Experiments on the Treatment of Animals Infected with Trypanosomes, by means of Atoxyl, Vaccines, Cold, X-rays, and Leucocytic Extract; Enumerative Methods Employed. (Preliminary Note.)

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These experiments were conducted with funds given by Sir Edwin Durning-Lawrence, Bart., for the purpose of testing the effect of cold on disease, and were suggested by Major R. Ross as a part of the studies made in connection with a case of Sleeping Sickness reported upon in an accompanying paper by him and Dr. David Thomson. Although many researches have been made on the effect of atoxyl and other drugs, we believe that these are the first in which that effect has been measured by regular daily counts of the parasites by measured thick film methods.

I. Atoxyl.

From a study of the patient W. A. at the Royal Southern Hospital by Major R. Ross and D. Thomson, it will be noted that atoxyl failed to be of any marked benefit to the patient, and in the doses administered there was no noticeable trypanocidal action. Various doses of atoxyl are recommended in treatment of human trypanosomiasis, and the patient (W. A.) received 4 grains as a maximum dose. It was impossible in this case to push the drug further, as the patient quickly showed signs of the toxic action of the drug.

Louis Martin and Henri Darré* believe that atoxyl can cure light forms

of the disease, but that it is often necessary to give large doses, namely, 1 grammé, which imperil vision. These authors, however, still admit that permanent cure is a doubtful matter, and Sir David Bruce says that so far as we know the death-rate from Sleeping Sickness seems to be 100 per cent.

In the experiments made by us we subinoculated rats with the Rhodesian strain of trypanosomes from the patient W. A. at the Royal Southern Hospital, in order that we might determine the effect of atoxyl on this particular form of trypanosomiasis, which, as will be seen from the accompanying paper on enumerative methods in untreated animals (Fantham and J. G. Thomson),* was of great virulence.

Eight rats were subinoculated with the Rhodesian strain from the patient (W. A.) at the Royal Southern Hospital. Four were treated with small doses of atoxyl, three were treated with large doses, and one was untreated.

(1) Small Doses of Atoxyl.—The weight of four rats treated with small doses varied from 139 grammés to 256 grammés. Take the body weight of a man as being 70 kilogrammes, and we find that the doses we administered to these rats would be represented in man by doses varying between 4·55 and 25·14 grains.

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<tbody>
<tr>
<td>34</td>
<td>Piebald</td>
<td>139</td>
<td>27</td>
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<td>35</td>
<td></td>
<td>145</td>
<td>26</td>
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<td>36</td>
<td>White</td>
<td>251</td>
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<td>37</td>
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<td>256</td>
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Conclusions.

Small repeated doses of atoxyl in this strain, i.e. the Rhodesian strain, prolong the life of the host, probably because of the tonic action of the atoxyl. Two of the above rats treated with atoxyl lived 27 days, and one lived 26 days. The last only lived 8 days, but, unfortunately, this animal contracted pneumonia. When we compare this with the life of the untreated animals inoculated with this strain we find that in 22 rats inoculated, the longest life recorded is 18 days, and the average life of the 22 controls was 11·3 days (Fantham and J. G. Thomson). Atoxyl, therefore, in small doses, undoubtedly prolonged the lives of Rats 34, 35, and 36.

Small repeated doses of atoxyl in this strain of trypanosomes actually stimulate division of the parasites. This conclusion is arrived at by the fact that in Rats 35 and 36 the number of parasites increased with great rapidity in the peripheral blood, and examination of the smears showed numerous trypanosomes in process of division. Many were found dividing into four.

It is interesting to note that the animal which died of pneumonia was living in the animal house. Rats 35 and 36 were treated in the cold chamber. As an explanation of the above, namely, the prolonged life of the animal and the rapid multiplication of the trypanosomes, we would venture to suggest the well-known tonic action of arsenic when given to man in small therapeutic doses. In small repeated doses arsenic in man, both in health and disease, increases the strength, weight, and appetite, and we would argue that small doses of atoxyl in rats raised the natural body resistance.

In Rat 34, which lived 27 days, treated with small doses of atoxyl, we find that the period between the crests of the waves in a graph representing the numbers of trypanosomes was certainly larger than in those of the untreated animals of this same strain. Here two high crests occurred in the graph corresponding to intervals of 5 days and 10 days respectively.

This may probably be explained by the action of the atoxyl in small repeated doses raising the resistance of the host. The doses given to this animal would represent in man approximately 8.38 grains, 16.76 grains, and 25.14 grains.

We find that Gies* treated young rabbits with arsenic and found that those treated weighed more, had larger bones and more developed muscles than the untreated controls. These facts would explain the prolonged life of the animal, and we could at the same time attribute the increased activity in division of the trypanosomes to the small doses of atoxyl acting as a tonic to the trypanosome cells.

(2) Large Doses of Atoxyl.—We now treated three rats with large doses of atoxyl. In this we were guided by the excellent work of Dr. Wolferstan Thomas in his investigations on the action of drugs in trypanosomiasis. We gave 0.5 c.c. of a 5-per-cent. solution of atoxyl subcutaneously, and we caused the trypanosomes to disappear from the blood of one rat in 24 hours. In this rat the weight was 202 grammes, and if we calculate this dose to the body weight of a man, it would be necessary to give over 130 grains of atoxyl to a patient weighing 70 kilogrammes.

* Cushny's 'Pharmacology.'
The trypanosomes at time of injection of the atoxyl were 21,736 per cubic millimetre of peripheral blood, and they all disappeared and were absent from the peripheral circulation for four days, when they suddenly reappeared (1600 parasites per cubic millimetre of blood).

This animal received a second injection of 0·5 c.c. of a 5-per-cent. solution of atoxyl, and the trypanosomes fell from 98,000 per cubic millimetre of peripheral blood to 89 per cubic millimetre. This animal lived 51 days.

In another rat weighing 129 grammes the trypanosomes also disappeared within 24 hours, but the animal evidently succumbed to the toxic action of the drug. The same dose was given. The third rat, weighing 113 grammes, succumbed to the toxic action of the drug. The control, weighing 238 grammes (untreated), lived 18 days. In large doses, therefore, atoxyl clears the trypanosomes from the peripheral blood, at least for a time. This is exactly in line with the discovery of Thomas and Breinl. These observers advocated the use of a large dose if treatment was to be successful.

Large doses of atoxyl are trypanocidal, but probably do not cure the animal, owing to the fact that atoxyl resistant forms round up in the spleen and bone marrow (Moore and Breinl).

Lastly we would like to state that in our opinion atoxyl is not a specific in this disease, but would appear to be as toxic to the body cells as to the trypanosomes themselves, and thus it is necessary to approach as near a lethal dose as possible if a permanent cure is to be expected. Small doses as given to man at present are in our opinion not trypanocidal, but might possibly prolong the life of the patient as it did in our rats.

II. Vaccine Treatment.

Previous work with vaccines and serums has given very indefinite results, and it seems that in animals little if any immunity is conferred by a previous attack of the disease (in *T. gambiense*).

We obtained a vaccine by inoculation of a rat with the Rhodesian strain. The disease was allowed to develop, and when the parasites were numerous in the peripheral blood the animal was killed.

The surface of the right auricle of the heart was seared with a hot needle in order to get a sterile portion, and through this the blood was drawn into a sterile pipette and mixed with normal saline. This mixture was placed for half-an-hour in an incubator at 55° C., and finally a little trikresol was added. A count was made of the parasites in 1 cubic millimetre before they were subjected to heat, and appropriate dilution was carried out with normal sterile salt solutions.

We now inoculated six rats with Rhodesian strain of trypanosomes, and
incubation was allowed to take place. Three rats were treated with vaccine, and three were kept as controls.

**Treated with Vaccine.**

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<th>Rat</th>
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<tr>
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<td>21</td>
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<td>43</td>
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**Controls Untreated.**

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<th>Rat</th>
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<tr>
<td>14</td>
<td>12</td>
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<td>15</td>
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Here an interesting point can be noted. It will be seen that Rat 42 treated with vaccine lived 21 days, whereas Rats 43 and 44, also treated with vaccine, only lived 9 and 11 days respectively.

We would suggest that this is explained by the fact that the vaccine in the case of Rat 42 was administered in two doses of 10,000,000, with an interval of one day between the doses, whereas Rats 43 and 44 received two doses each of 12,000,000, no interval of a day being allowed.

In the case of Rat 42, we have the life prolonged in a remarkable manner. The longest life of our three controls was 12 days, and the average life of 22 untreated animals, infected with the same strain, was 11.3 days, where the longest life recorded was 18 days (Fantham and Thomson).

When a vaccine is administered during a natural rise of trypanosomes we have on the following day a rise in the number of trypanosomes in the peripheral blood, and this represents a negative phase. A second dose of vaccine ought therefore not to be administered at this period, as was done in Rats 43 and 44, or more harm than good may be accomplished. On the other hand, if we allow a day to intervene, as was done in Rat 42, we give the vaccine at a favourable period, the negative phase having passed off.

We admit, however, that much more extensive experiments will require to be undertaken, and would suggest that failure in vaccine treatment in trypanosomiasis until the present time has been caused by the vaccine being administered at the wrong time. Probably the best time to administer a vaccine is when the trypanosomes are low in numbers in the peripheral blood, and a second dose ought not to be administered until the negative phase has passed off (see R. Ross and D. Thomson).*

We have said that the trypanosomes increased in numbers in the peripheral blood after a subcutaneous injection of vaccine. This is in all probability to be explained by the hypothesis of Dr. H. C. Ross, who holds that the extracts of dead tissues stimulate cell division.†

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III. Treatment by Means of Cold.

Here most interesting results have been obtained. Sir Edwin Durning-Lawrence, Bart., has taken great interest in the effect of cold in disease, especially tropical disease, and owing to his assistance these experiments have been made possible.

A chamber 12 feet long by 7 feet wide by 6½ feet high was constructed, and this is cooled by a refrigerator made by Sir Alfred Haslam. The lowest temperature reached in our experiments was 20° F. The humidity of the atmosphere in this chamber was low and varied between 50 per cent. and 60 per cent., but this varied with the humidity of the outside atmosphere, as the door had to be opened several times daily for the purposes of observation.

Preliminary experiments were carried out in this cold chamber by Major Williams, and his results have been published in ‘Annals of Tropical Medicine,’ Liverpool, July, 1910.

Major Williams compares the atmosphere of the cold chamber to the climate of the interior of Canada. The patient, W.A., suffering from Sleeping Sickness contracted in Rhodesia, several times visited the cold chamber for treatment; but, unfortunately, at this time no counts were made of the parasites in the peripheral blood. We have, however, the evidence of the patient himself, who emphatically declared that he felt much better after being for some time in the cold. As the patient became worse, treatment had to be discontinued, owing to the fact that the patient was considered too ill to travel from the Hospital to the University; and we had to resort to treatment of subinoculated animals.

We used two strains of trypanosomes:—

(1) The Rhodesian strain (T. rhodesiense, Stephens and Fantham).

(2) The old laboratory strain of T. gambiense.

The essential difference between these two strains has been discussed in an accompanying paper on enumerative methods in untreated animals by H. B. Fantham and J. G. Thomson.

Five guinea-pigs were kept in the animal house, and four were kept in the cold chamber. Of these seven were subinoculated with the old laboratory strain of T. gambiense, and two were inoculated with the Rhodesian strain. The average incubation period of the controls in the animal house was four days, whereas the average incubation period of those treated in the cold was 13·5 days.

The average duration of life of the controls was 64·2 days, whereas the average life of those in the cold was 97·25 days. In addition to this the
animals in the cold were livelier and took their food better than those in the animal house. We conclude that in our experiments the guinea-pigs had the incubation period delayed, and the life of the animal was prolonged in the cold.

We now experimented with rats. Again we used both strains of trypanosomes. Five rats were inoculated with the old laboratory strain of *T. gambiense*, three were treated in the animal house, and two were treated in the cold. Three rats were inoculated with Rhodesian strain. Two were treated in the animal house and one was treated in the cold. Here we find the average incubation period in the cold was 6·3 days, and the incubation in the animal house was 4·8 days. The average life in the cold was 16·3 days and the average in the animal house was 14·2 days. Daily enumerations were carried out in all the above animals. The conclusions to be drawn from the above are therefore in favour of treatment in the cold. The resistance of the animal body is evidently raised, for the incubation is delayed and life is prolonged.

In favour of the cold we have also the evidence of the patient W. A., who said he felt better in the cold chamber, and, lastly, all visitors to the cold chamber testified as to its bracing effect. What the physiological action of the cold is we are not prepared to state at present; but we certainly think that animals treated were beneficially affected, and that they were livelier. We think the cold dry atmosphere, which has been compared to the climate of Canada or Switzerland, raises the vitality of the whole animal organism and thus acted as a valuable therapeutic agent in treatment of Sleeping Sickness.

We did not cure the disease, but we were enabled to prolong life, and so we must regard the treatment as a valuable help in patients suffering from trypanosomiasis. Adding these results in animals to those previously reported by Major Williams, the *combined series of animals* experimented upon show very similar results.

IV. Treatment with X-rays.

A young piebald rat, weighing 77 grammes, was inoculated with the Rhodesian strain of trypanosomes. Here we have to thank Dr. Morgan, in charge of the Electrical Department at the Royal Southern Hospital, who kindly advised us in the administration of the rays. The exposures were given by Miss Wells, an experienced worker in electrical treatment, and we have to thank her for her kind assistance.

The rat lived 15 days, and we can therefore say that this animal’s life was
Treatment of Animals infected with Trypanosomes.

prolonged when compared with the average life of 22 controls, the average life of which was only 11.3 days.

In spite of five exposures, each of 20 minutes' duration, during which period the whole body of the rat was exposed to the direct action of the rays, the trypanosomes remained lively and increased steadily in numbers in the peripheral circulation. The rays were not therefore trypanocidal in the exposures given by us, but, curiously enough, the life of the animal was prolonged. The animal always seemed livelier during the exposure to the rays, and suffered no discomfort. There was no destruction of the trypanosomes, and this is comparable to the experiments made by one of us (R. R.) several years ago, who found that exposure of trypanosomes in vitro to the action of X-rays had no trypanocidal effect.

V. Leucocytic Extract.

This experiment was carried out by the suggestion of Dr. Moore Alexander, Pathologist to the Royal Southern Hospital, and he kindly advised us and supplied the leucocytic extract. A white rat, weighing 120 grains, was inoculated with the Rhodesian strain, and the disease was allowed to incubate. On the 12th day the trypanosomes numbered 89,000 per cubic millimetre, and we injected 0.5 c.c. of leucocytic extract. The leucocytes rose from 8,160 per cubic millimetre to 10,760 per cubic millimetre, and the trypanosomes rose from 89,000 to 220,800. The animal lived 14 days. We cannot, therefore, draw conclusions here, and much further work will require to be undertaken, before we conclude as to the value of leucocytic extract in trypanosomiasis.