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THE ACTION OF SOME TRYPANOCIDAL AND ANTIMALARIAL COMPOUNDS ON *BABESIA RODHAINI (PIROPLASMIDEA)*

BY

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This paper describes an investigation into the effects of a series of well-known trypanocidal and antimalarial compounds on the course of infection with the piroplasm *Babesia rodhaini* in white mice.

B. rodhaini was isolated from the blood of the rodent *Thamnomys surdaster surdaster* by van den Berghe, Vincke, Chardome, and van den Bulke (1950). The organism was transmitted to white mice and is now maintained in these animals. *B. rodhaini* promised to be a useful organism for the screening of drugs designed for the treatment of piroplasmosis; the results of some investigations in this field have already been published (Rodhain, 1951; Beveridge, 1953).

During the course of other work with *B. rodhaini* in this laboratory, it was discovered that infections could be cured with the trypanocidal compounds antrycide and 1-methyl-l-phenyldithiobiuret (Godfrey, 1955), whereas the antimalarial drugs mepacrine and proguanil were without effect. This result is surprising when it is considered that the *Piroplasmidea* are much more nearly related to the *Haemosporidiidea* than they are to the *Trypanosomidae*. Accordingly, it was decided to investigate the action upon *B. rodhaini* of a series of compounds which were known to have either trypanocidal or plasmodicidal properties.

METHODS

The strain of *B. rodhaini* used was the Antwerp strain, obtained through the courtesy of Miss E. Beveridge, of the Wellcome Laboratories of Tropical Medicine. The method of testing the drugs against *B. rodhaini* was adapted from the standard screening procedure used in our laboratories. White mice were inoculated intraperitoneally with three million parasitized erythrocytes contained in 0.2 ml. of citrated saline, and were then treated daily with the drug for four days, the first dose being given intraperitoneally four hours after infection. Thin blood films were made on the sixth day, stained with Giemsa, and the percentages of parasitized erythrocytes were recorded. The control animals generally showed a parasitaemia of 70–80% at this time and they usually died from the infection on or about the eighth day. The blood of mice which survived for eight days or longer was examined at weekly intervals for a further four weeks in order to follow the course of infection.

The drugs used were (*a*) antimalarial compounds: chloroquine diphosphate; mepacrine hydrochloride; pamaquin naphthoate; proguanil acetate; pyrimethamine; (*b*) trypanocidal compounds: antrycide methyl sulphate; "Berenil," di-(4-amidinophenyl)-triazene-(*N*-1:3) diaceturate-3H_2O (Milne, Robson, and Lwebandiza, 1955; Bauer, 1955; Enigk and Reusse, 1955); B314, 2-*p*-acetaminostyryl-6-methylaminoquinoline methosulphate (Browning, Cohen, Ellingworth, and Galbransen, 1929); "Congasin," bis-2-methyl-4-aminoquinolyl-6-melamine; ethidium bromide; RD1660, 1-methyl-1-phenyl dithiobiuret (Woolfe, 1953); the antibiotic "Stylomycin" (Porter *et al.*, 1952); suramin; tartar emetic; tryparsamide. All drugs were dissolved in sterile saline or sterile distilled water. The chronic toxicity of each drug was estimated by intraperitoneal injection into mice daily for four days. The maximum tolerated dose (M.T.D.) was taken as being the lower of two doses, one twice the other, of which the upper killed but the lower did not. Two mice were treated at each dose level. Six mice infected with *B. rodhaini* were treated with each drug at its M.T.D. When a drug was active, further batches of mice were treated at fractions of this dose. The minimum effective dose was taken to be the dose which limited the mean parasitaemia in the treated mice to 5% of the mean parasitaemia in the controls on the sixth day after infection. A drug was considered to have cured the infection when no parasites were seen in blood films from treated animals taken once weekly for four weeks after treatment.

RESULTS

The results of the tests carried out on five antimalarial and ten trypanocidal compounds are

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summarized in Table I. The antimalarial compounds were all without action, but six of the ten trypanocidal compounds showed activity. Five compounds, antrycide methyl sulphate, ethidium bromide, RD1660, "Stylomycin," and "Berenil,"

TABLE I

THE ACTION OF SOME TRYPANOCIDAL AND ANTIMALARIAL COMPOUNDS ON *B. RODHAINI*

Compound	Type	Max. Tolerated Dose mg./kg. Daily×4	Min. Effective Dose mg./kg. Daily×4	Curative Action
Chloroquine ..	Antimalarial	50	>50	−
Mepacrine ..	,,	100	>100	−
Pamaquin ..	,,	50	>50	−
Proguanil ..	,,	10	>10	−
Pyrimethamine	,,	350	>350	−
Antrycide ..	Trypanocidal	10	3·5	+
B314	,,	50	50	−
Berenil	,,	50	5·0	+
Congasin ..	,,	75	>75	−
Ethidium bromide	,,	20	3·0	+
RD1660 ..	,,	500	40	+
Stylomycin ..	,,	150	90	+
Suramin ..	,,	100	>100	−
Tartar emetic ..	,,	10	>10	−
Tryparsamide ..	,,	2,500	>2,500	−

cured the infection in all mice which were treated at the maximum tolerated dose. At lower doses they considerably delayed the progress of the parasitaemia but did not prevent the infection from killing some of the mice. The sixth compound which showed activity, B314, delayed the parasitaemia, but some of the mice died even when treated at the maximum tolerated dose. The remaining trypanocidal compounds, suramin, "Congasin," tartar emetic, and tryparsamide, were all without effect.

DISCUSSION

These results show that six of the ten trypanocidal compounds which were tested affected the course of infections of *B. rodhaini* in white mice; five antimalarial compounds were all without action. In a previous investigation, Beveridge (1953) found that the aromatic diamidines, stilbamidine, phenamidine, and pentamidine and 2:8-diamine-10-methylacridinium, a constituent of acriflavine, were all active against *B. rodhaini*. Thus *B. rodhaini* seems to be susceptible to drugs which also act against trypanosomes—especially *T. congolense*—but it is resistant to antimalarial compounds. These findings are in accordance with the views expressed by Goodwin and Rollo (1955). Congasin is an exception in that it is very active against *T. congolense* but does not affect *B. rodhaini*.

The exact systematic position of the *Piroplasmidea* is still a matter of some controversy, but it is generally agreed that they are fairly closely related to the *Haemosporidiidea*. It is therefore surprising to find that from the viewpoint of chemotherapy the affinities of *B. rodhaini* are with trypanosomes rather than with malarial parasites. In this respect it is interesting to recall that several attempts have been made to relate the piroplasms to the haemoflagellates, notably that of Léger and Dubosq (1910).

The trypanocidal compounds which are active against *B. rodhaini* are of widely differing structure and it seems unlikely that all these compounds affect any single enzyme system common to both *B. rodhaini* and trypanosomes. It is more reasonable to suppose that there is a closer resemblance between the metabolic systems of the parasites than might be expected from their different morphology and habits.

The results of these investigations indicate that trypanocidal compounds may prove to be useful agents in the chemotherapy of other piroplasmoses. According to recent reports, the trypanocidal compound "Berenil" has been successfully used in the treatment of bovine, ovine, and canine piroplasmoses in the field (Bauer, 1955; Enigk and Reusse, 1955).

SUMMARY

1. The following trypanocidal compounds were able to cure white mice infected with *Babesia rodhaini*: antrycide methyl sulphate, ethidium bromide, "Berenil," the dithiobiuret compound RD1660, and the antibiotic "Stylomycin."

2. A styryl quinoline compound (B314) delayed the progress of the infection but did not cure all mice treated at the maximum tolerated dose.

3. Four other trypanocidal compounds, "Congasin," suramin, tartar emetic, and tryparsamide, were without effect.

4. Five antimalarial compounds, chloroquine diphosphate, mepacrine hydrochloride, pamaquin naphthoate, proguanil acetate, and pyrimethamine, were inactive.

5. In many respects the chemotherapeutic reactions of *B. rodhaini* resemble those of trypanosomes, especially *T. congolense*, and are unlike those of malarial parasites.

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