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The interaction of chelating agents with methylmercuric chloride bound to erythrocytes

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Organic mercury compounds like methylmercuric chloride (MMC) are accumulated rapidly by erythrocytes, and bound very firmly within the cells. However, under certain conditions they are released and thus a transfer of MMC from the erythrocytes to the tissues becomes possible. It has been suggested that MMC is accumulated in erythrocytes because of the high intracellular concentration of SH-groups [1], and that its distribution on both sides of the erythrocyte membrane is determined by the relative sulfhydryl concentrations inside and outside the cell [2] and, therefore, can be influenced by the addition of SH-substances [1]. In our investigation several SH-containing chelating agents were tested for their ability to release MMC from erythrocytes in order to compare the effectiveness of these compounds in removing MMC from human and rat cells.

Human blood was obtained from the local blood bank. Some experiments were performed with blood from normal adult male donors mixed with 10 I.U. of heparin/ml. Rat blood was taken from the inferior vena cava of mature male Heiligenberg rats and mixed with 10 I.U. heparin/ml. After centrifugation, the plasma, white blood cells and platelets were discarded, the erythrocytes were washed 3 times with saline containing 1 mM glucose and buffered to pH 7.2 with 15 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris). The erythrocytes were then suspended in this buffer and mixed with MMC to give a final haematocrit of 40 per cent, and a final concentration of 0.04 μmole MMC/ml packed cell volume (PCV). Thirty minutes later the chelating agents were added in a small volume in order not to alter the haematocrit. During incubation with MMC and/or chelating agents the vials were slowly rotated at room temperature. At various intervals aliquots were removed, centrifuged, and two 0.25 ml aliquots of the resultant supernatant were counted in a Packard autogamma spectrometer. The radioactivity in the supernatant was compared with that of an equal volume of the cell suspension. In some experiments, after removal of the supernatant, the erythrocytes were washed twice with buffer and the washings and erythrocytes were counted separately. One set of experiments was performed with inactive MMC and ^{14}C-labelled 2,3-dimercaptopropane-1-sulfonate (DMPS). Methylmercuric chloride labelled with ^{203}Hg was supplied by The Radiochemical Centre, Amersham, (Sp. act. 50 μCi/mg Hg). To remove traces of inorganic ^{203}Hg it was purified according to the method of Norseth and Clarkson [3] and dissolved in 5 mM Na_2CO_3 [3]. Unlabelled MMC (E. Merck, Darmstadt) was treated in the same way. The stability of MMC in 5 mM Na_2CO_3 has been demonstrated by Norseth and Clarkson [3]. 2,3-Dimercaptosuccinic acid (DMSA) was obtained from E. Merck, Darmstadt, 2,3-dimercaptopropane-1-sulfonate (Dimaval®, DMPS), D-penicillamine (Metalcaptase®, PA) and *N*-acetylpenicillamine (NAPA) from Heyl & Co. Berlin. The chelating agents were dissolved in water, or an equimolar amount of NaOH and adjusted to pH 6–7. The ^{14}C-labelled DMPS, synthesized by the Institut für Radiochemie, Kernforschungszentrum Karlsruhe, had a specific activity of 0.1 mCi/mmole.

The results obtained with fresh human blood did not differ from those with stored (bank) blood.

Methylmercury was taken up very rapidly by human as well as rat erythrocytes. A fraction of 1–6 per cent of the ^{203}Hg remained in the supernatant and was constant for at least 6 hr. An increase in extracellular Hg after rotation of the erythrocyte suspension for 24 hr was found to be due to haemolysis. Rat erythrocytes took up slightly less MMC than human cells, but this difference may not be statistically significant.

All chelating agents tested caused a transient efflux of MMC from the cells. Figure 1 shows that the extracellular ^{203}Hg reached a maximum 5–10 min following addition of

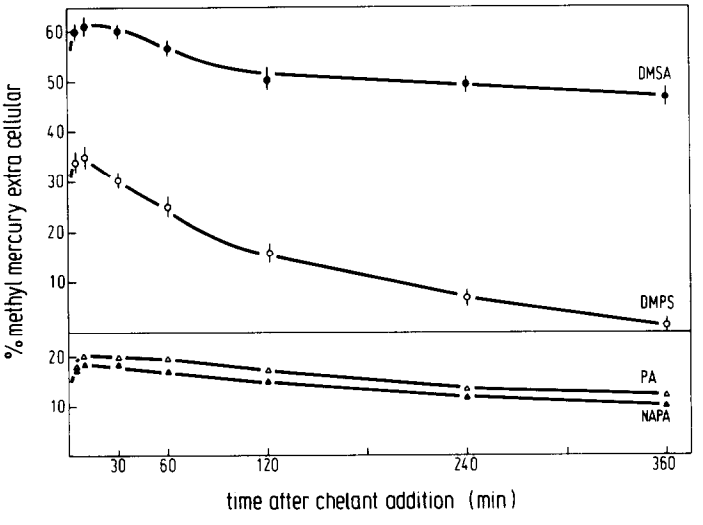

Fig. 1. Mobilization of MMC from human erythrocytes following addition of various chelating agents after 30 min. MMC concentration: 0.04 μmole/ml PCV. Chelant concentration: 0.4 μmole/ml PCV. Means of 6 experiments ± S.E. Control values were substracted (4.43 ± 0.16).

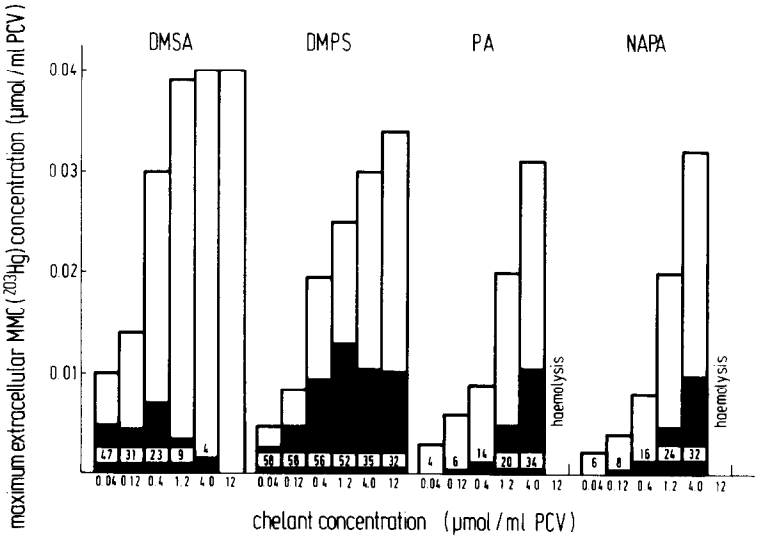

Fig. 2. Maximum [^{203}Hg]MMC in the extracellular fluid in the presence of different chelant concentrations. Black areas show the amount of MMC lost within 4 hr, the numbers indicate the lost MMC calculated as a percentage of the maximum represented by the columns.

the chelating agent and then again decreased. This loss of MMC from the extracellular fluid did not occur in the presence of high DMSA doses or low PA or NAPA doses, (Fig. 2), and it was most pronounced in the presence of DMPS. To elucidate the reason for this behaviour the same experiment was carried out with inactive MMC and ^{14}C-labelled DMPS. This showed that 30–40 per cent of the chelating agent was also lost from the extracellular fluid; however, 50–70 per cent of this lost chelating agent could be recovered again by washing the erythrocytes with buffer. In contrast, washing removed almost no ^{203}Hg.

The amount of MMC released from the erythrocytes increased with the dose of chelating agent (Fig. 3). Penicillamine and NAPA were equally effective, DMPS was more effective than NAPA and penicillamine only at doses up to 1.2 μmoles/ml PCV while DMSA was most effective at all doses. Much less MMC was removed from rat erythrocytes by all chelating agents (Fig. 3(b)). The effect of the highest dose of PA and NAPA could not be measured exactly as this concentration caused haemolysis of both human and rat erythrocytes, therefore, these figures are shown in parentheses in Fig. 3.

In contrast to White and Rothstein [1] we chose a haematocrit of 40 per cent in order to have a system that was as close as possible to that of normal whole blood but without potentially interfering substances in the plasma. Our results, therefore, cannot be compared quantitatively with those of White and Rothstein, but qualitatively they agree quite well. The initial rapid binding of MMC to the erythrocytes was reported by these authors as well as by others *in vitro* [2–4] and *in vivo* [7, 8]. This is ascribed mainly to the very high concentration of SH-groups in the erythrocyte which is reported to be between 8 μmoles/ml PCV 79] and 20 μmoles/ml PCV [5] for human erythrocytes. As the MMC concentration in our experiments was 0.04 μmoles/ml PCV there should have been a large excess of SH-groups in the red blood cells. A small amount of the MMC, however, remains outside the cells, and this appears to be in equilibrium with the intracellular Hg. The transfer of MMC from the blood to the organs and vice versa seems

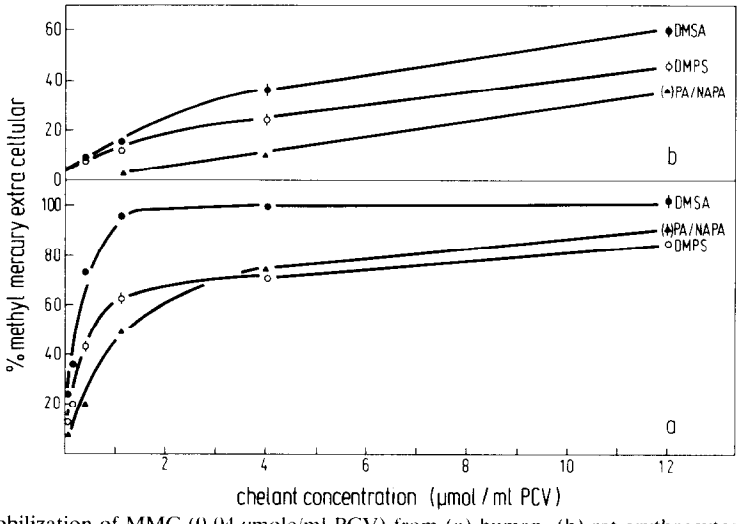

Fig. 3. Mobilization of MMC (0.04 μmole/ml PCV) from (a) human, (b) rat erythrocytes with varying chelant concentration 5 min after addition of the chelant. The chelants were added 30 min after MMC. Control values were not substracted. Mean values of 6 experiments ± S.E.

to be based on the possible disturbance of this equilibrium [10]. An efflux of Hg can be caused by the addition of SH-compounds to the extracellular fluid, as was shown by White and Rothstein [1], Giblin [2] and in this paper. The amount of Hg released would be expected to depend on the extracellular SH-concentration and the affinity of the added chelating agent for MMC. As shown in Fig. 3(a), only DMSA is able to remove all the MMC from human erythrocytes, the affinity of the other compounds apparently being smaller. Some of the MMC extracted by all the chelating agents tested is lost again gradually from the extracellular fluid. However, as Fig. 2 suggests, the underlying mechanisms may be different:

PA and NAPA. At low concentrations 5–12 per cent of the MMC was mobilized from the erythrocytes, but almost none of this was lost again during the observation period indicating that the complex between the mercurial and these substances, once formed, is quite stable. The loss of MMC from the extracellular fluid at higher PA or NAPA concentrations points to a damage of the cell wall which then permits diffusion of the complex into the cell. This is corroborated by the finding that haemolysis occurs soon after the addition of the highest dose of PA or NAPA, respectively.

DMSA. At low doses only part of the mobilized MMC is lost gradually. The reason may be that there are two binding sites on the DMSA-molecule of differing affinity for MMC.

DMPS. The loss of MMC from the extracellular fluid after mobilization by this chelating agent, at all concentrations, seems to reflect two superimposed effects: first, a dissociation of the mercurial from DMPS and back diffusion into the cell, and secondly, as shown by the study with the ^{14}C-labelled DMPS, an associaton of the whole complex with the erythrocyte. The order of effectiveness of the chelating agents for the removal of MMC from human and rat erythrocytes is the same. Recent experiments [15] have shown that in rats this is also true for the mobilization of MMC *in vivo*. We believe, therefore, that the *in vitro* system described in this paper could possibly serve as a test system to compare the effectiveness of chelating agents for the decorporation of MMC from the human body.

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Phagocytic activity of the reticulo-endothelial system in the rat and rates of *in vivo* excretion of metabolites of carbaryl and *in vitro* microsomal metabolism

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The role of the reticuloendothelial system (RES) in the toxicity and metabolism of xenobiotics is not well known. However, it has been shown that depending on the functional activity of the macrophages of the RES the toxicity of benzylpenicillin can be modified, RES inhibition increasing it and RES activation decreasing it [1]. In addition the RES is involved in the metabolism of sulfonamides [2]. For our part, we have previously observed that inhibition of the RES increases the duration of the anticholinesterase toxic effect of a carbamate pesticide, carbaryl [3], widely used as an insecticide in agriculture and veterinary medicine. Moreover, the administration of colloidal carbon decreases both the phagocytic activity of the RES and the blood clearance of carbaryl, whereas the administration of glyceryl trioleate increases phagocytic activity as well as the clearance of carbaryl [4].

From these data we wondered that if, parallel to the activation and inhibition of the RES, there were no perturbations in the metabolism of carbaryl. We measured the biliary, urinary and pulmonary excretions of ^{14}C-labelled metabolites *in vivo* after administration of [^{14}C]carbaryl in rats with the RES inhibited by colloidal carbon [5, 6] or activated by glyceryl trioleate [7], compared to control animals. In addition we evaluated in animals having undergone the same treatment, the *in vitro* *N*-demethylation of carbaryl and three other mixed function oxidase activities since it is known that hepatic drug-metabolizing enzymes metabolise, for the most part, this pesticide [8, 9].

Materials and Methods. [^{14}C]carbaryl (1-naphthyl *N*-[^{14}C]methylcarbamate) was prepared by reaction of freshly distilled naphthyl chloroformate with [^{14}C]methylamine, HCl (41.2 mCi/mmole) (CEA, Saclay, France) (10) and