Subcellular distribution and binding of heavy metals in the untreated liver of the squid; comparison with data from the livers of cadmium and silver-exposed rats

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Summary. In natural squid liver, about 30% of the total Cd present was found in the cytosolic fraction. A large portion of this Cd was bound to high molecular weight species (mol. wt > 70,000). In contrast to Cd, about 60% of the total Ag from the livers of cadmium and silver-exposed rats...
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ing 0.05 M NaCl and 0.003 M NaN₃. The column was eluted with the same buffer at a flow rate of 20 ml/h at 0–4°C. The elution was monitored at 280 nm using an ALTEX Model 150B Biochemical UV monitor. The effluent fractions (5 ml) were assayed for Cd, Zn, Cu, Fe and Ag by atomic absorption spectrophotometry, as described above, but without prior acid digestion.

**Results and discussion. Subcellular distribution of heavy metals in squid liver.** The subcellular distribution of Cd, Zn, Cu, Fe and Ag in untreated squid liver is shown in the table. Approximately 30% of Cd in the liver homogenates was found in the nuclear, mitochondrial and cytosolic fractions, respectively. In mammals, such as rats and mice, exposed to Cd, it has been observed in several laboratories that more than 60% of Cd in the livers or kidneys occurs in the cytosolic fraction in 24 h or more after a dose of Cd10,11.

We have also obtained similar results on Cd-exposed rats (unpublished). The experiments described here show that the subcellular distribution of Cd in untreated squid liver is notably different from that in Cd-exposed mammalian liver. The subcellular distribution of Zn was similar to that of Cd. In marked contrast to Cd or Zn, most of the Cu or Ag (about 60% of either) was found in the cytosolic fraction. Approximately 40% of Fe was localized in the microsomal fraction. The data show that there is a close resemblance between the subcellular distributions of Cu and Ag on the one hand, and of Cd and Zn on the other. This is probably related to the fact that Cd and Zn belong to group IIb and Cu and Ag to group Ib of the periodic table.

**Comparison of hepatic heavy metal-binding species in untreated squid and Cd and Ag-exposed rat.** Typical Sephadex G-75 gel filtration profiles of the liver supernatants from squid and from Cd and Ag-exposed rats are shown in the figure. In Cd and Ag-exposed rats (graph A), the major portion of Cd (about 90%) in the liver supernatant was associated with the metallothionein fraction, which was shown to have an apparent molecular weight of 11,000–12,000, as estimated from its elution volume from the calibrated column. These results are consistent with those obtained for Cd-exposed rat livers in the gel filtration experiments in several other laboratories11,13. A large proportion of the Zn in the liver supernatant was also found in the metallothionein fraction, while Ag was mainly bound to high molecular weight species (mol. wt > 70,000) eluting with the void volume (V₀, indicated by arrow 1).

Sephadex G-75 gel filtration of the liver supernatants prepared from Cd and Ag-exposed rat (A) and from untreated squid, Todarodes pacificus (B). The column (2.6 x 62 cm) was eluted with 0.02 M Tris-HCl buffer, pH 8.6, containing 0.05 M NaCl and 0.003 M NaN₃. Fractions of 5 ml were collected. Numbered arrows designate the positions of molecular weight standards: 1, Blue Dextran 2000 (V₀); 2, bovine serum albumin; 3, ovalbumin; 4, chymotrypsinogen; 5, ribonuclease; 6, glucagon; 7, K₂CrO₄. MT, rat liver metallothionein (as shown clearly in A).