not have anything like the corresponding effects on rabbits, guinea-pigs, and dogs. It is not only in the question of practical dosage that difficulties arise: each kind of animal has a personal equation, and their reaction to a given drug is not similar. This, and the relatively larger dosage in bigger animals, present considerable practical difficulties in the treatment of trypanosomiasis.

Our experiments have given the following results. Out of eight Surra rats of 180 to 200 grammes weight which were given one dose of 25 minims of a 1-in-80 solution of arsenophenylglycin, four died on the nineteenth day with living trypanosomes in their blood, the recurrences having taken place on the sixteenth to seventeenth day. Two were given three and five doses respectively of 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate on the seventeenth and following days, and they lived fifty-nine and fifty-one days. Of the two which are still living (ninety-five days), one has had five doses of 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate, beginning on the seventeenth day, and the other had one similar dose given on the day before the recurrences occurred in the other rats.

The following experiment shows the effect of this substance upon the trypanosomes in the blood, and how much longer it takes than the antimony salts to produce its effects.

A Surra rat on the fourth day of the disease was treated with 1 cc. of a 2-per-cent. solution of arsenophenylglycin (practically the same dose as given to the other rats).

Blood was taken and showed the following:—

Half hour after injection: Trypanosomes showed slight increase of motility.

One hour after injection: Trypanosomes showed slight increase of motility.

Two hours after injection: Trypanosomes, but more marked.

Three hours after injection: Trypanosomes not quite so active and fewer in number.

Four hours after injection: Trypanosomes now very few in number.

Four and a half hours after injection: Only one or two trypanosomes to be seen in a preparation.

Five hours after injection: No trypanosomes seen.

In these specimens no swollen, breaking up, or "battledore" forms were seen; the trypanosomes simply disappeared.

On the Effects of the Drugs used upon the Trypanosomes in the Living Body.

In studying the therapeutic effect of the various drugs tried, including metallic antimony in a state of finest division, repeated observations of the peripheral blood were made in order to observe the effect of the drug upon the trypanosomes, and to ascertain when the trypanosomes entirely disappeared from the blood. The first stage noticed of the effect of the drug was a great increase in the motility of the trypanosomes, followed by a gradual slowing down to movements slower than normal. At this stage there is a tendency for the whole trypanosome to swell and to become bloated in appearance. The swelling of the trypanosome continues until it becomes almost spherical in form, or oftener "battledore" shaped; the protoplasm becomes indistinct, and the flagellum appears to be attached to only one side of the periphery; the macro-nucleus is fairly distinct, but it eventually breaks up, and then the swollen mass disintegrates. The spleen at this time is full of these broken-up masses of trypanosomes, and as the nuclei will still stain, in films a plasmodial appearance is seen of bits of nuclei dotted about in a granular ground. These stages can be observed after treatment with all the salts of antimony used, and are well marked after the administration of the metal, in which case, however, the stages are slower. The soluble salts, lithium and sodium antimony tartrates, effect the total disappearance of the trypanosomes in about one hour. Metallic antimony, when given in the various media tried (Lambkin's medium, olive oil, cod-liver oil, heavy paraffin oil, egg-yolk), brings about this disappearance in from two and a half to four hours, according to the medium used; the first noticeable effects being produced in about half an hour. In the case of egg-yolk and olive oil the blood is free from trypanosomes in two and a half hours. This would seem to show that some portion of the metal introduced must be changed into some soluble form very rapidly; but apparently after the reaction of the tissues occurs the antimony becomes more or less shut off, and absorption must take place very slowly, as traces of the metal, apparently unaltered, have been found as late as six to seven weeks after the injection.

Sodium antimony lactate and antimony sodium calcium lactate were found to act rather more slowly than the above (see Table), the time at which the trypanosomes had completely disappeared varying from three to four hours.

It was noticed in these experiments that trypanosomes, though obviously drug-affected when the blood was taken, remained alive

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on the slide outside the body for a long time after all forms had disappeared from the circulating blood.

Further details of the time taken for the various drugs to act will be found in the sections upon sodium antimony lactate, antimony oil, antimony egg-yolk, and arsenophenylglycin.

On the Action of Trypanocidal Substances in vitro.

Experiments have been carried out with a view of throwing light on the more exact nature of the changes which are produced in trypanosomes when they are brought into contact with trypanocidal substances. The general principles we have observed in these experiments have been: (1) To dissolve the drug in some fluid so that when it is added to the infected blood it will not cause osmosis to occur in the cellular elements of, or trypanosomes contained in, the blood. (The various substances were dissolved in a 0.89-percent. salt solution, isotonic with rat's blood which was used in these experiments.) (2) To use always equal volumes of the solution and of the affected blood. (3) To use blood at the time when the trypanosomes are just becoming very numerous, so as to avoid the presence of old, feebly-moving forms, which are always present in the later stages of an acute infection. The method of observation has been to watch the behaviour of the trypanosomes when in contact with the various solutions of the drug under the microscope. A measured drop of blood and of the solution are mixed on a slide with care; the mixed drop is then covered with a sufficiently large cover-glass, and this is sealed with vaseline.

It has been found possible in this manner to exactly determine the dilutions at which the various drugs used cease to have an instantaneously trypanocidal action; further, in higher dilutions, by carefully watching the changes taking place in the trypanosomes, it is possible to determine the dilution at which no effect is produced, and between these two points the periods of time necessary to ensure immobility and death of the trypanosomes can be ascertained. By a comparison of the results obtained a very good estimate of the probable action of any drug when given to an infected animal can be arrived at.

For instance, sodium and lithium antimonyl tartrates were found to act, in the same dilutions, in a manner fairly comparable to their antimony content, and to their action on the trypanosomes in an affected animal. Again, with atoxyl a much higher concentration of the drug was necessary—it had to be about ten times stronger—in order to obtain the same destruction pictures, results corresponding with the rapidity of the disappearance of trypano-

somes from the peripheral blood of affected animals when treated with the above drugs.

In the case of the two new lactates mentioned above, their therapeutical value was accurately foretold by a preliminary study of their action *in vitro* in the manner described. In all these experiments controls have been carried out; it has been found that trypanosomes will live and retain their activity for hours when infected blood and the diluting fluid alone are mixed together.

The various changes taking place in trypanosomes on coming into contact with a dilute trypanocidal drug, commencing with their preliminary extraordinary increase of activity, and their subsequent swelling up, immobility, and disintegration, can be watched in all their different stages in this manner. These effects resemble very closely the changes which take place in the trypanosomes in the peripheral circulation of an animal treated with antimony.

The following tables show the effects produced by the different substances in their various dilutions.

Dilutions of sodium antimonyl tartrate in 0.89-per-cent. salt solution mixed with Surra rat's blood, in equal parts. The control in all cases is equal parts of blood and 0.89-per-cent. salt solution.

| Dilutions | | | | Time | Control |
|------------|------------|------------------|---------------------------------|---------|-------------|
| 1-500 | 1-1,000 | 1-5,000 | 1-10,000 | | |
| Motionless | Motionless | Few active forms | Trypanosomes active | 1 min. | Very active |
| " | " | Motionless | Few active forms; rest sluggish | 10 min. | " |
| " | " | " | All sluggish.. .. | 30 " | " |
| " | " | " | Motionless | 1 hour | " |

Dilutions of lithium antimonyl tartrate in 0.89-per-cent. salt solution mixed with Surra rat's blood in equal parts.

| Dilutions | | | | | Time | Control |
|------------|------------|--------------------------|--|---|---------|-------------|
| 1-500 | 1-1,000 | 1-5,000 | 1-10,000 | 1-20,000 | | |
| Motionless | Motionless | Some active trypanosomes | Active trypanosomes | Very active trypanosomes | 1 min. | Very active |
| " | " | Motionless | Some active trypanosomes | Many active trypanosomes | 10 min. | " |
| " | " | " | Practically no motile trypanosomes seen, only 1 or 2 in a slide: Tendency to clump | Few active trypanosomes seen. Tendency to clump | 30 " | " |
| " | " | " | Motionless .. | 1 or 2 active forms seen. Rest motionless | 1 hour | " |

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In a dilution experiment with lithium antimonyl tartrate made with the blood of a Surra rat after a second recurrence, after treatment with antimony (metal) and on first recurrence with lithium antimonyl tartrate, the trypanosomes *in vitro* appeared to have a greater resistance to the dilute drug than the stock strain.

A comparison of the following table with the previous one will demonstrate this:—

| Dilutions | | Time |
|------------------------------------|----------------------------------|---------|
| 1—1,000 | 1—5,000 | |
| A few active forms present | A number of active forms present | 1 min. |
| Motionless | A few active forms seen | 10 min. |
| „ | Motionless | 30 „ |

This bears out our experience that the recurrences become less and less amenable to antimony as they increase in number.

The following table shows the action of atoxyl and lithium antimonyl tartrate compared in the above manner:—

| | Dilutions of atoxyl | | | Time | Dilutions of lithium antimonyl tartrate | | |
|--------------------------|---|-------------------|-----------|--------|---|-----------------------|---------------------------------------|
| | 1—500 | 1—1,000 | 1—5,000 | | 1—500 | 1—5,000 | 1—10,000 |
| Trypanosomes, all active | Active .. | Active .. | Active .. | 1 min. | Trypanosomes, all motionless | All markedly affected | All fairly active. |
| Active but affected | „ .. | „ .. | „ .. | 5 min. | Motionless; commencing disintegration | Motionless.. | Less active. |
| Less active | Sluggish, but still many active | „ .. | „ .. | 15 „ | Only <i>débris</i> seen | „ .. | Some still moving; tendency to clump. |
| Practically motionless | Nearly all motionless; one or two active forms seen | Many moving still | „ .. | 2 hr. | „ .. | Disintegrated | Motionless; some disintegration. |

Concentrated decoction of quassia in 0·89-per-cent. salt solution mixed with Surra rat's blood in equal parts.

| Dilutions | | | | Time | Control |
|---------------|---------------|---------------|---------------|------------|--------------|
| 1-500 | 1-1,000 | 1-5,000 | 1-10,000 | | |
| Very active.. | Very active.. | Very active.. | Very active.. | 1 min. .. | Very active. |
| ” .. | ” .. | ” .. | ” .. | 10 min. .. | ” |
| ” .. | ” .. | ” .. | ” .. | 30 ” .. | ” |
| ” .. | ” .. | ” .. | ” .. | 1 hour .. | ” |
| Less active.. | Less active.. | Less active.. | Less active.. | 2 hours .. | Less active. |

The conditions of the dilutions and the control were precisely similar at the end of two hours. There was no swelling or clumping.

Dilutions of arsenophenylglycin in 0.89-per-cent. salt solution mixed with Surra rat's blood in equal parts.

| Dilutions | | | | | Time | Control |
|---|--------------------|-------------|-------------|-------------|---------|--------------|
| 1-100 | 1-500 | 1-1,000 | 1-5,000 | 1-10,000 | | |
| Very active | Very active | Very active | Very active | Very active | 1 min. | Very active. |
| Irritated : movements rapid and convulsive | Activity increased | ” | ” | ” | 10 min. | ” |
| Nearly motionless | Sluggish .. | ” | ” | ” | 30 ” | ” |
| Motionless | ” .. | ” | ” | ” | 1 hour | ” |

Experiments in vitro performed with the Blood of a Normal Rat which had been treated with Antimony.

Experiments were made in order to ascertain whether the blood of a rat which had been treated with antimony would show any active trypanocidal powers *in vitro*. Although in the case of an infected animal all the trypanosomes in the peripheral blood would have been destroyed in about an hour, no noticeable trypanocidal effects were shown by the blood of a treated rat in the following experiments.

A normal rat had 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate injected subcutaneously; its blood was taken at fifteen, thirty, sixty, and seventy minutes after the injection, and was mixed with an equal quantity of blood from a Surra rat containing many trypanosomes; the mixed bloods, taken at the times mentioned, were examined under the microscope at various intervals

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from five to thirty minutes after the mixing, and the trypanosomes were found to be entirely unaffected, so that the blood of the treated normal rat did not have any trypanocidal effect added to it by the dose of lithium antimonyl tartrate. The Surra rat, whose blood was used for this experiment, was then given 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate.

Blood was taken and showed the following :—

Ten minutes after injection : Trypanosomes affected ; movement very rapid.

Twenty minutes after injection : Many " battledores."

Forty minutes after injection : Trypanosomes greatly decreased in number all " battledores."

Sixty minutes after injection : Blood quite free from trypanosomes.

A normal rat was given four doses subcutaneously, one every other day, of 5 minims of a 1-per-cent. solution of lithium antimonyl tartrate; twenty-four hours after the last dose a drop of its blood was mixed with a drop of blood from a Surra rat in which trypanosomes were plentiful. The mixture was watched under the microscope for half an hour, but no effect was produced ; the blood of the treated animal behaving just as the blood of the control, an untreated rat.

A normal rat was given subcutaneously 10 minims (a lethal dose) of a 1-per-cent. solution of lithium antimonyl tartrate, and its blood was mixed at half an hour, one hour, and one and a half hours after the injection with an equal part of an emulsion of trypanosomes prepared from the lungs, liver, and heart's blood of a Surra rat just dead. Each of the mixtures was examined up to thirty minutes, but no effect whatever was produced on the trypanosomes. These experiments may be compared with those recorded on p. 4.

Experiments with Antimony upon Dogs.

Since the date of the last paper a large number of experiments have been made with antimony in various forms upon dogs suffering from Surra. Of the five dogs mentioned there, one remains alive and well at the present date, more than a year after inoculation.

Our experiences with dogs show that they are extremely susceptible both to the disease and also to antimony; they are therefore not quite suitable animals for these experiments, although they have all lived many times the length of the untreated disease—that is, fourteen days. Five of the dogs were treated with small doses of sodium antimonyl tartrate in their drinking water, but the disease is so acute in dogs that this method of giving the drug,

although it appeared to have some effect in postponing the reappearance of the trypanosomes in the blood, did not produce results sufficiently encouraging to warrant further experiments.

With regard to the experiments made with metallic antimony suspended in egg-yolk, the initial experiment was so encouraging as to make a further trial necessary. In this case the dog at the first relapse was given 20 minims of a 2½-per-cent. suspension; there was no local reaction, which in dogs is of frequent occurrence after the administration of antimony in any form, and the trypanosomes, which were very numerous, were entirely absent from the blood in twenty-four hours; the dog remained quite free from them for forty-eight days, and gained 3 lb. in weight and appeared perfectly well. The recurrence was very sudden, as the dog was perfectly well up to the moment when he was seized with a series of fits which ushered in the recurrence, from which he did not recover. A rat treated at the same time as this dog with 5 minims of the same suspension is alive and well more than 100 days after this one dose.

Many of the dogs mentioned in the table below have died with fits and paralyses and other nervous symptoms, but we are not certain whether these are due to the disease or to the antimony. In certain of the dogs the treatment has appeared to alter the acute disease into a chronic one, and in one of these more chronic cases there was a considerable excess of cerebrospinal fluid and a cellular exudation around the vessels in the brain, very similar in incidence and extent to that described and figured by one of us in rats dead from infection with *Trypanosoma gambiense*.¹

There is a curious uncertainty in the local effects produced in dogs by antimony, whether injected subcutaneously or intramuscularly, and they vary from time to time in the same dog; sometimes little or no effect is produced, and sometimes the suppuration and necrosis produced are sufficient to kill the animal.

We have recently given twenty-four injections of lithium antimonyl tartrate subcutaneously to three dogs in the greatest possible dilution. Of these, three places have suppurated slightly, although the conditions under which they were given were similar to those under which the twenty-one other doses were given. (These dogs are now living and well fifty-three days after inoculation, and they have had no recurrences.)

The following table gives a synopsis of the treatment, &c., of Surra dogs:—

¹ *Roy. Soc. Proc.*, B, vol. lxxix., p. 95.

| | | | | | | | | |
|----|-----|---|----|----|----|--------------------|---|--|
| 13 | 11½ | 1 | 20 | 2 | .. | lith. ant. tart... | 1 | Died on the 60th day with nervous symptoms. No trypanosomes seen for 29 days before death. |
| | | 1 | 15 | 2½ | .. | ant. oil | | |
| | | 2 | 15 | 5 | .. | " " | | |
| 14 | 10½ | 1 | 20 | 5 | .. | " " | | |
| | | 2 | 15 | 5 | .. | " " | 2 | Died on the 52nd day from septicæmia. No trypanosomes found after death. |
| | | 1 | 20 | 5 | .. | " " | | |
| 15 | 13¾ | 1 | 15 | 2 | .. | lith. ant. tart. | | |
| | | 2 | 20 | 2 | .. | " " | | |
| | | 2 | 20 | 2 | .. | " " | 5 | Died on the 50th day with living trypanosomes in blood. |
| 16 | 9¾ | 1 | 15 | 2 | .. | ant. oil " | | |
| | | 2 | 15 | 5 | .. | " " | — | Died on the 64th day from septicæmia. No trypanosomes found after death and no recurrence. |
| | | 3 | 20 | 2 | .. | lith. ant. tart. | | |
| 17 | 9¼ | 1 | 10 | 2½ | .. | egg ant. | | |
| | | 2 | 15 | 5 | .. | ant. oil .. | — | Died on the 48th day from pneumonia. No trypanosomes found after death. |
| | | 1 | 15 | 2 | .. | lith. ant. tart | | |
| 18 | 9¼ | 1 | 15 | 2 | .. | " " | 1 | Died on 50th day, possibly from ant. sod. lactate. No trypanosomes found after death. |
| | | 2 | 15 | 5 | .. | ant. oil | | |
| | | 1 | 20 | 1 | .. | ant. sod. lact. | | |
| 19 | 10¼ | 1 | 20 | 2 | .. | lith. ant. tart. | 3 | Died on 74th day with nervous symptoms. There were 48 days between the first and second recurrences. |
| | | 1 | 15 | 5 | .. | " " " | | |
| | | 1 | 10 | 5 | .. | " " " | | |
| | | 1 | 20 | 5 | .. | ant. cream | | |
| | | 1 | 20 | 2½ | .. | egg ant. | | |
| | | 1 | 10 | 2½ | .. | " " | | |
| 20 | 7½ | 3 | 15 | 2½ | .. | " " | — | Trypanosomes practically never out of blood. Died on the 37th day, paralysed. |
| | | 1 | 15 | 5 | .. | " " | — | Trypanosomes practically never out of blood. Died on the 44th day with fits and nervous symptoms. |
| | | 2 | 10 | 2½ | .. | " " | | |
| 21 | 13¼ | 3 | 20 | 5 | .. | " " | | |
| | | 1 | 15 | 2 | .. | lith. ant. tart. | — | Trypanosomes practically never out of blood. Died on the 55th day with fits and nervous symptoms. |
| | | 3 | 15 | 2 | .. | lith. ant. tart. | | |
| 22 | 8½ | 3 | 15 | 2 | .. | egg ant. | — | Trypanosomes practically never out of blood. Died on the 55th day with fits and nervous symptoms. |
| | | 3 | 15 | 2 | .. | lith. ant. tart. | | |
| | | 1 | 15 | 5 | .. | egg ant. | 3 | Died on the 55th day with fits and nervous symptoms. |
| 23 | 13¼ | 2 | 20 | 5 | .. | " " | | |
| | | 2 | 15 | 5 | .. | lith. ant. tart. | | |
| | | 4 | 5 | 5 | .. | " " | | |
| | | 1 | 15 | 5 | .. | egg ant " | 2 | Died on the 64th day with living trypanosomes in the blood. |
| | | 1 | 20 | 5 | .. | " " | | |
| | | 1 | 15 | 5 | .. | lith. ant. tart. | | |
| 24 | 12¼ | 1 | 10 | 5 | .. | " " | | |
| | | 5 | 5 | 5 | .. | " " | | |
| | | 1 | 10 | 5 | .. | " " | | |
| 25 | 9¾ | 1 | 10 | 5 | .. | " " | 2 | Died on the 56th day with nervous symptoms. |
| | | 1 | 20 | 5 | .. | egg ant. | | |
| | | 5 | 5 | 5 | .. | lith. ant. tart. | | |
| 26 | 8. | 1 | 10 | 5 | .. | " " | 2 | Died on the 47th day. Trypanosomes found in the cerebrospinal fluid. |
| | | 1 | 20 | 5 | .. | egg ant. | | |
| | | 5 | 5 | 5 | .. | lith. ant. tart. | | |