LETTERS TO THE EDITOR

MERCURY IN THE PLASMA AFTER SUBCUTANEOUS INJECTION OF SUBLIMATE OR MERCURIC NITRATE IN RAT

Mercury has been considered to be readily protein-bound in the plasma, since mercaptoalbumin, the fraction of serum albumin containing one free sulphydryl group, was found by *in vitro* experiment¹⁾. In reality, mercury can be found both in the red cells and the plasma protein following subcutaneous and intravenous administration of inorganic mercury compounds to experimental animals, and almost only in the red cells in case of alkyl mercury compounds^{2, 3)}.

There is also some dispute on the problem, whether or not, all the amount of mercury in the plasma are bound to the protein. The present authors reported that mercury in the plasma after subcutaneous injection of mercuric nitrate to rats was dialysable to the extent of about 50% 96 and 168 hours after injection, though only a several percentage of mercury was dialysable 48 hours after injection²⁾. The results by Berlin *et al*⁴⁾. was utterly different from ours: by *in vitro* addition of 20 μ g of Hg of sublimate to 2 ml of plasma of rabbit, less than 1% of mercury was dialysable and the percentage of dialysable mercury was the same for the plasma obtained by intravenous injection 5 hours after injection. By electrophoretic analysis on plasma added with sublimate *in vitro*, Lin⁵⁾ claimed that mercury did not bind to protein. Drevon *et al*⁶⁾., also using electrophoretic analysis, demonstrated that *in vitro* both mercuric chloride and mercuric salicylate were bound to albumin.

The present authors intended to trace the mercury in the plasma of rat injected subcutaneously with a solution of sublimate or mercuric nitrate by use of electrophoretic technique.

At first, four male albino rats of Donryu strain were subcutaneously injected with a solution of $\text{Hg}^{203}(\text{NO}_3)_2$, 5 μ C and 2.5 μ g of Hg per head. Twenty hours after injection, rats were sacrificed by heart puncture and the obtained blood was heparinized and divided into erythrocytes and plasma. Electrophoretical separation of plasma was carried out on cellulose acetate membrane with Veronal buffer, PH 8.6. After fixing and staining by a trichloroacetic acid and Ponceau 3R solution, each area of albumin and globulin was cut off and radioactivity on each area was estimated with a well-type scintillation counter.

The results are shown in Table 1. On both the areas of albumin and globulin about the same amount of radioactivity was detected. After electrophoresis on the same condition to those on plasma, inorganic mercury in an aqueous solution,

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Table 1. Hg Concentrations in the blood, erythrocytes and plasma, and distribution between albumin and globulin.

No. of rat	1	2	3	4
Hg concentration (cpm/ml)				
Erythrocytes	3142	3251	1955	3033
Plasma	3207	2822	1529	2161
Whole blood	3172	3045	1744	2645
Detected Hg on electrophoretic pattern (cpm)				
Albumin area	40	11	35	50
Globulin area	78	59	34	42

Note: $5\mu\text{C}$, $2.5\mu\text{g}$ of Hg was subcutaneously injected in an aqueous solution of $\text{Hg}(\text{NO}_3)_2$ to each rat and blood specimen was obtained by heart puncture on 20 hours after injection. The amount of plasma used for electrophoresis was about 0.04 ml.

pH 5, can be mainly found on the area of globulin, especially of beta-globulin and fibrinogen. As for the mercury detected on the albumin area, thus, it might be bound to protein, but the mercury on the globulin area was uncertain in its binding to protein.

In order to verify the binding of mercury to protein, the present author used an autoradiographical technique. Two male albino rats were subcutaneously injected with a solution of $\text{Hg}^{203}\text{Cl}_2$, $6\mu\text{C}$, $25\mu\text{g}$ of Hg per head, and the specimen of blood was harvested by cutting the tail vein on 5, 24 and 48 hours after injection. The electrophoresis of plasma was carried out as like as in the first experiment. The cellulose acetate membrane was placed on X-ray films for autoradiography (Fuji Co.), which was wrapped with a thin cellophane paper, for three weeks in a dark room.

As is shown in Fig. 1, the darkening on autoradiograph due to radioactive mercury is found clearly on the site corresponding to albumin and to less extent on the site corresponding to alpha-globulin. The darkening on the site of albumin became less in its degree on 24 hours after injection. In contrast, the darkening on the site of alpha-globulin unchanged or slightly increased.

The possibility of loss of mercury during the procedure of electrophoresis has not yet been excluded, for example, the radioactivity could not be found on the fixing and staining solution, but no estimation was done on the mercury which might vaporize during phoresis. But, as for the mercury existed on the membrane, it is probable to consider it being bound to protein. The most interesting finding is the fading of mercury only from albumin. This suggests the binding of mercury to albumin to have a nature of easy binding and easy splitting.

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Hg²⁰³Cl₂, 25 μ g as Hg, 6 μ C, subcutaneous injection

Fig. 1. Mercury in the plasma of rat after subcutaneous injection of radioactive sublimate solution.

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