THERAPEUTIC ACTION OF N-PHENYLGLYCINEAMIDE-\(p\)-ARSONIC ACID (TRYPARSAMIDE) UPON EXPERIMENTAL INFECTIONS OF TRYPANOSOMA RHODESIENSE.

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In a series of papers published in November, 1919 (1), we reported results which had been obtained from the treatment of various forms of experimental trypanosomiasis with \(N\)-phenylglycineamide-\(p\)-arsonic acid, or tryparsamide. The results were such as to indicate that this drug might prove of value in the treatment of the form of human trypanosomiasis which is due to infection with \(T.\ gambiense\). At the time this work was done, \(T.\ rhodesiense\) was not obtainable for experimental study, but as the therapy of the infections caused by this organism is becoming of increasing importance, it was deemed advisable to determine the effects which might be hoped for from the use of this drug in the treatment of Rhodesian sleeping sickness, and something of the method of treatment which might be employed in these cases.

Accordingly, a strain of \(T.\ rhodesiense\) was obtained and a series of experiments carried out upon various forms of the animal infection including that in mice, rats, and rabbits.

\(T.\ rhodesiense\) usually exhibits a higher degree of virulence for laboratory animals than \(T.\ gambiense\). The organism used in these experiments, however, was distinctly less virulent for all the animals used, except the rabbit, than the strains of \(gambiense\) with which we had worked. After serial passage through mice and rats, it developed a fair degree of virulence for both species of animals and was highly virulent for rabbits, but possessed very slight virulence for guinea pigs. When inoculated into mice and rats according to the procedure described in a previous paper (2), the incubation period of the infection
was from 2 to 3 days in the case of mice and 3 to 5 days in rats. Infected mice died in from 10 to 12 days, while rats survived a few days longer. Rabbits were also readily infected; well marked symptomatic manifestations of disease developed in less than a week after intravenous inoculation, and the infection terminated fatally within 2 to 4 weeks.

Attempts to transmit the infection to guinea pigs were at first unsuccessful, but eventually a low grade infection with a prolonged incubation period and a low mortality was produced and serial passages were maintained through several generations of transfers.

EXPERIMENTAL.

The general plan of the therapeutic experiments carried out was the same as that employed in our previous work; that is, the trypanocidal action of the drug was studied by the use of the simpler bloodstream infections of mice and rats, and the results thus obtained were applied to the treatment of the more complex disease as it appears in the rabbit. Mice and rats were inoculated intraperitoneally and treated 24 hours later by the intraperitoneal administration of a single dose of the drug. Repeated blood examinations were made to determine the effects upon the infecting organisms. Mice were kept under observation for 1 month after treatment and rats for 2 months, as a means of determining the probable results of treatment.

Rabbits were inoculated intravenously and treated by the same route when well marked symptoms of disease had developed; the period of observation was 3 months, although most of the animals in this series were held as long as 4 months.

Therapeutic Effects.

Mice.—Several series of mice were treated with doses of from 0.15 to 1.75 gm. per kilo of body weight. Doses below 0.25 gm. per kilo were found to exercise very little if any effect upon the general course of the infection and those of from 0.25 to 0.5 gm. merely served to delay its progress. No cures were obtained until the dose of drug given reached 0.75 to 1 gm. per kilo. As an example of the therapeutic effects obtained, the detailed results of one of the experiments are given in Table I.
TABLE I.
Results from the Treatment of a 24 Hour Infection of Tr. rhodesiense in Mice.*

<table>
<thead>
<tr>
<th>Dose per kilo.</th>
<th>No. of mice</th>
<th>No. of relapses</th>
<th>No. of probable cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
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<td></td>
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</tr>
<tr>
<td>1.75</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
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<tr>
<td>1.25</td>
<td>2</td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>2</td>
<td>1</td>
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</tr>
</tbody>
</table>

* Controls survived 11 days.

Rats.—It was found that the rat infection of Tr. rhodesiense was also difficult to influence with this drug. The results obtained from experiments carried out under the same conditions as with mice may be illustrated by the experiment given in Table II.

TABLE II.
Results from the Treatment of a 24 Hour Infection of Tr. rhodesiense in Rats.

<table>
<thead>
<tr>
<th>Dose per kilo.</th>
<th>No. of rats</th>
<th>No. of relapses</th>
<th>No. of probable cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>3</td>
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<tr>
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</tr>
<tr>
<td>0.35</td>
<td>3</td>
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<td>0</td>
</tr>
</tbody>
</table>

While smaller unit doses of the drug were capable of curing the infection produced in rats than in mice, when considered from the standpoint of the ratio of the curative to the lethal dose, the figures obtained in the two instances were in very close agreement, with a slightly better ratio for the mouse. Thus it was found necessary to administer upwards of two-thirds of the maximum dose in order to effect a complete sterilization in the case of the mouse, and while cures might be obtained with only two-thirds of the maximum dose in rats, the full dose was necessary to assure such a result.

These figures were quite different from those which had been obtained with Tr. gambiense under similar conditions. Comparable results were obtained in mouse and rat infections of this organism.
in doses as small as one-ninth and one-fifth respectively of the maximum dose for the two animal species. It appeared, therefore, that greater difficulty might be anticipated in the treatment of the more severe chronic infection of rabbits.

Rabbits.—In tests of the therapeutic effects against rhodesiense infection in rabbits, the animals used were inoculated intravenously with 1 cc. per kilo of body weight of a + to ++ suspension of trypanosomes prepared from rat blood. This produced a very intense infection, symptoms appearing in individual animals within 3 to 5 days after inoculation, and the majority of the animals showed well marked manifestations of disease by the end of the 1st week. The controls of the series survived for only 2 weeks. Three types of experiments were undertaken in this series: (1) the use of large single doses, (2) the use of repeated smaller doses, and (3) an intensive treatment of relapses.

Single Doses.—In view of the marked resistance exhibited by Tr. rhodesiense in mice and rats, the single dose treatment of rabbits was placed at the highest possible level consistent with safety. A small series of rabbits was treated, with the results recorded in Table III.

Repeated Doses.—The attempt was also made to determine whether any form of repeated dose therapy based upon the use of smaller doses than those described above might prove efficacious in this class of infection. Two rabbits were given doses of 0.4 gm. per kilo at intervals of 3 days. One of these received six doses, and after a rest
period of 1 week, the treatment was resumed with nine doses at intervals of 2 to 3 days. The animal thus received a total dose of 6.0 gm. of the drug per kilo of body weight within a period of 40 days. With the second animal the number of doses was reduced to six, or a total amount of 2.4 gm. of the drug per kilo was given within 15 days. Both these animals showed rapid improvement and remained in excellent condition throughout the course of treatment. Signs of the disease disappeared completely and there was no recurrence within the period of observation. They may, therefore, be regarded as probable cures.

The same procedure was followed with three other rabbits with a still smaller unit dose of the drug—0.3 gm. per kilo. One animal received six doses of the drug at intervals of 3 days, or a total of 1.8 gm. in 15 days; the second received five doses followed by a rest period of 1 week and then a second series of ten doses, or a total of 4.5 gm. was given in 43 days; the third rabbit was given fifteen consecutive doses at regular intervals, the 1 week interim between series being omitted.

The first of these animals did well under the treatment; symptoms of disease disappeared promptly and there was no recurrence. The initial effects were much the same in the second animal, but although the course of treatment was much more prolonged, it proved ineffectual. Within 4 days after administration of the drug had been stopped, signs of the disease reappeared, and the animal died from trypanosomiasis 15 days later. With the third rabbit, which was given the same amount of drug in consecutive doses, a cure was effected.

This small group of cases furnishes an excellent illustration of the variability of individual results which may be obtained in the treatment of infections of this type and possibly also the effects which may be produced by apparently minor variations in the course of treatment.

Relapses.—In view of the great difficulty experienced in influencing rhodesiense infections, little was to be expected from an attempt to treat these infections once they had relapsed. It was considered worth while determining, however, whether it was possible to obtain any effect from the use of the drug in this class of cases. Three experiments of this kind were carried out.
The first case treated was a rabbit which had relapsed from a single dose of 0.75 gm. per kilo. On the 3rd day after the symptoms of the disease reappeared, treatment was begun with 0.5 gm. per kilo, and nine doses of the drug were administered at intervals of 2 to 3 days, or 4.5 gm. per kilo in 21 days. This was considered to be as intensive treatment as could be undertaken and in this instance apparently effected a cure. The animal when inoculated weighed 1,625 gm.; when retreated after relapse, 1,600 gm.; when treatment was discontinued, 1,875 gm.; when discarded, 1,900 gm. This experiment furnishes an excellent example of the remarkable tolerance exhibited by animals towards the drug and the improvement in general condition which usually follows its administration.

A second animal which had relapsed from a single dose of 0.6 gm. was given five doses of 0.5 gm. and three doses of 0.6 gm. in 18 days, but the treatment was ineffectual and relapse occurred 1 week after it was discontinued.

In a third rabbit, the effect of the treatment was uncertain. This was likewise a case of relapse from a single dose of 0.6 gm. per kilo. The animal was in poor condition when relapse occurred and retreatment was instituted. Two doses of 0.5 gm. per kilo were given with 48 hours intervening. The signs of trypanosomiasis were but slightly improved, and the animal was weaker, so the dose of drug was reduced to 0.3 gm. and two doses of this size were administered. Death occurred 3 days later without complete disappearance of the manifestations of trypanosomiasis.

By comparing these results with those previously reported from the treatment of rabbits infected with Tr. gambiense, it is at once apparent that the rhodesiense infections were much more difficult to influence. Results comparable with those obtained with single doses of 0.6 to 0.75 gm. per kilo could be obtained in gambiense infections with approximately one-third of this amount of drug, and while cures were uniformly obtainable in gambiense infections with from 0.3 to 0.35 gm. per kilo, there appeared to be no single dose of the drug with which this could be accomplished with safety in cases of rhodesiense infection.
CONCLUSIONS.

With the three classes of animal infections studied, the trypanocidal action of tryparsamide was found to be much less for *Tr. rhodesiense* than for *Tr. gambiense*, and a correspondingly greater difficulty was experienced in the treatment of the chronic tissue infections of *Tr. rhodesiense*.

This is, of course, entirely in accord with past experience in the treatment of human cases of trypanosomiasis due to the two organisms. It is still possible that something may be accomplished by the use of tryparsamide in cases of Rhodesian sleeping sickness on account of the tolerance exhibited to the drug and the possibility of employing an intensive system of treatment. Much less is to be expected, however, than in cases of infection due to *Tr. gambiense*.

SUMMARY.

A series of experiments was carried out to determine the effects of tryparsamide upon the infections produced in various species of animals by *Tr. rhodesiense*.

The strain of trypanosome used was one which possessed a very low virulence for guinea pigs, was fairly virulent for mice and rats, and highly virulent for rabbits.

Therapeutic experiments carried out on 24 hour infections in mice and rats showed that cures could be obtained in this class of infections by the administration of a single large dose of the drug amounting to approximately two-thirds of the maximum tolerable dose, as contrasted with similar results in cases of *gambiense* infections from approximately one-ninth and one-fifth of this dose respectively.

With advanced infections in rabbits, there appeared to be no single dose of the drug capable of insuring a cure which could be administered with safety, although some cures were obtainable with doses approximating the maximum tolerable dose. Treatment of these infections could be carried to a successful conclusion, however, by an intensive system of treatment in which large doses of the drug were administered at short intervals of time, and even relapses yielded to this treatment in some instances. The employment of such a method of treatment was possible on account of the unusual tolerance exhibited by animals to this drug, a fact which was previously emphasized.
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These facts indicate that the outlook for the drug in the therapy of Rhodesian sleeping sickness is much less hopeful than in gambiense infections, though it is felt that some benefits may be derived from its use.

BIBLIOGRAPHY.