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Tissue Distribution of Trivalent Antimony in Mice Infected with *Schistosoma mansoni*

M. M. MOLOKHIA [1] & H. SMITH [2]

The work described in this paper is designed to use the very high analytical sensitivity of neutron-activation analysis. The antimony content of individual organs and pieces of organs have been analysed as part of an investigation of the chemotherapy of schistosomiasis. The results illustrate the great, and as yet relatively unapplied, value of this technique in dealing with the investigation of trace elements in biological systems. Values such as those given form firm bases on which further studies can be built and show that the single animal has the same metabolic reactions as those deduced from bulked samples, but of course with individual variations.

Since the discovery by Christopherson (1918) of the curative action on schistosomiasis of the trivalent antimony compound tartar emetic, many other antimonial and non-antimonial drugs have been introduced. However, tartar emetic is still the one that is most widely applied.

There has been a growing interest in the investigation of the fate of administered antimony in the body. It is useful to study the distribution patterns and the excretion rates of antimony in a variety of hosts in relation to different antimony compounds and different dose regimens. However, most of the previous work on this subject was concerned with antimony levels in only a few organs and the study period rarely exceeded 3 days' duration (Hassan, 1938; Bagdon & Zbinder, 1964; Thommen et al., 1964; Waitz, 1964).

The uptake of antimony by schistosomes also has received growing attention (Browne & Schulert, 1963, 1964; Khayyal, 1964; Schulert et al., 1966). The limiting factor in most instances has been the lack of sensitive analytical procedures with which to estimate the rapidly decreasing antimony levels usually found in host organs.

The need for further studies on the disposition of administered antimony in host and parasite, using newer methods of estimating antimony in biological specimens, has been emphasized by the WHO Scientific Group on Research in Bilharziasis (Chemotherapy) (1966).

In the present study, mice were given a single intraperitoneal injection of either tartar emetic or TWSb (sodium antimony 2,3-mesodimercaptosuccinate; Astiban),[3] with a view to finding the relative antimony uptake by mouse organs and tissues after different intervals. This was then compared with the uptake by schistosomes obtained from the same animal. The use of activation analysis for the estimation of antimony in the present investigation has made it possible to analyse most of the mouse organs and to extend the study period to 15 days.

METHOD

The mice used in this experiment were males of the BSVS type, weighing 25 g–30 g and about 6 months old. They were infected by an Egyptian strain of *Schistosoma mansoni*,[4] according to the method described by Standen (1949). After 9 weeks, 30 of these infected mice were divided into 2 groups with 15 mice in each group. The first group was given tartar emetic intraperitoneally, the dose being 5 mg per kg of body-weight. The second group was given TWSb, also intraperitoneally, the dose being 7.5 mg per kg of body-weight. Both these dosages contain the same amount of trivalent antimony.

[1] Research Fellow, Department of Forensic Medicine, University of Glasgow, Glasgow, Scotland.

[2] Lecturer, Department of Forensic Medicine, University of Glasgow, Glasgow, Scotland.

[3] Supplied by Hoffmann-La Roche, Basle, Switzerland.

[4] Obtained through the courtesy of Burroughs Wellcome Laboratories, London, England.

TABLE 1
RATIOS OF DRY WEIGHT TO WET WEIGHT IN MOUSE ORGANS AND IN SCHISTOSOMES

Sample	Ratio of dry weight to wet weight (%)
Mouse	
Blood	20
Bone	61
Brain	30
Eye	35
Gastrointestinal tract	25
Heart	26
Kidney	29
Liver	29
Lung	23
Muscle	29
Skin	31
Spleen	27
Teeth	98
Testis	25
Thyroid	28
Trachea	35
Urinary bladder	25
Schistome	
Female worms	30
Male worms	30

Two mice from each group were sacrificed ½ hour after the injection. Thereafter, 2 more mice from each group were sacrificed at 8 hours, 24 hours, 2 days, 4 days, 7 days and 15 days. The mice were all killed by cervical dislocation through applying pressure at the back of the neck and pulling the tail with a jerky movement. During the subsequent dissection of the mice, samples were taken from most organs and tissues with the minimum of handling and placed in clean polystyrene dishes. The mice were dissected over polyethylene-coated paper sheets and the adult worms were removed from the portal vein and mesenteric veins and washed in distilled water before being transferred to polystyrene dishes.

The samples were dried for 1 week in desiccators over self-indicating silica gel at a pressure of 0.1 mm of mercury. The dry : wet weight ratio was found by weighing duplicate samples from all the organs while wet and reweighing them after drying. The mean (Table 1) was used for correcting sample weights; this was also done for worms of both sexes. Tissues and organs from untreated mice were collected for analysis in order to correct for the normal antimony content in the treated mice. None of the instruments or materials used in the pre-irradiation handling of the tissues contained significant amounts of antimony, so contamination was avoided.

Samples were irradiated together with an antimony standard at a thermal neutron flux of 1.2×10^{12} neutrons/cm /s. A short irradiation lasting 2–4 hours was used for tissues and organs collected early in the experiment—namely, at the 30-minutes and 8-hours intervals. The rest of the samples, as well as all worm samples, were irradiated for 1–3 days, in order to obtain suitable antimony activities. All the worm samples were weighed and packed individually. The subsequent separation of antimony has been described elsewhere (Howie et al., 1965).

RESULTS AND DISCUSSION

The antimony content of the organs of untreated mice is given in Table 2. The lung, kidney, spleen,

TABLE 2
ANTIMONY CONCENTRATION OF UNTREATED MOUSE ORGANS

Sample	Antimony content (μg g of wet tissue)
Blood	0.001
Bone	0.006
Brain	0.003
Eye	0.001
Gastrointestinal tract	0.003
Heart	0.003
Kidney	0.010
Liver	0.007
Lung	0.012
Muscle	0.003
Skin	0.003
Spleen	0.010
Teeth	0.003
Testis	0.001
Thyroid	0.002
Trachea	0.006
Urinary bladder	0.003

liver and bone show the highest levels. The rest of the organs contain less than 0.005 μg per g. These values represent antimony naturally found in mouse organs. The origin of antimony is the food and drink consumed by the mice, and the environment they live in. It is interesting to note that the lung contains the highest level of antimony, since this was found in man also (Molokhia & Smith, 1967).

Antimony concentrations in the different tissues and organs of mice injected with tartar emetic and TWSb are given in Table 3 and Table 4, respectively. The values are expressed as μg of antimony per g of wet tissue and represent the average of 2 animals per group. A correction for the natural antimony concentration has been made. Empty places in the tables indicate that samples either have not been successfully obtained or were lost during analysis. Owing to the small number of animals sacrificed each time in each group, it was difficult to assess accurately the rate of accumulation or clearance of antimony in different organs. Instead, the relative antimony uptake by mouse organs in each group was found to reflect certain characteristics. During the first 48 hours, the highest antimony levels were recorded in the liver, gastrointestinal tract, kidney and urinary bladder. The antimony content of the stomach and oesophagus was significantly less than that of the duodenum and colon. The difference is probably due to antimony excreted *via* the bile into the duodenum.

In the later part of the experiment, the relative antimony uptake by the flat bones of the skull and by the teeth rises until it becomes higher than that of

TABLE 3
ANTIMONY CONCENTRATIONS [a] IN MOUSE ORGANS AT VARIOUS TIMES AFTER TARTAR EMETIC INJECTIONS

Organ	½ h	8 h	24 h	48 h	4 days	7 days	15 days
Blood	—	0.24	0.10	0.05	0.04	0.02	0.00
Bone	0.54	0.19	0.16	0.17	0.14	0.06	0.28
Brain	0.02	0.01	0.02	0.01	0.05	0.00	0.00
Colon	2.65	1.23	0.67	0.20	0.09	0.03	0.08
Duodenum	2.08	0.88	0.31	0.28	0.16	0.03	0.17
Eye	0.09	0.04	0.09	0.02	0.02	0.00	0.01
Heart	0.64	0.09	0.12	0.12	0.02	0.01	0.03
Kidney	0.63	0.25	0.23	0.15	0.06	0.05	0.05
Liver	7.12	2.19	3.14	1.25	0.50	0.14	0.19
Lung	0.54	0.16	0.25	0.19	0.04	0.01	0.10
Muscle	0.52	0.03	0.05	0.02	0.02	0.05	0.02
Oesophagus	—	0.09	0.31	0.15	0.08	0.03	0.06
Skin	0.17	0.01	0.15	0.07	0.08	0.03	0.03
Spleen	5.01	1.00	0.43	0.34	0.08	0.08	0.07
Stomach	1.07	0.36	0.35	0.11	0.25	0.08	0.08
Teeth	0.12	0.27	0.08	0.06	0.05	0.02	0.51
Testis	0.19	0.13	0.05	0.02	0.02	0.01	0.02
Thyroid	0.11	0.03	0.05	0.02	0.01	0.00	0.03
Tongue	0.03	0.15	0.13	0.07	0.03	0.01	0.01
Trachea	—	0.34	0.21	0.18	0.08	0.06	0.03
Urinary bladder	—	3.43	1.16	0.43	0.18	0.02	0.02

[a] Values are μg antimony/g wet tissue.

TABLE 4
ANTIMONY CONCENTRATIONS[a] IN MOUSE ORGANS AT VARIOUS TIMES AFTER TWSb INJECTIONS

Organ	½ h	8 h	24 h	48 h	4 days	7 days	15 days
Blood	—	0.28	—	0.02	0.03	0.02	0.01
Bone	1.53	0.42	0.03	0.16	0.20	0.14	0.49
Brain	1.10	0.01	0.02	0.01	0.01	0.01	—
Colon	3.38	1.37	0.09	0.10	0.13	—	0.04
Duodenum	7.19	1.30	1.41	0.14	0.31	—	0.01
Eye	0.68	—	0.02	0.09	0.02	0.03	0.01
Heart	1.54	0.26	0.03	0.05	0.02	0.03	0.01
Kidney	12.29	0.59	0.08	0.06	0.08	—	0.02
Liver	7.52	4.82	0.19	0.74	0.78	0.31	0.04
Lung	6.20	0.47	0.04	0.05	0.05	0.05	0.01
Muscle	2.07	0.12	0.02	0.03	0.02	—	0.01
Oesophagus	2.81	0.61	0.05	0.09	0.07	—	0.01
Skin	1.90	0.14	0.09	0.07	0.08	0.08	0.02
Spleen	6.38	1.29	0.06	0.09	0.10	—	0.03
Stomach	3.44	0.88	0.72	0.10	0.16	—	0.05
Teeth	0.69	0.65	0.10	0.05	0.09	0.09	0.41
Testis	2.49	0.60	0.02	0.01	0.01	—	0.01
Thyroid	1.05	0.05	0.01	0.03	0.01	—	0.01
Tongue	1.66	0.32	0.06	0.06	0.03	0.02	0.01
Trachea	4.35	—	0.05	0.08	0.08	—	0.02
Urinary bladder	—	2.45	—	0.05	0.06	—	0.03

[a] Values are μg antimony/g wet tissue.

other tissues. The brain, thyroid and male reproductive organs contained the lowest antimony concentrations throughout the experiment. The ventricular muscle of the heart, the tongue, and the adductor muscles of the thigh all gave nearly identical values, and these were comparable to the antimony level in blood. Skin levels were usually higher than those of muscle tissue and this is probably due to binding of antimony with sulfhydryl groups in the skin.

This pattern of relative antimony uptake by the different mouse organs was shown equally by both groups of mice.

The results of antimony analysis of schistosomes found in the mice are given in Table 5. Usually between 5 and 15 worms were found in each mouse. The average antimony content in a number of male worms, obtained from 2 mice per group, is given together with the corresponding average for females.

TABLE 5
ANTIMONY CONCENTRATION[a] IN SCHISTOSOMES AT VARIOUS TIMES AFTER INJECTION OF TARTAR EMETIC OR TWSb INTO HOST MOUSE

Drug used	Sex of worm	8 h	24 h	48 h	4 days
Tartar emetic	Female	2.63	3.01	1.58	0.58
	Male	0.81	0.75	0.25	0.20
TWSb	Female	3.14	2.71	1.59	1.70
	Male	1.96	0.52	0.40	0.44

[a] Values are μg antimony/g wet tissue.

FIG. 1

ANTIMONY CONCENTRATION IN THE ALIMENTARY TRACT OF MOUSE No. 1

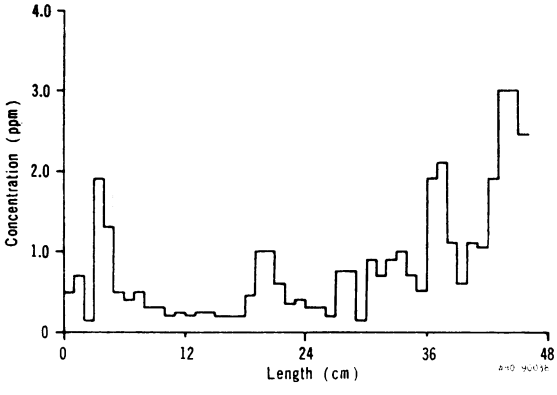
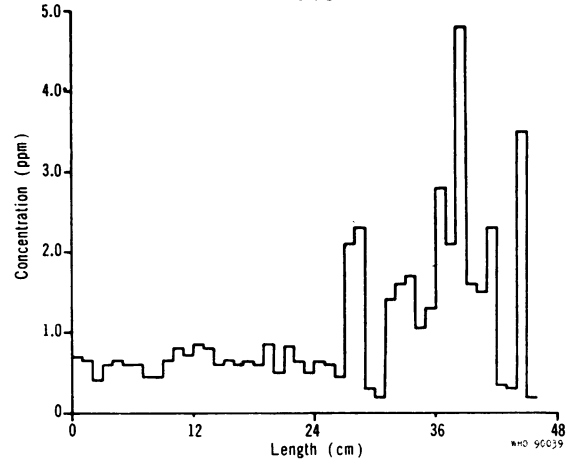


FIG. 2

ANTIMONY CONCENTRATION IN THE ALIMENTARY TRACT OF MOUSE No. 2



Again, it is difficult to estimate the rate of excretion of antimony in the worms owing to the small number of animals sacrificed at a time. However, the antimony level in female worms is generally between 3 and 5 times higher than that in male worms. This applies to the TWSb group as well as to the tartar emetic group. The antimony levels in females are comparable to those of livers from the same animals, although in the TWSb group the female worms seem to retain more antimony than the liver does. These findings are in agreement with those of Browne & Schulert (1963) who used labelled TWSb in infected hamsters and found that female schistosomes contained 3–5 times as much antimony as males. Khayyal (1964), working with ^{124}Sb-labelled tartar emetic and TWSb, found that the antimony level of female schistosomes was 10 times that of the males.

The antimony content of the alimentary tract was investigated in detail following the injection of TWSb in 2 uninfected mice. The 2 animals received a single dose of 7.5 mg/kg of body-weight and were sacrificed 24 hours later. The alimentary tract (oesophagus to large intestine, inclusive) was removed intact in both mice, dried, and divided into 1-cm segments which were weighed separately. A note was taken of whether segments contained food material or not. The findings of the analysis of these segments are illustrated in Fig. 1 and 2. The lower two-thirds of the stomach shows a higher antimony concentration than the upper third. This suggests that antimony enters the stomach from the duodenum, where it is excreted with the bile from the liver. The high antimony levels along the different parts of the alimentary tract were always associated with the presence of food material. The highest concentrations were found near the end of the large intestines and these could represent the earliest excretion of the element from the liver into the alimentary tract.

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RÉSUMÉ

RÉPARTITION TISSULAIRE DE L'ANTIMOINE TRIVALENT CHEZ LA SOURIS INFECTÉE PAR *SCHISTOSOMA MANSONI*

Les auteurs rendent compte d'une étude sur la destinée de l'antimoine trivalent administré à des souris infectées par *S. mansoni*. Ils ont utilisé la technique de l'analyse après activation neutronique, dont la sensibilité atteint environ 10^{-10} g lorsqu'elle est appliquée à l'antimoine. Entre autres résultats, ils ont déterminé les concentrations normales d'antimoine dans près de 20 tissus différents, ainsi que les concentrations à des intervalles de temps variables (jusqu'à 15 jours) après des injections uniques de tartre stibié et d'Astiban. Les auteurs indiquent également les teneurs en antimoine trouvées chez les schistosomes dans les mêmes conditions expérimentales et ils les comparent avec les concentrations tissulaires observées chez l'hôte. Il ressort de cette étude que l'antimoine est rapidement éliminé de la plupart des tissus, bien qu'il ait une certaine tendance à demeurer dans les os. Il apparaît également que les concentrations d'antimoine sont significativement plus élevées chez les schistosomes femelles quel que soit le délai après les injections. L'étude de sa répartition dans le tube digestif semble indiquer que l'antimoine pénètre dans l'estomac à partir du duodénum où il est excrété avec la bile provenant du foie.

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