IN VITRO MERCURY UPTAKE BY HYPOCATALASEMIC AND ACATALASEMIC MOUSE HEMOLYSATES AND HUMAN ACATALASEMIC HEMOLYSATES

Oxidation of elemental mercury produces the mercurous ion, $\text{Hg}^+$, and the mercuric ion, $\text{Hg}^{++}$. In biological fluids, elemental mercury is considered to be oxidized to the mercuric ion\(^1\).

Clarkson et al.\(^2\) demonstrated that elemental mercury was rapidly oxidized by human blood \textit{in vitro}. Kudsk\(^3\) found that ethyl alcohol inhibited the uptake of mercury by blood \textit{in vitro} and \textit{in vivo}. These findings raise the question of whether primary catalase-hydrogen peroxide complex is involved in the oxidation of mercury, due to its high affinity for these alcohols. Magos, Sugata and Clarkson\(^4\) reported that red blood cells preincubated with 3-amino-1,2,4-triazole in the presence of methylene blue, caused a decrease in catalase activity and in mercury uptake in air saturated with mercury vapor.

In order to confirm the function of catalase in the uptake of mercury, experiments on blood deficient in catalase activity are required. Acatalasemia is a rare congenital abnormality, first described by Takahara and Miyamoto in 1948\(^5\) and characterized by trace amounts of catalase activity\(^6\).

Radiation-induced hypocatalasemic and acatalasemic mice were reported by Feinstein et al.\(^7\).

The authors\(^8\) reported that erythrocytes, lung and liver homogenates prepared from acatalasemic mice exhibited an \textit{in vitro} decrease in their ability for mercury uptake from air saturated with mercury vapor as compared with normal mice, and acatalasemic mice had decreased levels of mercury in the lungs and blood, after exposure to elemental mercury. They also reported\(^9\) that as regards their ability for \textit{in vitro} mercury uptake from air saturated with mercury, human acatalasemic erythrocytes had only 0.01 to 0.06 times the uptake found in normal erythrocytes with hydrogen peroxide, and 0.06 to 0.24 times the uptake without hydrogen peroxide. However, the authors have not yet reported results with hypocatalasemia which has less catalase activity.

The present experiment concerns the relationship between catalase activity and uptake of mercury using normal, hypocatalasemic and acatalasemic hemolysates of mice and also the effects of catalase-inhibitor on the uptake of metallic mercury.

\textit{Blood specimens}: Radiation-induced hypocatalasemic (homozygotes) and acatalasemic (homozygotes) mice weighing 20–25 g (male) were obtained by courtesy of Feinstein, and Japanese human acatalasemic bloods were obtained by courtesy of Dr. Takahara. Human blood samples were taken from the cubital vein and mouse blood specimens from the orbital veins of normal, hypocatalasemic and acatalasemic mice, using glass capillaries coated with heparin after anesthesia with ethyl ether\(^8\). Hemolysates were prepared by the same procedure as described previously\(^9\), except that the hemoglobin concentration was adjusted to 0.50 g/dl in the mouse and 1.25 g/dl in the human hemolysates.
Pre-incubation of hemolysate with inhibitor: 3-Amino-1,2,4-triazole was preincubated with 4 mM ascorbic acid for 1 hour at 37°C, and potassium cyanide and sodium azide for 1 hour at 37°C.

Incubation experiment: Three ml of the hemolysate was placed in the main chamber of a 15-ml Warburg flask with 0.1 ml of metallic mercury in the side arm and with 0.1 ml of 3% hydrogen peroxide in the center well. The incubation was conducted at 37°C for 3 hours with shaking at 80 cycles/min.

Measurements: After the 3-hour, incubation, a sample (0.1–1.0 ml) was pipetted out from the hemolysate in the Warburg flask and digested with 5 ml of 6% KMnO₄ and 10 ml of 10 N H₂SO₄ by heating at 80°C for 1 hour. After removal of excess of KMnO₄ by adding several drops of aqueous 10% NH₂OH·HCl, the total mercury was reduced to elementary mercury with 2 ml of 10% stannous chloride solution in a total volume of 100 ml. Determination of the mercury count was carried out using an elemental mercury analyzer (Hitachi 207) with circulating air containing mercury vapor. The

![Fig. 1. Mercury uptake in vitro of hemolysate of normal, hypocatalasemic and acatalasemic mice, and the effect of 1 mM potassium cyanide.](image-url)
MERCURY UPTAKE BY BLOOD CATALASE

catalase activity estimation was performed by the perborate method\(^1\) and expressed as perborate units (PU). The hemoglobin (Hb) concentration was estimated with Drabkin cyanomethemoglobin reagent.

**Blood catalase activity:** The red blood catalase activity of the mice was \(937.0 \pm 60.0\) PU/gHb (mean \(\pm\) S.D.) in normals, \(150.0 \pm 33.3\) PU/gHb in hypocatalasemia and \(43.3 \pm 13.3\) PU/gHb in acatalasemia. That of humans was \(3220.0 \pm 70.0\) PU/gHb in normals and \(4.6\) PU/gHb in acatalasemia.

As shown in Fig. 1, as regards their ability for in vitro mercury uptake from air saturated with mercury vapor, mouse hypocatalasemic hemolysate had 0.40 times and mouse acatalasemic hemolysate only 0.03 times the uptake found in normal hemolysate with hydrogen peroxide. One mM potassium cyanide inhibited the uptake of mercury with hydrogen peroxide, and the inhibition ratios for normals, hypocatalasemia, and acatalasemia were 0.62, 0.82, and 0.41, respectively. As shown in Table 1, human acatalasemic hemolysate had only 0.02 times the uptake found in normal hemolysate as regards the uptake from air saturated with mercury vapor. Table 1 also summarizes the results for the uptake of metallic mercury with hemolysate which was preincubated with 3-amino-1,2,4-triazole at concentrations of from 5 to 20 mM, potassium cyanide at from 5 to 20 mM and natrium azide at from 0.5 to 2.0 mM. With increasing concentration of the above inhibitors, progressive inhibition of the mercury vapor uptake occurred up to a maximum inhibition of about 0.64 in 3-amino-1,2,4-triazole and ascorbic acid, 0.87 in

| Table 1. Effects of aminotriazole (AT), potassium cyanide (KCN) and azide (NaN\(_3\)) on the uptake of mercury in normal hemolysate and the uptake in acatalaseemic hemolysate. (Asc. A. = ascorbic acid) |
|---|---|---|---|
| **Addition** | **Inhibitor** | **4 mM Asc. A.** | **Hg uptake (ng/mg Hb)** | **Ratio of inhibition** |
| | | | | |
| Normal | 0 mM | + | 236.9 | 0.22 |
| | 5 | + | 195.4 | 0.36 |
| | 10 | + | 177.5 | 0.42 |
| | 20 | + | 110.2 | 0.64 |
| Hypocatalasemia | 5 mM | − | 55.2 | 0.82 |
| | 10 | − | 38.1 | 0.87 |
| | 20 | − | 40.2 | 0.87 |
| Acatalasemia | 0.5 mM | − | 169.1 | 0.44 |
| | 1.0 | − | 87.5 | 0.71 |
| | 2.0 | − | 82.4 | 0.73 |
| Acatalasemic | 5.4 | 0.98 |
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potassium cyanide and 0.73 in natrium azide.

The present study demonstrates a correlation between the catalase activity in hemolysates and the grade of uptake of metallic mercury, and the effect of catalase inhibitors in the reduced uptake of metallic mercury with human hemolysate. These findings indicate the participation of catalase related to its role in the degradation of H$_2$O$_2$ in the uptake of metallic mercury. This also suggests that the catalase in erythrocytes protects to some extent the transfer of metallic mercury to the brain through the blood-brain barrier$^{12}$.

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REFERENCES


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