PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 505-508 (1977)

Influence of Inhibitors of Protein Synthesis on Zinc Metabolism[1, 2] (39967)

MARK P. RICHARDS AND ROBERT J. COUSINS[3]

Department of Nutrition, Rutgers University-The State University of New Jersey, New Brunswick, New Jersey 08903

Drugs that inhibit specific stages of protein synthesis have recently been used to investigate various aspects of zinc metabolism (1-6). *In vivo* administration of actinomycin D (3-6) abolishes the apparent synthesis of both liver and intestinal metallothionein (MT) as well as the homeostatic regulation of zinc metabolism, probably mediated via the serum zinc content, that accompanies changes in the intracellular concentration of this regulatory protein (4-6). As yet, no attempt has been made to evaluate a number of inhibitors in one defined system. The experiments reported here were conducted to compare the relative influence of actinomycin D, chloramphenicol, cordycepin, and cycloheximide on zinc binding to soluble liver and intestinal components as well as on tissue and serum zinc concentrations 12 hr following parenteral zinc.

Materials and methods. Weanling male rats (Sprague-Dawley, Madison, Wisconsin) were housed individually in stainless-steel cages and fed *ad lib.* a standard natural diet that contained 50 ppm Zn until a weight of 150 g was attained, at which time the rats were used for experiments. Zinc was administered intraperitoneally (25 μmole of Zn^{2+} as $ZnSO_4$ in 0.9% NaCl). The rats were killed by decapitation 12 hr later and the blood was collected. Different groups, of three rats each, were given one of the four drugs investigated (actinomycin D, a gift from Merck, Sharp and Dohme; chloramphenicol, cordycepin, and cycloheximide from Sigma Chemical Co.) or the vehicle (50% propylene glycol in 0.9% NaCl). Actinomycin D was administered (sc) at 0.8 mg/kg either 4 hr before or 3 hr after the zinc. Chloramphenicol was administered (ip) at 600 mg/kg (as an aqueous suspension) 1 hr prior to the zinc. Both cordycepin at 150 mg/kg and cycloheximide at 1.5 mg/kg were administered (ip) every 2 hr starting 1/2 hr before the Zn^{2+} was injected. Mucosa was pooled because of its heterogeneous nature.

Serum, liver, and intestinal mucosal zinc concentrations were measured by atomic absorption spectrophotometry (5). Hepatic and intestinal cytosol was fractionated by gel filtration chromatography (5) to isolate MT. The purity of MT was greater than 90% for both the hepatic (7) and intestinal (8) proteins, as demonstrated by chromatography on DEAE ion-exchange chromatography which generates homogeneous MT (based upon disc gel electrophoresis).

The data were subjected to an analysis of variance, and the significance of the multiple comparisons was estimated by Duncan's multiple range test (9).

Results and discussion. The data presented in Table I substantiate the observation that parenteral zinc administration elevates the serum zinc concentration about two-fold. It was observed previously (3, 4) that this concentration is maximal by 8 hr postinjection and by 12 hr declined to only about twice that found in control rats. When actinomycin D, cordycepin, or cycloheximide was administered prior to zinc, the serum zinc content remained significantly ($P < 0.05$) elevated relative to that found when no drug was administered. Actinomycin D or cycloheximide administered after the zinc resulted in a similar effect, but of lesser magnitude. This suggests that inhibition of either transcription or translation blocks the synthesis or assembly of a transport mechanism that is involved in clearing zinc from the blood. It is evident that block-

[1] This work was supported in part by NIH Grant No. AM18555 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

[2] A paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University-The State University of New Jersey.

[3] To whom reprint requests should be addressed.

ISSN 0037-9727

ing either transcription or translation essentially abolished the uptake of zinc into liver (Table I). It is important to note that when actinomycin D or cycloheximide was administered after the metal it was less effective in blocking the uptake process. In contrast, chloramphenicol, which inhibits mitochondrial protein synthesis, had no significant inhibitory effect on zinc uptake by the liver.

The response of the serum zinc concentration to a sudden elevation of zinc status in chloramphenicol- and cordycepin-treated rats establishes two important points. Because chloramphenicol does not block the clearance of zinc from the serum, it is apparent that mitochondrial protein synthesis is not directly involved in the transport process. The fact that cordycepin acts in a fashion similar to actinomycin D strongly suggests that the addition of poly(A)-units to heterogeneous nuclear RNA is required at some stage of the process leading to transfer of zinc from serum to liver cells.

We have previously proposed that elevation of the serum zinc content, either by dietary or parenteral means (4, 5), acts directly or indirectly as a metabolic signal to activate a series of events that culminates in the homeostatically regulated absorption of zinc. Clearly, the mucosal zinc content (Table I) reflected changes in zinc metabolism as did the liver content. It appears that the uptake of parenterally administered zinc into mucosal cells can be blocked by all inhibitors of protein synthesis except chloramphenicol.

The increase in zinc content of liver and intestinal mucosa following parenteral zinc was accounted for primarily as MT (Figs. 1 and 2). When cytosol from equivalent amounts of tissue was fractionated, it was observed that the amount of zinc bound to the higher molecular weight proteins (>75,000 daltons; peak I) did not vary substantially. In contrast, it is evident that MT is the dynamic zinc-binding fraction of liver and mucosal cytosol. Administration of cordycepin before the zinc load nearly abolished the increase in zinc bound by the MT fraction, whereas chloramphenicol had no inhibitory effect on the increase in MT-bound zinc.

Data presented in Table II show that the changes in the amount of zinc bound to soluble proteins, and in particular the MT fraction, paralleled changes in the total tissue concentration of zinc for both liver and intestinal tissue. Although the relative percentage of cytoplasmic zinc remained about 50% of the total tissue quantity for all treatments, the amount of zinc in MT differed markedly among the treatments. MT accounted for about 50% of the soluble zinc in both the zinc-injected and chloramphenicol-treated groups in both hepatic and intestinal mucosa. Thus, approximately one-fourth of the total tissue zinc in both the livers and intestines of these rats can be accounted for as MT. MT accounts for a much smaller percentage of total tissue zinc in those groups administered actinomycin D, cordycepin, or cycloheximide prior to zinc administration.

The time course used in these experi-

TABLE I. EFFECT OF PARENTERAL ZINC AND INHIBITORS OF PROTEIN SYNTHESIS ON SERUM, LIVER, AND MUCOSAL ZINC CONTENT IN RATS.

Group	Serum Zn (μg/ml)[b]	Liver Zn (μg/g)[b, d]	Mucosal Zn (μg/g)[c, d]
Control	1.0 ± 0.1	30.4 ± 1.5**	17.6
+Zn[a]	2.6 ± 0.2	75.4 ± 11.2*	41.9
+Actinomycin D + Zn[a]	15.3 ± 0.4*,**	40.1 ± 3.8**	20.5
+Zn + actinomycin D[a]	9.1 ± 0.8*,**	60.1 ± 8.3*	37.0
+ Chloramphenicol + Zn[a]	3.3 ± 0.5	71.3 ± 11.0*	45.3
+Cordycepin + Zn[a]	5.2 ± 0.6*,**	33.4 ± 1.5**	20.1
+Cycloheximide + Zn[a]	14.7 ± 0.5*,**	31.8 ± 0.5**	17.6
+Zn + cycloheximide[a]	11.0 ± 0.8*,**	38.4 ± 2.5**	20.2

[a] Zinc (25 μmoles) was injected ip 12 hr before sacrifice.
[b] Mean ± SE of three rats.
[c] Value of pooled mucosa from three rats.
[d] Concentration in micrograms per gram of wet tissue.
* Significantly different from control group ($P < 0.05$).
** Significantly different from zinc-injected group ($P < 0.05$).

ments was 12 hr, which is sufficient time to allow the zinc-stimulated increase in the rate of MT synthesis to return to a basal rate (6). Therefore, it is significant to point out that both actinomycin D and cycloheximide were less effective when they are administered after the zinc. This observation, coupled with other recent data (6), suggests that MT-mRNA is synthesized shortly after

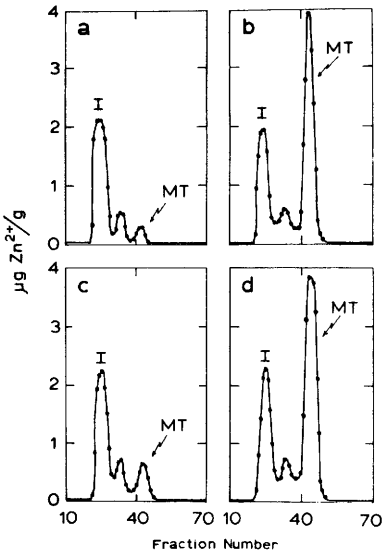

FIG. 1. Gel filtration (Sephadex G-75) elution profiles of cytosol-bound zinc from livers of rats fed adequate amounts of zinc. The rats were killed 12 hr after an injection (ip) of 25 μmoles of Zn^{2+} as $ZnSO_4$ or 0.9% NaCl (control). Cytosol was derived from pooled liver from three rats. Profile (a), control injection; profile (b), Zn^{2+} injections; profile (c), cordycepin (150 mg/kg) injected every 2 hr starting 1/2 hr before the Zn^{2+} injection; profile (d), chloramphenicol (600 mg/kg) injected 1 hr before the Zn^{2+} injection.

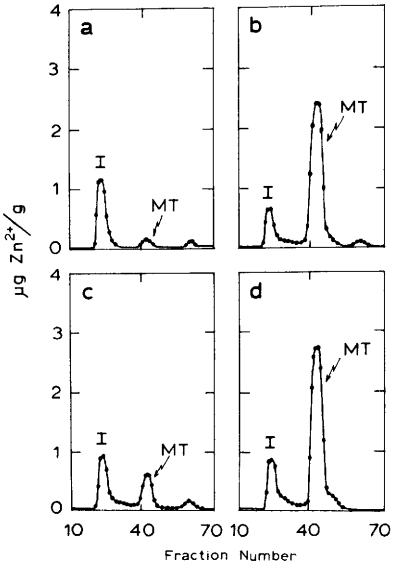

FIG. 2. Gel filtration (Sephadex G-75) elution profiles of cytosol-bound zinc from intestinal mucosa of rats fed adequate amounts of zinc. The rats were killed 12 hr after an injection (ip) of 25 μmoles of Zn^{2+} as $ZnSO_4$ or 0.9% NaCl (control). Cytosol was derived from pooled intestinal mucosa from three rats. Profile (a), control injection; profile (b), Zn^{2+} injections; profile (c), cordycepin (150 mg/kg) injected every 2 hr starting 1/2 hr before the Zn^{2+} injection; profile (d), chloramphenicol (600 mg/kg) injected 1 hr before the Zn^{2+} injection.

TABLE II. EFFECT OF PARENTERAL ZINC AND INHIBITORS OF PROTEIN SYNTHESIS ON CYTOSOL AND METALLOTHIONEIN ZINC CONTENT IN RATS.

Group	Cytosol Zn (μg/g)		MT-Zn[e] (μg/g)	
	Liver[b, d]	Intestine[c, d]	Liver[b, d]	Intestine[c, d]
Control	14.1 ± 1.1**	7.7	1.6 ± 0.5**	0.2
+Zn[a]	34.2 ± 1.8*	18.5	17.6 ± 3.6*	10.8
Actinomycin D + Zn[a]	19.8 ± 1.2**	10.3	2.4 ± 0.2**	2.7
Zn + actinomycin D[a]	27.0 ± 0.7*	16.9	9.6 ± 0.5*,**	9.1
Chloramphenicol + Zn[a]	35.9 ± 2.3*	19.1	19.8 ± 4.0*	12.0
Cordycepin + Zn[a]	17.1 ± 1.2**	11.6	2.5 ± 0.2**	2.8
Cycloheximide + Zn[a]	16.8 ± 1.2**	8.1	0.1 ± 0.1**	1.8
Zn + cycloheximide[a]	18.2 ± 0.8**	9.2	1.5 ± 0.1**	2.2

[a] Zinc (25 μmoles) was injected ip 12 hr before sacrifice.
[b] Mean ± SE of three rats.
[c] Value of pooled mucosa from three rats.
[d] Concentration in micrograms per gram of wet tissue.
[e] The MT-associated zinc was determined after chromatography of the cytosol on Sephadex G-75. MT-Zn is expressed as the total zinc content of all of the fractions that contained MT.
* Significantly different from control group ($P < 0.05$).
** Significantly different from zinc-injected group ($P < 0.05$).

zinc is administered. The inhibition observed is due in part to a decrease in the total amount of thionein message synthesized. In addition, the pronounced inhibition observed may indicate that thionein mRNA is relatively short-lived. Bryan and Hidalgo have shown that cadmium, which also induces MT synthesis, is detectable in the nucleus of liver cells within minutes following administration of the metal (10). We have recently shown that the rate of MT synthesis is maximal 5 hr after zinc is injected (6). These points emphasize that MT synthesis is rapid after augmentation of zinc status.

It appears that certain inhibitors of protein synthesis are useful tools that provide valuable information about zinc metabolism *in vivo*. From the present experiments the involvement of mitochondrial protein synthesis in the synthesis of MT can be ruled out, since chloramphenicol did not block MT formation. This is pertinent, since recently the similarity of MT to certain mitochondrial proteins has been demonstrated (11). It is unlikely that a direct binding of zinc to the inhibitors could explain the data obtained in these experiments, because the structures of these compounds vary widely, which would preclude uniform binding characteristics. Finally, the response of this system to cordycepin indicates that MT-mRNA contains poly(A)-units, a characteristic of the majority of eucaryotic mRNA (12, 13).

Summary. The drugs actinomycin D, chloramphenicol, cordycepin, and cycloheximide were evaluated for their relative effectiveness in blocking zinc binding by liver and mucosa following parenteral zinc. All the drugs, except chloramphenicol, were effective. The majority of the additional zinc bound following parenteral zinc was accounted for as metallothionein (MT) zinc. The inhibition of zinc binding by the drugs used was restricted to that associated with MT. The results collectively indicate that zinc uptake in liver and intestinal cells involves nonmitochondrial transcription and translation of poly(A)-containing RNA. Some of this RNA appears to code for MT.

1. Webb, M., Biochem. Pharmacol. **21,** 2751 (1972).
2. Richards, M. P., Bluestone, J. A., Newkirk, M. D., and Cousins, R. J., Fed. Proc. **33,** 699 (abstract) (1974).
3. Richards, M. P., and Cousins, R. J., Bioinorg. Chem. **4,** 215 (1975).
4. Richards, M. P., and Cousins, R. J., Biochem. Biophys. Res. Commun. **64,** 1215 (1975).
5. Richards, M. P., and Cousins, R. J., J. Nutr. **106,** 1591 (1976).
6. Squibb, K. S., Cousins, R. J., and Feldman, S. L., Biochem. J. **164,** 223 (1977).
7. Feldman, S. L., and Cousins, R. J., Biochem. J. **160,** 583 (1976).
8. Richards, M. P., and Cousins, R. J., Biochem. Biophys. Res. Commun. **75,** 286 (1977).
9. Hicks, C. R., "Fundamental Concepts in the Design of Experiments." Holt, Rinehart and Winston, New York (1965).
10. Bryan, S. E., and Hidalgo, H. A., Biochem. Biophys. Res. Commun. **68,** 858 (1976).
11. Prinz, R., and Weser, U., FEBS Lett. **54,** 224 (1975).
12. Darnell, J. E., Wall, R., and Tushinski, R. J., Proc. Nat. Acad. Sci. USA **68,** 1321 (1971).
13. Lee, S. Y., Mendecki, J. and Brawerman, G., Proc. Nat. Acad. Sci. USA **68,** 1331 (1971).

Received February 22, 1977. P.S.E.B.M. 1977, Vol. 156.