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CELLULAR DISCRIMINATION PROCESSES IN METAL ACCUMULATING CELLS

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SUMMARY

A technique is described for investigating cellular fluxes and the accumulation of metal ions in the hepatopancreas of the snail *Helix aspersa*. The method involves the simultaneous administration of double isotopes into the haemocoel and the subsequent detection of these metals in tissues and granule materials. By comparing *in vivo* and *in vitro* data it is possible to detect two separate accumulation systems which appear to correspond with metallothionein and pyrophosphate granules. It is also possible to obtain some information of the nature of possible cellular transport systems.

INTRODUCTION

Living systems have evolved in association with a wide variety of metal ions many of which are essential for the normal functioning of cells. It has, in fact, been argued that the fundamental involvement of iron- and copper-containing enzymes in respiratory pathways indicates that cells must have developed the ability to manipulate these ions before it was possible to evolve aerobic metabolism (Fridovich, 1978).

The association between metal ions and cells is, however, a complicated one involving a wide range of redox and ligand-binding activities. Thus, the occurrence of ferric ions is limited under physiological conditions by the solubility product of the hydroxide, which is 10^{-38} $(mol.dm^{-3})^4$, and cells have evolved a number of ways of overcoming this. Ferric ions are transported and metabolized in association with a variety of proteins such as transferrin and ferritin, which provide a ready source of this element for the haemoglobin or cytochrome oxidase systems. In fact the metal is so essential to all forms of life that withholding supplies from invading organisms is one of the primary defence mechanisms of the body (Weinberg, 1978).

Although metal ions are essential for life they frequently occur in trace amounts so that their study requires special techniques. In addition the ability of cells to handle metals is limited, and as the concentrations are increased a number of inhibitory and toxic effects become apparent. Organisms have a variety of ways of avoiding these effects. The metal may be excreted so that the concentration in the animal is independent of the environment (as with Zn^{2+} in *Carcinus*; Bryan, 1976), it may be excreted in proportion to the body load, so that the animal reflects the concentration

in the environment (as with Pb^{2+} in *Mytilus*; Schulz-Baldes, 1974), or it may be accumulated for some time so that the organism shows concentration factors thousands of times higher than environmental levels (as with Cd^{2+} in *Neiris*; Bryan, 1976).

Examples of this type of bioaccumulation have attracted much attention from environmentalists. There are a number of reasons for this. First, these systems are useful in that they may give direct indications of pollution. Secondly, they may have important influences on food chains since any concentrating effects that are shown by herbivores may become compounded in their predators. Finally, they give some easily measured indication of the ecological consequences of contamination.

Bioaccumulation has also attracted much interest from physiologists. First, it is a phenomenon that is relatively easy to detect but rather difficult to explain at the cellular level. Secondly, it provides, in a number of cases, a way to identify cellular sites of activity either by electron probe microanalysis (Walker *et al.* 1975; Brown, 1977) or by cell fractionation (Coombs & George, 1978). Finally, it has been implied that physiological processes exist specifically for regulating and removing such interfering cations so that the deposits represent the end-product of a detoxification system. If this is correct it would be an important cellular process, but it should be pointed out that there is as yet no direct evidence for such a clear function.

Recently, Coughtrey & Martin (1976, 1977) have shown that there is a massive bioaccumulation of metal ions in the hepatopancreas of the snail *Helix aspersa* collected from sites polluted by smelting works. An extensive range of metal ions are accumulated in this tissue in association with intracellular granules composed mainly of calcium and magnesium salts. These deposits are highly insoluble (Simkiss, 1976) and are formed via an unusual metabolic pathway which accumulates pyrophosphate ions (Howard *et al.* 1981). The granules containing the metal ions are easily extractable from the tissue and provide a method for studying some of the processes involved in the bioaccumulation of contaminating metal ions. As such, this tissue provides a unique opportunity for investigating the accumulated fluxes of trace elements across living cells.

MATERIALS AND METHODS

The snail *H. aspersa* was used throughout this work. Specimens were collected locally and maintained in the laboratory on a diet of carrots, lettuce and calcium carbonate for several weeks prior to use.

The object of the experiments was to attempt to characterize the metabolic pathways involved in removing a variety of metal ions from the blood and incorporating them into intracellular granules. The basic approach was to use a range of metal ions which would accumulate in the cells and also act as experimental probes. Thus, it was hoped that the properties of the bioaccumulation system would themselves be revealed by the pattern of the cellular interactions with the different metal probes.

In order to limit the various effects of several different discrimination sites in this process it is essential to eliminate as many epithelial transport systems as possible. For this reason the normal dietary route for metal ion uptake was rejected since the intestinal mucosa undoubtedly discriminates between different ions. All metal ions were therefore administered intravascularly. The optic tentacle of a snail was severed and a cannula introduced directly into the animal's haemocoel. This is a very simp

operation since the tentacle has circular muscles which immediately contract to seal the cut. It is therefore possible to sample the blood repeatedly by inserting and removing the cannula without anaesthetizing the animal or ligaturing it in any way.

The radio-isotopes used were $^{54}Mn^{2+}$, $^{59}Fe^{3+}$, $^{60}Co^{2+}$, $^{65}Zn^{2+}$, $^{85}Sr^{2+}$, $^{109}Cd^{2+}$ and $^{203}Hg^{2+}$ obtained from Radiochemical Centre (Amersham). All isotopes were made up to a concentration of 10 mmol.dm^{-3} with non-radioactive salts (as chlorides) and 200 μl were injected into the haemocoel of snails with an approximate blood volume of 2–3 cm^3, i.e. from snails weighing 7–8 g. Where an experiment involved injecting isotopes and subsequently sampling the blood, separate tentacles were used for each process to avoid any possible contamination. In order to avoid errors in absolute quantification and in order to compensate within the experiment for any differences in rates of granule formation or other physiological activity, all experiments were performed on mixtures of two metals and the results are expressed as ratios of the relative uptake of those two isotopes. All experiments were done in quadruplicate.

Three sets of measurements were made in each experiment. First, snails were injected *in vivo* with the radio-isotope and left for 6 h before sampling. A piece of hepatopancreas was then removed and used for counting the accumulated dose of each isotope. A second set of samples was taken simultaneously from the same animal by collecting a blood sample and by extracting the granules from the remaining piece of hepatopancreas. Granule extraction was performed by homogenizing the tissue and repeatedly centrifuging and resuspending the sample until a clear white pellet of pure granules was obtained (Howard *et al.* 1981). Blood and granules were both counted for radioactivity. Finally, a third set of experiments were performed by bleeding 'donor' snails and then homogenizing their hepatopancreas to extract the granules. The *in vitro* blood sample was spiked with an equivalent injection of two radio-isotopes, equilibrated with 5 % CO_2, 95 % O_2 and shaken continuously for 6 h with a sample of the isolated granules. They were then washed and counted with a separate blood sample.

Radioactivity was determined on an Ultragamma model 1280 (LKB Instruments). Windows were set manually by characterizing the counting conditions for each isotope. Automatic correction was used for 'spill over' between channels by using the spectra of individual pure samples of each radio-isotope. Injection solutions of dual isotopes were prepared so that there were approximately equal counts in each channel after these 'spill over' corrections had been made. Any specimens which gave less than twice the background levels after spectrum compensations were rejected as unreliable.

RESULTS

In physiological studies the discrimination ratios between two isotopes are normally expressed as the observed ratio (O.R.) of a sample to its precursor. Thus for two isotopes (X and Y) the observed ratio for a tissue (t) exposed to blood (b) would be

$$\text{O.R.} = \frac{X_t}{Y_t} \Big/ \frac{X_b}{Y_b}.$$

The O.R. for hepatopancreas tissue to blood is calculated in Table 1. In all cases the metal shown in the first column is expressed as a molar concentration factor over the

Table 1. *Observed molar ratios for the accumulation of metal ion pairs in hepatopancreas tissue treated* in vivo

(Note that the larger the number and the further the metal is to the right, the weaker the cellular accumulation. An 'iso metal' diagonal divides the table and theoretically all values to the left of this should be below 1·0 and all those to the right should be over 1·0. The values to the left of this 'iso metal' line are the reciprocals of the values for the same metal pairs to the right of this diagonal.)

	Mn	Fe	Cd	Hg	Zn	Co	Sr
Mn	—	3·4	35·7	35·5	63·5	92*	352
Fe	0·29	—	1·1	2·8	3·3	19·2	36·2
Cd	0·03	0·87	—	10·9	3·2	11·3	39·6
Hg	0·03	0·35	0·09	—	0·44	0·64	4·9
Zn	0·02	0·31	0·31	2·3	—	4·4	2·7
Co	0·01*	0·05	0·08	1·6	0·22	—	8·0
Sr	0·003	0·03	0·03	0·10	0·37	0·12	—

* Single samples.

Table 2. *Observed molar ratios for the accumulation of metal ion pairs in granules formed* in vivo

	Mn	Fe	Zn	Co	Cd	Hg	Sr
Mn	—	6·3	56	n.d.	35·7	265	4075
Fe	0·16	—	3·5	16·6	3·3	3·8	28·6
Zn	0·02	0·28	—	9·0*	17·8	12·8	2·9
Co	n.d.	0·06	0·11*	—	1·6	2·9	20·6
Cd	0·03	0·30	0·06	0·63	—	5·3	2·4
Hg	0·004	0·26	0·08	0·35	0·19	—	1·5
Sr	0·0002	0·03	0·34	0·05	0·42	0·66	—

n.d. = no data available.
* Single samples.

Table 3. *Observed molar ratios for the accumulation of metal ion pairs in granules exposed to labelled blood* in vitro

	Mn	Fe	Sr	Hg	Cd	Zn	Co
Mn	—	0·46	1·44	5·6	11·7	20·3	13·4
Fe	2·2	—	5·5	4·7	20·9	6·1	28·1
Sr	0·69	0·18	—	3·9	10·8	3·2	12·3
Hg	0·18	0·21	0·26	—	0·82	1·5	4·0
Cd	0·09	0·05	0·09	1·22	—	0·74	1·6
Zn	0·05	0·16	0·32	0·70	1·4	—	1·5*
Co	0·07	0·04	0·08	0·25	0·62	0·67*	—

* Single samples.

other metals. Thus in Table 1, Mn^{2+} is concentrated 3 times more than Fe^{2+}, 63 times more than Zn^{2+}, and 352 times more than Sr^{2+}. A comparison of these ratios enables one to produce a hierarchy for each of the ion pairs which indicates as a first approximation the preference of the system for handling each ion. This is the basis for the arrangement of the metals in Tables 1–3. Thus the series for the hepatopancreas tissues is

$$Mn^{2+} > Fe^{3+} > Cd^{2+} > Hg^{2+} > Zn^{2+} > Co^{2+} > Sr^{2+}. \quad (1)$$

Clearly this series is a mixture of several influences, including protein binding and

granule formation in the hepatopancreas cells. It is to be expected therefore that the series will vary somewhat with the individual treatments. Some of these interactions can be partially isolated by separating the granules which are formed *in vivo* and determining the O.R. of these samples. These results are given in Table 2 and produce the series

$$Mn^{2+} > Fe^{3+} > Zn^{2+} > Co^{2+} > Cd^{2+} > Hg^{2+} > Sr^{2+}. \quad (2)$$

The differences between these two series can be interpreted as due either to enhanced binding by proteins or to discrimination against certain metals by the cell in incorporating them into the mineralized interface of the granules. This latter effect can be quantified by exposing isolated granules to blood–metal ion mixtures *in vitro*. These results are given in Table 3 and produce the series

$$Mn^{2+} > Fe^{3+} > Sr^{2+} > Hg^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+}. \quad (3)$$

DISCUSSION

There are numerous difficulties in studying the cellular mechanisms involved in the bioaccumulation of metal ions. The extensive literature on the analysis of polluted animals cannot be interpreted in terms of mechanisms because the range of variables that are known to affect the data are so large. Thus the doses to which the organism are exposed are often unknown and variable. The routes of administration may be diverse and change from day to day, depending upon whether it is dietary, cutaneous, respiratory or a mixture of all these avenues. The relative accumulation of a number of ions will vary according to the selective permeabilities of the cellular membranes at each of the sites of uptake. The physiological state (e.g. feeding/starving, hydrated/dehydrated, acidotic/alkalotic, etc.) of the animal will also determine the relative loads of metals accumulated each day, and changes in turnover rates will determine what the apparent effect is for each accumulated ion. For most invertebrates these problems have barely been identified, let alone quantified, so that the interpretation of bioaccumulation data is virtually impossible despite the large amount of work on this subject.

In this paper, I have attempted to devise a relatively simple technique which overcomes many of these problems. A constant dose is given in an acute experiment. By using radio-isotopes that can be detected at very low concentrations and with short exposure times, it is possible to detect newly deposited material and also overcome the turnover problems of accumulated doses. The metals are given intravascularly so that there is no discrimination against particular ions by any cellular membranes, other than those of the accumulating tissue. Finally, by using dual labelling experiments and expressing the results as ratios it is possible to minimize most of the physiological variables between animals since relative uptakes are measured and no absolute values are involved.

The theoretical basis of this method is shown in Fig. 1. The mechanisms involved by cells in the uptake of metals from the blood are not known and may involve diffusion or binding to ligands. If, however, they are represented as first order reactions

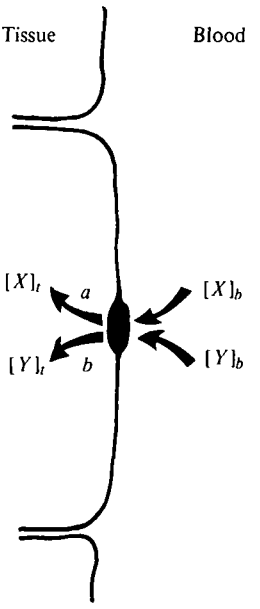

Fig. 1. The entry of metals (X and Y) from the blood (b) into the tissue (t) may be envisaged as simple proportionality constants (a and b). If two metals enter the same cell along a common route they may, however, interact.

then the accumulation of a radio-isotope (X) in tissue (t) depends upon the concentration of the metal in the blood (b), i.e.

$$[X_t] \propto [X_b],$$

therefore

$$[X_t] = a[X_b] \quad \text{or} \quad a = \frac{[X_t]}{[X_b]}.$$

The value a is a proportionality constant which will represent the properties of the uptake system. On its own this value is difficult to interpret because it is probably subject to the range of physiological variables already discussed, as well as a number of chemical constraints (e.g. K_m of binding sites, etc.). If, however, the conditions are kept as constant as possible (e.g. similar metal concentrations in blood) and the experiment is performed simultaneously on a second isotope (Y), then

$$b = \frac{[Y_t]}{[Y_b]}$$

and a comparison of the proportionality constants a and b gives a measure of the cellular discrimination system for those two systems, i.e.

$$\frac{a}{b} = \frac{[X_t]}{[X_b]} \Big/ \frac{[Y_t]}{[Y_b]}.$$

This expression can be rearranged into a more useful form, i.e.

$$\frac{a}{b} = \frac{[X_t]}{[X_b]} \times \frac{[Y_b]}{[Y_t]} = \frac{[X_t]}{[Y_t]} \times \frac{[Y_b]}{[X_b]} = \frac{[X_t]}{[Y_t]} \Big/ \frac{[X_b]}{[Y_b]}.$$

Table 4. *Percentage of metals that are ultrafilterable from blood samples compared with their relative uptake into hepatopancreas tissue*

	Mn^{2+}	Co^{2+}	Zn^{2+}	Cd^{2+}
Ultrafilterable (%)	32 %	27 %	9 %	8 %
Uptake relative to Mn^{2+}	100 %	1·1 %	1·6 %	2·8 %

Since X and Y can now be measured in the same piece of tissue (t) or blood (b) the size of the samples is not critical and a large number of experimental errors can be avoided. Thus the discrimination factor

$$\frac{a}{b} = \frac{X_t}{Y_t} \Big/ \frac{X_b}{Y_b}$$

is now simply the ratio of the accumulated isotopes in two samples.

The simplicity of this technique has obvious attractions but it involves a number of assumptions that need to be questioned. The first is whether the observed differences are due to cellular activities or whether they simply reflect the extent to which the metals are bound by blood proteins. This has been tested in two ways. Blood samples have been mixed with radioactive metals and then ultrafiltered through dialysis sacs in order to determine the extent of protein binding (Howard & Simkiss, 1981). Typical results are shown in Table 4 and it is apparent that there is no similarity between the extent of protein binding in the blood and the cellular discrimination factor. Thus, for example, the major difference in blood binding is a 4-fold difference between Mn^{2+} and Cd^{2+}, whereas the cellular discrimination factor for the same metals is 36-fold. Clearly the binding of the metals by the blood proteins is not the explanation for the discrimination at the cellular level. The protein binding effect has also been overcome in other experiments by comparing the discrimination factors for granules exposed to metals *in vivo* or *in vitro*. In this case the *in vitro* experiment was performed by using similarly labelled blood to the *in vivo* treatment. Again the differences between the two sets of results must be largely due to the activity of the cells *in vivo* since the metals were always presented in the same blood medium. The final problem is, however, the most serious. In comparing *in vitro* and *in vivo* uptake it is assumed that both sets of examples are exposed to similar ratios of metal ions. If, however, one metal is excreted or metabolized from the blood at a faster rate than the other then there is a constantly changing ratio in the *in vivo* data whereas the *in vitro* ratio is largely constant. In devising this technique it has therefore been necessary to measure the blood clearance rates of a variety of metals and choose appropriately short times to avoid major changes (B. Howard & K. Simkiss, unpublished). The only ion which is virtually cleared from the blood of the snail within a few hours is manganese, but since this ion is mainly lost to the granules it is a special case and comparisons between *in vitro* and *in vivo* rates can still be made.

The value of the technique can now be shown by discussing the results shown in Tables 1–3. When mixtures of metal ions are injected intravascularly into an animal they become partially ionized and partially bound to the blood proteins and other ligands and are circulated in those states within the organism. As a first approximation these conditions are the same for blood isolated and gassed with 5 % Co_2, 95 % O_2

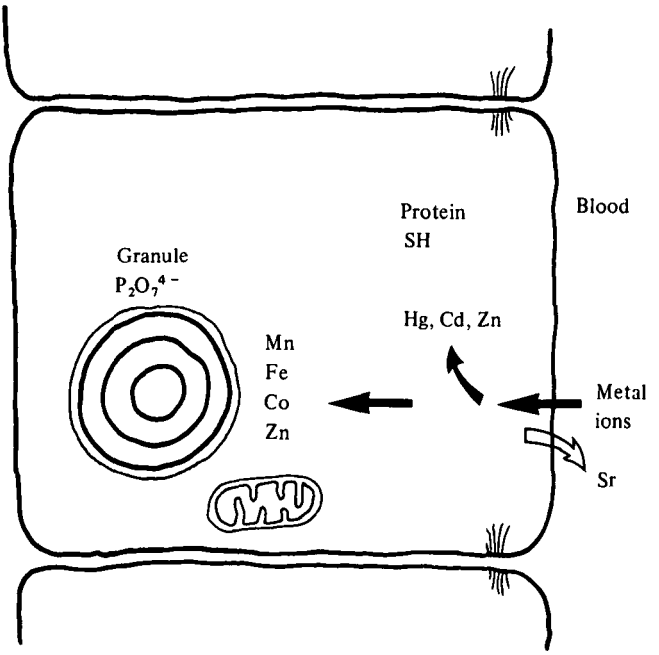

Fig. 2. A cellular interpretation of the double-isotope experiments. One group of metals accumulates in the cytoplasm but not in the granules. These are interpreted as binding to metallothionein-like proteins. A second group accumulate in pyrophosphate granules whereas some (e.g. Sr) are discriminated against by the cell.

and 'spiked' with the same metal ions *in vitro*. The major differences between the two sets of results should therefore be due to the fact that *in vitro* the granules are simply absorbing the metals on to their surfaces, whereas *in vivo* the ratios of the ions will be changed according to the activities of the cells.

It will be apparent that any cellular process that discriminates against a particular ion in these experiments will result in it occupying a lower place in the *in vivo* series whereas any preferential uptake will appear with a higher ranking. The series (1) obtained from the data in Table 1 is thus a measure of the total accumulating systems in the cell. Part of these effects are clearly due to the incorporation of metals on to granules but these deposits can be isolated and analysed separately to give the hierarchy shown in series (2) (Table 2). The differences between the positions of metals in these two series are therefore presumably due to some additional cytoplasmic accumulation system. Examining the two series

$$Mn^{2+} > Fe^{3+} > Cd^{2+} > Hg^{2+} > Zn^{2+} > Co^{2+} > Sr^{2+}, \quad (1)$$

$$Mn^{2+} > Fe^{3+} > Zn^{2+} > Co^{2+} > Cd^{2+} > Hg^{2+} > Sr^{2+}, \quad (2)$$

shows that the total tissue (series 1) preferentially takes up Cd^{2+} and Hg^{2+}, which have moved from behind Co^{2+} to in front of Zn^{2+} when compared with series 2. This is interpreted as evidence for a separate protein binding system that accumulated Cd^{2+}, Hg^{2+} and Zn^{2+} in some cells of the hepatopancreas.

The cellular process involved in the formation of metal granules can be furth

analysed by comparing the *in vivo* series (2) with the corresponding data obtained in the *in vitro* series (3). Any differences between these two series can as a first approximation be interpreted as due to selective cytoplasmic activities, i.e.

$$Mn^{2+} > Fe^{3+} > Zn^{2+} > Co^{2+} > Cd^{2+} > Hg^{2+} > Sr^{2+}, \quad (2)$$

$$Mn^{2+} > Fe^{3+} > Sr^{2+} > Hg^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+}. \quad (3)$$

It is clear that the hepatopancreas cells exert a very strong discrimination against Sr^{2+} and to a lesser extent against Hg^{2+}, although this may be partly due to the cytoplasmic binding of this ion already discussed. The hepatopancreas cells preferentially take up Zn^{2+} and Co^{2+} ions and deposit them in granules and this should be considered in addition to the strong preference which all the series show for Mn^{2+} and Fe^{2+}.

The interpretations of series (1), (2) and (3) are summarized in Fig. 2, which can now be considered in the light of what other studies have revealed about metal accumulating systems in the hepatopancreas of *H. aspersa*.

A wide diversity of organisms are known to produce metallothioneins, a group of small molecular weight, intracellular proteins that are rich in thiol groups. They are variously thought to be involved in zinc metabolism or to be part of a detoxification system (Kagi & Nordberg, 1977). Metallothioneins show strong binding activities towards Cd^{2+}, Hg^{2+}, Cu^{2+} and Zn^{2+} ions, and it is almost certainly these ligands that are responsible for the Cd^{2+} and Hg^{2+} accumulation obtained in these whole tissue experiments. This interpretation is supported by the work of Cooke *et al.* (1979), who isolated and characterized proteins with these general properties from the hepatopancreas of *H. aspersa*.

We have recently shown that the granular deposits in the hepatopancreas of *H. aspersa* are composed of a roughly equimolar mixture of Ca^{2+} and Mg^{2+} ions present as ortho (PO_4^{3-}) and pyrophosphates ($P_2O_7^{4-}$) (Howard *et al.* 1981). The pyrophosphate ion is produced by anabolic processes in most cells but it is normally rapidly hydrolysed by inorganic pyrophosphate, an enzyme that occurs in every cell that has so far been studied. The persistence of pyrophosphate ions in the hepatopancreas cells may be assumed therefore to have some functional significance.

Pyrophosphates have two remarkable properties that are important in terms of the binding of metal ions to ligands. First, they provide salts that are typically several thousand times less soluble than the characteristically insoluble orthophosphates. Secondly, they are relatively poor electron donors so that they show little change in binding strength from one metal ion to another. Both these properties would be ideal for a detoxification system since they would reduce the ion activities of a wide range of metals to a very low level. It is not surprising therefore that a great variety of metal ions are removed from the blood and immobilized on these deposits. The ions which appear to be particularly favoured are Mn^{2+}, Fe^{3+}, Co^{2+} and Zn^{2+} for the reasons that have already been discussed. There is some problem in interpreting the iron result since although ferric ion is injected into the animal and probably retains this form in the body fluids, cytoplasmic iron is often in the reduced form of ferrous ions once it is outside ferritin molecules. Cobalt may present a somewhat similar case in that it may be oxidized when it is exposed to 95 % O_2 during the *in vitro* treatment. Despite these complications, it is still possible to speculate that these experiments give some

Table 5. *Positions in the periodic table, ionic radii (bold figures) (nm) and electronegativity of the ions used in these studies*

Ca	Mn	Fe	Co	Zn
0·099	**0·080**	**0·064** (iii)	**0·078**	**0·074**
1·0	1·5	**0·076** (ii)	1·8	1·6
		1·8		
Sr	—	—	—	Cd
0·113	—	—	—	**0·097**
1·0	—	—	—	1·7
				Hg
				0·110
				1·9

insight into the cytoplasmic activities involved in favouring the deposition of these ions on the pyrophosphate granules. The relative positions in the periodic table of the various metals used in these experiments are shown in Table 5 together with their ionic radii and electro-negativity. It is apparent that Mn, Fe, Co and Zn all have ionic radii in the range of 0·064–0·080 nm (or 0·074–0·080 nm if iron is ferrous) and electro-negativities of 1·5–1·8. These two characteristics exclude all the other ions tested and could therefore provide a basis for the selectivity shown. In these experiments we have not included data for Ca^{2+} since it occurs in large quantities within the blood and this complicates both the calculation of specific activities and the interpretation of the chemical gradient into the cell. For these reasons the uptake of this ion needs to be considered separately but the indications are that it enters the granules relatively slowly and it has therefore also been included in Table 5 for comparison.

In recent years the term 'heavy metals' has led to considerable confusion and it is much more useful to classify them on the basis of the equilibrium constants for metal ion–ligand complexes. On this scheme there are two main classes variously called A or 'hard acids' and B or 'soft acids'. The former show a preference for ligands in the sequence

$$O > N > S$$

while the latter show the reverse order

$$S > N > O.$$

A 'borderline' group of metals show intermediate properties between A and B groups (Nieboer & Richardson, 1980). It is interesting to note therefore that the metal accumulating cells of the hepatopancreas of *H. aspersa* contain pyrophosphate ions, which with their charged oxygen atoms act as powerful ligands for class A metals, and a metallothionein-like protein which, with its abundant thiol groups, binds class B metals as tight complexes. It is not surprising therefore that the hepatopancreas is capable of acting as such a diverse site for metal bioaccumulation.

It would appear therefore that the technique which is described of using mixtures of metal ions is a powerful probe for investigating the metabolic activities of some cells. The results are often startling since they demonstrate cellular discrimination of several thousand-fold in the handling of different metal ions. These differences show up in a consistent pattern of interactions which all tend to produce the same discrimination series. These series can be interpreted as providing evidence for two types

of accumulation and also for indicating the types of the cellular transport systems involved. The technique is, however, potentially even more powerful than this for it will be obvious that a number of metals appear to be out of sequence in Tables 1–3. One explanation for this would be that in certain experimental combinations the two metals compete for a single pathway. A clear example of this is seen in Table 1, where the general series is Cd > Hg > Zn. When these metals are combined (e.g. in the Cd and Hg columns of Table 1) these series become reversed, presumably because both metals are then competing for the same binding site. In order to extend the analysis in this way, however, it will be necessary to refine further the methods that are used so as to increase the accuracy of the results.

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