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Changes of Some Properties of Blood of Rats administered with Methylmercuric Chloride

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Methylmercuric chloride (MMC, CH_3HgCl) was adminsitered to male Wistar rats weighing 150—160 g by subcutaneous injection in a daily dose of 1 mg or 0.4 mg per 100 g body weight to produce an acute (for 12 days, 20 rats) or a subacute intoxication (for 28 days, 20 rats). Hematocrit value decreased from 37% (control group) to 27% (MMC administered groups) with decreasing body weight.

About 90% of total mercury in the blood (*ca.* 95 μg Hg/ml) of rats of the two MMC-treated groups was distributed in the erythrocytes. The shapes of erythrocytes (acute and subacute intoxications) became the spicule cells like acanthocyte. The osmotic fragility of erythrocytes of rats with acute intoxication clearly decreased, but oxygen capacity per ml of the erythrocytes did not change.

Then, the properties of the erythrocytes incubated with various concentrations (44, 72, 170 μg Hg/ml) of MMC for 60 min at 37° were studied. Spicule cells like echinocyte II increased with increasing mercury amount into erythrocytes. However, the osmotic fragility and oxygen capacity per ml of the erythrocytes were unchanged.

Keywords——methylmercuric chloride; morphology of erythrocytes; osmotic fragility of erythrocytes; oxygen capacity of erythrocytes; erythrocytes; mercury incorporation into blood

Many reports were published on the toxicity of organic and inorganic mercury compounds for human and higher animals,[2,3] on their uptake,[4] metabolism,[5–7] accumulation in blood and other tissues,[8] and excretion patterns,[8] binding with SH groups of hemoglobin with alkylmercury compounds,[9] and morphological changes of human erythrocytes treated with *p*-chloromercuribenzoate.[10]

The authors investigated on the changes of shapes, oxygen capacity, osmotic fragility of erythrocytes of rats administered with methylmercuric chloride (MMC, CH_3HgCl), and compared them with those of erythrocytes incubated with MMC *in vitro*. The results in detail are communicated in this paper.

Materials and Methods

MMC——MMC purchased from Wakō Chemicals was recrystallized in pure ethyl alcohol (mp. 173°)[11,12] and used in the experiments.

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Animal Experiments——MMC dissolved in physiological saline solution (2.75 mg/ml) was administered by subcutaneous injection in a daily dose of 1 mg MMC per 100 g body weight to produce an acute intoxication, and of 0.4 mg MMC per 100 g body weight to produce a subacute intoxication to male Wistar rats, weighing 150—160 g. Blood of rats which had conspicuous neurological manifestations such as ataxia, convulsion after administration of MMC was immediately heparinized, and used for the experiments. On the other hand, rats injected physiological saline solution were used as control rats.

Scanning Electron Microscopic Observations of Blood[13]——One ml of blood tested was added to 5.0 ml of 0.1 M phosphate buffer (pH 7.4) containing 0.9% glutaraldehyde for fixation. The suspension was centrifuged, and the pellet was washed. The erythrocytes were coated with carbon and gold in a vacuum chamber, the specimen thus prepared were subjected to observations under scanning electron microscope, the JSM-SI instrument of the Japan Electron Optic Laboratory Co, Ltd., with accelerating voltage of 10 kV.

Centrifugation of Blood——The blood was separated to the plasma and erythrocytes by centrifugation at $1000 \times g$. The value of hematocrit of the blood tested was obtained by centrifugation using Kubota Hematocrit (KH-120 A).

Determination of Mercury Amount in Blood[14]——Blood of rat (0.2 ml) was homogenized with the same volume of 0.1 M phosphate buffer (pH 7.4) by Polytron (Kinematica, Switzerland), and the homogenate thus obtained (0.2 ml) was added to a mixture of 20 ml of 20% NaOH, 10 ml of 10% $NH_2OH \cdot HCl$, 1 ml of 10% $CuSO_4 \cdot 5H_2O$ and 5 ml of 10% $SnCl_2 \cdot 2H_2O$ dissolved in 0.5 N H_2SO_4, and put into the mercurial reduction apparatus. After organic and inorganic mercury compounds in the homogenate were reduced to free metal mercury, the vapour of mercury was introduced into the absorption cuvette, and the mercury amount was measured from the absorption of 253.7 nm by atomic absorption spectrophotometer (Model 303, Hitachi Perkin-Elmer Co, Ltd.).

Determination of Oxygen Capacity of Erythrocytes[15,16]——Oxygen capacity of 20 μl of rat erythrocyte fraction was determined in 1 ml-vial[17] in 2—3 min by our method using oxygen electrode (Model 777, Beckman Instruments Inc.). Potassium ferricyanide was used for release of oxygen from oxyhemoglobin.

Test for Osmotic Fragility of Erythrocytes[13]——To 4.0 ml of 10 mM phosphate buffer (pH 7.0) with varying NaCl concentration (20—120 mM) was added 0.2 ml of washed erythrocyte suspension (5% hematocrit) and the mixture was incubated at 37° for 5 min. After centrifugation at $2000 \times g$ for 5 min, absorbance at 543 nm of the supernatant was determined as a measure for the extent of hemolysis and the percentage of the absorbance of the supernatant from complete hemolysis was expressed as the hemolysis percentage.

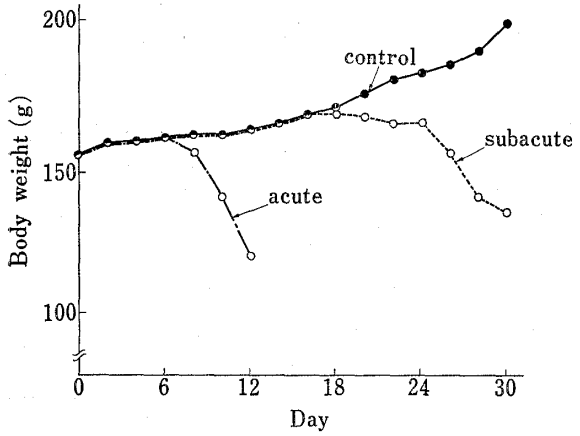

Fig. 1. Changes of Body Weight of Rats administered with CH_3HgCl

—●— : control (10 rats)
—○—: acute intoxication group (1 mg/100 g body weight/day, 20 rats)
---○---: subacute intoxication group (0.4 mg/100 g body weight/day, 20 rats)
Data for each point represent the mean of samples.

Results

I. Changes of Body Weight of Rats after Administration of MMC

Figure 1 shows changes of body weight of 10 rats (control group) and of 2 groups of rats after administration of different amounts of MMC; group (20 rats) of acute intoxication (1 mg/100 g body weight/day for 12 days), group (20 rats) of subacute intoxication (0.4 mg/100 g body weight/day for 30 days). The control group reached to *ca.* 200 g at the end of experiment on the 30th day. In the group of acute intoxication, the body weight increased as well as control group in the first 6 days, but began to decrease on the 7th day, and reached to 120 g on the 12th day. The body weight of rats with subacute intoxication increased, then gradually decreased,

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finally reached to 135 g on the 30th day. These two MMC treated groups showed some typical neurological symptoms such as ataxia, convulsion, crossing of hindlegs with decreasing body weight at the end of the experiments. These symptoms were almost the same as those observed on the rats administered with methyl methylmercuric sulfide (CH_3HgSCH_3) as reported by Takeshita and Uchida.[18]

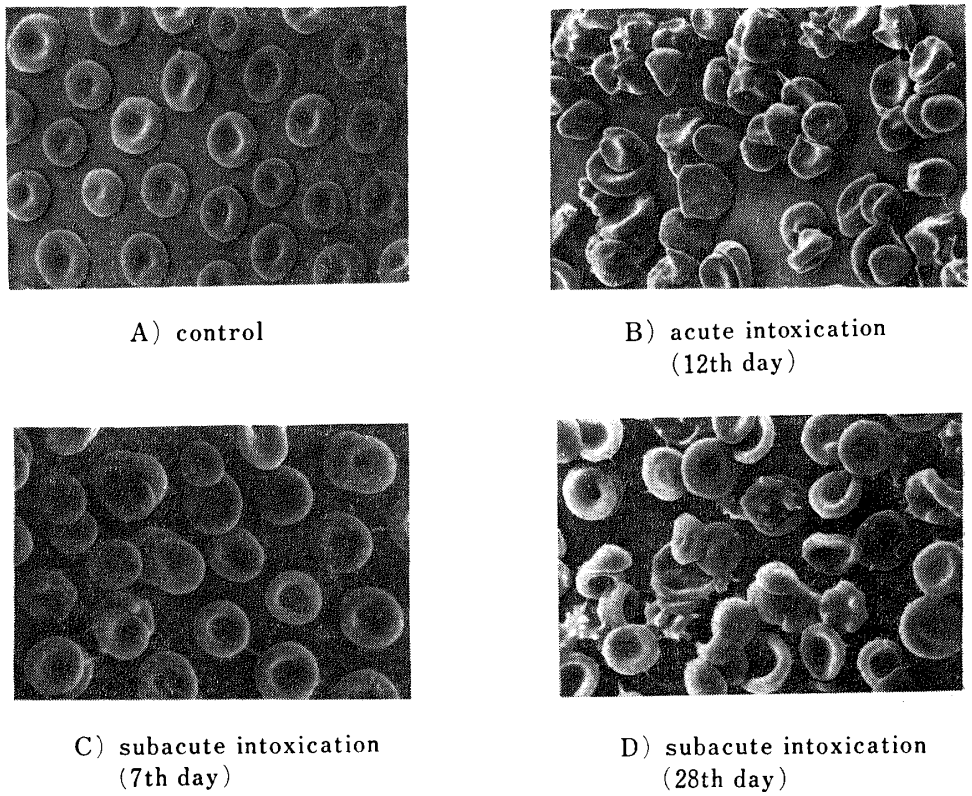
II. Changes of Some Properties of Blood of Rats after Administration of MMC

1. Changes of Hematocrit Values——As shown in Table I, both hematocrit values of the blood of rats with acute intoxication and that with subacute intoxication decreased from

Table I. Mercury Distribution and Properties of Blood from Rats administered with CH_3HgCl

	Control	Acute intoxication[a]	Subacute intoxication[b]			
Day of determination		12th	7th	14th	21st	28th
Hematocrit (%)	37	27	34	31	30	27
Mercury (μg/ml of blood)	0.5	95.3	26.7	45.2	69.6	94.3
Plasma (%)	20	5.2	7.9	3.1	4.7	5.0
Erythrocytes (%)	80	94.8	92.1	96.9	95.3	95.0
Oxygen capacity (μl O_2/ml erythrocytes)	280	279	280	281	280	279

a) for acute intoxication: 1 mg/100 g body weight/day
b) for subacute intoxication: 0.4 mg/100 g body weight/day
Each value represents the mean of samples (n=5).



A) control
B) acute intoxication (12th day)
C) subacute intoxication (7th day)
D) subacute intoxication (28th day)

Fig. 2. Changes in Erythrocyte Morphology of Rats (Acute and Subacute Intoxications) administered with CH_3HgCl

18) A. Takeshita and M. Uchida, *Kumamoto Medical J.*, **16**, 178 (1963).

37% (control group) to 27% on the 12th (acute group) and the 28th day (subacute group), respectively.

2. Changes of Shapes of Erythrocytes——Changes of shapes of erythrocytes from rats acutely intoxicated with MMC were studied on the 12th day by the scanning electron microscopy. As seen in Fig. 2A control erythrocytes of rat appeared to be smooth-surfaced, biconcave disc.[13,19] Erythrocytes of rats acutely intoxicated with MMC became characteristic shapes on the 12th day (Fig. 2B). Morphological changes of erythrocytes of rats with subacute intoxication were shown in Fig. 2C, and D. Some erythrocytes were transformed into the shapes with uneven surface on the 7th day, and the abnormal erythrocytes further increased with the lapse of administration period of MMC. Spicule cells like "acanthocyte"[20] were also seen in Fig. 2B, C, and D.

3. Incorporation of Mercury into Blood——As a large morphological change was observed in the erythrocytes of the MMC administered group, incorporation of mercury into the plasma and erythrocytes was studied. As shown in Table I, 95.3, and 94.3 μg of mercury were found in the blood of rats with acute (12th day) and subacute (28th day) intoxications respectively, and about 95% of the mercury in the blood was found in the erythrocytes.

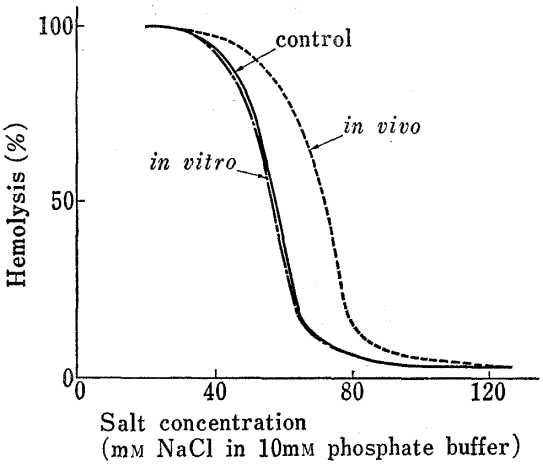


Fig. 3. Changes in Osmotic Fragility of Erythrocytes of Rats administered with CH_3HgCl

——: control
– – –: CH_3HgCl (*in vivo*) (Hg 90 μg/ml erythrocytes)
——: CH_3HgCl (*in vitro*) (Hg 90 μg/ml erythrocytes)

4. Oxygen Capacity of Erythrocytes——Oxygen capacity of erythrocytes in the blood of experimental rats (acute and subacute intoxications) was also estimated. Even when a considerable amount of mercury was incorporated in the erythrocytes, oxygen capacity per ml of erythrocytes did not change when compared with that of the control group (see Table I).

5. Changes of Osmotic Fragility of Erythrocytes——Changes of osmotic fragility of erythrocytes of rats administered with MMC (acute intoxication for 12 days) was studied. The results were shown in Fig. 3. The extent of hemolysis of erythrocytes of rats with acute intoxication was larger than that of control. From the result of this experiment, it is revealed that the membrane of erythrocytes of rat with acute intoxication became weaker than that of control.

III. Changes of Some Properties of Rat Blood after Incubation with MMC

To compare the properties of erythrocytes of rat after administration of MMC with those incubated *in vitro*, 2.0 ml each of control rat blood was mixed with 0.75 ml of the physiological saline solution containing three different concentrations of MMC (the final concentrations of mercury in the incubation mixture were 44, 72, and 170 μg of mercury/ml, respectively) and incubated at 37° for 30, and 60 min.

1. Changes of Shapes of Erythrocytes——The morphological changes of the erythrocytes of rats after 30 min incubation with MMC *in vitro* were studied. As shown in Fig. 4A, B, C, the shapes of erythrocytes became spicule cells like echinocyte II[20b] with increasing amount of MMC used. In Fig. 4D, the morphological changes of erythrocytes of the sample (170 μg

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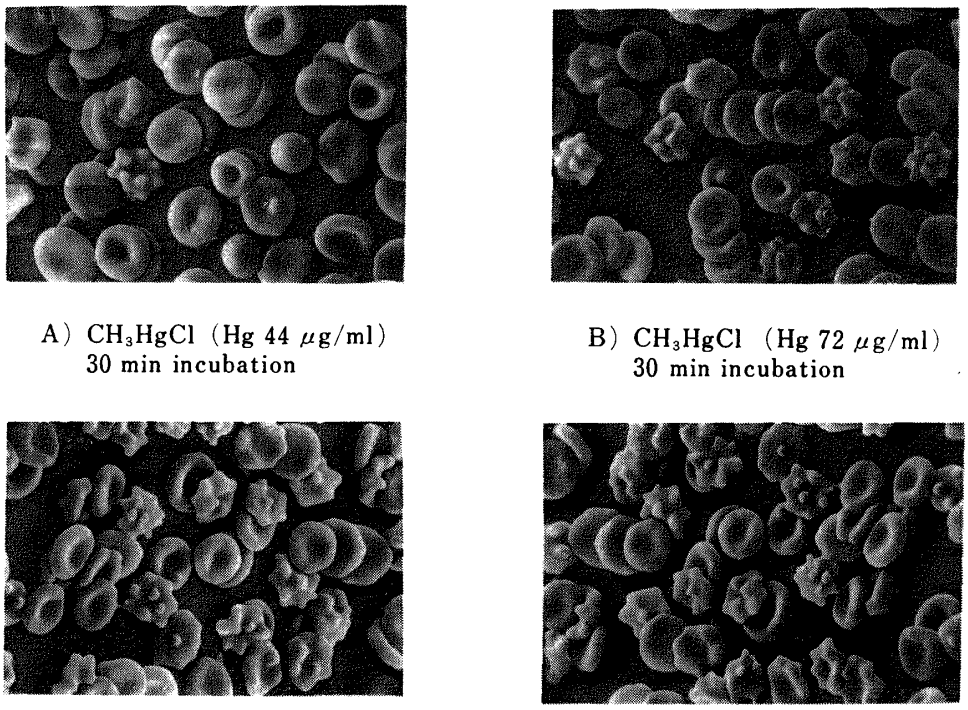

A) CH_3HgCl (Hg 44 μg/ml) 30 min incubation

B) CH_3HgCl (Hg 72 μg/ml) 30 min incubation

C) CH_3HgCl (Hg 170 μg/ml) 30 min incubation

D) CH_3HgCl (Hg 170 μg/ml) 60 min incubation)

Fig. 4. Morphological Changes in Erythrocyte incubated with CH_3HgCl

of mercury/ml) after 60 min incubation were also shown. In these samples, the spicule cells like acanthocyte as seen in the MMC group *in vivo* were not found during the incubation with MMC.

2. Incorporation of Mercury into Erythrocytes——As shown in Table II, MMC added to the blood was incorporated rapidly into the erythrocytes with increasing incubation time. When rat blood was incubated with MMC (170 μg as mercury/ml) for 60 min, 71.2% of the total mercury transferred into erythrocytes.

Table II. Distribution of Mercury into the Plasma and the Erythrocytes and Oxygen Capacity of Rat Blood incubated with CH_3HgCl

Mercury amount in blood[a] (μg/ml)		44		72		170	
		Pla	Ery	Pla	Ery	Pla	Ery
Incubation time	30 min mercury (μg)	21	23	33	39	80	90
	60 min mercury (μg)	14	30	21	51	49	121
Oxygen capacity (30, 60 min) (μl O_2/ml erythrocytes)		279		280		279	

a) hematorit: 27%
Each value represents the mean of samples ($n=5$).

3. Oxygen Capacity of Erythrocytes——Oxygen capacity of erythrocytes in the blood of rat after incubation with MMC was measured. The result showed no significant change as shown in Table II.

4. Changes of Osmotic Fragility of Erythrocytes——Changes of osmotic fragility of rat erythrocytes incubated with MMC was also studied. As seen in Fig. 3, the osmotic fragility of the erythrocytes in blood incubated with MMC (170 μg as mercury/ml) for 30 and 60 min was quite the same as that of control erythrocytes.

Discussion

The hematocrit value of the blood of rats after administration of MMC decreased from 37% (control group) to 27% at the end of the experiments of acute or subactue intoxication group as well as decrease in body weight.

In these experiments *in vivo*, about 90% of the total mercury in the blood of rats with acute or subactue intoxication was found in the erythrocytes. When rat blood was incubated with MMC *in vitro*, mercury was also rapidly incorporated into the erythrocytes (see Table I and II).

Takeda, *et al.*[8,21] also reported the results of their experiments *in vivo* and *in vitro* that ethylmercuric chloride (EMC) was rapidly transferred into the nonstromal fraction of erythrocytes through the stroma, and the EMC once combined with hemoglobin was transferred with difficulty through the stroma.

Our morphological studies by scanning electron microscopy revealed that erythrocytes of rats acutely and subacutely intoxicated became the spicule cells like acanthocyte. These abnormal cells were not observed in the erythrocytes incubated with MMC. However, erythrocytes incubated with MMC changed to the spicule cells like echinocyte II. The further investigations will be continued to study on the different localization of mercury on the membrane between the above two types of spicule cells.

Fujii, *et al.*[10] reported the incorporation of *p*-chloromercuribenzoate in the human erythrocytes resulted in the transformation of the shape into crenated spheres. However, when the human erythrocytes were incubated with N-ethylmaleimide, SH inhibitor, the membrane of erythrocytes showed some depression like pit. These and our results mean that each of SH inhibitors including MMC gives the different transformation of erythrocytes, and these differences of transformation may be attributable to the molecular size and hydrophobicity of SH inhibitors.

As described above, even when much incorporation of mercury (about 90 μg/ml of erythrocytes) into erythrocytes was demonstrated in the *in vivo* and *in vitro* experiments of MMC, capacity of oxygen per ml of erythrocytes in the blood did not change. This probably means that MMC neither interfere with the binding of oxygen to reduced hemoglobin nor with the release of oxygen from oxyhemoglobin.

Osmotic fragility of rat erythrocytes incubated with MMC were also examined, no change in the osmotic fragility of erythrocytes *in vitro* experiment was observed. The osmotic fragility of erythrocytes obtained by the acute intoxication with MMC *in vivo* clearly decreased when compared with those of control erythrocytes. Our results show that there may be some differences of distribution of mercury in the rat erythrocytes between *in vitro* and *in vivo* experiments with MMC. In 1962, Arbuthnott[22] described that hemolysis of erythrocytes by mercury compounds *in vitro* is dependent on both origin of the erythrocytes (animal species) and concentration of mercury compound used.

The anemia caused by administration of MMC to higher animals may be due to both the damage of bone marrow with mercury compound as reported by Takeuchi,[23] and the direct damage of erythrocytes (decrease of osmotic fragility) as described in our experiment. This anemia will surely result in anoxia in the peripheral tissues.

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