Three remarkable features make the study of this flagellate of great interest: its capacity to invade and multiply in the haemolymph and salivary glands of its chief vector *Rhodnius prolixus*; its pathogenicity to these invertebrate hosts in contrast to its harmlessness to man in whom it gives rise to an inapparent (occult) infection; and, finally, its doubtful taxonomic position, that is to say whether it should be placed in the Stercoraria or the Salivaria group (HOARE, 1972).

Previous observers have stated that the haemolymph is not invaded until several weeks after the infective meal (GROOT, 1954; GREWAL, 1957; D'ALESSANDRO, 1972). During the course of studies on a fresh strain of *T. rangeli* isolated by xenodiagnosis from infected dogs in Venezuela, the parasite has been seen in the haemolymph of *R. prolixus* as early as 24 hours after the insects had fed on mice with a parasitaemia of 600 trypomastigotes per mm^3.

15 fifth-instar nymphs of *R. prolixus* were fed on two mice which had been infected 48 hours previously by the bites of bugs with metatrypomastigotes in the salivary glands. A drop of haemolymph of each *Rhodnius* was examined daily from the first to the seventh day after infection. Two of 15 *Rhodnius* were found to have parasites in the haemolymph 24 hours later (i.e. first day), three more on the second day, another one on the third and another one on the fourth day. Five of the remaining eight bugs became infected later.

Two bugs died six days after infection; they showed numerous parasites in the haemolymph and a scanty number in the salivary glands. The other five infected bugs were still positive 12 days later.

Early dividing forms observed in the haemolymph resembled those seen by TOBIE (1970) after injection of culture forms of *T. rangeli* into the body-cavity of *R. prolixus*.

The examination of the faeces of the seven bugs with haemocoelomic infections failed to reveal parasites until 72 hours after the infective meal, although they were detectable in the haemolymph of the same bugs from 24 hours onwards. This is in contrast to the observations of GREWAL, 1856 and D'Alessandro, 1961 (see HOARE, 1972) who reported that the intestinal infection is usually well established by the time the flagellates begin to penetrate the gut wall and reach the body-cavity of *R. prolixus*.

These observations suggest that a primary characteristic of *T. rangeli* is its multiplication in the haemolymph rather than in the gut of its vectors, and support Hoare's opinion that this species is in process of adaptation to the anterior station.

I am, etc.,

N. ANEZ

Imperial College Field Station,
Ashurst Lodge, Ascot, Berks, UK

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Miconazole: an inhibitor of cyanide-insensitive respiration in *Trypanosoma brucei*

SIR—Aromatic hydroxamates are inhibitors of the glycerol-3-phosphate oxidase of *Trypanosoma brucei* bloodforms. By treating trypanosome-infected animals with one of these compounds—salicylhydroxamic acid (SHAM)—it was expected that the trypanosomes would be killed and the host cured. Salicylhydroxamic acid, however, has no effect on the course of parasitaemia in rats as the trypanosomes can survive under anaerobic conditions (OPPERDOES *et al.*, 1976a, 1976b). The action of SHAM can, however, be potentiated strongly by the simultaneous administration of glycerol (CLARKSON & BROHN, 1976; FAIRLAMB *et al.*, 1977), and such a combination leads to the complete cure of rodents infected with *T. vivax* (EVANS & HOLLAND, 1978) or *T. brucei* (van der Meer *et al.*, 1979). As the therapeutic levels of the SHAM plus glycerol combination are close to its LD_{50}, I decided to search for substitutes for SHAM and glycerol.

I found that miconazole nitrate (an imidazole derivative produced by Janssen Pharmaceutica), a compound in clinical use for the treatment of local *Candida* infections (SAWYER *et al.*, 1975), is an effective inhibitor of glycerol-3-phosphate oxidase in *T. brucei*. The respiration of intact trypanosomes was 50% inhibited at 10 μM and completely at 100 μM miconazole. Moreover, at the latter concentration the trypanosomes were completely immobilized and lysed within 30 min. This indicates that the mode of action of miconazole is different from that of SHAM, which upon complete inhibition only reduces motility (OPPERDOES *et al.*, 1976b).

According to LEVINE *et al.* (1975), levels of miconazole in mouse plasma of about 3 μg/ml (equivalent to 7 μM) could easily be achieved after i.m. injection. The effect of miconazole *in vivo* was

tested as follows. Mice were infected (i.p.) with 10^5 trypanosomes (*T. brucei* EATRO 427) and on the same day, or one day later a 44 mg/kg dose of miconazole (LD_{50} = 91 mg/kg) administered (i.p.). The drug was injected alone or in combination with glycerol (4·5 g/kg)—a curative dose when administered with SHAM (VAN DER MEER *et al.*, 1979). No significant effect of miconazole, alone, or in combination with glycerol, was observed on either the survival time of the infected mice or the motility of the trypanosomes. These results, in my opinion, indicate that a free plasma concentration of the drug, sufficient to suppress trypanosome respiration, cannot be reached in mice. Support for this conclusion comes from *in vitro* experiments. When increasing amounts of rat blood or bovine serum albumin were mixed with trypanosomes and respiration recorded in an oxygraph, increasing amounts of miconazole were required to obtain 50% inhibition of respiration; in 1:1-diluted blood a 100-fold increase of miconazole (up to 1 mM) was required.

It can be concluded that miconazole binds strongly to blood components so reducing its free plasma concentration, and this phenomenon renders miconazole unsuitable for the treatment of African trypanosomiasis.

I am, etc.,

FRED R. OPPERDOES

Research Unit for Tropical Diseases,
International Institute for Cellular and Molecular Pathology,
avenue Hippocrate, 74, B-1200 Brussels,
Belgium

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The use of enzymes in taxonomy of *Bulinus*—a reply

SIR—I refer to a recent letter (ROLLINSON, 1979) which criticized, unjustifiably, the description of *Bulinus browni* and *B. barthi* (JELNES, 1979).

The following facts should now be established:

(i) That Dr. Rollinson and his head of department were informed in some detail about the nature of the then unpublished isoenzyme data obtained on the subgenus *Bulinus* s.s. from East Africa.

(ii) The description of the two species was meant *only* as a taxonomic description and, as such, I take it to fulfil the requirements of the International Code of Zoological Nomenclature.

(iii) The description gives the best distinguishing (diagnostic) character for East African material of the species. As indicated (JELNES, 1979—discussion section), differences between the three species are found in enzymes other than alpha-glycerophosphate dehydrogenase, and a manuscript describing the full set of isoenzyme data obtained on the subgenus *Bulinus* was, at that time, in preparation.

(iv) That manuscript was submitted for publication in early June, 1979 (JELNES, 1980) and *all* the points raised by Rollinson are dealt with in detail therein.

A criticism might have been reasonable had my paper been fully understood. A recent paper (WRIGHT & ROLLINSON, 1979) does not, with respect to numbers of specimens surveyed, fulfil the requirements demanded of me.

I am, etc.,

J. E. JELNES

Dansk Bilharziose Laboratorium,
Jaegersborg Allé 1D,
DK 2920 Charlottenlund,
Denmark

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