BIOLOGICAL TRACE ELEMENT RESEARCH 5, 363–374 (1983)

Tumor–Host Zinc Metabolism
The Central Role of Metallothionein

ALAN J. KRAKER AND DAVID H. PETERING*
Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201

Received November 10, 1982; Accepted March 9, 1983

Abstract

Features of tumor and host zinc metabolism are described. Emphasis is placed on tumor–host interactions. Using the model of the Ehrlich ascites tumor in mice, one clear site of modulation of cellular zinc by the amount of nutrient zinc available in the host is a zinc-binding protein with the properties of metallothionein. The selective depletion of zinc from this protein is correlated with the loss of cell proliferation by tumors injected into zinc-deficient animals. The properties of isolated metallothionein are consistent with a role for it as a reactive pool of intracellular zinc which can be donated to apozinc proteins and other structures. The presence of the Ehrlich tumor in mice also perturbs their distribution of zinc: zinc leaves the plasma and is accumulated by liver in the form of newly synthesized zinc metallothionein. During host zinc deficiency, this redistribution is not observed. This may be caused not only by a lack of mobile plasma zinc, but also by an inhibition of the initiation of this host response at the site of the tumor in the peritoneum.

Index Entries: Zinc, metabolism in tumors and hosts; tumor, zinc metabolism in; metallothionein, in tumor–host Zn metabolism; inflammation, and tumor–host zinc metabolism; cell proliferation, and zinc metabolism; metabolism, tumor-host zinc.

1. Introduction

It is well-recognized that the nutritional state of an organism has a marked effect upon the onset and course of many diseases including cancer. Besides the influences of major components of the diet, it is now clear that trace elements are important determinants of carcinogenesis and the progression of cancer (*1*). Just as

†Abbreviations: Metallothionein, Mt; Zinc-normal, Zn(+); Zinc-deficient, Zn(−); ethylenediamine tetraacetate, EDTA.

0163–4984/83/1000–0363$02.40

the nutritional status of the host can affect the behavior of tumor cells, so too, the presence of a neoplasm can have a major impact on host nutrition and nutrient metabolism. It is the intent of this paper to review and explore aspects of this reciprocal tumor–host relationship as seen in the examination of tumor cell and host zinc metabolism.

2. Background

The importance of zinc for the growing animal was observed by Elvehjem and coworkers in 1934 (*2*). However, only in the past two decades have experiments shown the widespread and critical need that different proliferative tissues have for zinc. For example, not only does zinc deficiency inhibit the general growth of young animals, but in the growing rat it selectively prevents the growth of thymus tissue (*3*). Long periods of zinc deficiency during pregnancy of the rat lead to fetal resorption; shorter periods of depletion cause a variety of developmental abnormalities (*4*). Similarly, the proliferation of transplanted tumor cells is slowed or halted by host zinc deficiency (*5–8*). Figure 1 shows the comparative growth curves for Ehrlich cells growing intraperitoneally in adult female Swiss mice that were fed a zinc-deficient semipurified diet (<1 μg Zn/g diet) in the presence or absence of drinking water containing 80 μg Zn/mL. Zinc-normal animals sustain 10 times as many cells after log phase growth as do zinc-deficient mice. While the larger tumor kills the rodent in about 17 d, the zinc-depleted animal maintains the tumor cells in a viable, steady state for at least 5 weeks without severe effects in the host (*8*). Subsequent addition of 80 μg Zn/mL to the water supply induces rapid proliferation of the quiescent tumor cell population. Thus, although host zinc deficiency limits tumor growth, it is compatible with the long term survival of the cells.

Table 1 summarizes the properties of zinc-deficient Ehrlich cells. Of particular interest here are the facts (1) that zinc-deficient cells have 75–100% of their zinc-normal complement of zinc, (2) that RNA synthesis is much less affected than DNA synthesis as measured by precursor incorporation into macromolecules, and (3) that thymidine kinase appears to be the principal site of inhibition of thymidine incorporation into DNA. Although the RNA and DNA polymerases that have been examined are zinc metallonenzymes, there is no known requirement for zinc by thymidine kinase (*13–16*). From these results, it appears that cellular zinc depletion is selective, that there is not a general loss of zinc from zinc-metalloproteins and other macromolecular structures, and that the relationship between host zinc deficiency and the cellular response is subtle. What then might be the molecular site that links the zinc status of the host to the proliferative state of the Ehrlich cell? This question is addressed in Sections 3.1–3.4.

Turning to host zinc metabolism in response to the presence of a tumor, a number of studies have shown that extensive metal redistribution occurs in humans afflicted with a variety of cancers (*17*). This process is part of the general stress response of mammalian organisms to a number of inflammatory agents and conditions (*18*). For example, in the inflammatory stress response to bacterial in-

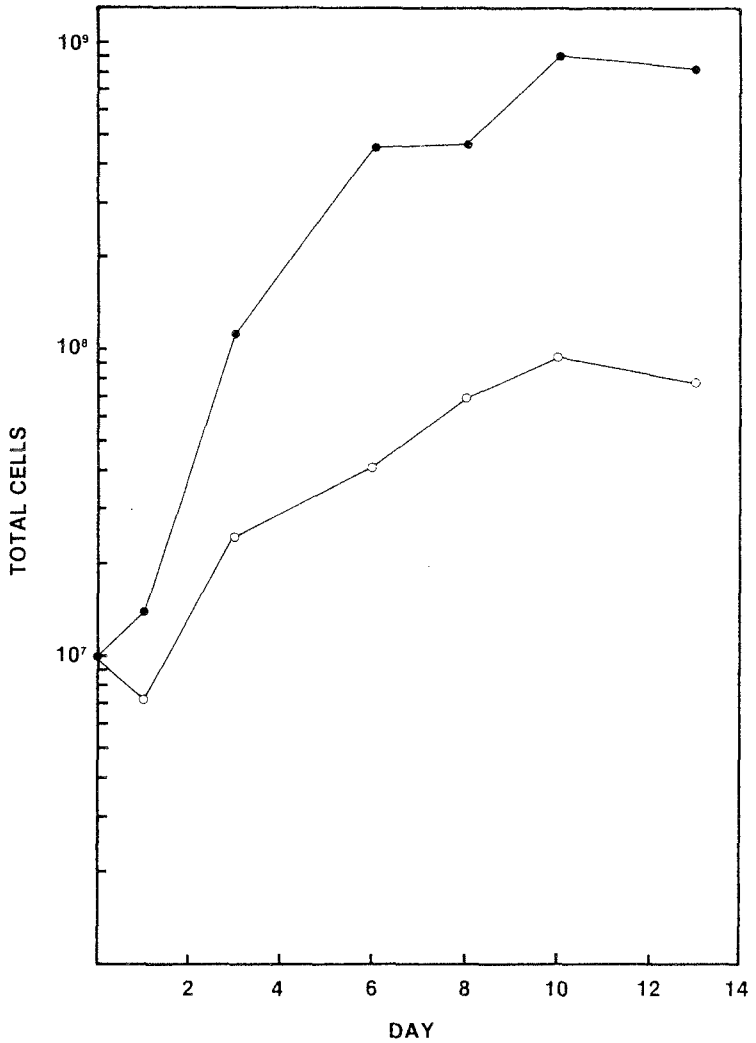

Fig. 1. Growth curves for Ehrlich ascites tumors growing in female Swiss mice. Mice, 10–14 weeks old, were injected with 10^7 cells. Animals were maintained on semipurified diet containing ≤ 1 μg Zn/g. Double distilled drinking water was supplemented with 80 μg Zn/mL (●) or 0 μg Zn/mL (○). Zn(−) animals had received Zn(−) water and diet for 2 weeks prior to day zero. Data are the average of three mice.

fection iron and zinc leave the plasma and enter the liver whereas copper bound to ceruloplasmin moves from liver into plasma. The zinc that enters the liver from plasma becomes bound to metallothionein. This is a small metal-binding protein, the synthesis of which can be induced by a flux of zinc into the liver (*19*). The link between the site of inflammation and liver is a hormone-like substance, leukocyte endogenous mediator (LEM), that is elaborated by macrophages and other host cells involved in nonspecific inflammatory response to foreign agents (*20*). The recent recognition that many secretory factors of monocyte-macrophages probably

TABLE 1
Properties of Zinc-Deficient Ehrlich Ascites Tumors

1. Non-proliferating cells remain viable for > 5 weeks in Zn (−) host animal (*8*)
2. Ascites fluid Zn depressed five-fold (*8*)
3. Ascites cell Zn depressed 0–25% (*8*)
4. Thymidine incorporation into DNA inhibited primarily at thymidine kinase, secondarily at DNA polymerase (*9, 10*)

$$\text{dThd} \xrightarrow{(-)} \text{dTMP} \longrightarrow \text{dTDP} \longrightarrow \text{DTTP} \longrightarrow \text{DNA}$$

5. RNA synthesis not greatly inhibited
6. Ehrlich cells contain a Zn,Cu-binding protein with properties of metallothionein (*11, 12*)
7. Conclusion: Zn deficiency causes specific, selective effect in cells not a generalized depletion of cell zinc for various metalloproteins.

represent a single molecular entity has led to the identification of the LEM activity with this common protein species called interleukin 1 (*21*). Some properties of the inflammatory stress response of mice to Ehrlich cells are described in Sections 4.1 and 4.2.

3. Tumor Metabolism

3.1. Tumor Cell Proliferation: A Molecular Site Sensitive to Zinc Deficiency

The cytosol of Ehrlich cells normally contain a zinc-binding protein (Fig. 2), which has the properties listed in Table 2. In all respects its characteristics are those of metallothionein, a protein apparently involved in zinc, copper, and cadmium metabolism, but one without carefully documented cellular function (*22–25*). Its zinc content accounts for about 20% of the total zinc in cytosol. When the distribution of zinc in the cytosol of zinc-deficient Ehrlich cells is measured by Sephadex chromatography, there is no measurable change in the high molecular weight pool from that seen in Zn(+)† cells. However, the band of Zn in metallothionein has been lost. In the absence of zinc the apoprotein is present at concentrations approximating that of Zn metallothionein in zinc-normal cells (*26*). From this result it is concluded that the apoprotein is a constitutive component of Ehrlich cells. It is not under the regulatory control of internal metal concentration as is observed with liver zinc metallothionein (*27*). Thus, in Ehrlich cells faced with a stringent host zinc deficiency, it is the pool of zinc in metallothionein that responds specifically to the external zinc status.

The labile nature of this pool of metallothionein zinc is further demonstrated in experiments to be reported elsewhere, in which cell proliferation is reactivated in zinc-deficient mice by the addition of 4 or 80 μg Zn/mL to the drinking water (*26*). At the lower concentration tumor growth is reestablished, but little or no zinc reappears in metallothionein as zinc becomes incorporated into new Ehrlich cells. At 80 μg Zn/mL there is a rapid shift from rest to proliferation accompanied by the

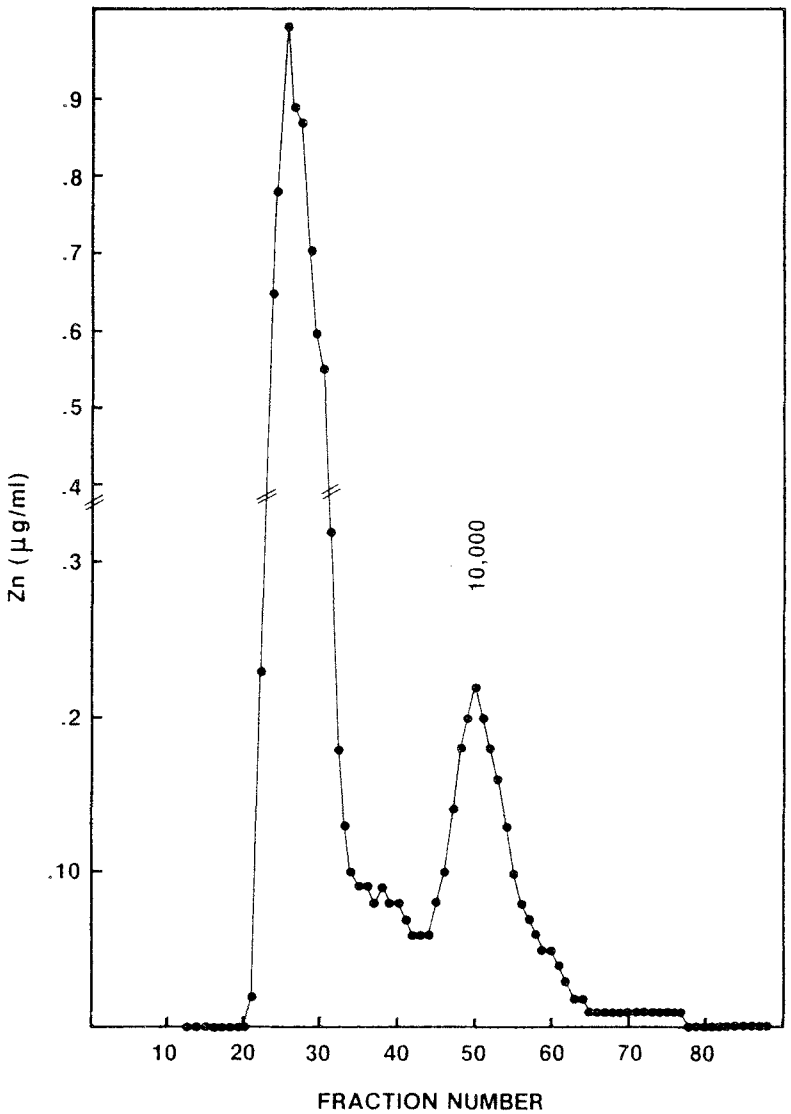

Fig. 2. Sephadex G-75 Chromatographic profile of Ehrlich cell cytosol from zinc-normal mice.

TABLE 2
Properties of Ehrlich Cell Zn-Binding Protein

1. Protein migrates on Sephadex G-75 as a 10,000 dalton protein (*11*)
2. Sephadex band separates into two isoproteins during DEAE-Sephadex chromatography, which elute under the same conditions as rat liver metallothionein (*12*)
3. Protein lacks A_{280} nm absorbance, and, thus, aromatic residues (*12*)
4. Protein SH/Zn ratio, 3.5 (*26*)
5. Ligand exchange reaction of Zn-binding protein with apocarbonic anhydrase has second-order rate constant of about 10^2 $s^{-1}M^{-1}$ (*26*)
6. Protein naturally contains Zn and Cu and binds almost all Cd after exposure of cells to this metal in vivo (*11*)

presence of metallothionein partially saturated with zinc. Apparently, at low levels of nutrient zinc the metal is preferentially bound into nonmetallothionein sites and the steady-state concentration of ZnMt is small; only when nutrient zinc is abundant does it also fill metallothionein-binding sites. Two models that are consistent with these findings are diagrammed in Fig. 3. Model A has ZnMt as an obligatory intermediate between extracellular zinc and final binding sites of zinc in cellular macromolecules. In Model B, apometallothionein and other structures receive their zinc in parallel from another pool in direct contact with extracellular zinc. Furthermore, in both models, ZnMt can donate zinc directly to apomacromolecules in a chemical exchange that does not require biodegradation of the protein to release metal (*23*). According to either model, the internal pool of zinc that is depleted first under zinc-deficient conditions, in which the concentration of Zn_E and Zn_I are drastically reduced is ZnMt. Both models require that the zinc in metallothionein be kinetically labile for metal transfers of the sort,

$$\text{ZnMt} + \text{apoLigand} \rightleftarrows \text{apoMt} + \text{Zn-Ligand} \qquad (1)$$

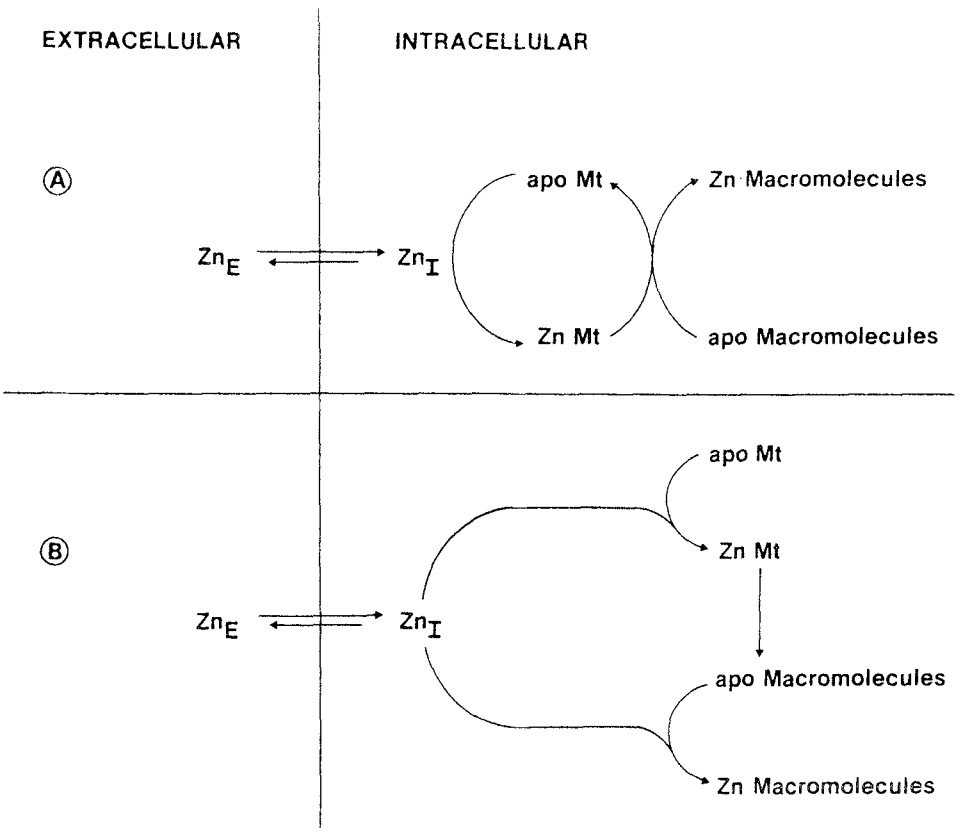

Fig. 3. Models for the role of zinc metallothionein in Ehrlich cells.

3.2. Properties of Metallothionein in Ligand Exchange Reactions (Reaction 1)

The kinetics and thermodynamics of ligand exchange reactions of zinc metallothionein have been under investigation (*12, 23, 28*). The ligands EDTA and a substituted bis(thiosemicarbazone) react slowly to remove Zn^{2+} from metallothionein (Table 3). These reactions are biphasic, probably indicative of the presence of two zinc-sulfhydryl clusters in metallothionein, each reacting as single cooperative units with the competing ligands (*29*). The reactions go to completion at ligand to metallothionein–zinc ratios of 10^3 to 1 at pH 7.4 and 25°C. Thus, the thermodynamic binding of zinc to metallothionein is of intermediate strength.

Zinc metallothionein does react rapidly with apocarbonic anhydrase. Thus, in contrast to these small, synthetic ligands, the apoprotein is an excellent ligand for reaction with ZnMt. In summary, model studies to date reveal zinc metallothionein to be a selectively and kinetically reactive structure in metal transfer processes. Combined with the modest thermodynamic stability of the metals in ZnMt, these results support the hypothesis that this protein in cells contains a labile pool of zinc that can be donated to newly synthesized apozinc structures as indicated in Fig. 3A or B.

3.3. Properties of Ehrlich Cell Cytosols in Ligand Exchange Reactions (Reaction 1)

Isolated Ehrlich cell metallothionein reconstitutes apocarbonic anhydrase (Table 2). When various concentrations of apocarbonic anhydrase are incubated with Ehrlich cytosol of known total zinc concentration, only 20% of the cytoplasmic zinc is available for reconstitution (*26*). Sephadex G-75 chromatography shows

TABLE 3
Ligand Exchange Properties of Rat Liver Zinc-Metallothionein[a]

$$\mathrm{ZnMt} + \mathrm{L} \overset{k.\mathrm{Keq}}{\rightleftharpoons} \mathrm{Mt} + \mathrm{ZnL}$$

Ligand	Rate constants for Reaction 1	Apparent stability constant of ZnL complex
EDTA,[b] *(ref. 23)*	(1) $-0.98\ s^{-1}M^{-1}[EDTA] + 4.4 \times 10^{-4}\ s^{-1}$ (2) $2.0 \times 10^{-4}\ s^{-1}$	10^{14}
3-Ethoxy-2-oxo-butyraldehyde bis(N^4-dimethylthiosemicarbazone)[b] (ref. *30*)	(1) $5 \times 10^{-3}\ s^{-1}$ (2) $1 \times 10^{-4}\ s^{-1}$	$10^{9.7}$ (ref. *31*)
Apocarbonic anhydrase[c]	$1.5 \times 10^3\ s^{-1}\ M^{-1}$	10^{12} (ref. *32*)

[a]Reactions carried out in 0.1*M* KCl and 0.01*M* Tris at pH 7.4 and 25°C.
[b]Reaction goes to completion at 10^3-fold excess of ligand.
[c]Reaction done at pH 5.4.

that zinc has left the metallothionein band and entered a new carbonic anhydrase band. Both results are consistent with the hypothesis that zinc-metallothionein is the major if not exclusive source of metabolically active, labile zinc. This view is strengthened by results of the reaction of apocarbonic anhydrase with cytosol from zinc-deficient cells. Here only about 4% of the supernatant zinc participates in reconstitution.

3.4. Relationship of Metallothionein to Cell Proliferation

The experiments described in Section 3.1 establish an association between zinc status, cell proliferation, and the binding of zinc to metallothionein. This relationship is strengthened by studies, summarized in Sections 3.2 and 3.3, that describe chemical properties of Mt consistent with its proposed role in Fig. 3. Whether or not metallothionein is the primary site relating nutrient zinc to cell division has yet to be determined. Nevertheless, there is a general correlation between proliferative state and the concentration of Mt in tissues. Thus, with the exception of kidney, mature, slowly proliferating tissues appear to contain only a very low, basal level of metallothionein (*33*). In contrast, Ehrlich tumor cells, and several other tumor lines according to preliminary data, fetal tissue, and regenerating rat liver contain much larger amounts of zinc and/or copper metallothionein (*34–36*).

What then might be the link between metallothionein and cell proliferation? A key feature of zinc-deficient Ehrlich cells is that at the level of present analysis, with the exception of metallothionein, the per cell concentration of macromolecular zinc is little changed from zinc-normal levels. There is also long-term viability of these cells. Thus, the effect of zinc deficiency is to take rapidly proliferating cells and place them in a resting condition. In this steady state, the cells husband their zinc content through many half lives of protein biodegradation. One may hypothesize that the metal content of metallothionein serves as a metabolic signal for the cell. In this model, zinc saturation of Mt is an indication that proliferation may proceed in the presence of adequate nutrient zinc to constitute new holozinc proteins and other structures in the cell; steady-state zinc depletion of metallothionein reflects insufficient nutrient zinc to support the synthesis of new cells. At present one may only imagine in general what the molecular basis for such a differential effect might be. Possibly apoMt could bind to a cellular control site for proliferation (S) to inhibit this process (Reaction 2). The binding of zinc to the protein would shift the equilibrium to favor free "S" and hence proliferation:

$$\begin{aligned} \mathrm{Mt} + \mathrm{S} &\rightleftharpoons \mathrm{Mt\ S} \\ \mathrm{Mt} + \mathrm{Zn} &\rightleftharpoons \mathrm{ZnMt} \end{aligned} \qquad (2)$$

Consistent with this general model is the finding that when Cd^{2+} is added to zinc-deficient and normal cells, ten times as much Cd^{2+} is needed to get binding to Mt in Zn(−) cells as in normal cells despite the unfavorable displacement of Zn^{2+} from ZnMt that must accompany cadmium binding to metallothionein in zinc-normal cells. This finding suggests that apoMt is also bound to another site in deficient cells, possibly the regulatory site envisioned in Reaction 2.

4. Host Zinc Metabolism

4.1. Redistribution of Zinc in Tumor-Injected Animals

Soon after the intraperitoneal injection of Ehrlich cells into Zn(+) adult mice, the plasma zinc level drops precipitously and remains low during the course of tumor growth (Fig. 4). Host zinc deficiency also rapidly depresses plasma zinc concentration. The two conditions do not have similar effects upon ascites fluid zinc. As seen in Fig. 4, this pool of nutrient zinc for the tumor cells is an order of magnitude larger in zinc-normal than in zinc-deficient animals. Thus, although plasma zinc level is commonly taken to reflect the readily available zinc being transported around the organism, there is still a sufficient steady-state movement of zinc about the organism in zinc-normal animals to support a much higher ascites fluid level of the metal.

The depression of plasma zinc in animals receiving a normal diet is accompanied by the uptake of zinc into the liver and its binding to metallothionein as indicated by the appearance of a band of zinc at 10,000 daltons on Sephadex G-75 chromatograms of liver supernatant prepared after tumor injection (Fig. 5). There is a progressive increase in metallothionein zinc over the 6–8-d log phase growth of the tumor. A similar increase does not occur in zinc-deficient mice. The simplest interpretation of this result is that there is not enough mobilizable zinc under deficient conditions to permit accumulation of the metal in liver metallothionein.

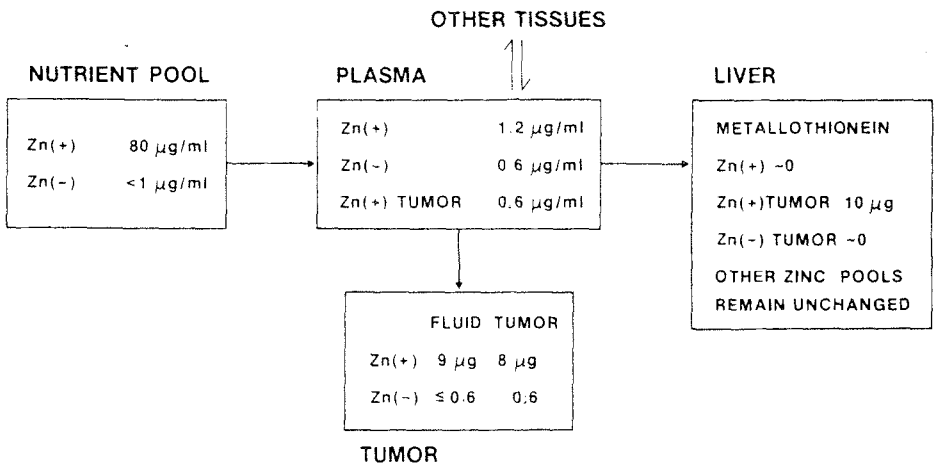

Fig. 4. Steady-state compartments of zinc in mice in the presence of 8–10 d tumor. Plasma and liver data are from ref. *37* and *38*. Tumor data were calculated as follows: Zn(+) fluid = 0.7 μg Zn/mL × 13 mL; Zn(−) fluid = 0.1 μg Zn/mL × (≤6 mL); Zn(+) tumor = 10^9 cells × 0.2 μg Zn/2.5 × 10^7 cells (ref. *11*); Zn(−) tumor = 10^8 cells × 0.15 μg Zn/2.5 × 10^7 cells.

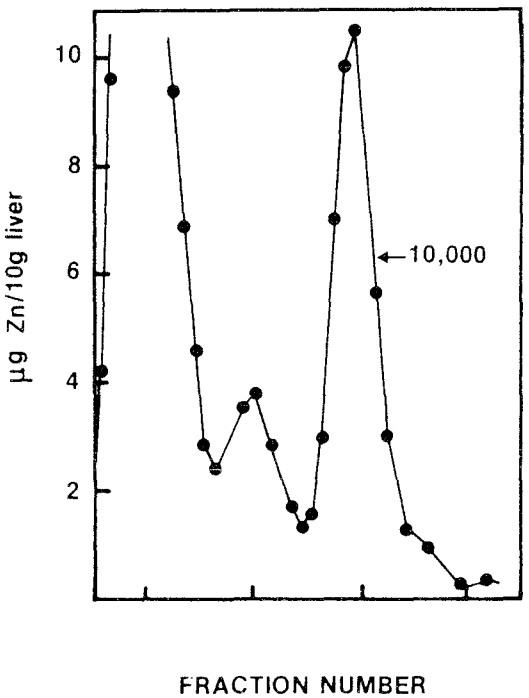

Fig. 5. Sephadex G-75 profile of mouse liver cytosolic zinc. Data taken 8 d after injection of Ehrlich cells into adult female Swiss mice.

4.2. Mechanism of Communication Between Tumor and Liver

The host response to the presence of tumor cells causes the shift of zinc from plasma to liver as seen in other examples of host inflammatory response. If the analogy is good, Ehrlich cells stimulate peritoneal macrophages to produce leukocyte endogenous mediator or interleukin 1. This material enters the blood stream, interacts with liver, and initiates the stress response as focused on liver. Preliminary studies have begun to test this mechanism (*37*). Thus, the injection of cell free ascites fluid from Zn(+) animals into zinc-sufficient mice causes the synthesis of new liver ZnMt. Ascites fluid from zinc-deficient mice causes little uptake of zinc into liver metallothionein. If this latter finding is confirmed, than the lack of accumulation of zinc in liver of zinc-deficient mice may result in part from inhibition of events in the peritoneum which trigger the host stress response.

Acknowledgments

The authors thank the National Institutes of Health (GM-29583-01) and the University of Wisconsin-Milwaukee Graduate School for financial support.

References

1. G. N. Schrauzer, Ed., *Inorganic and Nutritional Aspects of Cancer, Adv. Expl. Med. Biol.* **91,** Plenum, NY, 1978.
2. W. R. Todd, C. A. Elvehjem, and E. B. Hart, *Amer. J. Physiol.* **107,** 146.
3. R. W. Leucke, C. E. Simond, and P. J. Fraker, *J. Nutr.* **108,** 881 (1978).
4. L. S. Hurley, *The Johns Hopkins Medical Journal* **148,** 1 (1981).
5. H. G. Petering, H. H. Buskirk, and J. A. Crim, *Cancer Res.* **27,** 1115 (1967).
6. W. DeWys, W. J. Pories, M. C. Richter, and W. H. Strain, *Proc. Soc. Expl. Biol. Med.* **135,** 17 (1970).
7. J. T. McQuitty, Jr., W. D. DeWys, L. Monaco, W. H. Strain, C. G. Rob, J. Apgar, and W. J. Pories, *Cancer Res.* **30,** 1387 (1970).
8. D. Minkel, P. T. Dolhun, B. L. Calhoun, L. A. Saryan, and D. H. Petering, *Cancer Res.* **39,** 2451 (1979).
9. L. A. Saryan, D. T. Minkel, P. T. Dolhun, B. L. Calhoun, S. Wielgus, M. Schaller, and D. H. Petering, *Cancer Res.* **39,** 2457 (1979).
10. D. H. Petering and L. A. Saryan, *Biol. Trace Element Res.* **1,** 87 (1979).
11. J. Koch, S. Wielgus, B. Shankara, L. A. Saryan, C. F. Shaw, and D. H. Petering, *Biochem. J.* **189,** 95 (1980).
12. D. H. Petering, A. Kraker, G. Krakower, G. Bachowski, and C. F. Shaw, *Fed. Proc.* **41** (3); Abs. 2168 (1982).
13. J. P. Slater, A. S. Mildvan, and L. A. Loeb, *Biochem. Biophys. Res. Commun.* **44,** 37 (1971).
14. M. C. Scrutton, W. C. Wu, and D. A. Goldthwait, *Proc. Natl. Acad. Sci. USA* **68,** 2497 (1971).
15. K. H. Falchuk, C. Hardy, L. Ulpino, and B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **75,** 4175 (1978).
16. E. P. Anderson, in P. D. Boyer (ed.), *The Enzymes,* 3rd Ed., Vol IX, Academic Press, NY, 1973, pp. 69–93.
17. A. Askari, C. L. Long, and W. S. Blakemore, *J. Parenteral Enteral Nutr.* **4,** 561 (1980).
18. M. Powanda, in K. D. Rainsford, K. Brune, and N. W. Whitehouse, eds., *Trace Elements in the Pathogenesis and Treatment of Inflammation,* Birkhauser Verlag, Boston, 1981, pp. 121–135.
19. P.Z. Sobocinski, W. J. Canterbury, Jr., C. A. Mapes, and R. E. Dinterman, *Amer. J. Phsyiol.* **234,** E399 (1978).
20. R. F. Kampschmidt, *J. Reticuloendothel. Soc.* **23,** 287 (1978).
21. A. L. deWeek, F. Kristensen, and M. Landy, *Biochemical Characterization of Lymphokines,* Academic Press, New York, 1980, p. 417.
22. D. T. Minkel, K. Poulsen, S. Wielgus, C. F. Shaw III, and D. H. Petering, *Biochem. J.* **191,** 475 (1980).
23. T. Y. Li, A. J. Kraker, C. F. Shaw III, and D. H. Petering, *Proc. Natl. Acad. Sci. USA* **77,** 6334 (1980).
24. F. O. Brady, *Trends Biol. Sci.* **7,** 143 (1982).
25. M. Webb and K. Cain, *Biochem. Pharmacol.* **31,** 137 (1982).
26. A. J. Kraker, G. Krakower, C. F. Shaw III, and D. H. Petering, unpublished information.
27. R. J. Cousins, *Am. J. Clin. Nutr.* **32,** 339 (1979).

28. A. O. Udom and F. O. Brady, *Biochem. J.* **187,** 329 (1980).
29. J. D. Otvos and I. M. Armitage, *Proc. Natl. Acad. Sci. USA* **77,** 7094 (1980).
30. G. Bachowski, C. F. Shaw, and D. H. Petering, unpublished information.
31. D. H. Petering, *Biochem. Pharmacol.* **23,** 567 (1974).
32. S. Lindskog and P. O. Nyman, *Biochim. Biophys. Acta* **85,** 462 (1964).
33. A. J. Zelazowski and J. K. Piotroski, *Experentia* **33,** 1624 (1977).
34. A. J. Kraker and D. H. Petering, unpublished information.
35. S. R. Kern, H. A. Smith, D. Fontaine, and S. E. Bryan, *Toxicol. Appl. Pharmacol.* **59,** 346 (1981).
36. H. Ohtake, K. Hasegawa, and M. Koga, *Biochem. J.* **174,** 999 (1978).
37. B. Shankara, G. Krakower, G. Bachowski, C. F.Shaw III, and D. H. Petering, 13th International Cancer Congress, Seattle, Washington, USA, 1982, Abst. 2277.
38. B. Ujjani, G. Krakower, C. J. Shaw III, and D. H. Petering, unpublished information.