PARTIAL CHARACTERIZATION OF LIVER PROTEINS FOLLOWING EXPOSURE TO MERCURY

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1. Introduction

Molecular events which determine the biological tolerance for a substance such as mercury are not known; yet it is possible that protein induction, a selective means for regulating *in vivo* levels of proteins, is one cellular occurrence in which detoxifying proteins are increased, thus enabling an animal to adapt to higher levels of the (toxic) substance. Certain metals are known to function as activating or depressing agents in the regulation of protein synthesis and protein turnover [1–3]. Data presented in this report suggest that levels of mercury are tolerated under conditions which appear to involve induction-detoxification processes. Using the techniques of centrifugation, gel filtration, ultraviolet absorption and metal sulfide analysis, soluble proteins from mercury treated animals are shown to be increased and proteins known to contain specific binding sites for mercury are cited as possible candidates for the stimulated molecules.

2. Materials and methods

2.1. *Experimental design*

Male swiss albino inbred strain mice (weight 12–20 g and 1–1½ mon old at the beginning of each experiment) obtained from Dan Ralfsmeyer Co., Madison, Wisc., divided into groups of six, were given standard laboratory diets and water containing mercury chloride (table 1). The mice were sacrificed at the specified times, livers excised and quick frozen in an acetone–dry ice bath before storing at -5°.

All chemicals were reagent grades, water used in preparation of solutions (including drinking water and salt solutions) was deionized.

2.2. *Isolation of metal binding proteins*

Tissues were homogenized in 1 vol of 0.05 M K_2HPO_3 pH 7.0 using a Potter-Elvehjem homogenator [4]. After removing the major cellular debris with low speed centrifugation, the homogenate was centrifuged for 60 min at 105,000 *g* in a Beckman Spinco preparative ultracentrifuge. The pellet was discarded and the supernatant (soluble fraction) was taken for gel filtration. 1–2 ml of this supernatant was applied to a Sephadex G-75 column equilibrated with 0.001 M Tris and 0.001 M KCl at pH 7.0, and were eluted from the column with the same buffer. The presence of metal ions was determined qualitatively by use of thioacetamide spot tests (forming metal sulfide, highly insoluble precipitants). Ultraviolet absorption was measured on a Cary 15 spectrophotometer. Molecular weights were estimated by gel filtration using the Pharmacia Calibration Kit protein standards and procedures.

The results quoted are of a single experiment and the data given in table 1 and fig. 1 are from individual animals; however, two or more mice were analyzed

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Table 1
Analysis of partially purified liver soluble proteins.

Group	$HgCl_2$ in drinking H_2O (moles/l)	Time of exposure to Hg (wk)	Protein peak analyses after gel filtration[a]			
			Metal sulfide		UV absorption 257:280	
			A	B	A	B
Control	(deionized)	–	Neg.	Neg.	1.10	1.25
1	10^{-2}[c]	–	–	–	–	–
2	10^{-3}	3	Neg.	Pos.	1.11	2.05
Control	(deionized)	–	Neg.	Pos.	1.17	1.57
3	10^{-3}	15	Pos.	Pos.	1.19	2.08

[a] Sephadex G-75 equilibrated with 10^{-3} M Tris, 10^{-3} M NaCl buffer pH 7.0. A and B refer to protein peaks as shown in fig. 1.
[b] Thioacetimide spot test of peak fraction.
[c] Fatal to all animals after 10–14 days.

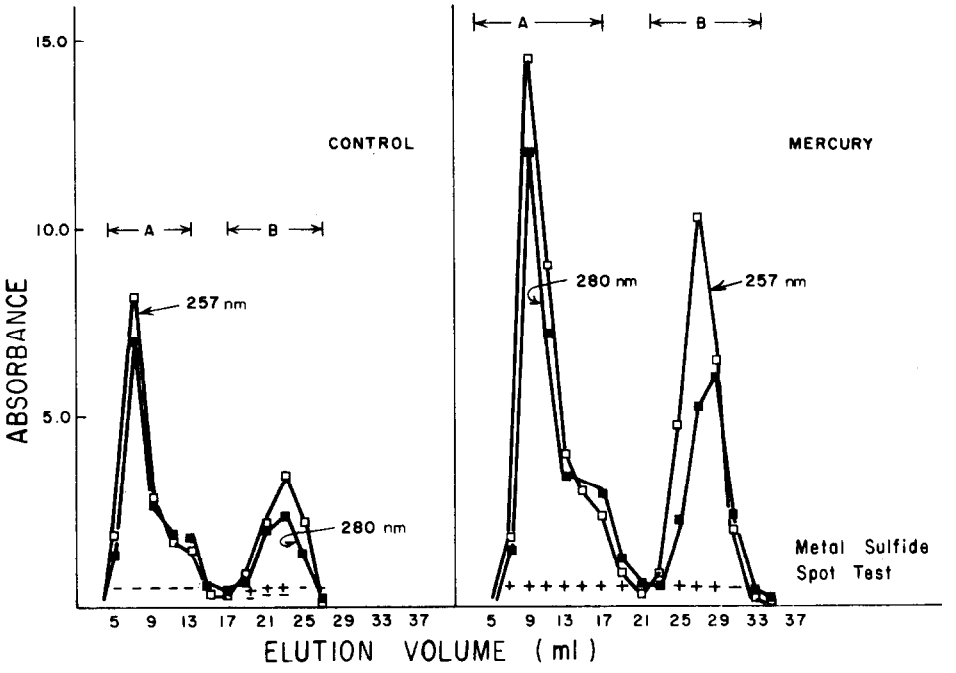

Fig. 1. Results obtained from gel filtration on Sephadex G-75. Thioacetimide spot tests for metal sulfides are shown as (+) positive and (–) negative. The two major protein fractions are indicated as A and B for control and mercury treated animals.

from each group and comparable results were obtained.

3. Results

Mice survived only 10–14 days when challenged with 10^{-2} M $HgCl_2$ but endured lower levels (10^{-3} M) up to four mon of constant exposure; in no case

did 10^{-3} M $HgCl_2$ proved fatal. Analyses of partially purified liver proteins after 3 and 15 wk of exposure with corresponding controls are given in table 1. A typical elution pattern showing the 257:280 profile is given in fig. 1 where 1.5 ml of the soluble fraction of control liver (1.77 g homogenized with 1.8 ml phosphate buffer) and 15 wk mercury treated liver (2.37 g homogenized with 2.4 ml phosphate buffer) were each applied to a Sephadex G-75 column (1 X 37 cm) equilibrated with 10^{-3} M Tris–10^{-3} M NaCl buffer, pH 7.0 (The flow rate 45.5 ml/hr and void volume 4.2 ml with blue dextran.) Thioacetimide spot tests were negative in all fractions (proteins A and B) of the 3 wk control (younger animals) but tests were slightly positive in peak fractions of protein B in 15 wk controls (older animals). As indicated in table 1 and fig. 1, metal sulfide tests were positive in both 3 and 15 wk samples for mercury treated animals. UV absorption analysis of each fraction was used to generate the 257:280 ratio (fig. 1 and table 1) which is clearly enhanced for protein B in mercury treated animals. Spectra of peak fractions from both protein A and protein B are given in fig. 2. The latter protein was estimated to correspond to a molecular weight of approx. 11,000.

4. Discussion

Mercury is known to bind and to be accumulated in metallothionein, a small (molecular weight, approx. 10,000) sulfhydryl-containing protein found in kidney cortex, liver and other tissues which also binds cadmium, zinc and copper [4–6]. Spectrophotometric and other studies indicate the metal(s) are bound to sulfhydryl groups since native metalloproteins have an absorption maximum near 250 nm, analogous to cadmium mecaptides. The employment of 250:280 ratios becomes a convenient criterion for measuring metallothionein which contains few or no aromatic amino acids and thus exhibits a low absorption at 280 nm. In this study, fractions (protein B) had an absorption maximum at 257 nm with the 257:280 ratio exceeding 2.0 for mercury treated animals; controls ranged from 1.2–1.5 which strongly suggests the stimulation (by mercury) of a mercapto-containing protein, possibly metallothionein. The fact that this protein occurs in the duodenum in addition to liver and kidney cortex (providing an effective shunt for the elimination of toxic levels of the metal) makes it a logical choice for the induced protein.

Analyses of A proteins were similar in both control mercury treated mice, showing ratios of 1.17 and 1.19, respectively. However the total quantity of these proteins was enhanced in mercury challenged animals (fig. 1). It is of interest that the broad UV band (peak A, fig. 2) is similar to the spectrum of cytocuprein (molecular weight 33,000, known also as erythrocuprein, heptacuprein and cerebrocuprein) a copper and zinc containing protein [7] which could conceivably bind mercury. The strongly positive thioacetimide test (presumably mercury sulfide) could result from interactions with cytocuprein or related metal binding proteins.

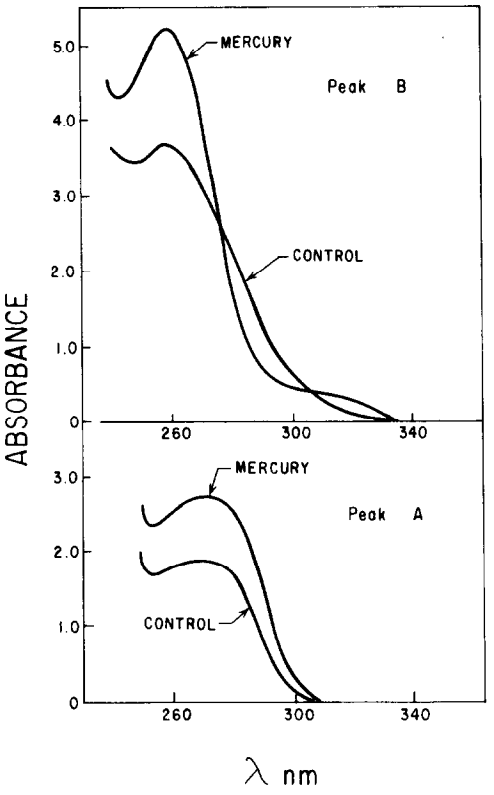

Fig. 2. Absorption spectra of protein fractions given in fig. 1. Peak A lower and peak B upper each diluted 10X with the elution buffer.

Studies are in progress to further identify proteins which appear to be stimulated and thus effective in increasing biological tolerance to mercury. However, assuming that the stimulation of detoxificating molecules (protein induction) accounts for the adaption process, the precise conditions of metal concentration and exposure time favoring protein induction are parameters which require further investigation.

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