Oxidation and Uptake of Metallic Mercury *In Vitro* by the Blood of Normal and Acatalasemic Mice

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(Received September 30, 1986)

ABSTRACT: The total concentration of mercury in the blood of normal mice exposed to metallic mercury vapor with hydrogen peroxide was significantly higher than that of acatalasemic mice. Metallic mercury in the blood of acatalasemic mice was significantly higher than that of normal mice. These data suggest that catalase play an important role in the oxidation of metallic mercury to mercuric ion. The concentration of metallic mercury in the red blood cell (RBC) of normal and acatalasemic mice tended to decrease with increased time of incubation. On the other hand, the concentration of metallic mercury in the serum of normal and acatalasemic mice increased with increased time of incubation. The result indicate that the lipid in the serum was related to the uptake of metallic mercury.

INTRODUCTION

The first oxidation of metallic mercury to mercuric ion inhaled, in the living-body, is considered that catalase in the lungs and blood play an important role. Ogata et al[1,2] demonstrated that the importance of catalase in the oxidation of metallic mercury *in vitro* using erythrocytes of acatalasemic mice and Japanese acatalasemic patients. Kudsk[3] demonstrated that ethyl alcohol depressed the pulmonary absorption of metallic mercury vapor. Kudsk[4] also repored that the inhibition of mercury uptake in the blood by ethyl alcohol might be related to the oxidation of mercury in erythrocytes by producing the primary hydrogen peroxide-catalase complex. Cohen et al[5] indicate that the

complex has a high affintiy with ethyl alcohol. Ogata et al[6] reported that the level of metallic mercury in the blood of acatalasemic mice after intraperitoneal injection of metallic mercury *in vivo* was significantly higher than that of normal mice.

The present report deals with the relationship between the concentration of metallic mercury and mercuric ion in red plood cell and serum of normal and acatalasemic mice exposed to metallic mercury vapor *in vitro*.

MATERIALS AND METHODS

The blood samples of normal and acatalasemic mice were taken from the orbital vein. Mercuric chloride ($^{203}HgCl_2$; specific activity = 0.52 mCi/mg) was purchased from New England Nuclear, Boston, M.A., USA.

The blood samples were placed in the main chamber of 15 ml Warburg flask. Metallic mercury vapor generated by adding a stannous chloride solution to an aqueous solution of mercuric chloride in the side arm and with or without 100 μl of 3 % hydrogen peroxide in the center well. Incubation was conducted at 37 ℃ for 90 minutes, while shaking at 40 cycles /min. After incubation, the blood samples were added to the mixture solution of 3 %-EtOH-heparin-sillcon and were bubbled with nitrogen gas for 2 minutes for removing metallic mercury in the blood. Metallic mercury was trapped with 6% $KMnO_4$-5N H_2SO_4 mixture solution. The concentration of metallic mercury and mercuric ion were determined by Multi-Mode Scaler scintillation counter (Aloka Co., TDC-601).

RESULTS AND DISCUSSION

Comparison with the concentration of mercry in the blood of mormal and acatalasemic mice

The concentration of metallic mercury and mercuric ion in the blood of normal and acatalasemic mice exposed to metallic mercury vapor with or without hydrogen peroxide for 30 minutes *in vitro* are shown in Table 1. The concentration of mercuric ion in the blood of normal mice with hydrogen peroxide was significantly higher than that of acatalasemic mice. On the other hand, the concentration of metallic mercury in the blood of acatalasemic

Table 1. Concentration of metallic mercury and total mercury in the blood of normal and acatalasemic mice with or without hydrogen peroxide.

Species	H_2O_2 (3%)	Metallic mercury (μCi/ml,m ± SD)	Total mercury* (μCi/ml,m ± SD)	Hg^0/Total * (%)	Test** (N-A)
Normal	+	0.0041 ± 0.0005	0.0544 ± 0.0019	7.7	+
	-	0.0094 ± 0.0003	0.0454 ± 0.0012	20.4	+
Acatalasemia	+	0.0106 ± 0.0010	0.0280 ± 0.0009	38.6	+
	-	0.0128 ± 0.0006	0.0300 ± 0.0007	43.5	+

* Total mercury: Hg^0+Hg^{2+}

** Level of significance for difference between normal and acatalasemic mice, using t-test[7] or Welchs test[8].

mice with hydrogen peroxide was significantly higher than that of normal mice. The result was similar to that in the blood of normal and acatalasemic mice exposed to metallic mercury vapor *in vivo* as described previously[6]. The total concentration of mercury in the blood of normal mice without hydrogen peroxide was higher than that of acatalasemic mice. The data suggest that the differences of catalase activity in the blood between normal and acatalasemic mice were related to the oxidtion of metallic mercury. The ratio of metallic mercury to total concentration of mercury in the blood of acatalasemic mice was extremely higher than that of normal mice.

Relationship between mercury uptake and incubation time

The concentration of metallic mercury and mercuric ion in the blood of normal and acatalasemic mice exposed to metallic mercury vapor without hydrogen peroxide are shown in Fig. 1 . The concentration of mercuric ion in the blood of normal and acatalasemic mice tended to increase with increased time of incubation. However, the concentration of metallic mercury in the blood of acatalasemic mice was higher than that of normal mice. The data suggest that catalase in the blood play an important role in the oxidation of metallic mercury to mercuric ion.

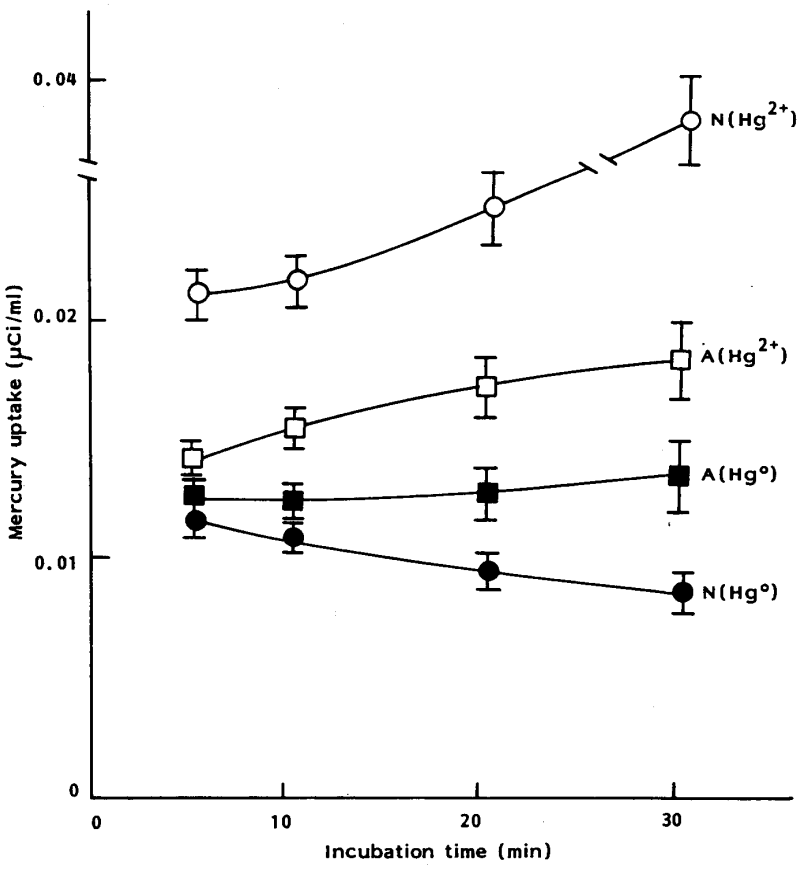

Fig. 1. Concentration of metallic mercury and mercuric ion in the blood of normal and acatalasemic mice.

Concentration of metallic mercury and mercuric ion in RBC and serum

In order to investigate in more detail of mercury concentration in the blood of normal and acatalasemic mice, the blood of both mice separated to red blood cell (RBC) and serum by centrifugation. The concentration of metallic mercury and mercuric ion in RBC and serum of normal mice exposed to metallic mercury vapor *in vitro* is shown in Fig. 2. The concentration of mercuric ion in RBC of normal mice tended to increase with increased time of incubation and in serum it decreased reversibly. On the other hand, the concentation of metallic mercury in RBC and serum of normal mice tended to decrease with increased time of incubation.

The concentration of metallic mercury and mercuric ion in RBC and serum of acatasasemic mice increased gradually with increased time of incubation. The result is shown is Fig. 3. The concentration of metallic mercury

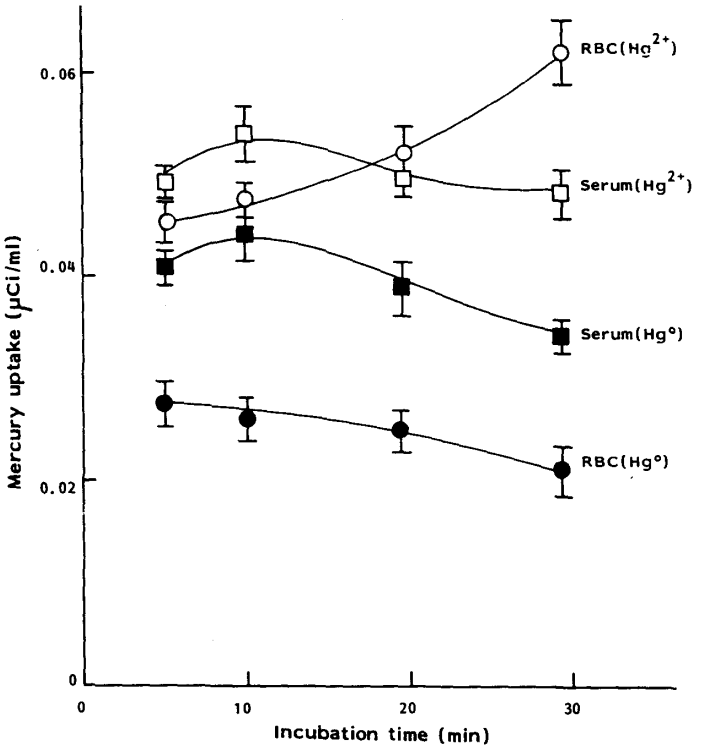

Fig. 2. Concentration of metallic mercury and mercuric ion in RBC or serum of normal mice.

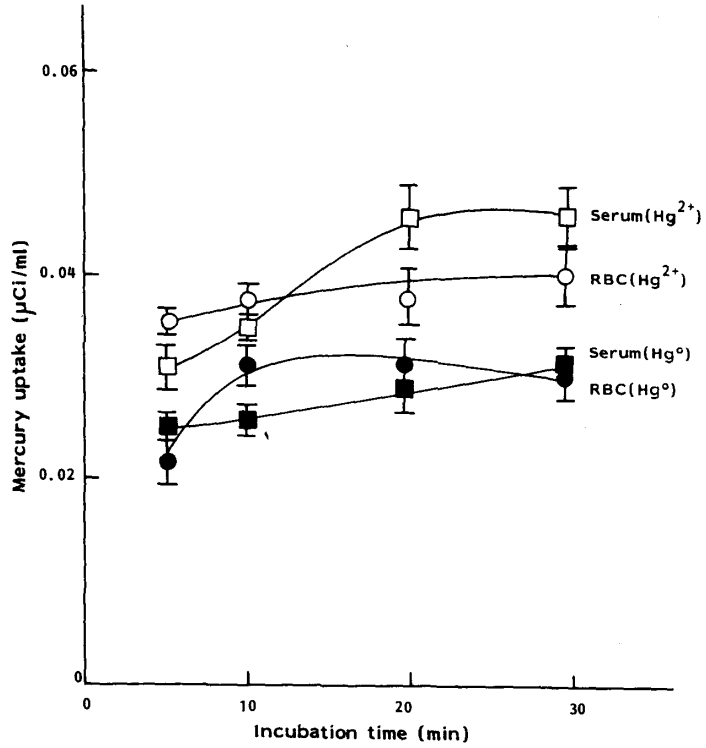

Fig. 3. Concentration of metallic mercury and mercuric ion in RBC or serum of acatalasemic mice.

in RBC of acatalasemic mice incubated for 30 minutes was significantly higher than that of normal mice. The data suggest that the oxidation of metallic mercury by catalase in RBC of acatalasemic mice is less than that of normal mice. The oxidation of metallic mercury by RBC is not solely due to the catalase activity, but also to the methemoglobin-hydrogen peroxide compounds and glutathione peroxidase in RBC.

Acknowledgement: I'm indebted to prof. M. Ogata, Department of Public Health, Okayama University Medical School, for his advice in preparing the manuscript.

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