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Behavior of Cadmium in Rabbit Blood

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Progressive distribution of ^{115m}Cd in the blood and the main ^{115m}Cd-binding protein in the red cells were investigated after a single intraperitoneal administration of ^{115m}Cd-Cl_2 to rabbit.

^{115m}Cd in blood decreased drastically on the 1st day after injection and increased sharply again on the 2nd day, then gradually decreased showing the regression equation of $Y=-0.019x+3.507$. The half life of ^{115m}Cd in the blood was calculated to be 158.4 days.

On the 14th day after injection of $^{115m}CdCl_2$ solution, 99.1% of the radioactivity in the whole blood was in the red cells, over 90% of the radioactivity in the red cells existing in the supernatant of stroma-free hemolysate.

From the results of gel-filtration, DEAE Sephadex column chromatography and gel-electrophoresis, it was elucidated that about 70% of the radioactivity of the stroma-free hemolysate was bound to thionein. But the ratio of the two components in metallothionein was different between the red cells and the liver.

It was also made clear that incorporation of metallothionein into red cells through cell membrane never occurred by incubating red cells with ^{115m}Cd-thionein.

Considering the observations mentioned above, it was presumed that the metallothionein in the red cells may have originated from some other organ than the liver.

Keywords——^{115m}Cd in rabbit red cell; cadmium-binding protein; ^{115m}Cd-thionein; β-counting of ^{115m}Cd; distribution of ^{115m}Cd in blood

It is well known that absorbed cadmium is transferred to the liver first, then to the kidney with lapse of time where it is accumulated for long duration.[2] According to Shaikh and Lucis,[3] ^{109}Cd in the liver of the rat subcutaneously given ^{109}Cd is distributed not to the nuclear, mitochondrial and microsomal fractions, but mainly to the soluble cytoplasmic fraction, where it forms metallothionein by combining with a soluble metal-binding protein. It is also reported that this soluble metallothionein is formed in mouse,[4,5] rat[6–8] and human[9] livers.

A study of the behavior and chemical form of cadmium in the blood may be important in tracing the fate of this metal in the body. It is reported that cadmium introduced into the blood disappears rapidly from the plasma and increases in blood cells up to 14 days after subcutaneous injection.[10] Carlson and Friberg,[11] in an early study of Cd-carrier protein in the blood, noted that almost all of the cadmium was bound to hemoglobin in the blood of rabbit

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exposed repeatedly to the metal salt. However, in the mouse repeatedly given $CdCl_2$, Nordberg, *et al.*[12] reported that the major part of cadmium in the blood was bound to a protein with the molecular weight of about 10000 which was separable from hemoglobin. Because the behavior of cadmium in the blood has not been fully investigated, a study of the progressive distribution of cadmium in the blood for 30 days after a single intraperitoneal injection of $^{115m}CdCl_2$ solution in the rabbit and of the main cadmium-binding protein in red cells is presented in this paper.

Experimental

Treatment of Animal——A solution of $^{115m}CdCl_2$ (New England Nuclear Corp.) was diluted with physiological saline to afford an isotonic solution of 43.78 μCi/0.5 mg Cd^{2+}/ml.

The solution of $^{115m}CdCl_2$ was injected once intraperitoneally in male albino rabbits weighing about 3 kg at a dose of 1 ml per kg body weight. The animals were housed in metabolic cages with food and water *ad libitum* under laboratory condition.

Distribution of ^{115m}Cd in Blood——The blood was sampled 14 times during a period of 30 days after injection. Blood was drawn through an ear vein using heparinized pipette, and about 0.1 ml each was put into two vials of known weight for measurement of weight and radioactivity. At the same time, about 0.5 ml of the blood sample was put in a polyethylene tube, separated to plasma and red cells by centrifugation at 2000 rpm for 5 min, and about 0.1 ml each of plasma and red cells poured into separate vials.

Preparation of Stroma-Free Hemolysate——On the 14th day after injection of the $^{115m}CdCl_2$ solution, a rabbit was bled to collect the blood sample. The plasma and red cells were separated by centrifugation at 2000 rpm for 5 min. The red cells were washed 3 times with cold isotonic saline.

Hemolysis was performed by adding distilled water to the red cells up to initial blood volume and allowing to stand overnight in a refrigerator. The stroma-free hemolysate was prepared by centrifugation at 15000 ***g*** for 20 min. The stroma was washed 5 times with cold isotonic saline, and the washings were combined to the stroma-free hemolysate.

Preparation of Liver-Soluble Fraction——On the 14th day after injection of the $^{115m}CdCl_2$ solution, the liver was taken out and minced in 4-fold volume of cold 0.25 M sucrose solution and homogenized in a Potter-Elvehjelm homogenizer under four strokes of the Teflon pestle driven at 1000 rpm.

A supernatant was collected by ordinary refrigerated centrifuge at 15000 ***g*** for 20 min. Preparative ultracentrifugation of supernatant mentioned above was performed by a Hitachi model 40p centrifuge at 105000 ***g*** for 60 min.

Gel Chromatography of Stroma-Free Hemolysate and Liver-Soluble Fraction——Sephadex G-75 column (2.5 × 42 cm) was equilibrated at 5° with 0.01 M Tris buffer (pH 8.6) and 3 ml each of the stroma-free hemolysate and the liver-soluble fraction was applied to the column.

Eighty fractions of effluent with 0.01 M Tris buffer (pH 8.6) were collected every 3.8 ml at a flow rate of 20 ml per hr and assayed for ^{115m}Cd contents by β-counting and for protein by determining the absorbance at 254 nm. The void volume (V_0) of the column was measured beforehand by eluting 3% blue dextran 2000 with 0.01 M Tris buffer (pH 8.6).

The combined fractions in the range from 1.6 to 2.3 of V_e/V_0 containing ^{115m}Cd were pooled and lyophilized.

Ion Exchange Chromatography of ^{115m}Cd-Binding Protein with DEAE Sephadex A-25——The ^{115m}Cd-binding protein fractions obtained from the stroma-free hemolysate and liver-soluble fractions by gel chromatography were dissolved in 3 ml of 0.01 M Tris buffer (pH 8.6) and dialyzed for 3 days against 1 l of 0.01 M Tris buffer (pH 8.6), changing the outer buffer 3 times. The content in the sac was applied to the DEAE Sephadex A-25 column (1 × 20 cm) equilibrated at 5° with 0.01 M Tris buffer (pH 8.6) and eluted with a linear gradient of 0.01 M to 0.3 M Tris buffer (pH 8.6) at a flow rate of 20 ml per hr. Forty fractions of both effluents were collected every 3.8 ml and assayed for radioactivity and for absorbance at 254 nm.

Disc Electrophoreses of ^{115m}Cd-Binding Proteins——Disc electrophoreses of ^{115m}Cd-binding protein fractions obtained by gel chromatography were carried out with 7.5% polyacrylamide gel in glycine-Tris buffer (pH 9.4) at a constant current of 2.1 mA per tube for 2 hr. Two gel-cylinders were used for every run. One cylinder was stained with amide black 10B for detection of protein band; the other was used for preparation of the segments for counting ^{115m}Cd by cutting the cylinder into 12 slices of 0.5 cm in width.

***In Vitro* Test on the Incorporation of ^{115m}Cd-Compounds to Red Cells**——0.1 ml each of isotonic $^{115m}CdCl_2$ solution (400000 cpm/ml) and a solution of ^{115m}Cd-thionein (250000 cpm/ml) isolated from rabbit liver by gel chromatography were added separately to vials containing 2 ml of healthy rabbit blood. The mixtures were incubated at 37° for 24 hr.

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The incubation mixtures were taken into heparinized capillalae 7 times in 24 hr, and each sample was centrifuged at 2000 rpm for 5 min to separate red cells and plasma. Radioactivities of both fractions were measured by β-counting.

The stroma-free hemolysates prepared from the red cells in the residual incubation mixture were fractionated by the same procedure of gel chromatography mentioned before. Next, the radioactivity of each fraction of effluents was also determined by β-counting.

Measurement of Radioactivity of ^{115m}Cd in the Samples——To about 0.1 ml of blood sample in a vial were added a few drops of toluene and 0.5 ml of 30% H_2O_2. The sample was digested by heating under an infrared lamp, and the procedure was repeated once or twice by adding 0.5 ml of 30% H_2O_2 until the blood was decolorized.

Every fractions (3.8 ml) of effluent obtained through the Sephadex and DEAE column were evaporated to dryness in a vial and digested under an infrared lamp after adding 1 ml of 30% H_2O_2.

Each segment of polyacrylamide gel was digested with 0.5 ml of 30% H_2O_2 at 60° in vial for 16 hr. The digested samples were dissolved by warming with 0.5 ml of Soluene-350 (Packard Instrument Corp.), and then neutralized by adding a small quantity of acetic acid.

Fifteen ml of the scintillation cocktail (4 g of PPO and 0.2 g of POPOP in 1 l of toluene) was added to each vial of all samples mentioned above, and the radioactivity was determined by a liquid scintillation counter (Aloka, model LSC 652).

Results and Discussion

Progressive Distribution of ^{115m}Cd in the Blood

The change in concentration of ^{115m}Cd in the blood during 30 days after an intraperitoneal injection of $^{115m}CdCl_2$ solution in the rabbit is shown in Fig. 1.

It is interesting to note that the radioactivity in the whole blood decreased drastically on the 1st day after injection but increased sharply again on the 2nd day, then gradually decreased with lapse of time. The regression line for whole blood gave an equation of $Y=-0.019x+3.507$ from the 2nd to the 30th day after injection, and the half life of ^{115m}Cd in the whole blood was calculated to be 158.4 days from the equation.

The radioactivity in the plasma decreased rapidly just after the dosing, but in sharp contrast that in the red cells rose remarkably on the 2nd day and increased continuously to the 9th day before decreasing very slowly.

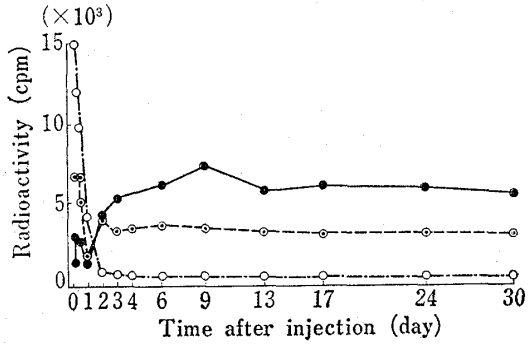

Fig. 1. ^{115m}Cd Contents in Whole Blood, Plasma and Red Cells after an *i.p.* Injection of $^{115m}CdCl_2$ Solution

—⊙—, whole blood; ·—○—·, plasma; —●—, red cells.

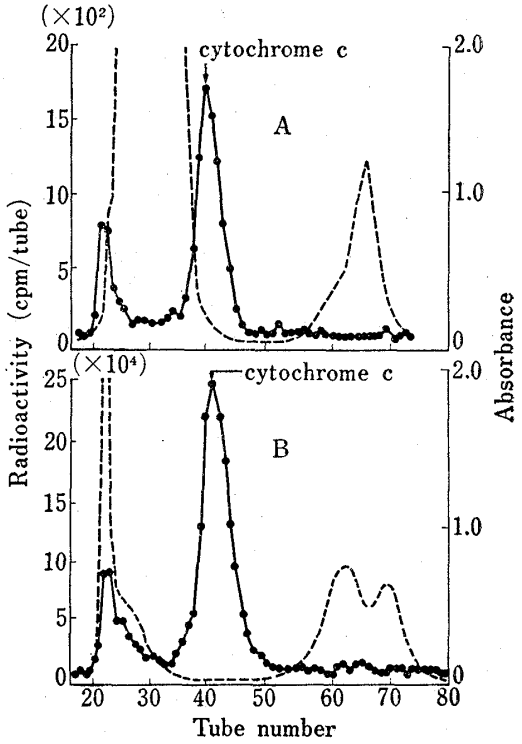

Fig. 2. Sephadex G-75 Column Chromatography of Stroma-Free Hemolysate and Liver Supernatant of Rabbit injected $^{115m}CdCl_2$ Solution

A, stroma-free hemolysate; B, liver-soluble fraction. —●—, radioactivity; ------, absorbance at 254 nm.

These findings suggest that administered cadmium might be once captured in some organs on the 1st day after injection and later redistributed to the red cells.

On the 14th day after injection of the $^{115m}CdCl_2$ solution, blood was separated into plasma and red cells. A large part (99.1%) of the radioactivity in the whole blood was in the red cells, over 90% of the radioactivity in the red cells existing in the supernatant fluid of stroma-free hemolysate and about 8% in the stroma.

^{115m}Cd-Binding Protein in the Red Cells

As illustrated in Fig. 2, about 70% of the radioactivity of stroma-free hemolysate was bound to the protein having the molecular weight of about 10000, which corresponds to the peak of cytochrome c processed by the same procedure.

Ion exchange chromatography using DEAE Sephadex A-25, on the ^{115m}Cd-binding protein in red cells and ^{115m}Cd-thionein in the liver showed a similar eluting pattern except that the ratio of the two components of metallothionein was reversed between the red cells and liver (Fig. 3).

Furthermore, both showed similar migration pattern by gel electrophoreses, meanwhile the same difference in ratio of the two components of metallothionein is observed in Fig. 4 as well as in Fig. 3.

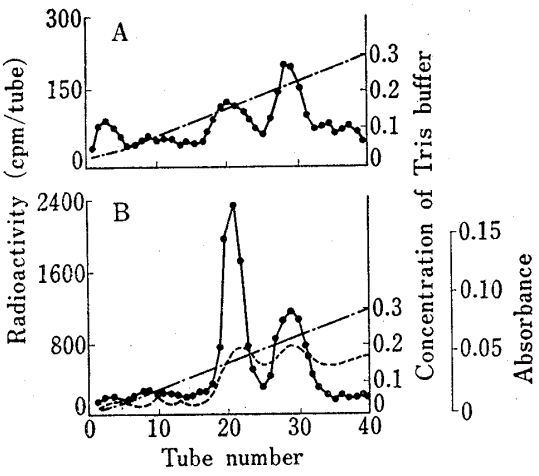

Fig. 3. DEAE Sephadex A-25 Chromatography of ^{115m}Cd-binding Proteins fractionated by Gel Chromatography of Sephadex G-75

A, stroma-free hemolysate; B, liver-soluble fraction.
—●—, radioactivity; ------, absorbance at 254 nm; ———. concentration of Tris buffer, M.

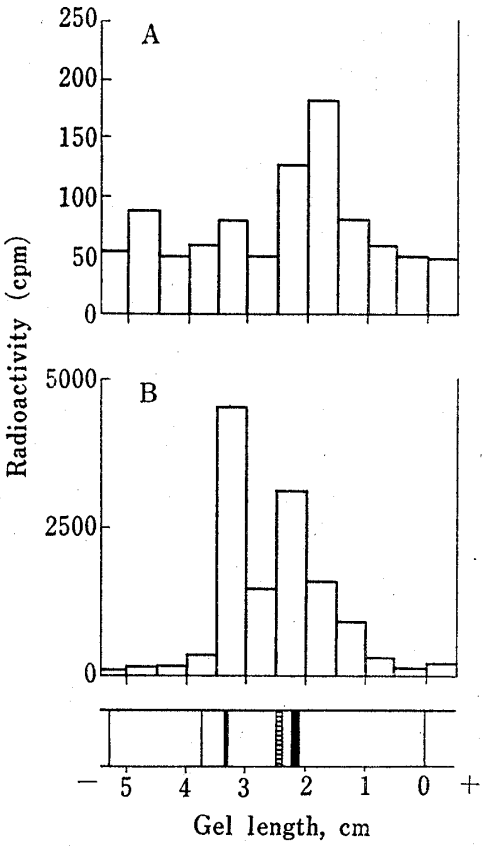

Fig. 4. Localization of ^{115m}Cd on Polyacrylamide Gel of fractionated ^{115m}Cd-binding Protein by the Gel Chromatography of Sephadex G-75

A, stroma-free hemolysate;
B, liver-soluble fraction.

From the results mentioned above, the main cadmium-binding protein in the red cells was identified to be thionein. However, the distribution pattern of ^{115m}Cd in the metallothionein of red cells was different from that of ^{115m}Cd-thionein obtained from liver.

In Vitro Test on the Incorporation of ^{115m}Cd-Thionein to Red Cells

^{115m}Cd was incorporated into red cells at almost equal ratio by incubating healthy rabbit blood with $^{115m}CdCl_2$ and ^{115m}Cd-thionein isolated from rabbit liver (Fig. 5).

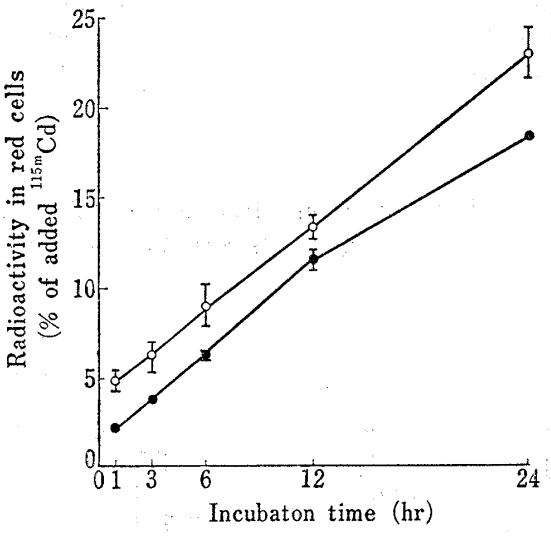

Fig. 5. Incorporation of ^{115m}Cd to Cells by *in Vitro* Test

—○—, $^{115m}CdCl_2$; —●—, ^{115m}Cd-thionein.

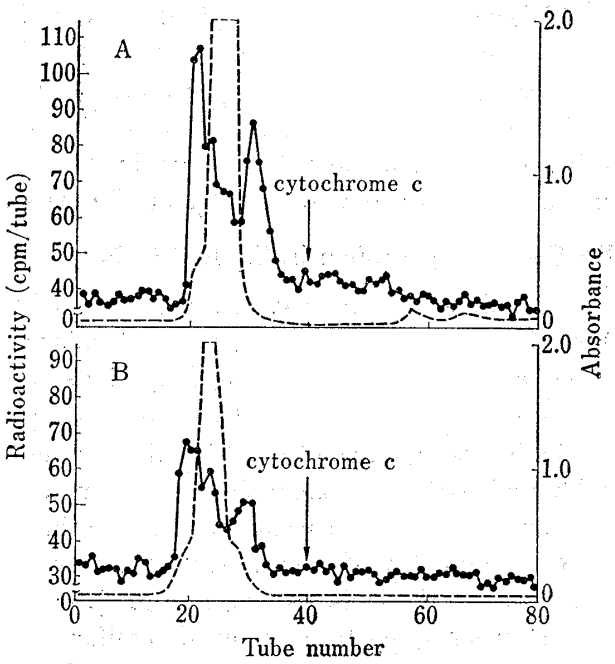

Fig. 6. Sephadex G-75 Column Chromatography of Hemolysate separated from Red Cells incubated with $^{115m}CdCl_2$ and with ^{115m}Cd-thionein

A, $^{115m}CdCl_2$; B, ^{115m}Cd-thionein.
—●—, radioactivity; ------, absorbance at 254 nm.

^{115m}Cd incorporated to red cells from ^{115m}Cd-thionein as well as from $^{115m}CdCl_2$ showed ^{115m}Cd to be bound to far macro-molecular protein rather than to thionein (Fig. 6). Therefore, the incorporation of liver metallothionein to red cells was not recognized.

On the Origin of ^{115m}Cd-Thionein in the Red Cells

As mentioned in the foregoing sections, the distribution pattern of ^{115m}Cd in the metallothionein in red cells differed from that of ^{115m}Cd-thionein obtained from the liver, and the incorporation of liver metallothionein to red cells through the cell membrane was not observed by *in vitro* test. Therefore, it is presumed that the metallothionein in red cells may originate from some organ other than liver. However, to verify the transferring mechanism of metallothionein to red cells, further studies are desirable.