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The Uptake of Tritiated Hycanthone by Male and Female *Schistosoma mansoni* Worms and Distribution of the Drug in Plasma and Whole Blood of Mice following a Single Intramuscular Injection *

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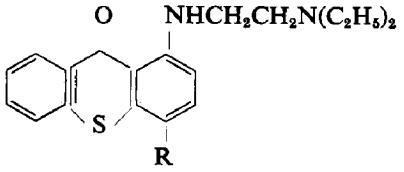
Mice infected with Schistosoma mansoni *were given an intramuscular injection of a single 80 mg/kg dose of randomly tritiated hycanthone. The uptake of the drug in male and female worms, as well as its concentration in the red blood cells and plasma of the mice, was followed for a period of 24 hours.*

Blood and plasma drug levels reached maximum values within 30 minutes. During the postmedication period, plasma concentrations were consistently higher than red blood cell concentrations. At 2–6 hours, when blood levels were declining, peak uptake was reached in the female schistosome worms; the concentration in the female worm was 5 times higher than in the male worm. At 24 hours the schistosomes contained appreciably more drug than was present in the blood.

Hycanthone (I), the hydroxymethyl analogue of lucanthone (II), has been reported to be schistosomicidal in the mouse, hamster and monkey (Rosi et al., 1965; Berberian et al., 1967; Pellegrino et al., 1967).



(I) R = CH_2OH (II) R = CH_3

Assessment of the drug in man by administration of daily oral doses (2.5 mg/kg ± 0.5 mg/kg) for 3–5 consecutive days or of a single intramuscular injection (3.0 mg/kg ± 0.5 mg/kg) has demonstrated its effectiveness against *Schistosoma haematobium* and *S. mansoni* (Katz et al., 1968; Maritz, 1968; Clarke et al., 1969).

A number of laboratory and clinical investigations are currently under way to elucidate the action of the drug and to provide information concerning its characteristics.

The experiment described herein was performed to determine the distribution of hycanthone between red blood cells and plasma in mice, and to determine the time relationship of drug levels in plasma and whole blood to drug levels in adult *S. mansoni* male and female worms during a 24-hour period after a single parenteral dose of drug.

MATERIALS AND METHODS

Female Swiss mice each weighing 32 g were infected with a Puerto Rican strain of *S. mansoni* according to the method of Berberian & Freele (1964); 48 days after infection the mice received an intramuscular injection of a single large dose (80 mg (base)/kg in 0.1 ml distilled water) of the methanesulfonate salt of randomly tritiated (173.1 mCi/mmol) hycanthone. The selection of the dose was based on an earlier observation that it caused the worms in copula to be separated, and initiated a shift of worms from the mesenteric veins to the liver within 24 h (A. Yarinsky & B. A. Jackson—unpublished data, 1968). The ED_{50} of hycanthone

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base in the mouse is only 18.5 mg/kg ± 2.4 mg/kg (Berberian et al., 1967).

At 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after the medication, heparinized blood was obtained from 4 infected mice and pooled. A sample of 0.1 ml of whole blood was pipetted on to a square of filter-paper and allowed to dry. The blood was then centrifuged and an aliquot of 0.1 ml of plasma was also placed on filter-paper. Following this, schistosome worms were removed from the mesenteric veins (except at 24 h) and rinsed briefly in 0.8% saline; the females were mechanically separated from the males and both sexes were given a second saline rinse. Eight hours after medication male and female worms were also collected from the hepatic sinuses because a major shift of worms to the liver had occurred. By 24 h, the worm shift to the liver was almost complete and all the males and females obtained were from the hepatic sinuses. The distribution of worms between the mesenteric veins and liver was recorded at 8 h and 24 h.

At each time period, 6 males were blotted to remove excess moisture, weighed [1] as a group, and placed on 2 in × 2 in (5 cm × 5 cm) squares of filter-paper; 6 or 7 females were placed directly into a weighing pan without blotting since the amount of residual moisture was small and evaporated prior to weighing. The females were weighed as a group and then placed on similar squares of filter-paper. The papers containing the samples of blood or worms were folded and made into pellets to ensure uniform and complete combustion in a Packard (Model 300) Tri-Carb Sample Oxidizer. After combustion the tritiated water formed and 10-ml samples of counting solution were automatically dispensed into counting vials. The system was purged with nitrogen to eliminate oxygen quenching and the samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 874). The counting solution used contained 100 g naphthalene, 5 g 2,5-diphenyloxazole (PPO) and 0.25 g 2,2-*p*-phenylenebis(5-phenyloxazole) (POPOP) dissolved in 1 litre of a mixture of 81% dioxane, 4% absolute ethanol and 15% toluene.

Under the conditions described above the range of counting efficiency was 30%–36%; the recovery for tritiated hycanthone added to blood or placed directly on filter-paper and then burned was consistently greater than 97%. Blanks were introduced between samples to check the residual radioactivity in the system. It was found that after rinsing and purging only traces of radioactivity remained in the machine from the previous run. In one instance a blood sample that yielded more than 10^6 counts per minute was followed by a blank in which only 100 counts per minute were recorded, indicating less than 0.01% residual contamination of the system.

For the determination of the nature of the radioactivity present in the worms, groups of 6 or 7 worms (randomly selected from among the worms taken from all mice in each batch of 4) were homogenized with a Potter-Elvelhjem homogenizer in 3 ml of 0.2 m borate buffer, pH 9.5. The homogenates were extracted with 3 volumes of dichloromethane. After separation by centrifugation, the organic phase was concentrated at 50°C under nitrogen. The concentrated extracts were spotted on 5 cm × 20 cm precoated silica gel F-254 thin-layer plates (E. Merck AG, Darmstadt, Federal Republic of Germany). The plates were developed in an ether–methanol–trimethylamine (8 : 1 : 1) system and the chromatographs were scanned in a Packard Radiochromatogram Scanner (Model 7201). Non-radioactive hycanthone and available metabolites were placed on one side of each plate for comparison with radioactive material(s) extracted from the worms.

RESULTS AND DISCUSSION

The data in Table 1 confirm earlier work in which it was found that within 24 h after a single intramuscular injection of hycanthone methanesulfonate at 80 mg/kg the worms normally found in copula become separated. In addition, there was a shift of worms from the mesenteric veins to the liver so that within 24 h 85% of the worms were in the hepatic sinuses. At the end of the experimental period all the worms found were still alive; however, they were sluggish and less resistant to mechanical separation than were the worms collected through the first 8 hours.

Peak concentrations of hycanthone in blood and plasma were reached within 30 min, after which they declined to the end of the experimental period (Table 2, and figure). The ratios of hycanthone concentrations of plasma : red blood cells were calculated (Table 2). It is clear that, although the ratios varied, plasma concentration levels were consistently higher at each point during the sampling period.

The uptake of the tritium label by the male worms was rapid; within 30 min hycanthone was found

[1] The male and female worms collected at 1 h were weighed in a Mettler balance. A Cahn electrobalance (Model M-12) was used for the other weighings.

TABLE 1

HEPATIC SHIFT OF *SCHISTOSOMA MANSONI* AFTER MEDICATION OF HOST MICE WITH A SINGLE INTRAMUSCULAR INJECTION OF 80 mg (base)/kg OF RANDOMLY TRITIATED HYCANTHONE METHANESULFONATE [a]

Time after injection (h)	No. of worms examined per group (and mean)	Average no. of worms per mouse [b]				Average no. of unpaired worms	
		Mesenteric veins		Liver			
		No.	%	No.	%	No.	%
8	3–31 (16.0)	8.4	52.5	7.6	47.5	10.8	67.5
24	12–23 (18.6)	2.8	15.0	15.8	85.0	5.0	27.0

[a] In contrast to the shift of the worms to the liver shown in the table, in untreated infections fewer than 2 worms are found in the liver, or less than 10% of the total number of worms harboured by the mouse.

[b] 5 mice were necropsied at each time period.

to the extent of 60 μg/g wet worm tissue. No distinct drug peak was observed although between 30 min and 6 h the concentration of the drug increased slowly in the male. By 24 h, the males retained a high drug level (72 μg/g wet worm tissue) in contrast to amounts detected in the blood (1.1 μg/ml) and plasma (1.4 μg/ml).

The female worms concentrated a larger amount of the drug than the males during the first 6 h after medication. A peak concentration of 392 μg/g was reached at 4 h which, on the basis of μg hycanthone/g wet worm tissue, was more than 5 times that in the males. The enhanced uptake of a schistosomicidal drug by female worms (compared with male worms) is in accord with the findings of Hess et al. (1966) for niridazole, of Khayyal (1964) and Molokhia & Smith (1968, 1969) for tartar emetic and of Browne & Schulert (1964) and Molokhia & Smith (1969) for sodium antimony dimercaptosuccinate (TWSb/6).

It is interesting to note that during the 30 min to 4 h postmedication period, during which time hycanthone levels in the blood and plasma were dropping, the female worms were accumulating the drug. By 6 h and continuing to the end of the experiment (24 h) the females showed a decrease

TABLE 2

CONCENTRATION OF HYCANTHONE [a] IN MALE AND FEMALE *SCHISTOSOMA MANSONI* WORMS AND IN PLASMA AND WHOLE BLOOD OF THE MOUSE HOST

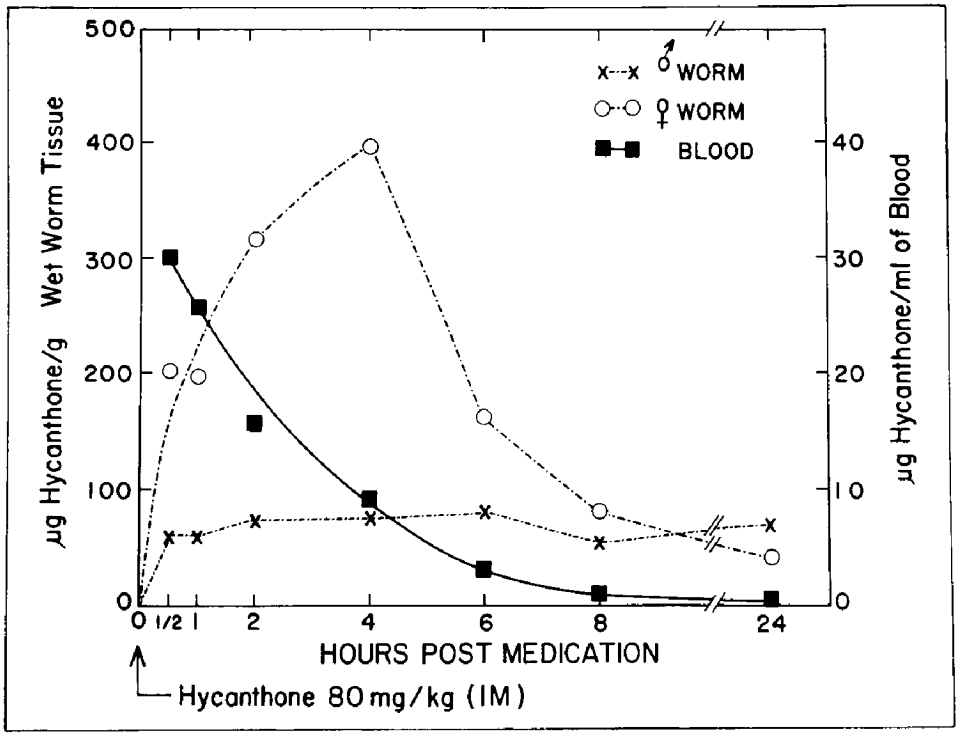
Postmedication sampling period (h)	No. worms collected [b]		Source of worms	Hycanthone conc. (μg/g wet worm tissue)		Hycanthone		
	Female	Male		Female	Male	Blood (μg/ml)	Plasma (μg/ml)	Plasma : RBC ratio [c]
0.5	7	6	Mesenteric veins	201	60	30.0	39.8	2.47
1	6	6	" "	197	62	25.5	37.3	4.18
2	7	6	" "	318	73	15.5	22.9	4.52
4	6	6	" "	392	75	9.0	10.5	1.53
6	6	6	" "	156	81	2.8	3.8	2.68
8	6	6	Mesenteric veins and hepatic sinuses	69	60	1.7	2.1	1.81
24	6	6	Hepatic sinuses	41	72	1.1	1.4	2.06

[a] Radioactivity expressed in terms of hycanthone base.

[b] Worms weighed in groups of 6 or 7.

[c] Adjusted for average haematocrit value of 41.5%.

HYCANTHONE UPTAKE AND RETENTION BY *S. MANSONI* RELATED TO CHANGING CONCENTRATION IN BLOOD OF HOST MICE



in their concentration of hycanthone. Nevertheless, at the latter period the concentration of the drug in the female (41 µg/g wet worm tissue) far exceeded that in plasma and whole blood. Khayyal (1964) reported that *S. mansoni* worms from mice which had received ^{124}Sb-labelled tartar emetic showed higher drug levels than that found in the blood. Brown & Schulert (1964) reported similar findings in the case of *S. mansoni*-infected hamsters treated with ^{124}Sb-labelled TWSb/6. Thus, it is probable that the ability of the worms to concentrate schistosomicidal agents is a contributing factor to the action of the drugs.

When the worms were examined to determine the nature of the radioactive material, it was found that the males contained only unchanged drug. At the 1-h postmedication period a sulfoxide derivative of hycanthone as well as unchanged hycanthone was found in the female worms. The sulfoxide derivative was present to the extent of approximately 20%–30% of the total radioactivity found in the worm. At subsequent sampling periods only unchanged hycanthone was detected in the female. These observations imply that most, if not all, of the toxicity of hycanthone for the adult schistosome may be attributable to the action of the unchanged drug.

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RÉSUMÉ

ABSORPTION DE L'HYCANTHONE MARQUÉ AU TRITIUM PAR *SCHISTOSOMA MANSONI* MÂLE ET FEMELLE ET RÉPARTITION DU MÉDICAMENT DANS LE PLASMA ET LE SANG COMPLET DE LA SOURIS APRÈS UNE INJECTION INTRAMUSCULAIRE UNIQUE

On a administré par voie intramusculaire à des souris infectées par *Schistosoma mansoni* une dose unique de 80 mg/kg d'hycanthone marqué au tritium, et suivi pendant 24 heures l'absorption du médicament par les parasites mâles et femelles ainsi que les variations de sa concentration dans le plasma et dans le sang complet.

La teneur du plasma et du sang complet en hycanthone est maximale après une demi-heure. Dans les schistosomes, les concentrations les plus fortes sont observées après 2 à 6 heures; à ce moment, et à poids égal, les vers femelles renferment approximativement cinq fois plus de médicament que les vers mâles.

Dans l'intervalle de 24 heures, plus de 95% du produit marqué est éliminé du sang et du plasma. La quantité d'hycanthone présente dans les vers femelles diminue pour n'être plus que du dixième environ de la valeur maximale enregistrée, mais elle reste très supérieure aux concentrations résiduelles dans le plasma et dans le sang complet. Chez les vers mâles, en revanche, la teneur en hycanthone ne s'abaisse que très faiblement durant la même période. La concentration d'hycanthone est constamment plus élevée dans le plasma que dans les érythrocytes.

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