On the Mechanism of Mercury-Induced Hemolysis

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The cytotoxic effect of mercury has been the subject of numerous investigations (Vallee and Ulmer 1972; Passow et al. 1961; Gatti et al. 1979). Nevertheless, the sequence of biological events leading to cell death has not been established. Detailed investigation on mercury toxicity should be carried out in biological systems in which the components and the functions are well described. The erythrocyte is an excellent subject for such studies, since it is both structurally simple and easily obtained. It has the additional advantage that it contains no internal membranes to complicate interpretations. The results obtained in this system show that mercury increases the cation permeability of red blood cell membrane and has a strong hemolytic effect (Valee and Ulmer 1972; Passow et al. 1961; Ohmiya and Koga 1977).

Our previous experiments have shown that mercury-induced hemolysis is associated with the development of peroxidative processes in erythrocyte membranes (Ribarov and Benov 1981). The level of the malondialdehyde products rises
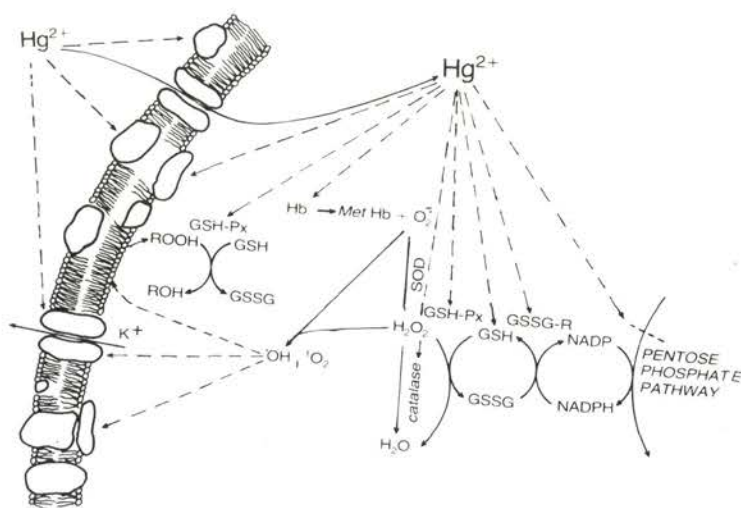

Fig. 1. Illustration of the key steps in mercury interactions with the erythrocyte.

before hemolysis occurs; this suggests that the development of peroxidative processes precedes hemolysis rather than results from it. However, we have found that Hg^{2+} has no prooxidant catalytic activity with respect to lipid peroxidation (Ribarov et al. 1982b), i.e. that mercury is not able to initiate peroxidation by direct action on the membrane lipids. It may, therefore, be concluded that an indirect mechanism of initiation of lipid peroxidation by mercury is very likely.

Recently, we found (Ribarov and Benchev 1982) that Hg^{2+} binds with high affinity to the major cytosolic components — hemoglobin and enzymes, and to the membrane proteins (Fig. 1). It was found, that mercury significantly inhibits the enzyme systems protecting erythrocyte from oxidative damage and decreases the reduced glutathione level (Ribarov et al. 1982a). Furthermore, mercury affects erythrocyte metabolism by inactivating the rate-determining enzymes (Ribarov, Benov and Benchev, unpublished data).

The inhibition of enzymes protecting the red blood cell from oxidative damage creates conditions for the development of peroxidation, but it seems unlikely that the peroxidation might be due to the mercury interaction with enzymes only. As it was mentioned, Hg^{2+} binds with high affinity to hemoglobin molecules. On the other hand, some metal ions are known to potentiate hemoglobin autoxidation by a mechanism involving superoxide production (Winterbourn et al. 1976; Ribarov et al. 1981). It may therefore be assumed that the mercury-hemoglobin interaction may also cause superoxide production, thus initiating a peroxidative destruction process in mercury-treated erythrocytes. Indeed, we have found that mercury stimulates hemoglobin-catalyzed peroxidation of model phospholipid membranes (Ribarov et al. 1982b). The inhibition of this effect by superoxide dismutase and catalase suggests that superoxide radical and hydrogen peroxide are involved. Also, we demonstrated that the mercury-induced hemoglobin autoxidation is the source of superoxide in this system. The superoxide radical can dismutate spontaneously to hydrogen peroxide. Numerous studies have suggested that superoxide radical and hydrogen peroxide can cooperate in the generation of species more potent in causing lipid peroxidation (Kellog and Fridovich 1977; Czapski and Ilan 1978).

In a normall cell, the superoxide radical will be broken down by superoxide dismutase, and the hydrogen peroxide by glutathione peroxidase and catalase. Any superoxide radical that bypasses this mechanism should react with other cell constituents possibly causing irreversible cell damage. In the erythrocyte which is unable to replace damaged constituents by resynthesis, the escape of even a very small amount of superoxide radical may have deleterious effects and may in fact lead to cell death. However, this mechanism is likely to become more significant if superoxide is produced in abnormally high amounts or if any of the protective systems become damaged.

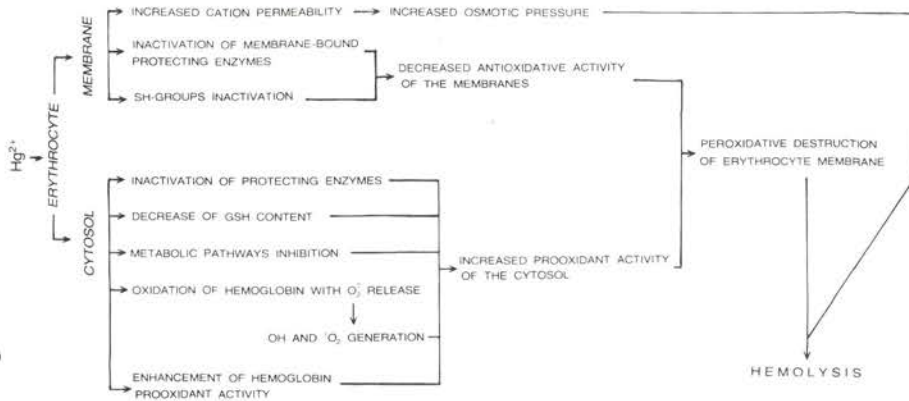Peroxidation produces further free radicals and other reactive compounds and

Fig. 2. Possible mechanism pathways of the mercury hemolytic action

it is conceivable that such compounds might result in further denaturation of hemoglobin and damage to red cell membranes. It has been demonstrated that lipid peroxidation products of a relatively stable nature are capable of hemolyzing the native erythrocyte (Kellog and Fridovich 1977; Goldstein et al. 1980).

Obviously, mercury induces a complex of reactions leading to cell damage and eventually to cell death (Fig. 2). On the time-scale the sequence of events must always proceed from the outside of the cell, towards its center. First, Hg^{2+} reacts with the cell, surface resulting in an increased cation permeability, inactivation of membrane-bound enzymes, inactivation of sulfhydryl groups etc. As the metal penetrates into the cell additional effects take place: inactivation of enzymes protecting the erythrocyte from oxidative damage, decrease of reduced glutathione content, inhibition of metabolic pathways, increased hemoglobin autoxidation with superoxide release, enhancement of hemoglobin prooxidant activity, etc. All these events summarize in three final effects: increased osmotic pressure within the cell, decreased antioxidant activity of the membranes, and increased prooxidant activity of the cytosol. The two latters lead to peroxidative destruction of the red blood cell membrane, which combines with the increased osmotic pressure to cause membrane rupture and hemoglobin release, i.e. hemolysis.

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