MINIREVIEW

Transport of Toxic Heavy Metals Across Cell Membranes (44486)

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Abstract. **Membrane transport of nonessential toxic heavy metals (type D heavy metals) not only controls their access to intracellular target sites but also helps determine their uptake, distribution, and excretion from the body. The critical role of membranes in the toxicology of class D metals has attracted the attention of many investigators, and extensive information has been collected on the mechanism(s) of metal transfer across membranes. Characteristics of metal transport in different cells, or even on opposite sides of the same cell, or under different physiological conditions, are not identical, and no unitary hypothesis has been formulated to explain this process in all cells. However, it seems possible that the mechanisms proposed for different cells represent variations on a few common themes.** [P.S.E.B.M. 2000, Vol 223]

Heavy metals, as loosely defined, include metallic elements of greater than 50 Daltons molecular weight that are capable of forming polyvalent cations. Although this definition covers uranium and transuranic metals, we are concerned in the present context with the chemical rather than radiological properties and effects of heavy metals. In general, the ions of these elements are chemically very reactive. Many, for instance, possess a high affinity especially for sulfhydryl groups in proteins and smaller biological molecules; uranium, in the form of uranyl ions, reacts preferentially with oxygen as in carbonate or in carboxyl groups. As a consequence, the metals can critically affect function in many biological systems, whether as essential components of enzymes, as powerful enzyme inhibitors, or in other ways.

From a biological perspective, and for the purposes of this review, the heavy metals can conveniently be assigned to four operationally distinct classes. Class A is represented by iron, which is essential for life in relatively high concentrations. Class B contains metals for which no biological role has been established, and which in low concentrations exhibit little or no toxicity; they include lanthanum, strontium, and others. A third class, C, consists of elements like zinc, copper, nickel, cobalt, molybdenum, and perhaps chromium, all essential in trace amounts for at least some living systems. At higher concentrations, class C elements can become very toxic, in line with the basic recognition by Paracelsus in the 16th century that "the right dose differentiates a poison from a remedy." Finally, heavy metals that are toxic even at very low levels, and for which no clear biological function has been established, are collected in class D. They include elements like mercury, lead, cadmium, and uranium. Metals from all four classes are present not only in occupational settings but also in the general environment, originating both from natural sources and from human activities. Industrialization and intense agricultural practices greatly increase environmental metal concentrations, as in the case of cadmium (1), and these metals therefore constitute potential or actual health risks to humans even outside occupational settings.

Although the mechanisms whereby these metals exert their toxicity at intracellular targets have been investigated extensively, many questions about their actions remain unanswered; among them is that of the chemical species of the

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metal actually crossing the cell membranes. In the body, most cells are potentially exposed to the diffusible metal species circulating in plasma. Many of these diffusible species, of course, also can react directly with various membrane constituents and thus cause functional lesions (2).

The ability of heavy metals to cross membranes into cells clearly forms a critical factor in many of their toxic actions. In addition, transmembrane transfer of metals may also be involved in their absorption, distribution in the body, and excretion, and thus transmembrane transfer helps determine metal toxicokinetics. Because of the importance of cell membranes in metal toxicology (2), the present review focuses on the question of how toxic and nonessential heavy metals (i.e., class D metals) cross plasma membranes, primarily in animal cells. A small number of representative metals are considered, primarily cadmium and mercury, sufficient to reveal both similarities and differences in their handling by different cells. It is not the purpose of this review to provide an in-depth analysis of the transmembrane movement of specific metals.

Some Basic Considerations

It will be useful to reemphasize here several important and characteristic properties of heavy metals and their reactions in biological systems. Failure to appreciate these characteristics fully may lead to invalid conclusions about membrane transport of heavy metals.

The Speciation of Metals Crossing the Membrane. A recent calculation led to the conclusion that the concentration of free (unbound) copper (Cu) in yeast is less than one ion/cell, in the face of an overall concentration of the metal of about 70 μM (3); in other words, essentially no unbound Cu exists in the cell. For class D metals, high affinity for proteins and many other biological molecules makes it similarly unlikely that their ions, except perhaps extremely transiently, can exist free in biological systems. Instead, the metals occur in complexes, bound to both high- and low-molecular-weight ligands. Transfer of metals across cell membranes, at least *in vivo*, therefore generally involves transfer of diffusible metal complexes. As further consequence of ready complex formation, class D metals, even when added to an inorganic cell culture medium, are not likely to cross a cell membrane passively without interacting with its constituents. Even passage through ion channels presumably involves control by the channel over ion movement (see next section).

Complexes involved in the transmembrane transport of metals *in vivo* need not be identical with those in which metals circulate in plasma. Thus, the affinity of metal binding sites on renal and intestinal cell membranes for mercuric mercury is much higher than is that of EDTA; in contrast, cadmium (Cd) is ligated more strongly by EDTA than by cell membranes. As a result, this chelator inhibits uptake of Cd by kidney or intestine, and probably other tissues, but exerts no effect on uptake of mercury (4). The mercury complex of EDTA dissociates in competition with metal binding sites on the membrane, and the chelator therefore plays no further role in mercury (Hg) uptake.

The rapidity of metal complex formation can be illustrated by the finding that following injection into the renal artery of a bolus containing chlorides of Cd, Hg, or Zn, the metals bind to plasma proteins and become nonfilterable within the fraction of a second required for the bolus to reach the glomeruli (5). Such a result raises the question to what extent *in vitro* findings (for instance, metal uptake by cells suspended in physiological saline) are always relevant to metal uptake *in vivo*. It is not certain, for instance, that cells will take up Cd in the same manner, whether suspended in a balanced salt solution or in the presence of the normal constituents of plasma or interstitial fluid.

The ability of the heavy metals to react with a great variety of molecules may also obscure actions of some metabolic inhibitors on metal movement. For instance, it has been observed repeatedly that dinitrophenol (DNP), in millimolar concentrations, depresses cellular uptake of Hg or Cd present in micromolar concentrations (6). Now, DNP readily forms metal salts, so that its inhibition of metal uptake could reflect binding of the metal; this would render it unavailable for reaction with the cell membrane and for transport. It follows that depression of energy metabolism in these experiments might have played little role in the inhibition of metal uptake. The unambiguous need for energy supply to support metal uptake, in any case, is not easily established. This fact is further considered in a later section.

Specificity of Metal Transport. Maintenance of homeostasis may require more or less specific mechanisms for mediating the cellular uptake of different essential metals (class C). In contrast, no obvious selective advantage would be gained in evolving such mechanisms for mediating uptake and maintaining homeostasis of nonessential and toxic class D elements (7). However, transfer of these elements could conceivably take advantage of partially specific mechanisms primarily controlling movement of essential class C and other elements.

There is strong evidence, for instance, pointing to a role for Ca channels in the uptake of Cd by some cells although no such interaction has been established in others. Thus, Souza *et al.* (8) reviewed findings that Ca channel blockers depress Cd uptake in several, though not all, cell types. Movement of Cd through Ca channels should result in competitive interaction between the two metals. They do, indeed, interact in many systems, but not consistently so in a competitive manner (see later section). An illustration of noncompetitive interaction was described in the rat duodenum, where Ca transport is irreversibly inhibited by Cd (9). If, nevertheless, Cd were believed to be absorbed through duodenal Ca channels, it would be necessary to explain the additional observation that stimulation of Ca absorption with vitamin D is not accompanied by increased Cd uptake.

Cd uptake in hepatocytes was reported to be competitively inhibited by Zn, as well as by several other heavy metals (10); Ca was relatively ineffective. Such interaction

points to possible use of Zn channels by Cd in this system. Intestinal uptake of Cd has similarly been ascribed to Zn transporters, but the evidence remains contradictory. Thus, the Zn inhibition of Cd uptake in rat jejunum was found to be independent of the Cd concentration, and could not be considered competitive under those experimental conditions (7). Upregulation of Zn uptake in Zn-deficiency was not accompanied by simultaneous stimulation of Cd uptake (11). However, Hoadley and Cousins (12), using the whole length of the intestinal tract rather than only jejunal segments, reported competition between Cd and Zn.

Separate processes for Cd and Zn uptakes were suggested by Corrigan and Huang for Chinese Hamster Ovary cells (13) on the basis of discrepancies between K_I (the constant characterizing the inhibition by one metal of the transport of the other) and the K_M for each metal. Similarly in rat jejunum, a high concentration of Zn was required to inhibit Cd transport, and a high concentration of Cd to inhibit Zn transport. Such observations are not easily reconciled with competitive inhibition, and it is doubtful whether simple Michaelis-Menten kinetics are applicable to these systems. An alternative explanation for metal-metal interaction at the cell membrane is offered later in this review.

Differences Between Transport Processes in Various Cell Types and Membranes. A frequent observation indicates that uptake of a metal across a cell membrane may involve more than one parallel mechanism. Souza *et al.* (8), for instance, described two such processes for Cd in hepatocytes, distinguished by their temperature dependence and sensitivity to inhibitors. Similarly, only 1/3 of Cd entering hepatocytes appears to use Ca channels (14). Separate processes clearly may coexist for transporting a particular metal into a cell.

Although mechanisms responsible in different cells for passage of heavy metals across membranes may resemble each other, there is no reason to expect that they will be identical. Indeed, differences have been pointed out, for instance, between the process of Cd uptake in hepatocytes and renal cortical epithelial cells (15). The reported temperature independence of Cd uptake by confluent cultures of renal cells was suggested to reflect uptake by simple diffusion. However, as already pointed out, it seems unlikely that a reactive ion could cross a tight membrane passively without reacting with its components. Cd uptake by voltage-sensitive Ca channels described in a pituitary cell line differs from the uptake in glial cells, where Ca channels do not appear to be involved (16), and from the process of Cd upake in jejunum.

Just as different mechanisms may be responsible for transferring heavy metals across membranes in various cell types, it is not surprising to find that transcellular metal movement across an epithelial cell barrier, for example, may result from different processes at the apical and basolateral cell membranes. Suggestive evidence for such differences has been found for the rat jejunum (17). However, the observation that apical uptake of Cd is inhibited by Zn, whereas basolateral extrusion does not appear to be, could be related to the presumably very low concentration of free as opposed to total Zn in the cell. In the kidney, metals complexed with the low-molecular-weight protein metallothionein are absorbed from the tubular lumen by a relatively specific carrier system at the brush border (18); Cd-metallothionein, in contrast, does not react with basolateral cell membranes (19).

The distinction between metal transport mechanisms in different cells may be complicated by specific effects of physiological variables such as age and diet. An example of such an effect is seen in the decrease of intestinal metal permeability with development.

Evidence for Active Transport and Participation of Sulfhydryl Groups. Since class D metals exist in the body, and particularly the cytoplasm, primarily as complexes, it is difficult to estimate their electrochemical activity gradient across the plasma membrane. Therefore, claims that cellular uptake of heavy metals requires energy expenditure need to be treated with caution. Further, as will be seen, a requirement for metabolic energy to take up metals cannot always be inferred simply from apparent inhibition of the process by metabolic inhibitors, or from its temperature dependence.

A possible role of complex formation in the action of inhibitors like DNP was already mentioned previously. The argument can probably be extended to other metabolic inhibitors like cyanide, and also to sulfhydryl-containing reagents. For instance, excess cysteine interferes with Cd transport in the renal tubule. Similarly, in the work of Souza *et al.* (8), excess dithiothreitol (DTT) was applied as a protector of thiol groups, and it counteracted the effect of Cd on Ca transport. This could reflect a role of SH groups in the action of Cd on the membrane. A more likely explanation for this observation is that excess DTT essentially completely binds Cd, thus making it unavailable for reactions with the cell membrane and consequent uptake into cells; this would prevent any further effects of the metal.

Possible explanations for temperature dependence and saturability of metal transport, not invoking mediation by energy-requiring carriers, are offered below. However, energy expenditure for metal transport may be implied by the discovery of P-type ATPases that pump heavy metals (20); however, it is not certain that the function of ATP here is to serve as a source of metabolic energy.

Temperature Dependence of Metal Movement. Several investigators reported that transfer of some metals across cell membranes varies with temperature. For instance, Endo *et al.*(6) observed that rat renal epithelial cells in culture take up Hg faster in the warm than in the cold. At the time this fact was interpreted in terms of active Hg transport, a conclusion overlooking an earlier suggestion that temperature effects on transport of heavy metals might reflect changes in membrane fluidity (21). Indeed, temperature dependence cannot prove participation of active transport in metal uptake. Instead, quite apart from the influence

of temperature on membrane properties such as membrane fluidity, the change in the rate of uptake with temperature here may simply reflect the well-documented and significant temperature dependence of physical diffusion in an aqueous medium; this exhibits a Q_{10} of around 1.2. Therefore, in general, a 50% decrease in activity associated with a drop in temperature from 37° to 4°C, equivalent to a Q_{10} of $\approx$ 1.2, is hardly characteristic of active transport. No kinetic evidence was found for a phase change in membrane lipids of jejunal epithelial cells between 0° and 37° C (22).

Saturability of Metal Uptake. Apparent saturation of metal transfer at higher metal concentrations is another observation that cannot automatically be equated to involvement of a saturable transporter. Reference in this connection may be made to studies of metal transport by jejunal epithelial cells of the rat, where apparent saturation could be attributed to electrostatic neutralization of fixed membrane charges. An excess of unlabelled Cd in a saline perfusate fractionally depressed ^{109}Cd absorption to the same extent as did an equal concentration of Ni (23) or Zn (22). Except for Hg^{++}, normally present in saline as polychloride anions (4), all polyvalent cations tested, including polylysine, inhibited Cd uptake (7). Specifically for La^{3+}, Zn^{2+}, and Ca^{2+}, the inhibition was independent of Cd concentration, and could therefore not result from competition between the metals.

Metal Transport in Various Cell Types

Transfer of heavy metals across plasma membranes has been studied in numerous cell types. Despite many similarities, the mechanisms involved frequently exhibit properties characteristic of different cells. Little evidence has been found for participation of pinocytosis. This can be deduced from the ready uptake of several heavy metals by purified membrane vesicles, as reported by several investigators (24), and by erythrocytes. In neither of these systems has significant pinocytosis been reported.

This section reviews and summarizes under appropriate headings some of the important information available on membrane transport of metals in different cells. No unitary hypothesis has been formulated to explain transport in all systems, but the similarities in these processes have been less emphasized than their differences.

Intestinal Mucosal Cells. Intestinal absorption and pulmonary uptake are the major routes of metal entrance into the body of higher organisms. The question to be considered here and under the next subheading will therefore be how metals cross the intestinal and alveolar cell barriers.

Intestinal absorption of class D metals has been studied in many laboratories, using especially isolated segments of the small intestine perfused *in vivo*, or everted sacs *in vitro*. The implicit assumption was generally made that the pathway of metal movement was identical in the two preparations, and that the metals followed a transcellular pathway. Metal absorption in that case would involve both apical and basolateral cell membranes.

The assumption of an invariant diffusion pathway may not always be valid. For instance, in jejunal segments perfused *in situ*, no significant contribution of passage of metals between cells (paracellular pathways) to total metal movement could be observed (23). The evidence against paracellular diffusion of these solutes included the demonstration that the rate of their transmural movement from the lumen into the portal vein is a direct function of their affinity for the intracellular metal-binding protein metallothionein. Also, the kinetics of Cd absorption indicate that the tissue pool of the metal through which the absorbed metal passes is coextensive with the total tissue Cd content; the major portion of the cadmium accumulated by the tissue is present in the cells, not adsorbed to their outside (23).

Preparation of everted sacs, in contrast, is accompanied by a large increase in the density of aqueous pores (4), whose possible role in transmural metal movement along paracellular pathways cannot be ignored. An additional disadvantage of everted sacs for the study of metal absorption specifically is the extensive metal binding in the submucosal tissues; this greatly diminishes transmural metal movement in sacs, and renders them unsuitable for such studies (25). It is therefore only Step 1 of the absorption process, the interactions of metals with apical cell membranes, that can be analyzed usefully in this preparation.

Removal of metals from the intestinal lumen, as expected, greatly depends on the composition of the luminal contents. This determines the nature of metal complexes presented for absorption. When a jejunal segment is continuously perfused with saline containing trace concentrations of Cd, for example, the first step in Cd absorption has been shown to consist of at least two reactions: Step 1A represents the binding of the metal to the membrane, and Step 1B describes internalization of a portion of the bound metal into the cells. The cationic metals react with anionic sites on the cell membrane. In contrast, mercuric mercury is present in solution as anionic polychloride and reacts with a membrane site possessing greater affinity for the metal than does EDTA (26); this is most likely a sulfhydryl group. Uptake of Hg (20 μM) into cells in rat jejunum is not inhibited by up to millimolar concentrations of the anion transport inhibitor diisocyanatostilbene disulfonate (DIDS), suggesting that the polychloride complex is not the form in which Hg crosses the jejunal brushborder; a role of the anion channel in metal uptake has been observed in some other tissues. The absence in jejunum of any effect of even a large excess of DIDS also makes it unlikely that DIDS inhibition in other systems can be attributed to formation of complexes binding the metal in an unavailable form. Such a possibility was raised for the inhibitory effect of the metabolic inhibitor DNP.

Perhaps half of the Cd or Hg bound to the outside of the membrane is subsequently taken up into the cell (21). A similar observation was made by Templeton in renal tubule cells (27), where binding of Cd to the exterior of the cell was not necessarily followed by internalization of the metal.

The internalization step (Step 1B) is temperature sensitive, an observation attributed to changes in membrane fluidity (21). Extrusion of metals from intestinal epithelial cells (Step 2 of metal absorption) may consist of downhill movement of diffusible complexes with compounds like glutathione or cysteine (28).

An additional observation of interest in relation to Cd and Hg uptake in the rat jejunum is that the rate of their removal from the lumen per unit absorbing area in immature animals greatly exceeds that in adults (4), possibly because of contributions from paracellular pathways. Even in one specific epithelial layer, characteristics of metal transport may be influenced by physiological variables such as age. In the present context, this finding emphasizes that transport processes for heavy metals may not be immutable in any one tissue.

Evidence for and against passage of class D metals in intestine and other tissues through homeostatically controlled channels for transporting essential metals like Ca and Zn was already considered previously.

Alveolar Epithelium. Inhalation exposure to class D metals is accompanied by potentially adverse effects, especially because metal uptake across alveolar epithelium is much more efficient than across the intestinal wall (1). Reasons for the greater permeability of alveolar cell barriers have not been clarified. Possible explanations include greater paracellular metal movement in the alveoli and smaller concentrations of metal-binding ligands in alveolar than in intestinal fluids.

Renal Tubule Cells. Transport of heavy metals by renal cells has been investigated in many laboratories; a summary of the results was presented previously (29). The process has been studied in whole animals, in tissue slices, perfused tubules, isolated cells, and purified membrane fractions. Advantages and disadvantages of the various preparations are presented elsewhere in some detail (2).

Class D metals can enter renal tubule cells across both apical and basolateral cell membranes. Fractions of metals circulating in plasma may be filtered and presented to the brush border as a reabsorbable complex with low-molecular-weight proteins like metallothionein (19). Alternatively the filtered metal is likely to be ligated to small molecules like cysteine and glutathione, or to bicarbonate in the case especially of the uranyl ion. These complexes may be transported across the brush border as such, as is the case with the transporter-catalyzed uptake of the cysteine-methylmercury complex discussed below. Other complexes may be reabsorbed provided the metal can still react with binding sites on the membrane. In that case, an excess of the ligand may depress metal absorption. These facts are well illustrated in the work of Felley-Bosco and Diezy (30) who observed in tubular microinjection studies that 70% of Cd injected as $CdCl_2$ was reabsorbed from the tubular lumen. In the presence of equimolar L-cysteine, fractional reabsorption of Cd rose to 82%; however, a five-fold molar excess of L-cysteine reduced reabsorption to 52%.

Basolateral uptake of heavy metals in the kidney has also been reported frequently. In the dog kidney, for instance, Hg, Cd and Zn, injected in an arterial bolus, crossed the basolateral membranes but only in the presence of a low-molecular-weight thiol compound like mercaptoethanol; the function of this thiol appeared to consist of minimizing sequestration of the metals by nondiffusible plasma proteins (5). Zalups (31), in contrast, suggested a specific role for cysteine and glutathione in the basolateral uptake of Hg in the rat kidney. An alternative and perhaps simpler explanation for the influence of the two thiol molecules on metal uptake was not considered, namely that they increase the diffusible fraction of the metals in plasma, as described for mercaptoethanol above (5). However, the results of Felley-Bosco and Diezy (30) on metal transport at the brush border also pointed to a specific role of cyteine at the basolateral membrane. At that membrane, involvement of the organic anion transport system was described in transport of Hg-conjugates of cysteine and N-acetyl cysteine (32).

Significant similarities are found between metal uptake mechanisms at the jejunal and renal brushborder, and at renal basolateral cell membranes. Differences, however, have also been described. For instance, as mentioned above, metallothionein is transported across apical but not basolateral renal membranes. The similarities include the finding that in all three systems Cd uptake is a relatively slow (i.e., diffusion rather than flow-limited) process, and that basolateral renal as well as apical jejunal uptake are inhibited by Zn (33).

The results of Templeton (27) on Cd binding to membranes of renal cells, followed by internalization of a portion of the bound metal, recall the findings discussed previously on jejunal Cd transport. No role was found for Ca channels in Cd uptake, confirming the results of Hinkle *et al.* (16) on LLC-PK1 cells. In the same cells, Endo *et al.* (34) found evidence for involvement of the anion channel in Hg uptake. The previous observation of the temperature dependence of Hg uptake by renal cortical epithelial cells was confirmed, but now was attributed to changes in membrane fluidity, in agreement with the earlier conclusion of Foulkes (21). Shaikh *et al.* (15) attributed uptake of Cd in confluent cultures of LLC-PK1 cells to simple diffusion, as pointed out previously. One may, however, raise the question whether a reactive ion like Cd could diffuse across the membrane without reacting with it, and the suggested interpretation cannot readily account for the Zn sensitivity of the process as described above.

Hepatocytes. Metal accumulation by hepatocytes in tissue culture has been investigated extensively. Stacey and Klaassen (35) described the efficient uptake of Cd by these cells. According to Blazka and Shaikh (14), about one-third of Cd entering hepatocytes use Ca channels. Partial interaction between Cd and Ca was also described by Souza *et al.* (8): Cd inhibited Ca uptake, but only to a maximum value of 40%. The significance of this kinetic analysis is obscured by the reported time dependence of the fractional

inhibition of Ca transport. It is not clear why calculation of kinetic constants was based on the 30-min incubation time point. The proposed conclusion that Cd competitively inhibits Ca transport in this system therefore remains unresolved. Similarly, the evidence for a role of sulfhydryl groups is subject to alternative explanations.

Erythrocytes. A major fraction of Pb and Cd, for example, in circulating blood is carried in erythrocytes. These cells clearly effectively accumulate the metals. The mechanism of this accumulation has been studied in several laboratories. For instance, Garty *et al.* (36) found that metal transport in erythrocytes is inhibited by N-ethylmaleamide, suggesting a role of sulfhydryl groups in the process.

The erythrocyte membrane differs, of course, in many ways from that of other cells; a distinguishing property is its great anion permeability. It is interesting to note that metal transport in these cells is sensitive to diisocyanatostilbene disulfonate (DIDS) (37, 38), a classical inhibitor of the anion channel. Uptake of a number of metals, including Cd, Pb, Zn, and Cu, is depressed by μM concentrations of this compound. The metals can apparently cross the membranes through the anion channel in the form of anionic complexes with carbonate, bicarbonate, hydroxyl, or chloride ions.

Other Animal Cells. Metal uptake has been followed also in a number of other cell types. The results of Hinkle *et al.* (16) on Cd transport by pheochromocytoma cells have already been cited. Uptake of the metal in these cells is competitively inhibited by Ca and appears to use voltage-sensitive calcium channels. In contrast, Ca channels do not seem to be involved in transport of Cd by mouse neuroblastoma or rat kidney cells. The sensitivity of brine shrimp to cadmium is decreased by the Ca channel inhibitor diltiazem (39), indicating a possible role of the Ca channel in Cd uptake in that organism.

A role of neutral amino acid carriers in the transfer of methylmercury-cysteine has been observed in intestine (40) and the blood-brain barrier (41). In Chinese Hamster ovary cells separate mechanisms have been proposed for Cd and Zn transport (13). An overall conclusion from these experiments with animal cells must take into account both the similarities and the differences between the processes described. Perhaps the metal transport mechanisms found represent variations on some common themes.

Microorganisms. Metal transport has also been studied in microorganisms where it plays a role especially in metal extrusion and the consequent development of metal resistance. Families of transport proteins have been described and implicated in this process (42). These include the so-called cation diffuser facilitator proteins from yeast and other microorganisms; observation of their presence also in mouse and rat tissues raises the possibility of yet additional mechanisms of metal transport in animal cells. In this connection, reference may be made to a yeast protein involved in protecting cells against cadmium, most likely by mediating its extrusion across the cell membrane. This has been shown to resemble the human cystic fibrosis transmembrane conductance regulator (43).

Summary and Conclusions

Many investigators have studied the transport of nonessential and toxic heavy metals across cell membranes. Cells in, or from, a variety of tissues in many species ranging from vertebrates to microorganisms have been obtained for this work. Although many gaps remain in our understanding of the mechanism(s) of metal transport, much useful information has been obtained, and it seems hardly justified to continue to introduce new papers on the subject with the rationale that the process is little understood. What clearly emerges from the work is that no one mechanism has been identified and is likely to exist that can fully explain this process in all cells. Even in one cell type, separate and parallel transport processes have occasionally been observed for metals, while the mechanism of apical metal uptake and basolateral extrusion may differ significantly. A complicating factor is that physiological variables like age can alter the characteristics of metal transport.

Because of their high affinity for proteins and many other biological molecules, heavy metals do not exist in biological systems in a free or unbound form. As a result, *in vitro* studies with free metals may not always yield information directly relevant to reactions of metals *in vivo*. In any case, their high reactivity makes it unlikely that free metals, or partially bound metals like mercury in monomethylmercury can cross membranes without interacting with membrane constituents. However, there is no compelling support for the suggestion that active transport mediates movement of free metals across membranes, and some of the evidence for action of metabolic inhibitors or participation of thiol groups remains subject to alternative explanations.

Highly lipid-soluble metal complexes like dimethylmercury, on the other hand, rapidly and passively cross cell membranes and are extremely neurotoxic. Latex gloves, as recently described in a news report, did not protect an investigator against the lethal effects of this compound.

It seems biologically unlikely that carrier systems should have evolved specifically for mediating uptake of nonessential and toxic heavy metals. In some cells, these metals appear to compete for transport systems designed to maintain homeostatic concentrations of essential trace elements like Ca or Zn. However, evidence for such competition could not be obtained in all cell types. For rat jejunum, where the interaction between Cd and Ca is noncompetitive, a detailed mechanism has been proposed to account for Cd absorption (21). The temperature dependence of the process, and its saturability, can be explained without having to invoke saturation of a conventional carrier system.

In erythrocytes, yet other mechanisms are involved in mediating uptake of heavy metals. Here the sensitivity of the process to DIDS, a classical inhibitor of anion pores, suggests that metals cross the membrane as polyanionic

complexes with chloride and other anions. DIDS-sensitive transport has also been observed in other tissues. Specific carriers for certain metal complexes have been identified in some tissues. They include a renal brushborder system for anionic low-molecular-weight proteins like Cd-metallothionein, a neutral amino acid transport system in the intestine with affinity for Cd-L-cysteine, and a similar system in the blood-brain barrier, capable of reacting with monomethylmercury-cysteine. Families of membrane proteins facilitating transport of metals have been described especially in microorganisms.

In conclusion, heavy metals are transported across cell membranes by a number of complex mechanisms. Although these mechanisms in different cells share many characteristic properties, they may differ significantly in other ways. However, it is attractive to speculate that the various mechanisms reported perhaps represent variations on a few common themes whose details remain to be worked out.

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