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Effects of Hycanthone Against two Strains of *Schistosoma japonicum* in Mice

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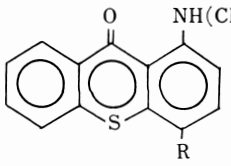
The chemotherapy of *Schistosoma japonicum* consists of a series of intravenous injections of tartar emetic or intramuscular injections of stibophen (Schmidt and Peter, 1938; Cheng, 1971). The length and difficulty of treatment with the antimonials and the production of undesirable side effects, in particular cardiotoxicity, have precluded extensive use of these drugs thereby limiting their utility to a relatively small number of the more than thirty-three million persons estimated to be infected with this schistosome (Wright, 1968).

The success of Miracil D (I) in treating experimentally-induced infections with *S. mansoni* (Kikuth and Gönnert, 1948) prompted its testing in monkeys, rabbits and hamsters against *S. japonicum* for which the drug proved to be inefficacious even at near toxic doses (Vogel and Minning, 1948). Poor results of a clinical evaluation confirmed the laboratory findings (Pesigan *et al.*, 1951).



I－R＝CH_3
II－R＝CH_2OH

In 1965 hycanthone (II), a 4-hydroxymethyl metabolite of Miracil D, was reported to be a more potent schistosomicide (*S. mansoni*) in hamsters than the latter compound (Rosi *et al.*, 1965). In addition, hycanthone was active by both oral and parenteral routes. Subsequent reports confirmed hycanthone's activity in mice, hamsters and Cebus monkeys (Berberian *et al.*, 1967; Pellegrino *et al.*, 1967; Khayyal *et al.*, 1969). On the basis of structural similarity, it was anticipated that hycanthone, like Miracil D, would be ineffective against *S. japonicum*; however, because of the greater efficacy of the newly isolated metabolite against *S. mansoni*, the testing of this compound against *S. japonicum* was indicated.

As discussed below an initial test (Experiment I) showed that hycanthone administered to mice either orally or intramuscularly was ineffective against *S. japonicum*. Successive evaluations (Experiments II and III) were aimed at determining whether varying the test procedures and dosage regimens of hycanthone would result in efficacy. Hycanthone was also co-administered with antimonials (Experiment IV) to determine whether enhancement of activity would be obtained. In none of these tests did hycanthone show activity against *S. japonicum*. In search of an explanation for the absence of schistosomicidal activity of hycanthone, a final experiment (V) was performed to determine the uptake and distribution of tritiated drug in the blood of the mouse host and *S. japonicum* worms.

Materials and Methods

The mesylate salt of hycanthone (HM), which readily dissolves in distilled water, was injected intramuscularly into the vastus lateralis muscle in a volume of 0.1 ml. For oral testing, the less soluble base (HB) was prepared as an aqueous suspension and given by stomach tube, in divided daily doses in 0.2 ml of diluent, eight hours apart

and continued for five consecutive days. All solutions were prepared fresh daily and dosages were calculated to base.

Female Swiss mice from Carworth Farms and weighing approximately 20 grams each were individually infected percutaneously with 25 cercariae of the Japanese or Philippine of strains *S. japonicum* in the laboratory of Drs. Henry van der Schalie and Elmer Berry at the University of Michigan. The infected mice were air shipped to us and held until the schistosomes had matured, as judged by examination of a few mice for the presence of mated pairs and ova deposited in visceral tissue. For Experiments I through IV, three or more weeks after initiation of medication, test animals were sacrificed by inhalation of chloroform vapor and the liver and intestines with their adhering mesenteric blood vessels were removed from the abdominal cavity. The liver was crushed between rectangular glass plates and examined under 30 × magnification for living and dead schistosomes. The intestines, cut into loops so that their mesenteric veins converged to the center of the loop, were similarly examined.

Experiment I.

Hycanthone (HM and HB) was administered at 50 and 100 mg/kg intramuscularly or orally to groups of 10 mice with a forty-day old infection of the Japanese strain. A group of nonmedicated, infected mice served as controls. Surviving animals were sacrificed and examined for schistosomes twentyone days after initiation of treatment.

Experiment II.

It was considered that the three week holding period between medication and autopsy might be too short to allow for therapeutic efficacy. Therefore, mice harboring mature forty-two day old infections of the Philippine strain were medicated with a single intramuscular injection of HM at 100 mg/kg. At periods of four, five and six weeks after medication four to five mice were necropsied and searched for worms as described earlier.

Experimeut III.

Hycanthone mesylate was given intramuscularly to four groups of mice with forty-two day infections of the Philippine strain. Each group, consisting of four to five animals, received 1, 2, 3 or 4 injections at 50 mg/kg at weekly intervals. Three weeks after the last medication the animals were sacrificed and the liver and intestinal veins examined for schistosomes.

Experiment IV.

Hycanthone at 50 mg/kg/day was co-administered for five consecutive days with subcurative doses of stibophen and tartar emetic to mice with a forty-eight day old infection of the Japanese strain to determine if the combination of drugs would increase the efficacy of the antimonials. Tartar emetic and stibophen were each given in a volume of 0.5 ml distilled water as single daily intraperitoneal injections for the five day medication period. Each drug was also given alone for reference purposes and a control group of mice received no medication.

Experiment V.

Forty mice infected forty-nine days previously with 25 cercariae of the Philippine strain of *S. japonicum* were given single 80 mg/kg intramuscular injections of tritiated HM with a specific activity of 194 mC/m mole. At 0.5, 1, 2, 4, 6, 8 and 24 hours after medication, three to seven mice were sacrificed by cervical dislocation, blood was pooled, collected in heparinized tubes and six to seven male and female worms were removed from the mesenteric veins for radioactivity determinations. An additional five to ten male and female worms were obtained for radiochromatographic analysis. The biological procedures as well as the measuremnts of radioactivity, quantitation of hycanthone and qualitative analysis by radiochromatography have been reported in detail (Yarinsky *et al.*, 1970).

For autoradiographic studies three pairs of worms in copula were removed from the mesenteric veins at 1, 4, 8 and 24 hours, washed in saline and immediately frozen in

a horizontal plane in a block of carboxymethyl cellulose gel. Cryostat sections 8 μ thick, made under safelight illumination, were placed on −20°C slides previously coated with Kodak NTB-2 nuclear track emulsion for autoradiographic localization of radioactive hycanthone within the *S. japonicum* worm. Exposures of the autoradiographs were carried out in light and vapor tight slide boxes at −20°C with silica gel drying agent for a period of three weeks. The slides were fixed in four percent formalin buffered to pH 7.4 prior to processing by the method outlined by Rogers (1967). Silver grain densities were examined in photomicrographs of hematoxylin-eosin stained *S. japonicum*.

Results

The results of the several experiments are given in Tables 1 through 4 and Figures 1 through 3.

Experiment I (*Table 1*). Following a single intramuscular injection of HM at 50 or 100 mg/kg an average number of 18.7 and 19.0 live worms, respectively, were found. Neither group harbored any dead worms. The five-day oral regimen of 50 or 100 mk/kg of HB, likewise, was not lethal to the worms; the mice harbored an average of 17.4 and 17.5 live schistosomes, respectively.

Experiment II (*Table 2*). No dead worms were observed at intervals of 4, 5 and 6 weeks following a single intramuscular injection of HM at 100 mg/kg. The average numbers of live schistosomes found at autopsy were 16.0, 12.0 and 10.3, resepctively.

Experiment III (*Table 3*). Hycanthone administered in a single intramuscular dose of 50 mg/kg at weekly intervals for four

Table 1 Effect of a single intramuscular injection of hycanthone mesylate and a five-day course of oral medication with hycanthone base against *S. japonicum* (Japanese strain) infections of mice.

Route of Medication	Medication Dose (mg/kg)	No. Mice at Necropsy / No. Mice given Medication	Avg. No. Worms/Mouse at Necropsy	
			Live	Dead
Intramuscular	100	8/10	19.0	0
	50	10/10	18.7	0
Oral	100	6/10	17.5	0
	50	10/10	17.4	0
Nonmedicated Controls	—	9/10	13.2	0

Table 2 Effect of a single intramuscular injection at 100 mg/kg of the mesylate salt of hycanthone against *S. japonicum* (Philippine strain). Host mice autopsied 4 to 6 weeks after medication.

Autopsy Period (weeks) Post-treatment	No. Mice	Avg. No. Worms/Mouse		Percent Dead Worms
		Live	Dead	
Medicated Animals				
4	5	16.0	0	0
5	5	12.0	0	0
6	4	10.3	0	0
Nonmedicated Control Animals				
4	5	16.0	0	0
5	5	13.8	0	0
6	5	14.0	0	0

Table 3 Effect of 1 to 4 weekly spaced intramuscular injections at 50 mg/kg of the mesylate salt of hycanthone against *S. japonicum* (Philippine strain) in mice.

No. Medications*	No. Mice	Avg. No. Worms/Mouse		Percent Dead Worms
		Live	Dead	
1	5	13.0	0	0
2	5	14.4	0	0
3	4	12.0	0	0
4	5	12.8	0	0

* Mice necropsied 3 weeks after last medication. In nonmedicated groups of infected mice carried as controls no hepatic shift of worms occurred and no dead worms were found.

Table 4 Effect of antimonial schistosomicides given in conjunction with hycanthone mesylate against *S. japonicum* (Japanese strain) in mice.

Drug*	No. Mice	Medication Dose (mg/kg)	Route of Medication	Percent Dead Worms
Stibophen	4	100 (13.6)**	IP	10.1
Stibophen	9	200 (27.2)	IP	25.0
Tartar Emetic	10	30 (11.2)	IP	51.0
Tartar Emetic	Toxic	60 (22.4)	IP	—
Hycanthone + Stibophen	6	50 100 (13.6)	IM IP	5.8
Hycanthone + Stibophen	6	50 200 (27.2)	IM IP	9.0
Hycanthone + Tartar Emetic	5	50 30 (11.2)	IM IP	37.7
Hycanthone + Tartar Emetic	Toxic	50 60 (22.4)	IM IP	—
Hycanthone	5	50	IM	0
Non medicated Controls	5	0	—	0

* Drugs were administered as single daily injections for 5 consecutive days.

** Figures in parentheses give the drug dose expressed in terms of antimony content (mg/kg).

consecutive weeks failed to kill any schistosomes. Mice given one dose of the drug at forty-two days post-infection had an average of 13.0 worms; after two doses at 42 and 49 days post-infection, 14.4 worms were found; after three doses at 42, 49 and 56 days, an average of 12.0 worms were observed and the animals receiving four doses of drug at 42, 49, 56 and 63 days post-infection, harbored an average of 12.8 worms.

Experiment IV (Table 4). Stibophen administered intraperitoneally alone once daily for five days at 100 mg/kg/day killed 10.1 percent of the schistosomes and at 200 mg/kg/day the drug killed 25 percent of the worms. When similar doses of the antimonial were given in conjunction with intramuscular injections of HM at 50 mg/kg/day once daily for five days, 5.8 percent and 9.0 percent of the worms were killed.

Intraperitoneal injections of tartar emetic at 60 mg/kg/day for five days proved to be lethal. Likewise, the group of mice that were given similar dosages of tartar emetic in conjunction with a five-day course of intramuscular injections of hycanthone at 50 mg/kg also died. Tartar emetic at 30 mg/kg/day for five days killed 51.0 percent of the schistosomes and when the antimonial was given in conjunction with hycanthone only 37.7 percent of the worms were killed.

Experiment V (*Figures 1-3*). The data shown in Figure 1 indicate that following a single intramuscular injection of 80 mg/kg HM the concentration of drug per gram of wet worm tissue at each of the time periods is greater than that per milliliter of blood of the mouse host. It can also be seen at each of the time periods that the female worms accumulated higher concentrations of hycanthone than male worms; peak concentration in the former was noted at two hours post-medication at which time it was approximately five times that in the males. That the radioactivity in both the male and female worm could be accounted for as unchanged hycanthone is shown in Figure 2 which is representative of the radiochromatographic results obtained at the various examination times during the twenty-four hour post-medication period. Thus it is evident that minimal, if any, biotransformation of hycanthone by the worms occurred.

Autoradiography revealed that at one hour post-medication tritiated hycanthone was present in the intestinal tract and musculature surrounding the intestine of the male and female worms. Rovdioactive drug was present in the eggs of the female at the four hour time interval, but there was no apparent preferential uptake compared with surrounding tissue; tritiated drug appeared to be fairly uniformly distributed throughout the worms. Uniform distribution was also observed at the eight and twenty-four hour examination times, the only difference seen was decreased silver grain density at the later time periods which correlated with a reduction in the level of the drug in the worms as shown in Figure 1.

Further information about the distribution of hycanthone in the worms was obtained in a supplementary experiment in which a comparison was made of adult *S. japonicum* and *S. mansoni* worms. The method employed was the same as in the previous experiment

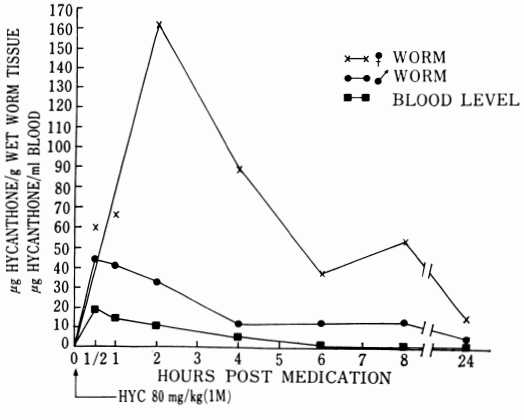


Fig. 1 Hycanthone uptake and retention by *S. japonicum* related to changing concentration in blood of host mice.

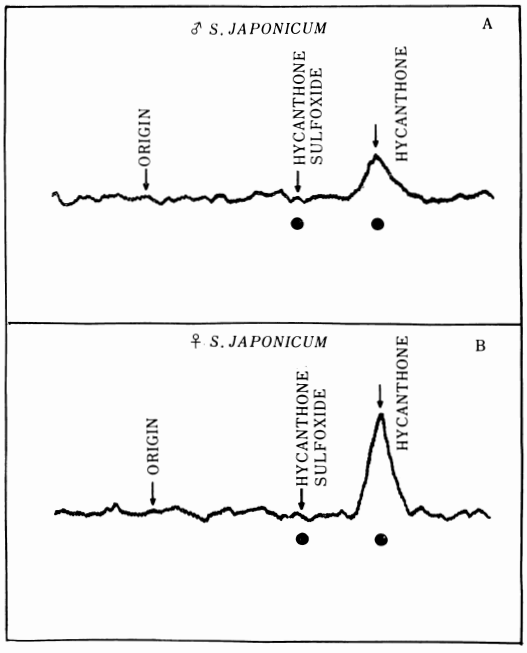


Fig. 2 Radiochromatographs of extracts from male (A) and female (B) *S. japonicum* obtained from infected mice that received tritiated hycanthone.

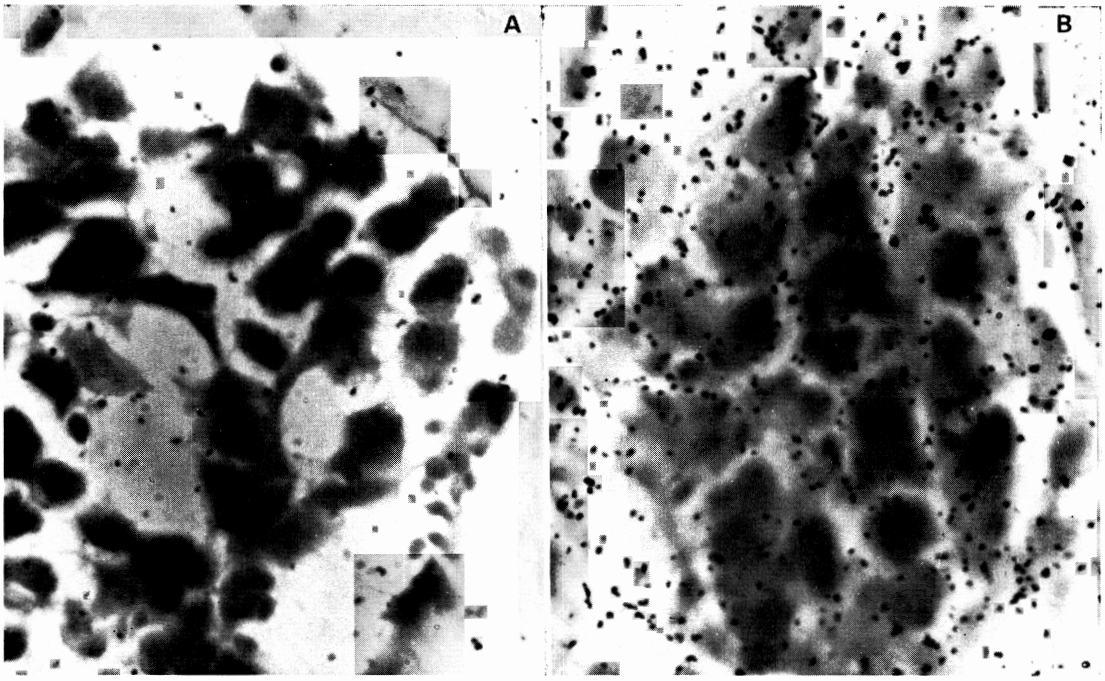


Fig. 3 (1800× magnification). Differential uptake of tritiated hycanthone by testicular tissue of *S. japonicum* (A) and *S. mansoni* (B), as indicated by silver grain density, three hours after intramuscular injection of 40 mg/kg of the drug.

but the doses of HM given to the infected mice ranged from 10 to 80 mg/kg and observations were made at three hours after drug administration. Differences were obscured at the 80 mg/kg dose due to uniformly high silver grain density. However, as shown in Figure 3, at the 40 mg/kg dose the testes in *S. japonicum* contained markedly fewer silver grains than testes in *S. mansoni* observed at the same magnification (1800×). In contrast, silver grain density did not differ within the eggs of the two species. At 10 mg/kg, a decreased density of silver grains was also observed in the testes of *S. japonicum* but to a lesser degree.

Discussion

Despite various dosage regimens and medication schemes, hycanthone, *per se*, was not lethal to Japanese and Philippine strains of *S. japonicum* in experimentally-infected mice. Moreover, no hepatic shift of worms was observed. The drug also failed to enhance the antischistosomal activity of either tartar emetic or stibophen.

It is apparent, under the experimental conditions reported herein, that *S. japonicum* responds differently than *S. mansoni* to therapy with hycanthone. It was clearly demonstrated that the drug was present in the tissues of the worms. A comparison of the uptake and distribution of hycanthone in this experiment with another experiment in which an infection with *S. mansoni* was treated under identical conditions (Yarinsky *et al.*, 1970) revealed that in the latter the concentration of the drug on a microgram per worm weight basis was approximately three times greater than in *S. japonicum*. Confirmation for a difference in concentration of the drug in the two species of worms was demonstrated by autoradiographic studies in which a greater amount of hycanthone was found in testicular tissue of *S. mansoni* worms. Furthermore, as shown by radiochromatographic determinations, the proba-

bility of biotransformation of the drug by *S. japonicum* to a less active derivative is unlikely and may be excluded as a reason for the lack of sensitivity of *S. japonicum* to hycanthone therapy. Our findings suggest, therefore, that the ineffectiveness of hycanthone against *S. japonicum* may be due, at least in part, to the lower degree of uptake of the drug by this species.

Summary

Hycanthone, the 4-hydroxymethyl analog of lucanthone, was ineffective against mature infections of the Japanese and Philippine strains of *Schistosoma japonicum* in experimentally-infected mice when administered orally at 100 mg/kg/day for five consecutive days, when injected intramuscularly in a single dose at 100 mg/kg or when injected intramuscularly at 50 mg/kg weekly for four consecutive weeks. The drug also failed to enhance the activity of the antimonials, tartar emetic and stibophen.

Despite the absence of antischistosomal efficacy of hycanthone, radioactivity, radiochromatography and autoradiography measurements with tritiated drug confirmed its presence within the reproductive and somatic tissues of the worm. However, the uptake of the drug by *S. japonicum* particularly in testicular tissue was significantly less than that observed for *S. mansoni*.

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Addendum

After submission of this manuscript, Dr. Muneo Yokogawa kindly provided us with additional information relating to oral therapy with hycanthone of *S. japonicum* infections in mice and hamsters (Yokogawa, M., Sano, M. and Kojima, S., Jap. J. Parasitol., **18** : 416, 1969). The authors reported that the drug was ineffective at 100 mg/kg/d×5 and 10 days in 64 and 110 day old infections, respectively, in mice and when given at 100 mg/kg/d×10 days in 90 day old infections in hamsters.

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