A High-Molecular-Weight Cadmium-Binding Fraction Isolated from the Liver Cytosol of Trout Exposed to Environmentally Relevant Concentrations of the Metal

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Sulphur-rich metal-binding proteins (metallothioneins) have now been isolated from a variety of tissues and species (Cherian & Goyer, 1978) including the liver of marine fishes (Overnell et al., 1977). The protein, with a molecular weight in the range 6000–10000, is only present in very low amounts in normal tissues, but its synthesis can be stimulated in a variety of species, particularly in the liver, by injection of sub-lethal amounts of cadmium salts (Cherian & Goyer, 1978). The dose regimes used to induce the cadmium-binding metallothionein are not, however, related to the concentrations of the metal now thought to be of pathological significance in the environment. By contrast, when low chronic doses, compatible in concentration and route of administration with the environmental metal hazard, were administered to rats, for example, cadmium was found to bind to proteins of molecular weight greatly in excess of 10000 (El-Gazzar et al., 1978). The present communication reports the presence of a cadmium-binding high-molecular-weight fraction isolated from the livers of trout previously exposed to low, environmentally significant doses of cadmium in the aquarium water.

Rainbow trout (Salmo gairdneri Richardson) and brown trout (Salmo trutta fario L.) were exposed to nominal concentrations of cadmium in the water of 9 and 27 μg/litre respectively for up to 3 months in aquaria at the Water Research Centre, Stevenage. These concentrations were the maximal values permitting survival of the fish used for the periods studied (Ball, 1967). Under these conditions, cadmium accumulates in the liver and gills of the fish, but induces little or no change in the activity of a variety of metabolically significant enzymes in the fish tissues, including the liver (Roberts et al., 1979).

After anaesthesia with MS 222 and injection with heparin (100 units/kg body wt.), the livers of both species of fish were removed and perfused with 0.9% NaCl. The fresh livers were then homogenized in an iso-osmotic medium (0.154 M-KCl or 0.25 M-sucrose) under conditions of minimal organelle damage. After filtration through cheese-cloth, cytosolic fractions were prepared from the homogenates by centrifugation at 100000 g for 90 min. The cytosolic fractions were assayed for protein and cadmium content and were found to contain approx. 90% and 70% of the metal originally present in the liver homogenates of rainbow and brown trout respectively. Both fresh fractions were concentrated in an Amicon ultrafiltration cell fitted with either a PM 10 (nominal molecular-weight retention of 10000) or a PM 30 (30000-mol.wt. retention) membrane. For the two species, 90% of the cadmium present in the cytosols was retained within the ultrafilter with both of the membranes. After 15-fold concentration of the two cytosolic fractions by ultrafiltration with the PM 30 membrane, the recovery of the original homogenate cadmium was approx. 80% for the rainbow trout and 70% for brown trout.

For both species, the material thus obtained by concentration over a PM 30 membrane was applied to a column of Sephadex G-75 (2 cm x 60 cm) equilibrated with 0.01 M-sodium phosphate buffer (pH 7.4)/0.15 M-KCl. Elution was continued with the same buffer and the protein and cadmium content of each fraction was determined. Only the fractions emerging at the void volume, with the major protein peak (mol.wt. greater than 70000) contained the metal. Similar elution profiles were obtained for the two species. The recovery of cadmium in this void-volume fraction was 100% of that applied to the column. This in turn represented 80% and 70% of that metal originally present in the tissue of rainbow and brown trout, respectively. There was no evidence, from these experiments, of a protein in the low-molecular-weight region that absorbed specifically
at 250 nm and/or bound cadmium. Thus the presence of cadmium-metallothionein in the liver cytoplasm of these animals seems unlikely.

When PM 10-concentrated cytosol fractions were dialysed against 10 mM-sodium phosphate buffer, pH 7.4, none of the cadmium passed through the dialysis membrane. By contrast, after attempts to fractionate the material with \((\text{NH}_4)_2\text{SO}_4\), dialysis of the redissolved pellets resulted in the loss of much of the cadmium through the dialysis membrane. Similarly, adjustment of the PM 10-concentrated fraction to a final concentration of 10% (v/v) trichloroacetic acid also caused the cadmium to become freely soluble in the aqueous supernatant. After removal of the trichloroacetic acid-insoluble material, these supernatant fractions were neutralized. When they were subjected to ultrafiltration with a PM 10 membrane, none of the cadmium was retained by the membrane. These results are consistent with the formation of a complex between cadmium and one or more components of high molecular weight, distinct in character from the lower-molecular-weight protein, metallothionein.

A similar distribution of cadmium was observed when gill tissue from the same animals was fractionated in an analogous manner to that described above for liver tissue.

The concentration of cadmium administered to the fish in these experiments was considerably lower than those used in other investigations in which a metallothionein response has been observed (Overnell et al., 1977; Marafante, 1975).

Marafante, E. (1975) *Experientia* 31, 149-150

### Effect of 5-Fluorouracil on Liver and Plasma Vitamin A in Female Rats

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Several vitamins play an important role in hepatic oxidative metabolism. Vitamin A deficiency in rats significantly decreases microsomal protein and oxidative enzymes. Vitamin A decreases, and deficiency enhances, the carcinogenic properties of certain chemicals. Large doses of vitamin A are toxic and tend to destabilize membranes.

5-Fluorouracil is used either alone, or in combination with other drugs, in the treatment of metastatic cancer, but as with all cytotoxic drugs the margin between therapeutic and toxic doses is small.

In the present study female Wistar albino rats (200 g body wt.) were given an intraperitoneal injection of 5-fluorouracil on 3 consecutive days. Blood and liver samples were taken on day 4.

Plasma vitamin A was measured by the method of Hansen & Warwick (1969) with the fluorescence technique of Van Steveninck & De Goeij (1973); liver vitamin A was measured by the method of Thompson *et al.* (1971).

Table 1 shows that the human therapeutic dose of 15 mg/kg body wt. decreased the haematocrit and caused a slight decrease in liver and plasma vitamin A concentrations.

At the higher doses both liver weight and terminal body weight were greatly decreased. The animals had diarrhoea, and the haematocrit increased with the loss of body fluid.

Both liver and plasma vitamin A concentrations were elevated, but in each case the increase was greater than could be accounted for by the decrease in liver weight or loss of body fluid. The amount of vitamin A per whole liver was decreased significantly in 15 mg/kg-dose group and significantly increased in the other groups.