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THE EFFECTS OF ANTIMONY UPTAKE ON THE LOCATION AND PAIRING OF SCHISTOSOMA MANSONI

BY

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The rate of uptake and elimination of antimony by both male and female schistosomes has been measured. It has been confirmed that the female schistosome is more susceptible to antimony therapy than the male since she absorbs more drug. The changes in location of the worms and the separation of the sexes following one or more injections of antimony potassium tartrate have been related to the different rates of uptake and elimination of the drug by the two sexes. The suppressive treatment of schistosomiasis is discussed in the light of these observations.

It is wellknown that active schistosomicides cause the parasites to move from the mesenteric veins where they are normally found, in animals infected with *Schistosoma mansoni*, towards the liver (Bang & Hairston, 1946 ; Vogel & Minning, 1947 ; Watson, Azim & Halawani, 1948 ; Kikuth & Gönnert, 1948 ; Schubert, 1948 ; Standen, 1953). This change of habitat or "worm shift" occurs at different times after treatment, according to the drug used. With antimonials in general, and antimony potassium tartrate in particular, it takes place rapidly after a single injection (Buttle & Khayyal, 1962). This observation led to a closer examination of the schistosome shift, both after a single injection and after multiple injections of antimony potassium tartrate, especially with a view to assessing any differences in behaviour between the male and female worms. The present paper deals with these differences and correlates them with the respective antimony concentrations of both sexes. Up to the moment, very little work has been done on the drug concentrations of the schistosomes themselves, because of the minute levels involved and the difficulty of measuring them. The use of the ^{124}Sb-labelled compound of antimony potassium tartrate helped in overcoming these difficulties. Since ^{124}Sb is a strong β- and γ-emitting isotope it is easily detectable and it is possible to measure antimony concentrations in schistosomes with a high degree of accuracy.

METHODS

Male Swiss mice (Agricultural Research Council) were each infected with 150 cercariae of an Egyptian strain of *Schistosoma mansoni*. The mice were used 8 to 9 weeks after infection by which time adult schistosomes had developed and were inhabiting the mesenteric veins. In one experiment, antimony potassium tartrate (25 mg/kg of body weight), containing ^{124}Sb to give a radiation dose of 1 μc/animal, was injected intraperitoneally into mice weighing 24 to 26 g. These mice were divided into five groups of seven animals each. The groups

were autopsied 30 min, 2 hr, 8 hr, 24 hr and 1 week after injection respectively. A further group was used as controls. The portal vein was ligatured immediately after death and the worms in the portal vein, mesenteric veins and liver were counted separately. In the latter procedure, the liver was crushed between two glass slabs, as described by Standen (1949).

For measuring the level of antimony in the schistosomes, the worms removed from each mouse were treated separately in order to be able to measure accurately the degree of scatter within the group. The use of mice of uniform weight reduced this scatter.

Worms were removed from the mesenteric veins and/or liver, washed for 10 min in 0.9% saline, and then in distilled water. Males and females were transferred separately to an aluminium planchet. They were dried and their radioactivity was measured using a Panax anticoincidence unit Type AU 460 coupled with the low-background counter assembly Type MX-157. With this equipment it was possible to measure accurately the activity of one or two worms at a time. From the measure of radioactivity the mean level of antimony per worm for each group was calculated.

In another experiment five groups of mice were treated with 1, 2, 3, 4 and 5 injections of unlabelled antimony potassium tartrate (25 mg/kg) given at daily intervals. A separate group was used for controls. The mice were autopsied 24 hr after the last injection, in the same way as before, special note being made of the distribution of paired worms. The same procedure was then repeated using three groups given respectively 1, 2 and 4 injections of ^{124}Sb-labelled antimony potassium tartrate intraperitoneally. The levels of antimony in the male and female parasites were again measured separately, as already described.

RESULTS

Worm habitat and incidence of pairing

The distribution of schistosomes after a single injection of tartar emetic is shown in Fig. 1. As previously reported (Buttle & Khayyal, 1962), the worms were swept

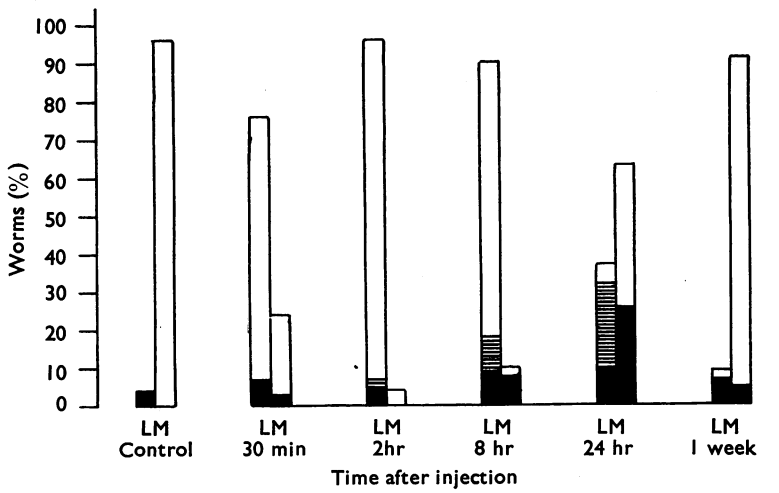

Fig. 1. Distribution of schistosomes in the hepatic portal system of infected mice following treatment with antimony potassium tartrate (25 mg/kg of mouse) given intraperitoneally. L, liver, and M, the mesenteric and portal vein levels. Black areas, males; hatched areas, females; and empty areas, paired worms. Schistosomes are moved rapidly to the liver following the injection of antimony potassium tartrate. Sex separation starts at about 2 hr and increases during 24 hr. Many males return to the mesentery before the females.

on towards the liver soon after the injection and returned slowly to the mesenteric veins in the course of a week.

Closer observation of this movement revealed a difference in the behaviour of male and female worms. Separation of the sexes began about 2 hr after injection; at about 8 hr, when the worms were beginning to return to the mesenteric veins, there was a predominance of male worms in the mesenteric and portal veins, leaving a more or less equivalent number of single females in the liver. This effect was more pronounced after 24 hr, but after a week the worms were again paired in the mesenteric veins as before treatment. The extent of sex separation became more marked with each successive daily dose of antimony potassium tartrate, until, after five doses each of 25 mg/kg, there was no pairing 24 hr after the last injection (Table 1).

TABLE 1

DISTRIBUTION OF PAIRED WORMS IN MICE AFTER THE INJECTION OF ANTIMONY POTASSIUM TARTRATE

Values give the distribution of worms 24 hr after the last injections of antimony potassium tartrate (25 mg/kg of mouse)

No. of injections	No. of mice per group	No. of worms per mouse	$\frac{\text{Paired worms} \times 100}{\text{Total worms}}$
0	5	15	96
1	5	17	41
2	6	18	25
3	7	10	11
4	7	14	6
5	6	13	0

Antimony levels of schistosomes following treatment

Each point on the curves in Fig. 2 represents the average antimony levels of schistosomes obtained from seven mice. Initially, both male and female worms took up nearly the same amount of antimony. The difference between the sexes at 30 min and at 2 hr was not significant. After 2 hr, the level of antimony in the males fell rapidly in contrast to that in the females, so that at 8 hr the males contained only half as much antimony as the females. This difference was sustained up to 24 hr and was still highly significant. At 1 week the drug levels in the worms were too small to be determined accurately.

The interpretation of the results in terms of amount of antimony per worm, as described above, was not satisfactory owing to the differences in weight between the male and female parasites. Hence the above results were recalculated on a weight basis to give the antimony levels in μg/g of schistosomes (Fig. 3). The antimony concentrations in the blood of the hosts are also included for comparison. On this basis, then, it was evident that the females absorbed about five-times as much antimony as the males and that they eliminated the drug at a slow but steady rate. There was a sharp drop in the antimony concentration of the males between 2 and 8 hr from the time of injection, but the drug elimination after that time ran more or less parallel to that in the females.

Table 2 shows that the difference in antimony concentrations between the male and female schistosomes increased with the increasing number of injections of

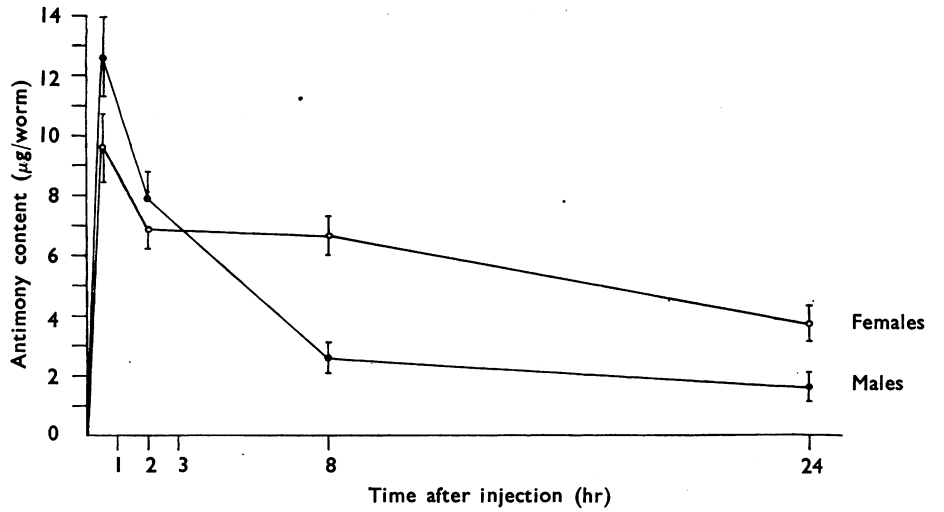

Fig. 2. Trivalent antimony levels in schistosomes following treatment with antimony potassium tartrate (25 mg/kg of mouse, intraperitoneally). The ordinate shows the average antimony content (ng/worm) and the abscissa the time (hr) after the injection. ○, females; ●, males. Males and females initially took up nearly equal amounts of antimony but there was a sharp drop in the antimony level of the males in the first 8 hr. Vertical lines represent standard errors.

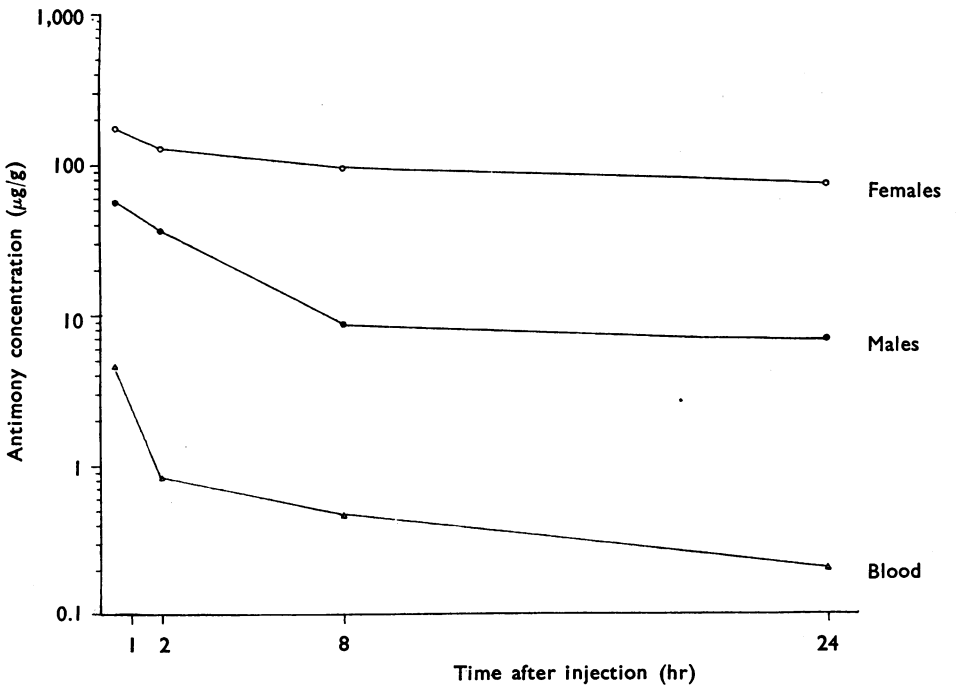

Fig. 3. Trivalent antimony levels (μg/g; ordinate, log scale) in schistosomes following treatment with antimony potassium tartrate (25 mg/kg of mouse, intraperitoneally). This figure shows the results of Fig. 2 recalculated per unit weight of worm. On this basis females take up about four-times as much antimony as the males but after 8 hr the rate of elimination from both sexes runs parallel. Schistosome levels are much higher than the mouse blood levels. ○, females; ●, males; and △, blood.

TABLE 2

ANTIMONY LEVELS IN SCHISTOSOMES AFTER THE INJECTION OF ANTIMONY POTASSIUM TARTRATE INTO MICE

The levels were measured 24 hr after the last daily injection of the drug. Values are means with standard errors

No. of injections	Antimony level (ng/worm) in Males	Females
1	1·57±0·44	3·72±0·58
2	2·80±0·73	7·69±1·42
4	6·09±0·93	14·84±1·52

antimony potassium tartrate, but that the ratio of male to female concentrations was always 1:2.5, as measured 24 hr after the last injections. This ratio would have been higher had the results been expressed per unit weight of worm.

DISCUSSION

The mode of action of antimonials has been extensively studied and reviewed by various authors (Bang & Hairston, 1946 ; Vogel & Minning, 1947 ; Watson *et al.*, 1948 ; Kikuth & Gönnert, 1948 ; Schubert, 1948 ; Standen, 1949 ; Bueding & Swartzwelder, 1957 ; Nagaty & Rifaat, 1960 ; Buttle & Khayyal, 1962). In appropriate doses (25 mg/kg of host) the effect on the worms was a very rapid paralysis causing them to lose their grip on the walls of the blood vessels and to be swept on towards the liver soon after the injection. Smaller doses had less effect whereas higher doses had a quicker and longer lasting action at the expense of a higher toxicity to the host (unpublished observations).

From the present findings, it seems highly probable that a threshold level of antimony is necessary to paralyse the worms, which are subsequently swept on towards the liver. Once the muscular control is impaired, the males can no longer enclose the females and a certain degree of separation occurs. The effect lasts as long as there is a concentration in the worms sufficient to keep them immobilized. Below that concentration the worms regain their muscular control and travel slowly back to the mesentery. The level of antimony in the male worms is much less than that in the females and will therefore fall below the threshold sooner, since the rate of elimination from both sexes is nearly the same per unit weight of worm. This fact, coupled with the greater agility of the males, facilitates their early remigration to the portal and mesenteric veins.

It is not clear whether the females, after losing sufficient drug, travel singly to pair eventually with the males in the portal vein, or whether the males keep swinging to and fro in the region of the portal and large hepatic portal veins till eventually they manage to pair with the females inside the liver. Standen (1953) observed that females are not capable of motion on their own. They might, however, still be capable of a limited movement, and under the influence of the presence of males in the portal vein they might be urged to migrate towards and pair with them, a process which might take up to 1 week for its accomplishment.

The degree of sex separation increases with the increasing number of doses (Table 1) and it has been shown that this is accompanied by a linear increase in

the antimony levels of the parasites themselves (Table 2). As previously mentioned, sex separation is caused by the paralysis of the paired worms so that the males can no longer embrace the females. The higher the antimony level in the worms, therefore, the greater the degree and duration of paralysis and hence the greater the chance of sex separation.

As to whether the difference in absorption and elimination rates between males and females occurs with other antimonials and with other classes of drugs, little is as yet known. Browne & Schulert (1963), working with ^{124}Sb-labelled TW Sb/6 (sodium antimony dimercaptosuccinate) in infected hamsters, found that the females contained three- to five-times as much antimony as the males, measured 24 hr after three daily injections of antimony potassium tartrate. This finding is similar to that described in this paper. The anion combined with the antimony may possibly influence its rate of absorption and/or elimination by the male and female schistosome, but it is clear that, at least with these two antimonials, the female is much more susceptible to drug action than the male and there is no reason to believe that other antimonials would behave differently. That the female worm is the weaker sex and the most affected by drugs has been observed already by Fairley (1926) and thereafter by many others, but all observations were done on a qualitative rather than on a quantitative basis. In effect, therefore, these findings substantiate those of other workers.

Many authors (Christopherson, 1920; Nishi, 1923; Vogel & Minning, 1947; Gönnert, 1947) have shown evidence that schistosomicides and particularly antimonials affect the reproductive organs of the parasite so that oviposition is impaired. The ease with which antimonials can be used for suppressive treatment in schistosomiasis may well depend on the larger uptake and longer retention of antimony by the female worms, which thus suppresses ovulation for a long time until the following dose of the drug is given. The uptake of antimony by the male worms during such therapy would be less relevant since the primary objective really is to impair ovulation.

It may therefore well be possible to adjust the dose level of the antimonial drug in such a way that only the female schistosomes would be affected. Such dose-levels would be smaller than the dose affecting both male and female worms. Moreover such a course would further reduce the toxicity to the host. The potentialities of such a line of treatment, however, need yet to be more fully investigated.

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REFERENCES

BANG, F. B. & HAIRSTON, N. G. (1946). Studies on schistosomiasis *japonica* IV: chemotherapy of experimental schistosomiasis *japonica*. *Amer. J. Hyg.*, **44**, 348–366.

BROWNE, H. & SCHULERT, A. R. (1963). Disposition of Sb^{124} labelled Astiban in animals with bilharziasis. *Fed. Proc.*, **22**, 666.

BUEDING, E. & SWARTZWELDER, C. (1957). Anthelmintics. *Pharmacol. Rev.*, **9**, 329–365.

BUTTLE, G. A. H. & KHAYYAL, M. T. (1962). Rapid hepatic shift of worms in mice infected with *Schistosoma mansoni* after a single injection of tartar emetic. *Nature (Lond.)*, **194**, 780–781.

CHRISTOPHERSON, J. B. (1920). Bilharzia disease: the sterilization of the ova during the course of cure by antimony (tartrate). *J. trop. Med. Hyg.*, **23**, 165–167.

FAIRLEY, N. H. (1926). Studies in the chemotherapy and immunity reaction of schistosomiasis (*Schistosoma spindalis* and *S. haematobium*). *Trans. roy. Soc. trop. Med. Hyg.*, **20**, 236–273.

GÖNNERT, R. (1947). Zur Frage des Wirkungsmechanismus von Miracil. *Naturwissenschaften*, **34**, 347–348.

KIKUTH, W. & GÖNNERT, R. (1948). Experimental studies on th therapy of schistosomiasis. *Ann. trop. Med. Parasit.*, **42**, 256–267.

NAGATY, H. F. & RIFAAT, M. A. (1960). Treatment of schistosomiasis, past and present. I. Historical review of the use of antimony in the treatment of bilharziasis. II. Clinical investigations on the treatment of urinary and intestinal bilharziasis with TWSb (antimony dimercaptosuccinate) with a critical study of its toxic side-effects. III. Synergism between antimony (tartar emetic) and thioxanthone compounds in the treatment of schistosomiasis. *J. Egypt. med. Ass.*, **43**, 659–677, 678–695, 696–707.

NISHI, M. (1923). Experimental study on the treatment of schistosomiasis *japonica* with tartar emetic. Clinical observation, histological investigation and pathological changes on animals intoxicated with tartar emetic. *Sci. Rep. Inst. infect. Dis. (Tokyo Univ.)*, **2**, 485–501.

SCHUBERT, M. (1948). Conditions for drug testing in experimental schistosomiasis *mansoni* in mice. Screening of drugs in experimental schistosomiasis *mansoni*. Effect of drugs during earliest stages of experimental schistosomiasis *mansoni* in mice. *Amer. J. trop. Med.*, **28**, 121–136, 137–156, 157–162.

STANDEN, O. D. (1949). Experimental schistosomiasis. 2. Maintenance of *Schistosoma mansoni* in the laboratory, with some notes on experimental infection with *S. haematobium*. *Ann. trop. Med. Parasit.*, **43**, 268–283.

STANDEN, O. D. (1953). Experimental schistosomiasis. 3. Chemotherapy and mode of drug action. *Ann. trop. Med. Parasit.*, **47**, 26–34.

VOGEL, H. & MINNING, W. (1947). Über die Einwirkung von Brechweinstein, Fuadin und Emetin auf *Bilharzia japonica* und deren Eier im Kaninchenversuch. *Acta trop. (Basel)*, **4**, 21–56, 97–116.

WATSON, J. M., AZIM, M. A. & HALAWANI, A. (1948). Investigations on the antibilharzial action of miracil D (Nilodin). *Trans. roy. Soc. trop. Med. Hyg.*, **42**, 37–54.