The selective uptake of mercury by myocardial infarcts in the rat

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Rats with myocardial infarcts, sham operated and control animals were treated with methoxyethyl mercury chloride (MEMC) intraperitoneally or with mercamphamide intravenously or intraperitoneally and the mercury content in the hearts determined spectrophotometrically with dithizone after various time intervals. After MEMC treatment there was no difference between the amounts of mercury found in the hearts of rats with myocardial infarcts and controls. After injection of mercamphamide, mercury was taken up by hearts of rats with myocardial infarcts while those of sham operated and control animals remained free of mercury. Optimal results were yielded by 1 h experiments made within the first 24 postoperative hours. The selective uptake of mercury from organomercurials is much dependent on the organic part of the molecule present in the given individual compound.

Experimentally induced myocardial infarcts have been precisely localized using [²⁰³Hg]chlormerodrin by Gorten, Hardy & others (1966). Organically bound mercury accumulated in the infarcted areas but not in undamaged heart tissue. We wished to make a quantitative determination of the mercury accumulated in the heart and as the first step experiments were undertaken to reproduce the findings of Gorten & others in rats, by chemical determination of non-labelled mercury. Rats with myocardial infarcts, sham operated and unoperated control animals were treated with mercamphamide or methoxyethyl mercury chloride and after appropriate time intervals the content of mercury in their hearts was determined.

METHODS

Myocardial infarcts were produced by surgical coronary ligature (Selye, Bajusz & others, 1960) in male albino rats, 155 ± 5 g (n = 315) of an inbred colony maintained on standard semisynthetic pellet diet, with free access to food and drink water throughout the experiment.

The descending branch of the left coronary artery was tied near to its origin. Mortality rates considerably exceeded those reported by Selye & others, and were similar to those of Dušek & Jezdinská (1965).

The hearts of sham-operated animals were lifted from the opened thorax and replaced after a short time and the wound closed.

The organomercurial compounds, methoxyethyl mercury chloride (MEMC; mol wt 295·15; Berk Ltd, London) and mercamphamide (N-3-hydroxy-mercury-2-methoxy-propylcamphoramidic sodium; mol. wt 509·95; Novurit inj. Chinoin, Budapest) were used. Mercamphamide was injected in 25 mg/kg intravenous or intraperitoneal doses, diluted with distilled water to give 0·1 ml/100 g weight; MEMC

in 10 mg/kg intraperitoneal doses was suspended with 1% methylcellulose to give 0·1 ml/100 g giving the animals treated with mercamphamide 0·986 mg Hg/100 g and those with MEMC 0·686 mg Hg/100 g.

Organomercurial treatment was given 3 to 5, 18 to 22 or 42 to 46 h after surgery and the animals were killed 5 min, 1, 5 or 16 h after injection by ether. The hearts were excised, opened and the ventricles cleaned from atrial and other adherent tissues. They were thoroughly washed from blood with tap water, then with distilled water, weighed, cut into small pieces with scissors and then homogenized in a Potters flask with 3 volumes of distilled water.

The homogenized hearts were digested and the amount of mercury present in them was spectrophotometrically assessed by the dithizone method. Graded reagents and water distilled twice from glass were used. Carbon tetrachloride was freshly purified according to method recommended for chloroform by (Iwantscheff, 1958a). Dithizone (G. R.; Reanal, Budapest) was dissolved according to Grusz-Harday (1969). The concentrated solution could be stored in a dark glass in a refrigerator for about one month.

For digestion, the method of Grusz-Harday (1969) was adopted for the rat hearts by the following modifications. The homogenate was transferred to a 25 ml Erlenmeyer flask, 0·15 g of potassium chlorate added and the mixture heated on a boiling water bath for 5 min. One ml of concentrated hydrochloric acid was then added and the flask was removed from the bath. After the intensive gas production had ceased, 1 ml of concentrated hydrochloric acid was added again and the flask, covered with glass, was left on the water bath switched off overnight. Distilled water (10 ml) was then added to the mixture which was heated on the bath until the chlorine had evaporated (about 8 to 10 h). Lost water was replaced. The chlorine-free liquid was filtered made up to 80 ml with distilled water to keep the concentration of chloride ions below 0·2N (Iwantscheff, 1958b).

Mercury was determined as mercury dithizonate according to Fischer & Leopoldi (1935). Accidental metallic contamination was removed as described by Iwantscheff (1958c). The absorbance was measured at 485 nm against pure carbon tetrachloride. The blank value of untreated rat hearts, determined in each experimental series and subtracted from the measured absorbance, was 0·035 ± 0·003 (n = 23). The mercury content was calculated on the basis of a calibration curve made with pure mercury chloride solution treated according to the above schedule. These modifications increased the sensitivity of the method, originally described for mercury concentrations about 2 μg/ml, by one order of magnitude.

Data in numbers throughout this paper are given as mean values ± standard error of the mean ($\bar{x} \pm s_{\bar{x}}$). Significance of differences was assessed by the t or, if the "F" test revealed significant heteroscedasticity, the "d" test.

RESULTS

Table 1 shows the mercury content of rat hearts after MEMC treatment. The amount of mercury in the hearts of control animals and those with myocardial infarcts increased as a function of time elapsed after MEMC treatment, but although the figures of each group differed significantly from zero there was no significant difference between animals with infarcts and controls.

With mercamphamide (Table 2), the hearts of unoperated control animals contained relatively large amounts of mercury a few minutes after injection, but 1 or more h later

Table 1. *Mercury content in the hearts of rats injected with MEMC intraperitoneally.*

| | Time (h): | | | | Significance of the difference from: | |
	between surgery and MEMC injection	after MEMC treatment	Number of animals	Mercury content (μg/heart)	zero P	respective controls P
Control animals	—	1	18	0.58 ± 0.167	< 0.01	—
		5	27	0.95 ± 0.202	< 0.01	—
		16	9	1.23 ± 0.281	< 0.01	—
Animals with myocardial infarcts	18 to 22	1	23	0.23 ± 0.092	< 0.05	> 0.05
		5	24	1.16 ± 0.154	< 0.01	> 0.20
	3 to 5	16	10	1.53 ± 0.265	< 0.01	> 0.20

the mercury content in the hearts did not significantly differ from zero, with the only exception of the group tested 1 h after the intravenous treatment. Large amounts of mercury were found in the hearts of the rats with myorcardial infarcts at each time. One to five h after the intravenous treatment given within 24 h after surgery there was more than $2\,\mu$g of mercury in the hearts. This amount decreased as the time elapsing between surgery and mercamphamide treatment or that between the injection and killing the animals was prolonged to 48 and 16 h, respectively. After intraperitoneal treatment, the mercury content after 1 h was higher, while that after 5 h was much lower than, the respective values found after intravenous mercamphamide. All these values significantly differ from zero and, apart from the

Table 2. *Mercury content in the hearts of rats injected with mercamphamide.*

| | | Time: | | | | Significance of differences from: | | |
	Route	between surgery and mercamphamide inj.	after mercamphamide treatment	Number of animals	Mercury content (μg/heart)	zero P	controls P	sham operated animals P
Control animals	i.v.		5 min	5	3.52 ± 0.941	<0.05	—	—
			1 h	14	0.31 ± 0.143	<0.05	—	—
			5 h	12	0.27 ± 0.146	>0.05	—	—
			16 h	8	0.52 ± 0.26	>0.05	—	—
Sham-operated animals		18 to 22 h	1 h	12	0.22 ± 0.123	>0.05	>0.20	—
			5 h	12	0.60 ± 0.185	<0.01	>0.10	—
		3 to 5 h	16 h	6	0.43 ± 0.274	>0.10	>0.20	—
		42 to 46 h	5 h	7	0.37 ± 0.259	>0.10	>0.20	—
Animals with myocardial infarcts		3 to 5 h	1 h	11	2.38 ± 0.293	<0.01	<0.01	—
		18 to 22 h	1 h	13	2.52 ± 0.479	<0.01	<0.01	<0.01
			5 h	14	2.28 ± 0.449	<0.01	<0.01	<0.01
		3 to 5 h	16 h	16	1.18 ± 0.257	<0.01	>0.10	>0.10
		42 to 46 h	5 h	11	1.55 ± 0.610	<0.05	>0.20	>0.10
Control animals	i.p.	—	1 h	9	0.37 ± 0.186	>0.05	—	—
			5 h	13	0.11 ± 0.074	>0.10	—	—
Sham-operated animals		18 to 22 h	1 h	10	0.07 ± 0.070	>0.20	>0.20	—
			5 h	12	0.07 ± 0.067	>0.20	>0.20	—
Animals with myocardial infarcts			1 h	12	3.93 ± 0.215	<0.01	<0.01	<0.01
			5 h	23	0.94 ± 0.209	<0.01	<0.05	<0.01

16 h experiments, also from those of the respective unoperated controls. Thus, after mercamphamide treatment, selective uptake of mercury by rat hearts with myocardial infarcts takes place.

This conclusion is supported by the results of experiments on sham-operated animals. Amounts of mercury, approaching those found in rats with myocardial infarcts, could not be detected.

DISCUSSION

The experiments were made with two different organomercurials. MEMC was chosen because it is a relatively small molecule and its structure is similar to that of chlormerodrin used by Gorten & others (1966). Mercamphamide, also has a structure similar to, and the same biological diuretic action as, chlormerodrin. But the results were totally divergent. Both the dynamics of changes in heart mercury levels and the selectivity of uptake were different. In those rats given MEMC the amount of mercury present in the hearts increased as a function of time elapsed after the organomercurial injection. There was, at the same time, no significant difference between parallel values of groups with myocardial infarcts and unoperated rats. With the animals treated with mercamphamide, after intravenous injection, the mercury content was very high even in the hearts of unoperated control animals in the first 5 min. An hour later, however, it had decreased to near zero and remained there. In those rats with myocardial infarcts the amount of mercury was equally high 1 or 5 h after intravenous injection but decreased by 16 h after the treatment. In those rats with myocardial infarcts that received mercamphamide intraperitoneally, the decrease between the first and fifth h was faster but the conditions were also in this case fundamentally different from those seen after MEMC. This difference can be explained by different resorption rates.

Different resorption rates, however, fail to offer any reasonable explication for the lack of selective uptake of mercury from MEMC. Selective uptake of mercury takes place, in the theory of Gorten & others (1966) because there is increased permeability of capillaries in the ischaemic areas where a large amount of organomercurial passes into the interstitium and is bound by SH groups of cell proteins. After MEMC treatment, the hearts of unoperated animals also contained large amounts of mercury. This may be perhaps explained by the relatively small size of the MEMC molecule, enabling it to pass more readily into the interstitium. It may also be because MEMC is among those organomercurials metabolized to inorganic mercury (Report of an International Committee, 1969). The degradation of MEMC according to Ulfvarson (1962), is a relatively slow process and would not be important in our experiments.

The uptake of mercury according to Gorten & others (1966) is not due to haemorrhages, hyperaemia or inflammation. This view is supported by the fact that in hearts of our sham operated animals, potentially exposed to the action of all these factors, no large amount of mercury could be found.

If mercamphamide was injected 48 h after the coronary ligature, the uptake of mercury was less than in the first 24 postoperative h. Thus, the facility of selective mercury uptake is impaired by progression of the process of tissue necrosis in the heart. Optimum results were yielded by 1 h experiments made within the first 24 postoperative h. In the experiments of Gorten & others (1966) the greatest contrast between healthy and infarcted areas was found 3 to 5 days after surgery. The results were worse and scarcely available on the 6–8 and 9–12 postoperative days,

respectively. Their result agrees with ours. Apparent discrepancies are well explained by differences in the surgical technique and the species used. In the rat, a small animal, biological processes are quicker, and the genesis and cicatrization of myocardial infarcts passes off more rapidly than in the pig.

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