Cadmium Accumulation and Protein Binding Patterns in Tissues of the Rainbow Trout, *Salmo gairdneri*

by John Kay,* Dafydd G. Thomas,† Michael W. Brown,* Anthony Cryer,* David Shurben,‡ John F. deL. G. Solbe,‡ and Justine S. Garvey†

Rainbow trout were exposed to defined levels of cadmium in their aquarium water for differing periods at a variety of near-lethal concentrations that ensured the survival of the majority of the fish. The gills, liver and kidney together accounted for 99% of the accumulated load of body cadmium in the fish under these conditions. Although the proportion of total cadmium present in the liver remained relatively constant throughout, the distribution of the remainder between gill and kidney altered with the time of exposure. The cadmium in all three organs was bound by two low molecular weight proteins distinct in character from metallothionein. The isoforms of metallothionein were also present but were found to bind only zinc and copper. By contrast, when trout were injected with cadmium intraperitoneally, most of the metal accumulated in the liver where it was sequestered by the two isoforms of metallothionein.

Pre-exposure of the trout to either a low concentration of cadmium (for several months) or to an elevated concentration of zinc (for 5 days) allowed the animals to survive a subsequent exposure to a high, otherwise lethal concentration of cadmium.

The proteins responsible for sequestration of the two metals were identified, but two different mechanisms seemed to be involved in the protection of the animals. The significance of these observations in terms of the induction of proteins and the prevention of the toxic effects of cadmium is considered.

The occurrence and distribution of cadmium in the environment have been well documented. Assimilation of the metal from the environment by a variety of species has also been well studied. Absorption through the skin is believed to have little significance, and it is considered that the most dangerous routes of intake and absorption are by inhalation or by ingestion in the diet, with the former having a much greater acute effect.

The concentration of cadmium in unpolluted surface fresh water is usually below 1 µg/L. When higher levels are detected, they have usually arisen as a consequence of the widespread utilization of the metal in a number of industrial processes. Surveys in the U.S.A. and in Belgium have indicated that, of the freshwater samples taken, 40–50% contained cadmium at concentrations ranging from 1 to 20 µg/L. Thus, the industrial discharge of cadmium is felt keenly in the aquatic environment and the toxic nature of the metal poses a considerable threat to aquatic species.

Although a substantial proportion of the cadmium in river water is absorbed onto solids in suspension, much of it remains in solution, where it proves toxic to fish (1). Some fish species, trout for example, are especially sensitive to its presence in their environment. For these reasons, then, the work in this laboratory has been based on the use of fish, and trout in particular, as the experimental vehicle for investigations into the cellular aspects of cadmium toxicity.

In this work, rainbow trout and brown trout have been exposed to environmentally relevant concentrations of cadmium by the physiologically important route of inhalation (2). A variety of concentrations and times of exposure have been investigated and the accumulation of the metal in the tissues and organs of each species has been determined. Because identical patterns were obtained for each species, only the salient features for rainbow trout will be reported here.

More than 99% of the total body loads of cadmium accumulated by all fish was always accounted for by the liver, kidney, and gills collectively (Table 1). The mortality

---

*Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.
†Water Research Centre, Medmenham, Marlow, Bucks, SL7 2HD, U.K.
‡Department of Biology, Syracuse University, 120 College Place, Syracuse, NY 13210 (U.S.A.).
data indicate that only relatively low levels of cadmium, such as are found in many polluted waters, are required to kill the rainbow trout. Thus 9 μg Cd/L was selected as a near-lethal concentration of cadmium which, in longer term exposures, might nevertheless permit the majority of the fish to survive. During 8 months of exposure to this concentration of cadmium (Table 2), again greater than 99% of the total body burden in the fish was located, at all times, within the liver, kidney, and gills. The metal accumulated progressively in the liver where a constant proportion (around 50%) of the increasing total body load of cadmium was present at all times of exposure (8). The distribution between the other two major organs of accumulation altered with time, with the gills containing between 40 and 50% of the total body cadmium in fish exposed for brief periods. On the other hand, in animals exposed for longer than 16 weeks, the bulk of the total body burden (approx. 65%) was present in the kidney (4).

For trout exposed to 9 μg Cd/L for a fixed period (3 months) at different times of the year (5), the load of cadmium in the liver followed closely the seasonal variation in the ambient temperature of the water together with other parameters such as length of day light, state of sexual maturation, and amount of food ingested. The highest concentrations of cadmium were measured in liver samples analyzed during the summer months. Because the hepatic weight (as a proportion of total body weight) did not change, the observed alterations in hepatic cadmium were not related to a seasonal change in the weight of the liver and must, therefore, reflect seasonal variation in the amount of cadmium accumulated.

In fish exposed to either a range of cadmium concentrations (as in Table 1) for a short period (8 weeks) or to a defined concentration (Table 2) over a 32-week period, no other organ or tissue with the exception of the spleen and gonads (4,5) showed any significant concentrations of cadmium. Although cadmium did not accumulate appreciably in bone, degeneration of the tail, dorsal, and ventral fin filaments was observed in the long-term exposed fish. When the amount of osseous calcium (expressed as a percentage of that present in the bone tissue of nonexposed fish) was measured, a steady decline was observed with increasing time of exposure to 9 μg Cd/L until, after 9 months, the amount of calcium present was reduced to approximately 60% of that in the bones of nonexposed fish (5). Thus, although cadmium does not appear to replace the calcium in the bone, prolonged exposure to cadmium seems to bring about loss or reabsorption of calcium from bone.

Cadmium was never found in significant amounts in the gonads of female trout exposed to the metal as described above. However, attempts to fertilize eggs stripped from these females after 4 months of exposure to 9 μg/L resulted in the maturation of only 10% of the eggs to the alevin stage (5). By contrast, small amounts of cadmium, increasing with time, were measured in the testes of male fish. Indeed when trout were exposed to 54 μg Cd/L for 2 months (Table 1), of the 30% that survived the exposure, all were females (4). The spermatozoa from fish exposed to cadmium (9 μg/L) for 3 months were also unable to fertilize eggs from nonexposed fish. Although less than 1% of the total cadmium in the bodies of these fish is found in these peripheral tissues, it clearly exerts some effect in the long term on the vitality of the trout.

It was considered of greater importance, in the first instance, to examine whether the metabolism of the major organs of accumulation was affected under the conditions of exposure described. Accordingly, the specific activities of a number of enzymes (metallo- and nonmetal-dependent) from several cellular compartments (membrane-bound, limited or soluble) were measured in the liver, kidney, and gills of rainbow trout (and brown trout) (2). No discernible differences were obtained when Cd-exposed and control fish were compared, nor were there alterations in the specific activity of certain red blood cell enzymes nor activities in blood plasma which might have reflected tissue damage (2).

Attention was then focused on the nature of the proteins responsible for the sequestration of cadmium. Homogenates of the liver, kidney, and gills were prepared in 0.01 M sodium phosphate buffer, pH 7.4/0.15 M KCl and centrifuged for 40 min at 30,000g (6). The resultant supernatants were then recentrifuged for 120 min at 100,000g in order to remove further particulate material but primarily to ensure complete flotation of the lipid material present. Omission of this centrifugation step (6) which allowed the complete exclusion of lipid, resulted in anomalous behavior of the cadmium-containing moieties apparent on subsequent gel filtration (7). After concentration by ultrafiltration and further centrifugation for 120 min at 100,000g, the supernatants were subjected to gel filtration on columns of Sephadex G-75 (3,4). In all three tis-

---

Table 1. Accumulation of cadmium in the tissues of rainbow trout as a function of increasing concentration of the metal in their aquarium water.*

| Organ   | 0  | 9 μg/L | 18 μg/L | 36 μg/L | 54 μg/L |
|---------|----|--------|---------|---------|---------|
| Liver   | 0.05 ± 0.002 | 0.4 ± 0.1 | 1.2 ± 0.1 | 1.3 ± 0.5 | 1.9 ± 0.6 |
| Gill    | 0.02 ± 0.001 | 0.5 ± 0.2 | 2.9 ± 0.4 | 2.7 ± 1.2 | 6.1 ± 3.8 |
| Kidney  | 0.01 ± 0.007 | 0.7 ± 0.2 | 6.6 ± 3.3 | 10.4 ± 3.4 | 13.2 ± 3.8 |

Fish surviving, % | 100 | 90 | 80 | 70 | 30

*Groups of approximately ten fish were exposed for 2 months at each concentration of cadmium.

b Values given are the means ± SD of six independent observations.
Table 2. Accumulation of cadmium in the tissues of rainbow trout as a function of increasing time of exposure to 9 μg Cd/L of aquarium water.*

| Organ | Cadmium concentration, μg/g wet weight after exposure to 9 μg/L | 0 | 2 weeks | 12 weeks | 22 weeks | 32 weeks |
|-------|-------------------------------------------------------------|---|--------|---------|---------|---------|
| Liver | 0.05 ± 0.002                                               | 0.2 ± 0.004 | 0.4 ± 0.1 | 0.7 ± 0.2 | 1.1 ± 0.4 |
| Gill  | 0.02 ± 0.001                                               | 0.3 ± 0.02 | 0.5 ± 0.2 | 0.7 ± 0.2 | 1.2 ± 0.4 |
| Kidney| 0.02 ± 0.007                                               | 0.2 ± 0.1  | 0.6 ± 0.2 | 5.2 ± 0.6 | 8.6 ± 1.2 |

*Groups of approximately ten fish were exposed to 9 μg Cd/L for each length of time.

Values are the means ± SD for six independent observations.

FIGURE 1. Ion-exchange chromatography on DEAE-cellulose of the low molecular weight fraction obtained after gel filtration on Sephadex-75 of the extract of the liver of rainbow trout exposed to 54 μg Cd/L of aquarium water for 2 months. The low molecular weight pool of protein was dialyzed against 20 mM Tris-HCl buffer, pH 7.4, and applied to a column of DEAE-cellulose (1.2 x 14 cm), equilibrated in the same buffer. After extensive washing, a linear gradient of 20-100 mM Tris-HCl buffer (200 mL of each) was used for desorption: (●) Cd; (×) Zn; E260 and E280 values were monitored also but are omitted from the figure for the sake of clarity.

FIGURE 2. Ultraviolet absorption spectra of the cadmium-binding proteins obtained after further purification of peaks 1 and 3 resolved by ion-exchange chromatography on DEAE-cellulose. Extensively dialyzed samples of the homogeneous proteins from peak 1 (upper) and peak 3 (lower) isolated from the liver of rainbow trout exposed to cadmium in their aquarium water for 3 months were analyzed between 180 and 350 nm (——) at pH 7.0 and (— —) at pH 2.0.

high amounts of aromatic (phenylalanine, three and five residues per mole, respectively) and hydrophobic amino acids and low cysteine (five and three residues per mole, respectively). The two proteins were not identical, although both displayed very similar ultraviolet absorption spectra (Fig. 2). Both proteins had E280/E250 of between 0.6 and 0.8. Acidification of solutions of the two proteins did not cause any substantial alteration in the absorption spectra (Fig. 2). Analysis of the metal content of each protein indicated that cadmium was the sole metal present (2 and 1 g-atom/mole, respectively). Neither protein was able to bind zinc, copper, calcium, nickel, or iron.

By contrast, when the proteins present in peaks 2 and 4 (Fig. 1) were purified to homogeneity and characterized (3,4), their properties were considerably different from those of the proteins responsible for the sequestration of environmental cadmium. Isoelectric points of 4.9 and 4.7

sues, the cadmium was eluted (recoveries > 90%) as a single, low molecular weight peak coincident with a peak of zinc. This position is consistent with the metals being associated with proteins of approximate molecular weight 10,000.

In order to establish the identity of the protein moieties responsible for binding cadmium and/or zinc in the tissues of rainbow trout, the low molecular weight pools of material from liver, kidney, and gills were concentrated (separately) by ultrafiltration, dialyzed, and subjected to ion-exchange chromatography on separate columns of DEAE-cellulose (4). Figure 1 illustrates a representative elution profile obtained with the low molecular weight pool from liver from trout exposed to 54 μg Cd/L for 2 months. Identical distributions of the metals were observed with every liver, kidney, and gill sample analyzed from all of the trout exposed to cadmium under the conditions described in Tables 1 and 2.

The cadmium-binding moieties in peaks 1 and 3 (Fig. 1) were further purified and characterized (3,4) and shown to be acidic proteins with isoelectric points of 4.0 and 3.4, respectively. The proteins were shown to be homogeneous by N-terminal analysis and by electrophoresis in three separate gel systems. Amino acid analysis indicated that each of the proteins contained relatively.
were measured for peaks 2 and 4, respectively, and amino acid analysis indicated a lack of hydrophobic and aromatic amino acids and a relative abundance of cysteine (20 residues/mole) in each of these proteins. Analysis of the metal content of these two proteins isolated from liver, kidney, or gills revealed that, although no cadmium was present, both proteins contained zinc and copper (at about 2.5 and 4.5 g-atoms/mole, respectively). These properties are all consistent with the identification of the proteins in peaks 2 and 4 (Fig. 1) as isoforms of metallothionein.

It appears that in the experiments described here, none of the cadmium was ever sequestered by metallothionein despite the presence of the isoforms of this protein in the major organs of accumulation. However, metallothionein has been implicated in the detoxification of cadmium as a result of the efforts of many other investigations. Frequently, however, such experiments have involved administration of the toxic metal by the nonphysiological route of intraperitoneal injection, to both fish (8) and other species (9). Furthermore, concentrations greatly exceeding those ever likely to be encountered in the environment have been used.

As a comparative exercise, cadmium was therefore introduced by intraperitoneal injection of the metal (total 2800 μg/kg body weight over 10 days) into rainbow trout (3). Under these circumstances, analysis of the organ contents revealed that liver alone (31 μg/g) contained almost 90% of the total body load, with most of the remainder being located in the kidney (3.1 μg/g). This distribution is in complete contrast to those observed with all of the environmentally dosed animals described previously (Tables 1 and 2). Most interestingly, despite much higher body loads of cadmium overall, none of the IP-injected fish died (Table 1).

Supernatants from the livers of the IP-injected trout were prepared and chromatographed on Sephadex G-75 as described above. A very similar elution profile to those from the environmentally exposed fish was obtained (9). However, when the low molecular weight pool of material from this gel filtration was subjected to ion-exchange chromatography on DEAE-cellulose, while a small amount of cadmium was still present in peaks 1 and 3, most co-eluted with zinc in peaks 2 and 4. Purification and characterization of the proteins from the latter two peaks showed them to fulfill all of the criteria for qualification as metallothioneins: isoelectric points of 4.9 and 4.7; a high content of cysteine with low hydrophobic amino acids. The metal contents of the two proteins are shown in Table 3 and the ultraviolet absorption spectra in Fig. 3. In both cases, these display an extensive shoulder from 300 to 240 nm which can probably be attributed to the summation of the individual absorbances due to the metal–mercaptoine complex with copper (280 nm), cadmium (250 nm), and zinc (212 nm). Acidification to pH 2.0 abolished the shoulders in both spectra (Fig. 3), and subsequent neutralization resulted in their reappearance. These spectral properties are characteristic of metallothionein.

Both proteins were tested for their cross-reactivity in a radioimmunoassay for mammalian metallothionein (10). Different amounts of the two proteins (10⁶–10⁷ pg) were incubated with a known amount of rat ¹²⁵I-metallothionein and rabbit anti-rat liver metallothionein antiserum. The amounts of ¹²⁵I-metallothionein precipitated were then quantitated using the logit-log regression and the results indicated that although both fish proteins did cross-react partially with the anti-rat metallothionein, they differed from the 50% bound (pg) value for the native rat metallothionein by a factor of approximately 600. This indicates a partial immunological similarity between the piscine and mammalian metallothioneins. By contrast,

![Ultraviolet absorption spectra of the isoforms of metallothionein obtained after further purification of peaks 2 and 4 resolved by ion-exchange chromatography on DEAE-cellulose. Extensively dialyzed samples of the homogeneous proteins from peak 2 (upper) and peak 4 (lower) isolated from the liver of trout injected intraperitoneally with cadmium were analyzed between 180 and 350 nm at (—) pH 7.0 and (——) pH 2.0.](image-url)

Table 3. Metal content of the two isoforms of metallothionein (peaks 2 and 4) purified from the liver of trout injected intraperitoneally with cadmium sulfate or from the organs of trout exposed to 100 μg Zn/L of aquarium water for 5 days.

| Peak | Tissue and source | Cd | Zn | Cu |
|------|------------------|----|----|----|
| 2    | Liver; Cd-injected | 3.9 | 1.5 | 2.2 |
| 4    | Liver; Cd-injected | 2.8 | 1.9 | 2.4 |
| 2    | Liver; Zn, 5 days | 0   | 2.7 | 4.1 |
| 4    | Liver; Zn, 5 days | 0   | 2.5 | 4.5 |
| 2    | Kidney; Zn, 5 days | 0   | 2.5 | 4.5 |
| 4    | Kidney; Zn, 5 days | 0   | 2.8 | 4.4 |
| 2    | Gill; Zn, 5 days  | 0   | 2.8 | 4.3 |
| 4    | Gill; Zn, 5 days  | 0   | 2.3 | 4.6 |
however, the proteins (peaks 1 and 3) from the environmentally exposed trout did not compete at all with purified rat metallothionein for the antibody. These results suggest that cadmium is not sequestered by metallothionein in the tissues of rainbow trout unless the metal is introduced in vivo in amounts considerably greater than those which the fish could encounter and survive in their environment. In order to examine this further, extraneous cadmium was introduced in vitro into the low molecular weight pools of unresolved proteins obtained by chromatography of extracts from liver, kidney and gills on Sephadex G-75. Aliquots of each pool were dialyzed against buffer containing either 10 or 1000 μg CdSO₄/mL and then chromatographed on identical columns of DEAE–cellulose (4). With all three tissues, upon dialysis against 10 μg CdSO₄/mL, the pattern of Cd/Zn elution observed previously (Fig. 1) was not altered. This concentration of cadmium in vitro is apparently not sufficient to promote binding of the metal to the isoforms of metallothionein in (peaks 2 and 4). By contrast, when the concentration of the metal (salt) was increased to enormously high levels (1,000 μg/mL), it was found that the cadmium was distributed among all four peaks in the elution profile (4).

Thus, it would appear that, at levels which prove fatal to rainbow trout, cadmium cannot displace endogenous zinc/copper from pre-existing metallothionein in the tissues of the animals nor can it induce (at such low concentrations) the synthesis of apotheionein de novo. The specific nonmetallothionein-binding proteins seem to sequester all of the cadmium which is present in all three of the major organs under all of the conditions of environmental exposure that have been studied. These metal–protein interactions may well be of significance in determining the toxicity of cadmium at the cellular level.

It was possible to investigate these aspects further since it was known that exposure of a variety of species including fish to a low concentration of cadmium affords protection against the toxic manifestations associated with exposure to a much higher concentration of the metal (11). A similar protective effect is obtained upon brief pre-exposure of the animals to elevated levels of zinc before the cadmium administration is commenced. Rainbow trout were acclimated, then, under these two distinct sets of conditions: (a) by pre-exposure to a low level of Cd (9 μg/L) for increasing lengths of time (up to 10 months) before being transferred to tanks equilibrated with water containing 54 μg Cd/L for 2 months or (b) by pre-exposure to zinc at 100 μg/L of tank water for 5 days before transfer to 54 μg Cd/L (11).

By contrast to the situation described previously in which 70% of the trout died upon direct immersion in water containing 54 μg Cd/L (Table 1), under condition (a) it was indeed found that exposure to 9 μg Cd/L for either 2 weeks or 3 months resulted in an increase in the number of fish able to survive subsequent exposure to 54 μg Cd/L over 2 months. When the animals were conditioned for 6 to 10 months at 9 μg/L, complete protection was afforded against the higher concentration of cadmium (11). The nature of the proteins responsible for sequestration of cadmium and zinc in the liver, kidney, and gills was then investigated in these trout in which a tolerance of cadmium had been developed.

Extracts from all three organs from trout exposed to all of the increasing time periods of “double” cadmium exposure were processed through the gel filtration on Sephadex G-75 stage, as described above. The elution profiles obtained upon subsequent ion-exchange chromatography on DEAE–cellulose of each of the low molecular weight pools of protein displayed exactly the same distribution of cadmium and zinc (viz., Cd in peaks 1 and 3 and Zn in peaks 2 and 4) as was observed previously with fish exposed only to single concentrations of cadmium over various time periods (Fig. 1). Thus, in the tissues of the Cd-resistant trout, all of the cadmium in these double-dosed animals was still sequestered by the specific cadmium-binding proteins and not by the metallothioneins (peaks 2 and 4).

In order to examine the specificity of interaction of Cd and Zn with the mixture of binding proteins and metallothioneins, attempts were made to introduce the metals at different concentrations in vitro. Thus, aliquots of the low molecular weight pools of protein from the tissues of fish exposed to the two concentrations of cadmium in vivo, were dialyzed against buffer containing 10 or 1000 μg CdSO₄/mL or 1000 μg ZnSO₄/mL. Upon subsequent ion-exchange chromatography on DEAE–cellulose, it was observed with all three of the major organs of accumulation that dialysis against cadmium sulfate at 10 μg/mL did not change the intrinsic pattern of Cd/Zn elution in any way (from that depicted in Fig. 1). Once again, this concentration of cadmium applied in vitro is insufficient to promote binding of the metal to rainbow trout metallothionein. By contrast, when the concentration of the metal (salt) was raised to the extremely high level of 1000 μg/mL, the two isoforms of metallothionein (peaks 2 and 4) were found to be able to sequester a proportion of the toxic metal. Conversely, the cadmium-binding proteins (peaks 1 and 3) showed no affinity for zinc, since dialysis against 1000 μg ZnSO₄/mL did not result in the appearance of any of this essential metal in the elution positions of peaks 1 or 3 (11). The isometallothioneins (peaks 2 and 4) did, however, contain elevated amounts of zinc.

Similar experiments were carried out to establish the nature of the proteins responsible for sequestration of the two metals in rainbow trout pre-exposed to zinc (for 5 days) before immersion in cadmium-containing water [condition (b) above]. This conditioning resulted in survival of all of the animals through the 2 months exposure to 54 μg Cd/L (Table 1). Tissue extracts were processed for ion-exchange chromatography on DEAE–cellulose as described above.

In complete contrast to all of the previous observations described above, in the tissues of these trout in which resistance to cadmium had been induced by brief pre-exposure to elevated levels of zinc, a distinctive pattern of metal elution was obtained. Although some of the tissue cadmium was still found associated with the binding proteins (peaks 1 and 3), much of the metal in liver, kidney, and gills was associated with the isoforms of me-
tallothionein (peaks 2 and 4) together with zinc (and copper).

These experiments confirm that, in the rainbow trout, cadmium is unable to displace zinc or copper from endogenous metallothionein except when absurdly high concentrations are introduced: in vivo, by intraperitoneal injection, or in vitro, by dialysis against 1000 μg/mL. At the much lower concentrations of cadmium (by 5–6 orders of magnitude) which kill the fish when inhaled from the animal’s aqueous environment, all of the toxic metal seems to be sequestered in the major organs of accumulation by the specific cadmium-binding proteins despite the presence of Zn/Cu containing isom metallothioneins in these tissues. Apparently, also, cadmium at such (lethal) levels in the environment is unable to induce the synthesis of new apothionein since prolonged exposure of the fish to 9 and then 54 μg Cd/L still resulted in the toxic metal being sequestered by the specific binding-proteins. If metallothionein is unable to sequester cadmium once it is bound to these other proteins, then it is necessary to account in another way for the presence of cadmium in metallothionein in the tissues of fish exposed first to zinc and then to cadmium. Since no protection is afforded when the Zn is administered simultaneously with the cadmium, it is conceivable that cadmium may be bound by metallothionein only in the apoprotein form, i.e., by sequestration as the nascent polypeptide and is being newly synthesized. Cadmium itself is unable, at least at the concentrations which prove lethal to the rainbow trout, to induce synthesis of apoprotein de novo, whereas during the brief pretreatment with zinc, induction of thionein synthesis by zinc may take place, thus allowing the sequestration of cadmium by apothionein upon subsequent exposure to the toxic metal.

Further experiments are in progress to test this hypothesis and to investigate whether at low concentrations, cadmium may switch on the production of the specific nonmetallothionein proteins. Currently, it is not possible to quantitate the amounts of cadmium-binding proteins or metallothioneins present in the fish tissues (e.g., by immunoassay), since appropriate antisera are not available. Analysis of each experimental situation is then dependent on interpretation of the elution profiles obtained in the types of experiment described above. However, Cd-binding proteins do appear to be present (in as yet undetermined amounts) in the tissues of rainbow trout that have not been deliberately exposed to either cadmium or zinc (11). Similarly, these proteins have been shown to be present in the organs of fish exposed only to the high concentration of zinc since they can be loaded up with cadmium in vitro by dialysis of the low molecular weight protein mixtures from Sephadex G-75 against buffer containing 10 μg CdSO₄/mL (11). All of the cadmium introduced artificially at this high concentration was eluted with peaks 1 and 3, and none was found in association with the isoforms of metallothionein in peaks 2 and 4. Indeed, when the metallothionein peaks from these Zn-exposed fish were purified to homogeneity, analysis of the metal content (Table 3) of the two isoforms from all three tissues indicated that zinc and copper were the only metals present; 7 g-atom of metal was bound per mole of protein in all cases (Table 3). This is further confirmation that Cd in vitro, at concentrations as high as 10 μg/mL cannot replace the Zn or Cu in pre-existing metallothionein, so that introduction of the toxic metal in the environment at 10 μg/L of aquarium water is most unlikely to promote interaction with metallothionein. Thus, while metallothionein undoubtedly does have an important role in supplying zinc and copper for incorporation into newly synthesized cellular proteins (19), it appears to have little involvement in the detoxification of cadmium in the rainbow trout under physiologically relevant conditions. It is possible, however, that the binding proteins may act in such a fashion although it is uncertain presently whether cadmium sequestration by these proteins results in the generation of a toxic entity or whether the Cd-protein interaction prevents the proteins from fulfilling their normal cellular function. Investigations are in progress to examine these possibilities, the implications of which may not be restricted solely to fish. Specific cadmium-binding proteins distinct from metallothionein have now been found to occur in the tissues of organisms as widely separated as mushrooms (13), whelks (14), rats (15), and rabbits (16).

This work was supported by the award of two SERC/CASE studentships (for K. S. Roberts and D. G. Thomas) and by a grant from the Natural Environment Research Council (Number GR3/4970A). It is a pleasure to acknowledge also the superb secretarial and administrative contributions made by Barbara Power.

REFERENCES
1. Alabaster, J. S., and Lloyd, R. Water Quality Criteria for Freshwater Fish. Butterworth, London, 1980.
2. Roberts, K. S., Cryer, A., Kay, J., Solbe, J. F. del. G., Wharfe, J. R., and Simpson, W. R. The effects of exposure to sub-lethal concentrations of cadmium on enzyme activities and accumulation of the metal in tissues and organs of rainbow and brown trout. Comp. Biochem. Physiol. 62C: 135–140 (1979).
3. Thomas, D. G., Solbe, J. F. del. G., Kay, J., and Cryer, A. Environmental cadmium is not sequestered by metallothionein in rainbow trout. Biochem. Biophys. Soc. Commun. 110: 584–586 (1983).
4. Thomas, D. G., Cryer, A., Solbe, J. F. del. G., and Kay, J. A comparison of the accumulation and protein binding of environmental cadmium in the gills, liver and kidney of rainbow trout. Comp. Biochem. Physiol. 76C: 241–246 (1983).
5. Thomas, D. G. Cellular aspects of cadmium toxicity in fish. Ph. D. thesis, University of Wales, 1984.
6. Roberts, K. S., Cryer, A., Kay, J., and Solbe, J. F. del. G. A high molecular weight cadmium-binding fraction isolated from the liver cytosol of trout exposed to environmentally relevant concentrations of the metal. Biochem. Soc. Trans. 7: 650–651 (1979).
7. Thomas, D. G., Cryer, A., and Kay, J. The effect of endogenous lipids on the apparent size of the cadmium-binding proteins in the liver of rainbow trout. To be submitted.
8. Overnell, J., and Coombs, T. L. Purification and properties of plaice metallothionein, a cadmium-binding protein from the liver of the plaice. Biochem. J. 183: 277–283 (1979).
9. Kagi, J. H. R., and Nordberg, G. F. (Eds.). Metallothionein. Birkhauser-Verlag, Basel, Switzerland, 1979.
10. Vandemalle, R. J., and Garvey, J. S. Radioimmunounassay of metallothioneins. J. Biol. Chem. 254: 4368–4372 (1979).
11. Thomas, D. G., Brown, M. W., Shruben, D., Solbe, J. F. del. G., Cryer, A., and Kay, J. The sequestration of cadmium and zinc in the tissues of rainbow trout following exposure to the metals singly or in combination. Comp. Biochem. Physiol. 82C: 55–62 (1985).
12. Brady, F. O. The physiological function of metallothionein. Trends Biochem. Sci. 7:143–145 (1982).
13. Meisch, H.-U., Beckman, I., and Schmitt, J. A. A new cadmium-binding phosphoglycoprotein from the mushroom, *Agaricus macroporus*. Biochim. Biophys. Acta 745: 259–266 (1983).
14. Dohi, Y., Ohba, K., and Yoneyama, Y. Purification and molecular properties of two cadmium-binding proteins from the hepatopancreas of a whelk, *Buccinum tenuissum*. Biochim. Biophys. Acta 745: 50–60 (1983).
15. Waalkes, M. P., Chernoff, S. B., and Klaassen, C. D. Cadmium-binding proteins of rat testes. Characterisation of a low molecular mass protein that lacks identity with metallothionein. Biochem. J. 220: 811–818 (1984).
16. Post, C. D., Squibb, K. S., Fowler, B. A., Gardner, D. E., Illing, J., and Hook, G. E. R. Production of low molecular weight cadmium-binding proteins in rabbit lung following exposure to cadmium chloride. Biochem. Pharmacol. 31: 2969–2975 (1982).