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Host zinc metabolism and the Ehrlich ascites tumour

Zinc redistribution during tumour-related stress

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Zinc redistribution between plasma and liver has been examined in mice injected with Ehrlich-ascites-tumour cells. Within 24 h of injection plasma Zn levels decrease and Zn appears in newly synthesized liver metallothionein. This response is dependent upon the number of tumour cells injected into the host. Uptake of Zn into liver and its specific accumulation in a Zn-binding protein, identified as metallothionein, continues for a number of days and reaches a plateau as tumour growth ceases. Over this time period, plasma copper rises. This redistribution also occurs in mice pretreated with cadmium in their drinking water for 1 month at levels of 20, 50, and 100 μg/ml. However, in each case there is a lag of 3 days before Zn increases in the livers of these animals which already contain substantial amounts of Cd/Zn-metallothionein. When Ehrlich cells are injected into mice previously placed on a Zn-deficient diet for several days, plasma Zn is already low and no net uptake of Zn into liver metallothionein is apparent. Finally, it is shown that ascites fluid can itself stimulate a transient shift of host Zn into liver. Heat-inactivated fluid loses this property. It is suggested that, in the peritoneum, tumour cells initiate a stress response mediated by an ascites-fluid factor.

INTRODUCTION

A variety of stresses upon mammalian organisms, such as bacterial infection, burns, surgery and cancer, cause metal redistribution between plasma and tissues (Beisel *et al.*, 1976; Askari *et al.*, 1980). Frequently, decreases in plasma zinc and iron occur in conjunction with an increase in plasma copper (Powanda, 1981). Particularly in the study of bacterial infection, a hormone-like substance called 'leucocyte endogenous mediator' (LEM) has been isolated that can initiate metal redistribution (Kampschmidt, 1978). Recently, it has been recognized that during different stresses to the host metallothionein (MT) synthesis is induced in liver and that Zn entering liver cells binds to this protein (Sobocinski *et al.*, 1978; Oh *et al.*, 1978; Ohtake *et al.*, 1978). These observations demonstrate that this protein has a fundamental, though undefined, role to play in the normal metabolism of Zn during stress.

The present study was stimulated by reports that various human cancers cause metal redistribution and by the observation that the Ehrlich ascites tumour in mice causes Zn to accumulate in the liver (Askari *et al.*, 1980; Minkel *et al.*, 1979). The present paper characterizes the redistribution of Zn that occurs in zinc-replete (normal) and zinc-deficient mice bearing this tumour. A preliminary report of some of this research has been published (Kraker & Petering, 1983).

MATERIALS AND METHODS

Supplies and chemicals

Sephadex G-75 was purchased from Sigma, St. Louis, MO, U.S.A. 2-Mercaptoethanol was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Eagle's minimal essential medium plus Earle's salts (MEM) was a product of Grand Island Biological Co., Grand Island, NY, U.S.A. The Zn-deficient diet was prepared by Zeigler Brothers, Gardiner, PA, U.S.A., and contained less than 1 μg of Zn/g of diet. All other chemicals were the highest purity available.

Animals and animal care

Female Swiss HA/ICR mice were purchased from Harlan Sprague Dawley, Madison, WI, U.S.A. Animals weighing 28–30 g and 8–10 weeks old were used for the experiments. Groups of five animals were kept in stainless-steel wire cages over wood-chip bedding and maintained at 25 °C with a 12 h light/12 h dark cycle. Zinc-replete animals were fed Purina lab chow containing 70–90 μg of Zn/g and tap water. When animals were exposed to Cd, it was given as $CdCl_2$ in their drinking water.

Zinc-deficient mice were maintained in cages that had been soaked overnight in 1% EDTA and rinsed in distilled water to minimize metal contamination. The feeders were washed in a similar way. Glass water bottles were acid-soaked before the experiment and were used with silicone stoppers. These mice were given zinc-deficient diet together with glass distilled water.

Maintenance of the Ehrlich ascites tumour

The Ehrlich ascites mammary adenocarcinoma was maintained in mice and transferred weekly to new hosts. For experiments *in vivo*, cells were washed free of fluid, suspended in Eagle's MEM, and injected intraperitoneally into mice.

Abbreviations used: BP, binding protein; MT, metallothionein; MT-I and MT-II, two isoproteins of metallothionein; LEM, leucocyte endogenous mediator.

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Preparation and chromatography of mouse liver cytosol

After animals had been killed, the livers were removed, cleaned and weighed. Each liver was homogenized with 3 ml of ice-cold Ringer's solution and 5 μl of 2-mercaptoethanol with a power-stirred Teflon/glass homogenizer for 2 min. The homogenate was centrifuged for 30 min at 20000 rev./min and the supernatant was applied to a 2.5 cm x 60 cm column packed with Sephadex G-75 gel equilibrated with 5 mM-Tris/HCl, pH 7.8. The fractions obtained were analysed for Zn, Cu, and Cd.

To explore the MT nature of the 10 kDa protein, isolated from the Sephadex G-75 eluate, fractions were directly added to a DEAE h.p.l.c. column (75 mm x 7.5 mm) and chromatographed by using a linear gradient of 0.01–0.25 M-Tris/HCl, pH 7.2.

Analysis of superoxide dismutase activity of column fractions

The presence of superoxide dismutase in column fractions was determined by using the method of Beauchamp & Fridovich (1971). This technique measures the inhibitory effect of superoxide dismutase on the generation of O_2^- in the reduction of Nitro Blue Tetrazolium by a xanthine/xanthine oxidase generating system. A stock solution containing 150 μM-xanthine, 37.5 μM-Nitro Blue Tetrazolium and 150 μM-EDTA in 75 mM-sodium carbonate, pH 10.2, was prepared immediately before the reactions were initiated. A 2 ml portion of this solution and 0.1 ml of sample were mixed and the reaction initiated with 5 μl of a suspension of xanthine oxidase (20 units/ml). This was quickly mixed and the formation of blue colour at 560 nm measured. The initial change in absorbance/min for each sample was compared with the reaction rate in the absence of superoxide dismutase and reported as percentage inhibition of the reaction.

Induction of Cd,Zn-Mt

Induction of Cd,Zn-Mt was accomplished by using published methods (Petering *et al.*, 1984). Animals were exposed to three concentrations of Cd as $CdCl_2$ in their water for 30 days: 20, 50 and 100 mg of Cd/ml. The Cd treatment was then discontinued and the animals were given distilled water for 7 days and, finally, they were injected with tumour cells.

Measurement of plasma Zn

After mice were anaesthetized, approx. 1 ml of blood was collected from the eye socket. The heparinized blood was centrifuged in a table-top centrifuge for 5 min at 2000 rev./min to separate the serum from the blood cells. The serum was diluted five times and analysed for metal.

Metal estimation

The Zn, Cu and Cd content of the samples was determined by atomic-absorption spectrophotometry. A Perkin–Elmer 360 or Instrumentation Laboratory IL-357 atomic-absorption spectrophotometer was used for this purpose. Standard curves were developed for each element and used to convert instrumental readings into metal concentration (μg/ml).

RESULTS

Host Zn redistribution after tumour-cell injection

Zn redistribution from plasma into liver occurs in response to the injection into female mice of 1.2×10^7 Ehrlich cells suspended in Eagle's minimal essential

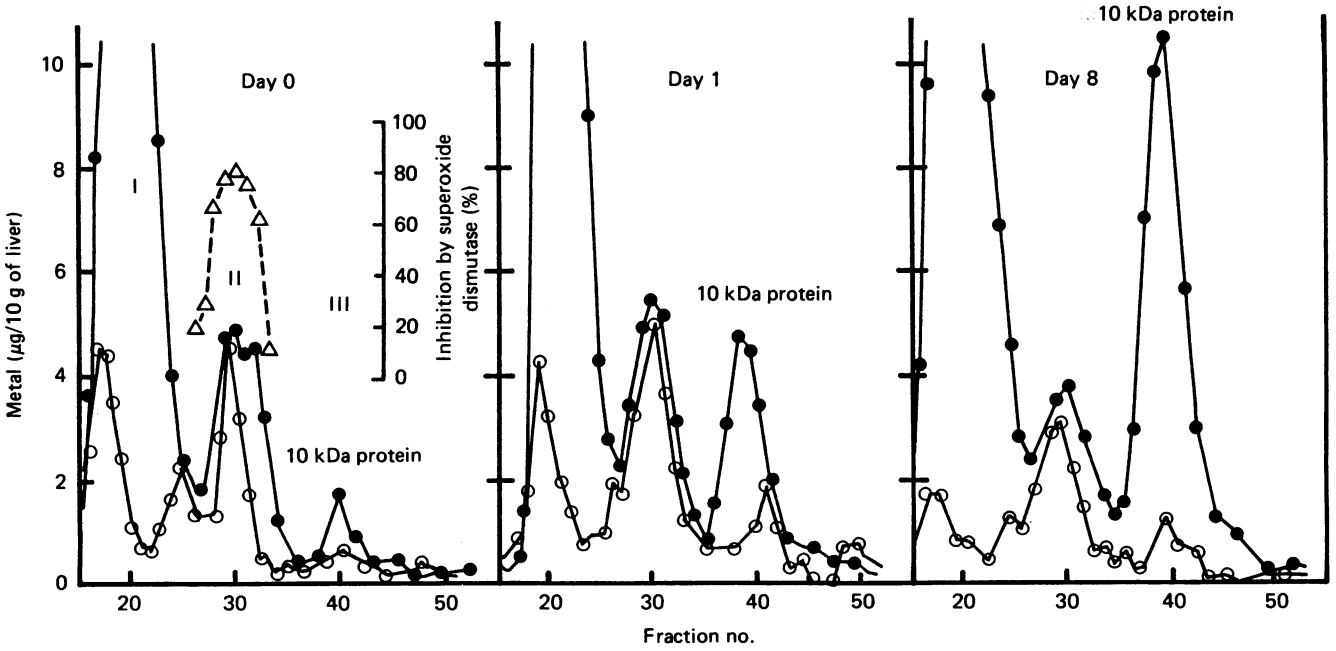

Fig. 1. Sephadex G-75 profiles of liver cytosol Zn and Cu after tumour-cell injection

Cells (1×10^7) were injected at day 0. Profiles were obtained on day 0 (control), day 1, and day 8. ○, Cu; ●, Zn; △, superoxide dismutase activity.

medium plus Earle's salts (Fig. 1). It can be seen that qualitatively the amount of Zn in band III increases with time. This band moves with an apparent M_r of 10000 and is designated 'zinc-binding protein' (Zn-BP). It has been isolated and chromatographed over a DEAE-cellulose ion-exchange h.p.l.c. column (Fig. 2). Two bands of Zn emerge, which are eluted at conductivities of 3.9 and 6.9 mS. Authentic Zn-MT isolated from mice injected with 20 mg/kg of $ZnCl_2$ shows identical behaviour on this column. It is interesting that the bulk of Zn-BP that is found in the liver of mice with advanced Ehrlich tumour moves like Zn-Mt-II, the second isoprotein of Zn-MT.

Returning to Fig. 1, within 24 h Zn-BP is readily observed in liver. Additional Zn enters the protein for several days before a plateau is reached. As illustrated in Fig. 3, Zn uptake into Zn-BP levels off as the tumour itself completes its phase of rapid growth. The rest of the cytosolic profile of Zn observed by Sephadex G-75 chromatography is relatively unaffected by the presence in the host of proliferating tumour cells (described below).

After 1.2×10^7 cells had been injected into mice, zinc was rapidly lost from plasma as it is concomitantly accumulated in liver Zn-BP. According to Table 1 there is a dependence of both plasma and liver binding-protein Zn upon the number of tumour cells in the host. Thus 24 h after injection of 1.1×10^6 cells, no Zn-BP was synthesized in liver, and there is only a small decrease in plasma Zn. In contrast, injection of 10^7 or 10^8 cells stimulates significant Zn-BP production in conjunction with a large decrease in plasma zinc. There is a narrow range of cell counts over which the maximal 24 h production of BP occurs. This probably represents a rate limitation in the stress-response system of the organism,

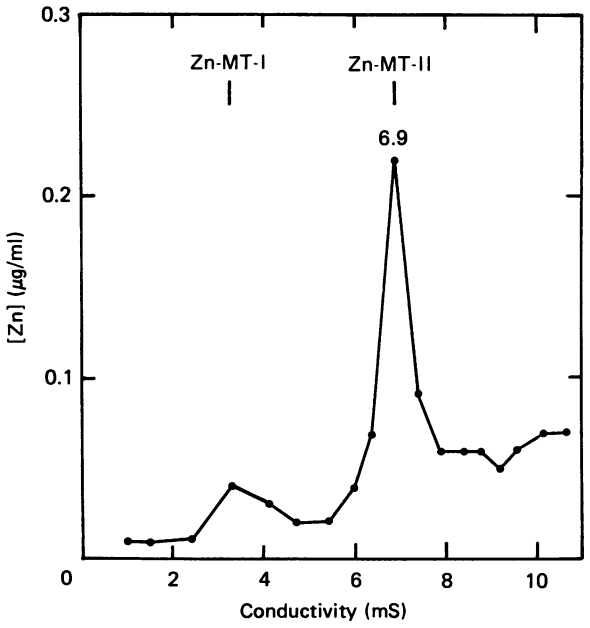

Fig. 2 H.p.l.c.-DEAE column chromatography of band III (Fig. 1) Zn-BP

The elution profile is plotted as the conductivity of the eluate in mS. Elution positions of Zn-MT-I and Zn-MT-II are shown. A 30 min linear gradient was run from 0.01 to 0.25 M-Tris/HCl, pH 7.2, at a flow rate of 1 ml/min. Fractions (1 ml) were collected and their conductivity and Zn content were measured.

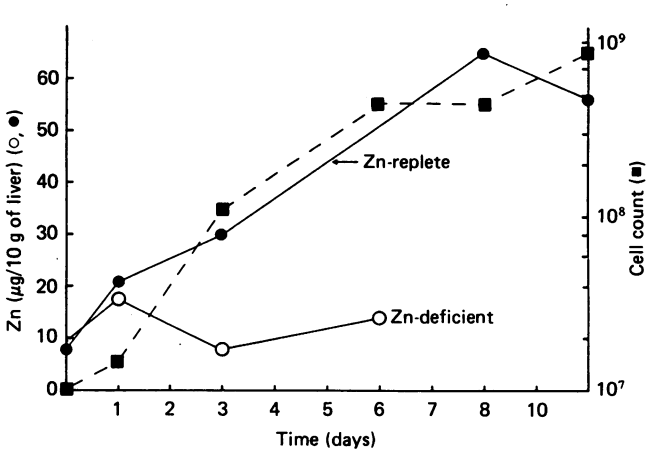

Fig. 3. Kinetics of growth of the Ehrlich ascites tumour and accumulation of Zn in liver MT after Ehrlich-cell injection

Cells (1×10^7) were injected on day 0. One animal was killed at each time point for column chromatography, three for all cell counts.

Table 1. Zn redistribution in mice 24 h after tumour-cell injection

No. of cells*	MT Zn (%)†	Plasma Zn (μg/ml)‡
0	0.5	1.2
1.1×10^6	0.5	0.98
9.9×10^6	11	0.69
1.0×10^8	13	0.53

* Number of Ehrlich cells injected into mice; cells were suspended in Eagle's minimal essential medium.

† Percentage of total supernatant Zn in MT 24 h after intraperitoneal injection of Ehrlich cells; calculations were made after Sephadex G-75 chromatography of liver supernatant from two mice.

‡ Average plasma Zn levels for two mice measured 24 h after injection of cells.

for, as shown in Fig. 3, more Zn-BP is made with time as the tumour grows. Interestingly, although plasma Zn falls to levels similar to those seen in Zn deficiency within 24 h (as discussed below), there is still sufficient circulating Zn to supply the liver with the metal for many days thereafter in order to make more Zn-BP.

Specificity of Zn and Cu redistribution in liver cytosol

Table 2 summarizes data on the metal contents of components of liver cytosol from animals injected with tumour cells. Cytosolic fractions I–III are defined in Fig. 1. Fraction I comprises the high-M_r peak of Zn, whereas II centres on Cu/Zn-superoxide dismutase, which elutes at M_r 32000, as shown by the enzymic-activity profile for the enzyme in Fig. 1(*a*). Metal bound to Zn-BP constitutes the third fraction. In these studies no significant amounts of Zn or Cu were found in later tubes.

Liver binding-protein zinc increases markedly between days 0 and 8 among Zn-replete control animals. Other fractions show no progressive change in zinc concentration. Copper levels remain relatively unchanged over the

Table 2. Metal contents of components of the G-75 Sephadex chromatographic profile of liver cytosol

One animal was killed each day for chromatographic analysis. When no progressive change in metal content was observed, mean ± S.E.M. in μg of metal/10 g of liver is reported for the group of animals in the experiment. The data showing time-dependent changes in metal content are provided. Group designations: Zn(+), Zn-replete control animals fed stock diet and distilled water; Zn(−), mice fed Zn-deficient diet for 4 days before injection of tumour cells on day 0; Cd (20, 50, 100 μmol), animals fed stock diet and distilled water containing Cd at these concentrations for 30 days before tumour injection. During the experiment, mice were fed stock chow diet and distilled water. Values in parentheses are means ± S.E.M. for the metal content between days 0 and 3.

		Content (μg/10 g)								
		Band I			Band II			Band III		
Group	Day	Zn	Cu	Cd	Zn	Cu	Cd	Zn	Cu	Cd
Zn(+)	0	110±4	19±3		32±5	15±1		8	9±2	
	1							21		
	3							30		
	8							65		
	10							56		
Zn(−)	0	166	21±5		42±1	14±1		12±2	4±0.4	
	1	152								
	3	125								
	6	94								
Cd										
20 μg/ml	0	136±18	18±4	0	34±7	10±2	0	33	8±0.5	10±1
	1							28		
	3							18		
								(26±4)		
	6							84		
50 μg/ml	0	132±8	13±2	4±1	41	14	3±2	43	11	40±2
	1				43	20		60	14	
	3				53	23		48	12	
					(46±4)	(19±3)		(50±9)	(12±1)	
	10				15	4		102	4	
100 μg/ml	0	123±4	13±1	6±2	70	39	7±3	85	31	99±9
	3				57	21		90	23	
					(64±7)	(30±7)		(88±2)	(27±4)	
	10				33	1		146	12	

10-day period. However, in a companion experiment, plasma Cu increases by day 6 (Table 3). The elevation of plasma Cu in caeruloplasmin is commonly observed after imposition of a stress upon the host (Askari *et al.*, 1980; Beisel *et al.*, 1976; Powanda, 1981).

Table 3. Plasma Cu levels in serum of mice injected with tumour on day 0

Time after tumour injection (days)*	[Cu]† (μg/ml)
0	0.82±0.06
1	0.88±0.03
3	0.96±0.06
6	1.33±0.10

* On day 0, 5×10^6 cells were injected into each mouse.
† Means ± S.E.M. of data from three animals.

Response of liver to injection of tumour cells in animals fed a Zn-deficient diet

Table 4 shows that clearance of plasma Zn occurs quickly in mice after they are placed on a semi-purified Zn-deficient diet. When tumour cells are injected into animals previously fed such a diet for several days, slow tumour growth still occurs (Minkel *et al.*, 1979). The cytosols of livers gathered from animals made Zn-deficient for 4 days before tumour-cell injection were chromatographed over Sephadex G-75. The metal contents of fractions I–III were determined and are summarized in Table 2. In contrast with the results obtained with mice fed a chow diet throughout the experiment, these animals show no increase in binding-protein zinc over time (Fig. 3). Interestingly, however, a small background amount of Zn in fraction III remained as seen in Zn-replete cells before injection (Fig. 1 and Table 2). Although Zn in fractions II and III remained approximately constant, the Zn content of fraction I fell throughout the experiment. Within error, Cu levels in the various fractions did not change. These results suggest that mild Zn deficiency affects Zn, but not Cu, metabolic redistribution.

Response of liver to injection of tumour cells in animals exposed to Cd

According to Fig. 1 and Table 2, the accumulation of liver Zn-BP after injection of tumour cells is a function of the number of cells and is not limited by the ability of liver to respond to the stress. It was of interest to see if the presence of a pre-existent level of MT would affect the

Table 4. Plasma Zn levels in mice maintained on a Zn-deficient diet

Days on Zn-deficient diet	[Zn] (μg/ml)*
0	1.79±0.14
1	0.91±0.09
2	0.77±0.08
3	0.72±0.06

* Mean±S.E.M. for three mice.

response. Thus Cd,Zn-MT synthesis was stimulated with low levels of Cd to generate a sustained level of Cd/Zn-MT with a small Cd,Zn ratio. The results are summarized in Table 2. In contrast with the Zn-replete group there is no immediate increase in the Zn content of band III in any of the groups of animals exposed to Cd. Only after day 3 does the characteristic redistribution occur. In each of the three Cd-treated groups the ultimate increase in Zn bound to MT or binding protein approximates that in the control, Zn-replete, animals. Thus this part of the stress response is independent of the initial concentration of Zn in MT, which varies from 26 to 88 μg of Zn/10 g of liver, or Cd in the protein, which increases from 10 to 99 μg of Cd/10 g of liver among the three groups of mice.

There are other notable features of the metal profiles of livers from mice exposed to 50 and 100 μg of Cd/ml. There is an elevated level of Cu in band III, which decreases after day 3. Similarly, the Zn and Cu content of band II appears to be higher than in Zn-replete controls or among mice drinking 20 μg of Cd/ml. Again, both Zn and Cu decreased after day 3.

Stimulation of binding-protein synthesis by cell-free ascites fluid

Reasoning that the host, stimulated by the injection of tumour cells, releases a factor into ascites fluid to cause the effects reported here, ascites fluid alone was injected into mice. Fig. 4(*a*) shows a Sephadex G-75 chromatographic profile of liver cytosol from such an animal injected with 0.5 ml of fluid 24 h previously. A substantial amount of Zn-BP is present. This volume of fluid contains 5×10^7 cells. Interestingly, the concentration of Zn in BP, about 30 μg/10 g of liver or about 20% of the cytosol, is similar to that stimulated by 10^7 cells (Table 1). When assessed again at 3 days after fluid injection, little or no Zn-BP was detected.

To show that this effect probably represents a specific response of the mice to a native component of ascites fluid, animals were injected with fluid heat-treated at 60° and 100 °C (Figs. 4*b* and 4*c*). Fluid incubated at the lower temperature does stimulate a decreased amount of binding-protein synthesis. But after heat treatment of the fluid at 100 °C and its injection into mice, no Zn-BP is found in livers of the animals killed 24 h later. This is consistent with the hypothesis that the heat-sensitive tertiary structure of a macromolecular substance in the fluid is needed to elicit the metal redistribution.

DISCUSSION

Part of the process of cancer development is the progressive independence which tumour cells gain in their growth characteristics within the host. Nevertheless, many reciprocal host–tumour relationships exist (Kraker & Petering, 1983). One clear example is that the tumour obtains its nutritional requirements from the host. Some of these may be needed by the tumour more than by the host. Zn is such a requirement. During nutrient Zn limitation, a variety of transplantable tumours are inhibited from growing (Petering *et al.*, 1967; McQuitty *et al.*, 1970; DeWys & Pories, 1972; Minkel *et al.*, 1979). Just as the conditions of the host affect tumour growth, so the presence of the tumour stimulates host responses. Again, in a variety of cancers, the tumour induces metal redistributions within the host. This includes a significant decrease in plasma zinc (Askari *et al.*, 1980). Such effects

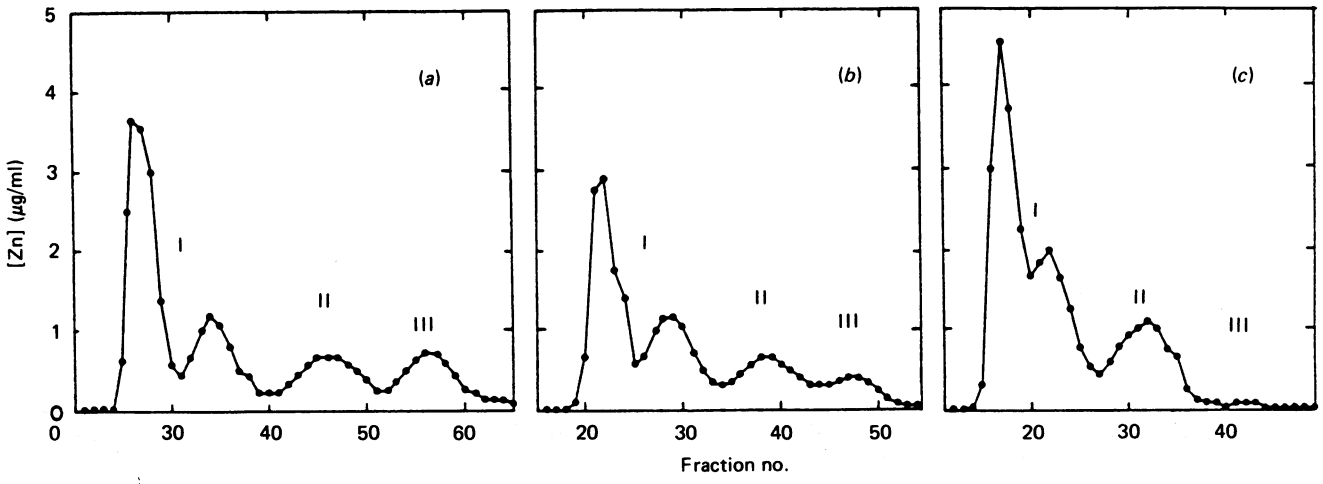

Fig. 4. Sephadex G-75 chromatographic profile of Zn in liver cytosol

(*a*) Mouse injected with 0.5 ml of ascites fluid from donor animal bearing the Ehrlich tumour for 10 days. The mouse was killed 24 h after injection. (*b*) Mouse injected with 0.5 ml of fluid heat-treated for 30 min at 60 °C before injection. (*c*) Mouse injected with 0.5 ml of ascites fluid heat-treated for 10 min at 100 °C before administration. Each profile was normalized to represent 1 g wet weight of liver. The profile is divided into three segments, I, II, III, as in Fig. 1. The larger column used for these livers resolves I into two components. As in Fig. 1, III represents MT.

Table 5. Identification of Band III of Fig. 1 as liver MT

1. Runs as a 10 kDa protein on Sephadex G-75 (Minkel *et al.*, 1980).
2. Binds most of the cytosolic Cd (Minkel *et al.*, 1980).
3. Chromatographs as Zn-MT-I and -II of authentic mouse Zn-MT on DEAE.
4. Rapidly induced and binds Zn as plasma Zn level declines.
5. Rapidly lost without stimulus of induction (Oh *et al.*, 1978; Sobocinski *et al.*, 1978).
6. Sensitive cellular pool in presence of Zn deficiency (Petering *et al.*, 1984).

on circulating metal levels have the appearance of a stress response, which has been studied in detail as a part of the host inflammatory response to infection (Powanda, 1981). The present study considers the properties of the metal-centred response of mice to the Ehrlich tumour and the relationship of the response to other examples of stress responses.

After Ehrlich tumour cells are injected into mice, the plasma Zn concentration rapidly decreases and a new pool of Zn appears in liver in the form of Zn-BP. Table 5 lists the properties that support the identification of this band of metal as MT. It behaves like MT chemically, having an apparent M_r of 10000, being present as two isoforms, with mobility in ion-exchange chromatography identical with those of Zn-MT-I and Zn-MT-II. Biologically, its rapid synthesis in the presence of tumour cells, coupled with the rapid decline in plasma Zn and slower rise in plasma Cu, all indicate that tumour cells act as a stress agent which induces liver MT synthesis. This is supported by the rapid loss of Zn-MT if the stress (ascites fluid) is not continued. Finally, its absence in Zn-deficient animals is consistent with the known sensitivity of Zn in MT to such conditions. Thus Zn-BP is identified as Zn-MT in the rest of the discussion. One interesting feature is that, in mice with larger tumours, liver primarily contains Zn-MT-II. Similar findings were made by two groups who examined partially-hepatectomized-rat liver for the presence of Zn-MT (Ohtake & Koga, 1979; Webb & Cain, 1982). Further studies need to address the question of whether this isoform of metallothionein is preferentially induced throughout the period of tumour growth. Quantitative studies, summarized in Table 2, show that, within error, all of the increases in Zn appear in the MT band. In addition, plasma Cu levels slowly increase. Thus the host response to tumour cells has the appearance of a continual stress response, in which plasma hypozincaemia and the formation of liver Zn-MT are common features (Oh *et al.*, 1978; Sobocinski *et al.*, 1978). The syntheis of MT is dependent on the number of tumour cells injected (Table 1). The first-day response is saturated by the injection of 10^7 cells, but does not represent the maximal amount of MT that can be made with time as the tumour grows (Figs. 1 and 3). Interestingly, the amount of MT made in response to the proliferating tumour reaches a plateau 8–10 days after cell injection. This is also the end of the rapid growth phase of the tumour. Their coincidence suggests that the magnitude of the response over time is related to the total number of cells present and to the proliferative state of the cells. From numerous other studies it is known that Zn in MT, in contrast with Cd, does not reinduce the synthesis of more MT protein as biodegradation occurs (Feldman & Cousins, 1978). Thus the continual presence and increase of Zn-MT in liver also implies that the stimulus for induction of the protein exists and grows with time as the tumour-cell population increases. In keeping with this view, the injection of a small amount of ascites fluid in the absence of cells produces only a temporary increase in liver MT.

According to previous studies, the inflammatory stress response involves the activation of macrophages and perhaps other cells by the foreign agent to release a hormone-like substance, LEM, which is now thought to be the same as endogenous pyrogen or interleukin I (Kampschmidt, 1978; Oppenheim *et al.*, 1982). This, in turn, stimulates liver to take up Zn from plasma. If so, then as Ehrlich cells activate peritoneal macrophages to secrete LEM, the ascites fluid should become a rich source of the factor. As summarized in Fig. 4, ascites fluid does mimic Ehrlich cells in its ability to cause new Zn-MT synthesis in liver. However, the response is transient, since liver Zn-MT was detected on day 1, but not day 3, after fluid injection. The fact that heat treatment inactivates the ability of ascites fluid to stimulate Zn redistribution suggests that this host response is due to a specific heat-sensitive factor in fluid and not to the general presence of foreign protein in the peritoneal cavity. The relative sensitivities of the fluid to temperature qualitatively match those for interleukin I (Lachman, 1983). Further studies are needed to isolate the factor responsible for the effects of fluid in Zn redistribution and to see if its properties match those of LEM or interleukin I.

Because of the centrality of Zn in the stress response and the ease with which nutrient Zn deficiency depletes animals of plasma Zn, it was of interest to see how the host response would be modified under conditions of mild host Zn deficiency. Thus tumour cells were injected into mice that had eaten a Zn-deficient diet for 3–4 days. Given the rapid depletion of 'loosely' bound plasma Zn under these conditions (Table 4), it was not surprising to find the absence of Zn-MT in liver (Fig. 3). That is, plasma was already apparently lacking in metabolically available Zn from the low-M_r pool and albumin, and had no Zn to donate to liver. However, in a study in chicks it was shown that endotoxin, but not cortisol, can induce Zn-MT in the Zn-deficient bird (Sas & Bremner, 1979). Thus decreased plasma Zn may not provide the only explanation for the findings in Fig. 3. Results in Tables 1 and 4 show that plasma Zn decreases precipitously to about 50% of the normal level in both normal mice exposed to tumour cells and in Zn-deficient animals. Yet only in Zn-replete normal mice can Zn be readily accumulated in large amounts by the liver. This comparison demonstrates that plasma containing a low level of Zn can still act as an effective transport medium for Zn. Low plasma Zn does not necessarily signal impaired Zn metabolism and does not prevent Zn uptake into liver over a period of at least 7–10 days as long as a dietary source for the metal exists.

Another perturbation of the stress response system was to pretreat animals with chronic low levels of Cd, which localize in the MT fraction of liver. According to Table 2, the effectiveness of the tumour-cell stress to cause Zn uptake into liver MT was not compromised by the presence of Cd,Zn-MT in large excess of the total incorporation of Zn (50–60 μg of Zn/10 g of liver) due

to the stress. Thus the stress response was not limited by the ability of liver to maintain synthesis of the entire pool of MT in this short-term experiment. However, the kinetics of response of liver to the stress were modified by pre-exposure of animals to Cd. In such mice, livers do not begin to take up Zn until after day 3 at all levels of Cd pretreatment (Table 2). This unexpected finding suggested that Cd may interfere with some aspects of the stress-response mechanism under conditions of low-level oral injection of Cd.

The biochemical basis of the lag effect of Cd is unknown. Two general sites suggest themselves: the site of synthesis of the ascites factor which links the tumour to the liver, and the mechanism of MT induction in liver. Because the metal redistribution is only delayed by Cd, it appears to raise the threshold of the biological response and not the extent once the threshold has been reached. Considering the second site, perhaps the presence of the inducer Cd in liver inhibits the effects of a second inducer of MT synthesis until its concentration reaches a large threshold value. To our knowledge possible interaction between promotor sites for MT has not been studied to test this hypothesis.

The results in Table 2 for the metal content of MT on day 0 in mice treated with Cd are also interesting in light of recent studies of the organization of Cd and Zn among the two metal clusters of MT. As seen in Table 2, the g-atom ratio of Zn to Cd varies between 5.7 and 1.5. Furthermore, significant Cu exists in each sample. These metal ratios differ significantly from those observed in animals injected with large doses of Cd (Minkel, 1980; Otvos & Armitage, 1981). In these cases, there is more Cd than Zn in the protein. Recent studies of the cluster structure of MT have utilized the Cd-injection method. Cd/Zn ratios between 1:1 and 2:1 have been shown to correlate with a preponderance of Cd in the four-metal cluster and Zn in the three-metal cluster (Armitage & Otvos, 1981). If, as inferred, these are strong cluster preferences, then the livers in the present study must contain a number of species of MTs with different metal compositions, including all-Zn MT for the lowest dose of Cd. On the other hand, it is possible that MTs with vastly different metal ratios from those previously studied for their cluster preferences may display other distributions of metals among the two clusters (Otvos *et al.*, 1984).

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