THE EFFECT OF COBALT CHLORIDE ON SERUM PROTEIN ELECTROPHORETIC PATTERNS IN MICE

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1. Introduction

Metals can produce diverse and varied effects on biochemical systems. Certain metals (such as cobalt) are known to be indispensable in trace quantities to some organisms yet will become toxic at higher concentrations [1, 2]. However, the precise response elicited by various levels of this metal (and others) has been only partially investigated [3] and mechanisms by which metals function in order to produce these responses remain obscure.

A diversity of biochemical effects can also be observed among classes of metals. Recently compounds of platinum and of rhodium were cited as potent anti-tumor agents [4] whereas a barium compound was noted to induce cancer development in human cervical cells [5]. It appears that the largely unexplored questions of how multivalent metal ions influence normal and pathological conditions in the cell are pertinent.

The present study was undertaken to determine the *in vivo* effect of cobalt chloride on mice serum protein electrophoretic patterns following short and long-term exposure to various levels of the salt.

2. Materials and methods

2.1. *Animals*

Male -AKR-J albino inbred strain mice (weight 12–20 g and 1 to 1½ month old at the beginning of each experiment) were obtained from Jackson Laboratory, Bar Harbor, Maine. Mice from a Swiss strain (all male, weight 12–20 g and 1 to 1½ month old) obtained locally were used in one experiment.

All chemicals were reagent grade. Water used in the preparation of solutions, and drinking water given to animals, was deionized.

2.2. *Blood samples*

Serum was obtained by centrifugation of blood collected in capillary tubes from the tip of the tail 2–3 days prior to electrophoresis. The sera were stored at 4°.

2.3. *Starch gel electrophoresis*

Starch gels were prepared from hydrolyzed starch (Connaught Medical Research Laboratories, Toronto, Canada) using an apparatus and the general procedure described by Smithies [6] and Poulik [7]. Serum samples were applied by use of 2 × 6 mm strips of Whatman no. 1 filter paper dipped in the serum and inserted into a slit 5 cm from the cathode edge of the gel. Electrophoresis was performed horizontally using an electrode buffer containing 0.294 M boric acid and 0.06 M sodium hydroxide at pH 8.0 (Modified after Poulik). Each gel was run with constant 220 V at about 18°. After approximately 5 hr of running time (migration of Bromophenol blue front to 6–7 cm from origin), marker holes were aspirated in the gel above and below the sample strips of paper. The gels were sliced horizontally into four 2-mm layers (the top layer was discarded) and incubated in appropriate fixing and staining solutions (Bromophenol blue) [8]. The stained gels were photographed (panatomic x film, fig. 1) and the film negatives analyzed with a microdensitometer (figs. 2, 3).

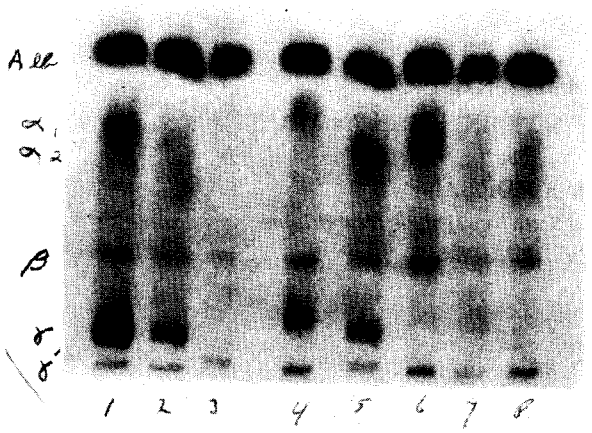

Fig. 1. Starch gel electrophoretic patterns. Samples 2, 3 and 4 are from mice receiving 10^{-4}, 10^{-3} and 10^{-2} M cobalt chloride solutions respectively for 3 months; patterns 6, 7 and 8 after animals had received 10^{-2} M salt for 1, 2 and 3 weeks respectively. Samples 1 and 5 are from the control group. (Tracings of the two controls were quite similar with 1 (AKR) showing a slighly more intense γ band. Sample 5 (Swiss) is shown in figs. 2 and 3).

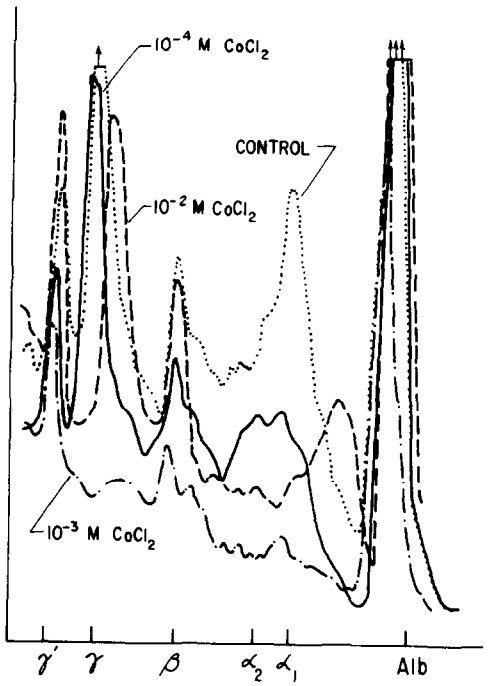

Fig. 2. Microdensitometer tracings of patterns 2, 3, 4 and 5 shown in fig. 1.

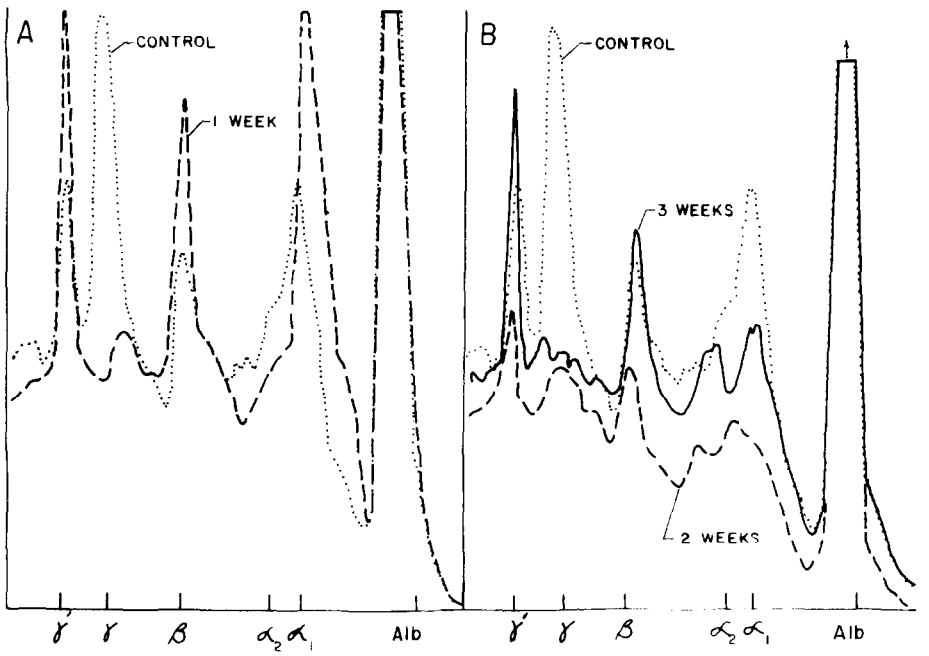

Fig. 3A, B. Microdensitometer tracings of patterns 5, 6, 7 and 8 shown in fig. 1.

3. Results

3.1. *Experiment I*

Five groups each containing 5–6 AKR male mice were given standard laboratory diets and water containing levels of cobalt chloride as indicated in table 1. Blood samples were collected at specified times (see figs. 1–3) from members of each group, the samples pooled and serum analyzed by starch gel electrophoresis. The gel in fig. 1 is an example of a run in which concentration of metal salt (samples 2–4) and time of exposure (samples 6–8) were varied. The microdensitometer tracings in fig. 2 (samples 2–4 from fig. 1) show the effect of salt concentration on protein patterns after animals had received the indicated levels for three months.

Table 1

Group	Concentration of $CoCl_2$ in drinking water (moles/liter)	Estimate of [c] $CoCl_2$ ingested (moles/week/mouse $\times 10^3$)
I [a]	10^{-1}	–
II	10^{-2}	1.2
III	10^{-3}	0.15
IV	10^{-4}	0.015
V [b]	(deionized water)	control

[a] Animals survived only 6–10 days apparently dying from dehydration since they drank very little of this concentrated cobalt salt water. Mice in groups II, III and IV appeared to be healthy and lived as long as the control group.
[b] Controls were given deionized water with no added metal.
[c] Based on daily intake measurements.

3.2. *Experiment II*

Following similar procedures, two groups of Swiss mice were studied with one group serving as a control (receiving deionized water) and the other group 10^{-2} M cobalt chloride solution. Electrophoretic patterns in fig. 1 (samples 6–8) and fig. 3A and 3B were obtained from serum collected at the end of 1, 2 and 3 weeks, along with the control (sample 5).

In other experiments AKR mice were given 10^{-2} M and other levels of cobalt chloride for various lengths of time to confirm the results reported in this communication.

4. Discussion

Striking alterations appear in serum protein electrophoretic patterns following ingestion of high levels (10^{-2} M) cobalt chloride for relatively short periods of time. Severe depression of γ globulins and subsequently a decrease in both α_1 and α_2 (fig. 3A, B) can be observed after 1 and 2 weeks of exposure. At the end of 3 months, these protein peaks reappear (fig. 2) however, both γ and α, show an increased mobility to suggest that these globulins have now been at least partly replaced by different proteins.

Prominent changes can also be seen after animals have received lower levels of the salt for longer periods of time (fig. 2). After three months, the group receiving 10^{-3} M salt showed markedly depressed globulins while the 10^{-4} M group appeared only slightly different from the control. However in other experiments after approximately 7 months, this group (10^{-4} M) also showed depressed globulins with the 10^{-3} M group showing an enhancement. Thus the same effect (depression followed by a stimulation of γ and α globulins) can be elicited by ingestion of less concentrated salt over longer periods of time. The animals appear to become adapted to these levels upon long-term exposure. This adaptation could result from the synthesis of different (perhaps specific metal binding) proteins. In all experiments both β and γ^1 globulins and albumin retained electrophoretic characteristics very similar to the control and could thus serve as markers.

Two strains of mice were used to show that the phenomenon of depression followed by stimulation of certain globulins in response to ingestion of cobalt chloride is not restricted to AKR mice (a tumor bearing strain). Furthermore, changes in the protein patterns in rat serum sensitive to cobalt chloride concentrations have previously been reported [3]. (Unpublished data in our laboratory confirm these observations). Therefore, it is possible that the response produced by cobalt intake in mice and rats may be a very general one.

Preliminary experiments using chloride salts of other divalent metals (zinc and manganese) appear to show protein changes similar to those induced by cobalt. However, more detailed studies may show that different metals alter serum proteins patterns in subtle yet distinctive ways.

Acknowledgments

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