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# SHORT COMMUNICATION

## Cadmium and mercury binding to metallothionein as influenced by selenium*

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It has been reported in numerous papers that selenium plays a protective role in cadmium poisoning, abolishing toxic effects in the testes, preventing haemorrhagic necrosis of nonovulating ovaries, placental necrosis, teratogenic effects, lethal effects in non-pregnant rat and mouse females and elevation of blood pressure in rats [1, 2]. It is well known that in rats multiple administration of cadmium results in its deposition mainly in the liver and in an efficient binding to metallothionein therein. The high efficiency of this binding seems to be due to the inductive effect of cadmium on the biosynthesis of this protein [3–8]. The data of Chen [9] from a single dose of both cadmium and selenium indicate more distinct changes in the type of cadmium binding in the testis and in the kidneys than in the liver.

The aim of this paper was to establish if such changes could be found following a multiple exposure to cadmium and selenium, especially if selenium affects the induction of biosynthesis of cadmium metallothionein. For comparison, a pilot experiment was performed under analogous conditions with selenium and mercury since serious changes in the mechanism of the organ binding were found for the latter metal [10].

Female rats of the Wistar strain weighing 150–200 g, fed with a standard LSM diet were used in the experiments. The animals were administered subcutaneously with cadmium chloride labelled with $^{115m}$Cd (activity of about 4–2 $\mu$Ci per dose) 0.5 mg Cd/kg body weight every other day for a fortnight. Selenium was administered orally as sodium selenite ($^{75}$Se activity of about 12 $\mu$Ci per dose) every day for a fortnight, 0.5 mg Se/kg daily. The control group received subcutaneously physiological saline. The animals were sacrificed in ether narcosis 24 hr after the last administration. The kidneys and the liver were withdrawn for analysis. The organs were ground in 0.9% (w/v) NaCl, the homogenate was spun for 10 min at 3,500 rpm, and the resultant supernatant was subjected to a Sephadex G-75 chromatography. The column was calibrated with: dextran blue, cytochrome *c*, albumin and insulin; 0.1 M formate buffer, pH 8.0 was used the eluent. Activities of both the isotopes were determined by the scintillation method using a Tesla SPF-35 plastic crystal for $^{115m}$Cd measurements, and a NaJ/Tl crystal for $^{75}$Se measurements. In the doubly labelled samples activities were corrected for the presence of the second isotope.

Metallothionein was determined in full homogenates according to the radiochemical method [11, 12] based on estimation of this protein with a $^{203}$Hg radiotracer following the TCA-precipitation of high-molecular weight proteins and a selective precipitation of metallothionein-like proteins with tannin. Horse kidney metallothionein (molec. wt = 10,500, 3 $\mu$moles SH groups per mg), binding about 210 $\mu$g Hg/mg under the applied conditions was used as a standard [13].

Administration of $^{115m}$Cd in the absence of selenium resulted in deposition of 7.4 per cent of the total dose in the liver, while in the presence of selenium this value decreased down to 4.9 per cent. On the other hand, $^{75}$Se retention amounted to 2.9 per cent of the cumulative dose in the absence of cadmium, increasing up to 7.6 per cent in the presence of cadmium. The chromatographic pattern of binding of both the elements in the postnuclear fraction of the liver after independent and combine administration is presented in Fig. 1. It is evident that selenium did not change the binding of cadmium by proteins significantly except for a slight tendency for cadmium localization in high-molecular weight protein fractions. Cadmium administration did not affect selenium binding either.

It is noteworthy that in the case of selenium–mercury interaction an evident shift of mercury from the metallo-

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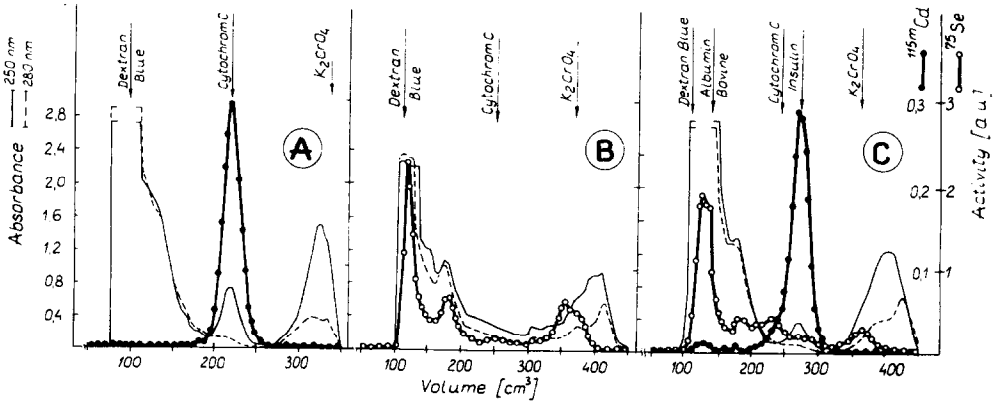


Fig. 1. Chromatograms of liver postnuclear supernatants; rats exposed to: A) $^{115m}CdCl_2$, B) $Na_2{}^{75}SeO_3$, C) $^{115m}CdCl_2$ and $Na_2{}^{75}SeO_3$. Conditions: Sephadex G-75, 2.8 × 75 cm column, ammonium formate buffer, pH = 8.0, flow rate of about 1.2 cm$^3$/min, 5 cm$^3$ fractions collected.

Table 1. Metallothionein levels in the kidney and in the liver of rats administered with the metals and with the metals plus selenium. Means and range values. For details see text

| Metal | Number of animals | Metallothionein mg/g tissue Kidney | Liver |
|---|---|---|---|
| Control | 12 | 0.20 (0.15–0.28) | 0.11 (0.08–0.15) |
| Cd | 8 | 0.39 (0.30–0.52) | 0.77 (0.64–0.91) |
| Cd + Se | 5 | 0.42 (0.26–0.49) | 0.89 (0.87–0.90) |
| Hg | 3 | 0.55 (0.51–0.61) | 0.09 (0.07–0.10) |
| Hg + Se | 3 | 0.17 (0.15–0.18) | 0.07 (0.06–0.09) |

thionein fraction into the high-molecular weight protein fraction was found, especially in the kidney [10].

Table 1 shows the levels of metallothionein in the liver and in the kidneys of rats administered with cadmium, selenium and both these metals together. For comparison, results of the pilot experiment with mercury are also presented. It results from the Table that multiple cadmium administration resulted in an increase of the metallothionein level in the liver and in the kidneys by factors of seven and two, respectively, and that selenium administration did not affect this phenomenon. On the other hand, the effect of selenium is evident in animals administered with mercury. In the latter case selenium abolished the stimulative effect of mercury on metallothionein in the kidney. This observation is in line with the previous report indicating the abolition of mercury binding to metallothionein in the presence of selenium [10].

Chen *et al.* [9] found 1 hr following administration of cadmium that pretreatment with selenium resulted in almost complete abolition of cadmium binding to metallothionein in the kidneys and testis in favour of proteins of higher molecular weight. This was not the case, however with the liver, where metallothionein still played a dominant role.

Our studies indicate that in the course of repeated exposure, where multiple doses of both cadmium and selenium are introduced over a longer time period, the influence of selenium on binding cadmium by metallothionein is much less pronounced, and the stimulatory effect of cadmium on the biosynthesis of metallothionein of both liver and kidney is not affected.

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