Notes

Aspects of the effect of thioxanthone on *Schistosoma mansoni* in mice and *in vitro*

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The efficacy of the thioxanthone drugs, lucanthone and hycanthone, in the treatment of experimental and clinical *Schistosoma mansoni* infections appears to be well established. Until recently (Lee & Cheever, 1970; Foster et al., 1971) the numerous laboratory studies of these agents had produced few data concerning possible differential effects on male and female parasites. Hill (1956) briefly stated that female parasites preferentially survived subcurative lucanthone treatment in mice, but most investigations into thioxanthone chemotherapy either were not concerned with such an effect or did not find it. Another striking aspect of the published work on experimental thioxanthone chemotherapy is the variation in dose–response relationships, even within a single host species, in different laboratories. The study described herein was designed to investigate the parasiticidal and sublethal effects of these thioxanthone agents on both male and female *S. mansoni* in infected mice and *in vitro*.

Materials and methods

The NIH Puerto Rican (NIH-PR) strain of *Schistosoma mansoni* was employed in all experiments. However, strains of different geographical origin were occasionally used for comparison with NIH-PR (Lee et al., 1971). Shedding of cercariae was generally induced from pooled infected snails. When unisexual infections were desired, cercariae from single snails previously exposed to one miracidium (NIH-PR strain) were used. Female Swiss albino mice weighing 18–20 g were exposed to a measured number of cercariae by tail-immersion using a modification of the technique of Olivier & Stirewalt (1952). Mice were later randomly assigned to control and treatment groups. Chemotherapy consisted of one of the following regimens: 5 daily doses of lucanthone by gavage, 5 daily doses of stibophen intraperitoneally, or, a single intramuscular dose of hycanthone base dissolved in castor oil. Except as noted in the results, treatment of mice was begun 48 days after exposure to cercariae, and mice were sacrificed and perfused by the method of Duvall & DeWitt (1967) 21–25 days later.

The *in vitro* assays were performed with hycanthone methanesulfonate (Sterling–Winthrop), which is water-soluble. The method of Lee & Michaels (1968) was employed with the exception that the culture medium generally lacked serum enrichment and the period of observation was extended to 7 days. Serial two-fold dilutions of drug were employed in each trial.

Results

The lethal effects of lucanthone on parasites of each sex in two selected groups of mice are shown in Table 1. The results of a similar study using hycanthone treatment are given in Table 2. The parasiticidal effects of hycanthone methanesulfonate *in vitro* are shown in Table 3.

Tables 1 and 2 show that male worms were more susceptible to the lethal action of lucanthone and hycanthone than were females. Males of the St Lucia, Liberia, and Belo Horizonte (Brazil) strains were likewise more susceptible than females to both drugs. This difference was independent of the initial worm burden and was not a feature of the response to stibophen or niridazole. Entirely comparable results were obtained in an experiment in which mice were perfused 60 days after treatment rather than after the customary 21–25-day period. From several trials (NIH-PR strain) it appeared that 25 mg of hycanthone per kg of body weight killed about 70% of male worms and that 50 mg/kg killed about 30% of females regardless of whether infections were unisexual or bisexual. Females surviving doses lethal for males were stunted and lacked gut pigment (Fig. 1). It was interesting to note that in mice treated with 50 mg of hycanthone per kg of body weight, female NIH-PR worms had returned to their

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Table 1. Examples representative of the parasiticidal effect of lucanthone (gavage daily for 5 days) by sex *

Parasite strain	Daily dose (mg/kg)	Mean no. of live worms per mouse (± SE)		Worm survival as percentage of control		No. of mice
		Male	Female	Male	Female	
NIH-PR	control	11.7 (0.8)	18.2 (1.4)	100	100	17
NIH-PR	25	9.4 (1.2)	14.7 (1.6)	80	81	10
NIH-PR	50	7.9 (1.5)	12.8 (1.6)	68	70	9
NIH-PR	75	3.6 (1.2)	14.4 (1.4)	31	79	10
NIH-PR	100	1.8 (0.6)	15.6 (1.3)	15	86	10
Liberia	control	12.2 (1.5)	6.9 (0.7)	100	100	16
Liberia	75	3.0 (0.8)	6.1 (1.1)	25	88	8
Liberia	100	0.83 (0.17)	5.7 (1.0)	7	83	6
Liberia	150	0.33 (0.17)	4.9 (1.1)	3	71	9
Liberia	200	0.29 (0.18)	3.7 (0.4)	2	54	7

* The experiments represented here are the same as those for which results on the male worms have been previously reported (Lee et al., 1971).

Table 2. Examples representative of the parasiticidal effect of hycanthone (single dose, intramuscularly) by sex *

Parasite strain	Daily dose (mg/kg)	Mean no. of live worms per mouse (± SE)		Worm survival as percentage of control		No. of mice
		Male	Female	Male	Female	
NIH-PR	control	10.8 (1.4)	10.0 (0.83)	100	100	17
NIH-PR	20	8.0 (0.90)	6.9 (0.71)	74	69	10
NIH-PR	25	3.7 (1.1)	8.0 (0.75)	34	80	10
NIH-PR	30	3.2 (0.73)	6.4 (0.76)	30	64	9
NIH-PR	40	0.9 (0.18)	5.6 (0.65)	8	56	10
NIH-PR	50	0.7 (0.35)	7.1 (0.64)	6	71	10
St. Lucia	control	8.8 (0.69)	6.5 (0.69)	100	100	17
St. Lucia	20	7.3 (1.3)	7.0 (1.0)	83	108	10
St. Lucia	25	7.4 (1.0)	5.9 (1.0)	84	93	10
St. Lucia	30	7.9 (1.2)	8.6 (1.2)	90	134	10
St. Lucia	40	4.9 (0.94)	7.0 (0.97)	56	108	9
St. Lucia	50	5.1 (1.1)	7.4 (1.0)	58	114	9

* The experiments represented here are the same as those for which results on the male worms have been previously reported (Lee et al., 1971).

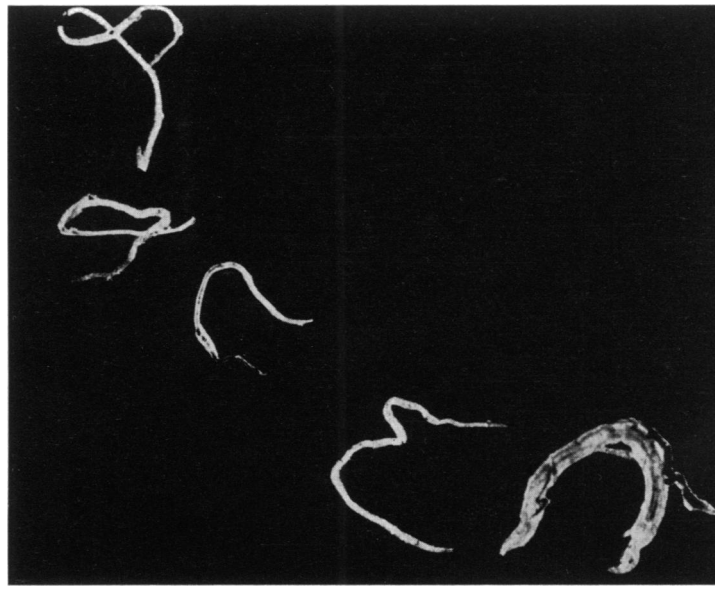

Fig. 1. Stunted worms recovered from mice treated with 50 mg of hycanthone/kg body weight 58 days previously and not reinfected.

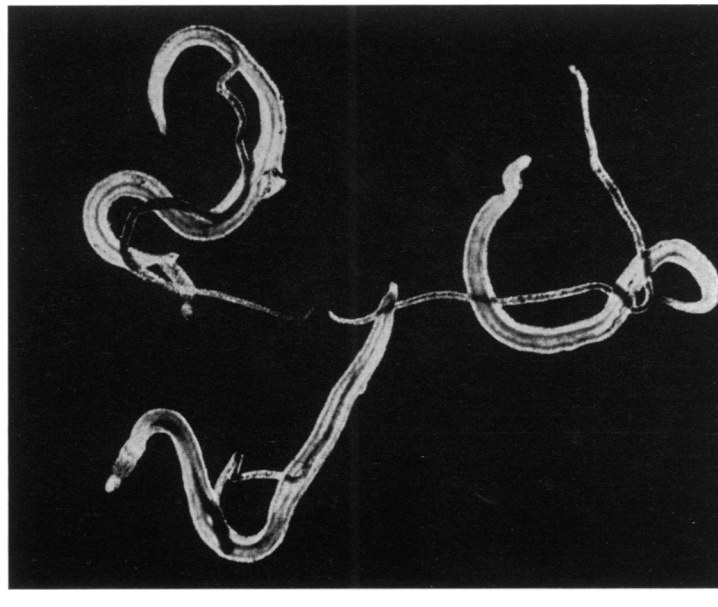

Fig. 2. Worms that appear normal recovered from mice treated with 50 mg of hycanthone/kg body weight 58 days previously and reinfected with male cercariae 53 days previously. Most male worms in these mice are presumed to be from the challenge infection by male cercariae. The females, however, are equivalent to those shown in Fig. 1 except that new males were supplied by the second exposure.

normal adult appearance by 4 months after a challenge infection with male cercariae (Fig. 2), but there was no microscopical evidence of ova in host viscera or stools. In contrast, surviving NIH-PR male worms in mice given a dose of 25 mg/kg stimulated production of viable ova by 2 months after a challenge infection with female cercariae.

In vitro, hycanthone methanesulfonate proved to be highly active against *S. mansoni* (Table 3). At the higher effective drug concentrations, worms were stained yellow by the drug even while they were still actively motile. Death of worms was observed after an exposure of as little as 24 hours to the higher concentrations, and at lower drug levels most worms were dead after exposure for 7 days. However, worms exposed to low drug concentrations occasionally died in the second week of incubation while control worms always survived for at least 2 weeks. Enrichment of the basic medium with fetal calf serum decreased the effectiveness of the drug. A remarkable and consistent feature of the drug's activity *in vitro* was the greater killing effect on male worms.

Discussion

Experimental. It appears that the thioxanthone drugs, lucanthone and hycanthone, exert a much greater effect on male *S. mansoni* than on females and that the effectiveness of these agents on mouse infections is less than has been generally reported (Azim et al., 1948; Kikuth & Gönnert, 1948; Gönnert & Vogel, 1955; Berberian & Freele, 1964; Berberian et al., 1967). Of the many studies published, only the recent work of Foster et al. (1971) agrees qualitatively and quantitatively with the present data. As pointed out by the above authors, the use of techniques other than portal perfusion to study drug effects seems the best explanation of the earlier failure to demonstrate that some or all of the female worms survived treatment. By the technique of Duvall & DeWitt (1967) these stunted depigmented female worms (Fig. 1) rarely, if ever, escape detection. Hosts infected intraperitoneally (Berberian & Freele, 1964) must be perfused after rinsing away immature peritoneal worms (Moore & Meleney, 1955), which closely resemble the widowed females. It is not clear why the results of Thompson et al. (1962) with lucanthone did not indicate a preferential survival of females.

Table 3. Effect of hycanthone methanesulfonate on worm survival *in vitro*.

Trial	Replicate [a]	Medium [b]	Exposure (h)	Minimum lethal concentration (μg/ml)	
				Male	Female
1	1	199	168	0.63	1.3
	2	199	168	0.63	1.3
2	1	199	168	0.16	1.3
	2	199	168	0.32	1.3
	3	199	168	0.32	1.3
	4	199 + 10 % FCS	168	2.5	5.0
	5	199 + 10 % FCS	168	1.3	5.0
3	1	199	24	5	20
	2	199	24	5	40
	1 [c]	199	24	1.3	10
	2 [c]	199	24	0.63	10
4	1 [c]	199	24	0.9	7.1
	2 [c]	199	24	0.9	7.1

[a] One pair of worms at each drug concentration in each replicate.
[b] Medium 199 was obtained from Grand Island Biological Co., Grand Island, N.Y., USA. FCS = fetal calf serum.
[c] Read 6 days after transfer to drug-free medium.

At least some of the males that survived a dose of 25 mg/kg were able to stimulate normal females to lay fertile ova. With the exception of one infection, it appears that the female parasites that survived in mice treated with 50 mg of hycanthone per kg of body weight, and that were supplied with male partners shortly thereafter, produced no eggs for 4 months after treatment. Apparently this loss of fertility may not be a permanent effect of this regimen, as parasite fertility in mice treated with hycanthone methanesulfonate has been destroyed temporarily only to reappear 3–12 months later (Rogers & Bueding, 1970) in association with inheritable resistance to the drug on the part of the parasite (Rogers & Bueding, 1971).

It is not known, at present, why female worms are less susceptible to the lethal action of the thioxanthone drugs. The foregoing results *in vitro* suggest that the differential susceptibility of males and females is innate and not host-mediated. It is interesting to note that the *in vivo* uptake of hycanthone methanesulfonate is actually greater in female than in male worms (Yarinsky et al., 1970).

Epidemiological implications

Since the preferential effect of hycanthone on male *S. mansoni* exists both in mice and *in vitro*, it seems appropriate to speculate in terms of natural hosts such as man. Patients treated with hycanthone generally show a 90–100% decrease in egg passage, and

one would suspect persistence of numbers of unpaired female worms.

While a degree of immunity to reinfection is generally conceded to exist in populations with a high prevalence of *Schistosoma mansoni* infections, the stimulus to such immunity is not well defined. The significance of premunition in several parasitic infections is widely appreciated. The " concomitant immunity " in the rhesus monkey against *S. mansoni* (Smithers, 1968) suggests that, perhaps in man as well, an established infection with adult worms may be the essential stimulus for immunity against reinfection.

Since infections with worms of one sex are expected to be of limited pathogenicity (Warren, 1961) and yet are able to confer immunity to reinfection in the rhesus monkey (Smithers, 1962; Hsü, 1969) by maintaining an immunogenic stimulus, persistent females in thioxanthone-treated patients might conceivably play a beneficial role when reinfection is likely. Although depreciation of the immune status of patients after effective treatment has only been a subject of speculation, reinfection remains a major threat to the concept of effective mass therapy. In comparison with other anti-schistosomal drugs, thioxanthone treatment may warrant scrutiny in terms of its effect on prevalence and magnitude of reinfection.

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