Meso-dimercaptosuccinic acid, a chelating agent for the treatment of mercury poisoning

BAL is able to form a pentatomic ring with metals which have a high affinity for sulphhydryl groups, and was successfully introduced into the treatment of poisoning with sublimate (Longcope & Luetscher, 1949). One of the disadvantages is the limited water solubility of the reaction product (Gilman, Allen & others, 1946) which explains why the urinary excretion of mercury is low (Fitzsimons & Kozelka, 1950). Another untoward effect of BAL is that in certain circumstances it is able to transport some mercury to the brain (Berlin, Jerksell & Nordberg, 1965), or increase significantly the blood concentration of mercury (Magos & Stoytchev, 1969). The third disadvantage of BAL is its relatively high toxicity (Longcope & Luetscher, 1949). It has been previously reported (Friedheim, da Silva & Martins, 1954) that the inconveniences of BAL as well as the lack of water solubility of its chelates are overcome by incorporating the BAL principle, i.e. two thiol groups attached to adjoining carbon atoms, in a symmetric dicarboxylic acid. The resulting meso-2,3-dimercaptosuccinic acid (HOOC-HCSH-HCSH-COOH) (DMS) forms water soluble metal-dimercaptides, has low toxicity and with arsenic (Friedheim & de Jongh, 1959) and antimony (Friedheim & others, 1954) compounds, significant trypanocidal and schistosomocidal activity. The present paper presents data on the effect of DMS on the tissue distribution of mercury in mice and guinea-pigs treated with methylmercuribromide and mercury dichloride. D-Penicillamine served as reference.

DMS was synthesized according to Owen & Sultanbawa (1949). It is a white crystalline powder, m.p. 192–194°. The disodium salt is soluble in 10 parts of water w/v at 20°. A 0·1 equimolar solution of DMS and $HgCl_2$ remains clear over a pH range of 2–9, while an analogous solution of BAL and $HgCl_2$ forms a precipitate at pH below 7, which is dissolved by DMS. This observation supports the view that under the stated conditions DMS has a higher affinity for mercury than BAL.

I. The toxicity of DMS was investigated in white Swiss mice of 18–20 g by intraperitoneal injections of 5 or 10% solutions of DMS in distilled water adjusted with sodium bicarbonate to pH 7·2. The acute toxicity was explored with single intraperitoneal doses of the 10% solution applied to groups of 10 mice in doses of 1000, 2000, 3000 and 4000 mg kg^{-1}. Survival was recorded after 10 days. The subacute tolerance was tested in a group of 15 mice for a dose of 1000 mg kg^{-1} (i.p.) in two series of 5 daily injections, the two series being separated by an interval of two days. After the last treatment the animals were observed for a further two weeks.

II. Groups of 5 mice each received one intramuscular dose of $HgCl_2$ (0·1 and 1·0 mg kg^{-1}) or CH_3HgBr (1·0 and 2·5 mg kg^{-1}) and were treated intraperitoneally 24 or 48 h later with DMS (100, 250, 500 and 1000 mg kg^{-1}) and penicillamine (100 and 250 mg kg^{-1}) on two or three consecutive days. The mercury compounds were injected dissolved in distilled water in concentrations of 0·002–0·02% w/v, and the thiol-compounds were dissolved in distilled water adjusted to pH 7·2 in concentrations of 1·0–10·0% w/v. The volumes injected ranged from 0·1 to 0·25 ml. All mice, including untreated controls, were anaesthetized with ether and decapitated 24 or 48 h after treatment. The same organs of 5 mice were pooled and homogenized.

III. Analogous procedures were applied to groups of two guinea-pigs, with the difference that the organs were analysed individually and the results averaged. Schedules of doses and timing are in the Tables.

IV. Mercury was determined in 0·2–0·3 g samples of the fresh homogenates or organs by cold vapour atomic absorbtion with the "Cold-Vapour Analyser Kit, part number 790557" of Pye Unicam, Ltd., according to the procedure of sample prepara-

Table 1. *Effect of meso-dimercaptosuccinic acid (DMS) and D-penicillamine (PA) on mercury in organs of mice after a single i.m. dose of 1·0 mg kg^{-1} methylmercury bromide and 0·1 and 1·0 mg kg^{-1} mercuric chloride.* Mercury concentrations in treated animals are reported as % of mercury in controls. The absolute value of mercury concentrations in the controls in ng g^{-1} dry weight are given in brackets.

Exposure		Methylmercury bromide 1·0 mg kg^{-1}			Mercuric chloride 1·0 mg kg^{-1}	0·1 mg kg^{-1}
		Kidney	Liver	Brain	Kidney	
Schedule		A	A	A	B	C
Controls		100%	100%	100%	100%	100%
ng g^{-1} dry weight		(30·2)	(5·8)	(1·6)	(13·2)	(4·6)
Compound	mg kg^{-1}					
DMS	100	32%	34%	68%	66%	47%
PA	100	53%	38%	94%	68%	87%

A. day 1 1·0 mg kg^{-1} $HgCH_3Br$ (i.m.)
2,3 treatment (i.p.)
4 pause
5 necropsy

B. day 1 1·0 mg kg^{-1} $HgCl_2$ (i.m.)
2 pause
3,4 treatment (i.p.)
5 necropsy

C. day 1 0·1 mg kg^{-1} $HgCl_2$ (i.m.)
2 pause
3,4 treatment (i.p.)
5 necropsy

Table 2. *Effect of meso-dimercaptosuccinic acid (DMS) and D-penicillamine (PA) on mercury in organs of mice and guinea-pigs after a single i.m. dose of 2·5 mg kg^{-1} methylmercury bromide.* Mercury concentrations in treated animals are reported as % of mercury in the controls. The absolute value of mercury concentrations in the controls in ng g^{-1} dry weight are given in brackets.

		Mice					Guinea-pigs		
		Kidney		Liver		Brain	Kidney	Liver	Brain
Schedule*		D	E	D	E	D	D	D	D
Controls		100%	100%	100%	100%	100%	100%	100%	100%
ng dry weight		(45·9)	(40·4)	(5·6)	(4·8)	(2·4)	(42·2)	(20·4)	(5·2)
Compound	mg kg^{-1}								
DMS	1000	15%	8%	34%	13%	12%			
	500	17%	13%	32%	15%	37%			
	250	24%	20%	41%	26%	46%	24%	33%	42%
PA	250	68%	24%	82%	33%	50%	77%	62%	86%

*D. day 1 2·5 mg kg^{-1} $HgCH_3Br$ (i.m.)
2, 3 treatment (i.p.)
4 pause
5 necropsy

E. day 1 2·5 mg kg^{-1} $HgCH_3Br$ (i.m.)
2, 3, 4 treatment (i.p.)
5 pause
6 necropsy

tion and analysis described by Braun & Husbands (1971). Dry weights of the homogenates of mice or organs of guinea-pigs were determined in 0·2–0·3 g samples dried for 3 h at 120°. Mercury concentrations in organs were recorded in ppm/dry weight and are reported as the mean of duplicate analysis.

Toxicity. The LD50 (i.p.) mouse was in excess of 3000 mg kg^{-1}. Of 15 mice receiving 10 doses of 1000 mg kg^{-1}, i.p. over 12 days, 15 mice survived 7 days and 13 mice survived 14 days after the last dose.

Tables 1 and 2 show the effect of the complexing agents DMS and penicillamine on the tissue concentration of mercury in mice and guinea-pigs treated with mercury compounds. It can be seen that: (1) after a single dose of $HgCl_2$, both DMS and

penicillamine decrease the mercury content of kidneys in mice, DMS more than penicillamine (Table 1, schedules B and C); (2) after a single dose of CH_3HgBr, and the variations of doses and timing in schedules A, D, E, both DMS and penicillamine decrease the mercury content of kidneys, liver and brain in mice and guinea-pigs, DMS consistently more than penicillamine (Tables 1 and 2).

Mass law is to be expected to affect the change-over from mercury bound to constituents of the organism to the mercaptans. This aspect has been explored in mice in the methylmercury series by an increase in the number of mercaptan doses from 2 to 3 (schedules D and E) and with three levels of DMS increasing geometrically by a factor of two (Table 2). This results in the mercury content of kidneys, liver and brain dropping as the DMS dose increases, but ink idneys and liver the decrement becomes smaller for successive doubling of the DMS dose. This is in keeping with the notion that mercury is bound in the tissues in two forms or sites, one more, the other less, prone to release mercury (Clarkson & Magos, 1966).

Compared with penicillamine, DMS in equal doses was consistently more effective in preventing the accumulation of mercury in the three organs tested. The efficiency of DMS over penicillamine increased in mouse kidneys when the dose of $HgCl_2$ was decreased from 1·0 to 0·1 mg kg^{-1} (Table 1). With methylmercury bromide, DMS decreased the kidney concentration of mercury with both levels of mercury doses investigated. This effect was present in mice and guinea-pigs. After the injection of 1·0 mg kg^{-1} methylmercury bromide to mice the brain mercury concentration was decreased five times more by two days of treatment with 100 mg kg^{-1} DMS (Table 1), than by two days of treatment with 100 mg kg^{-1} penicillamine; conversely, this effect was marginal in mice given 2·5 mg kg^{-1} methylmercury bromide and two days of treatment with 250 mg kg^{-1} DMS or penicillamine respectively (Table 2). However, even in this case DMS was more effective in decreasing mercury concentrations in kidney and liver.

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