COBALT DEPOSITION IN RAT ERYTHROCYTES AND CARDIAC TISSUE AS EVIDENCE FOR THE BIOSYNTHESIS OF COBALT PORPHYRINS

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1. Introduction

Ferrochelatase, the enzyme which catalyses the incorporation of Fe(II) into porphyrins has been prepared from a variety of tissues [1–3]. However, *in vitro* studies with ferrochelatase extracts have shown that cobalt-porphyrins are readily formed. Furthermore, substrate specificity for this enzyme system has been tested using various divalent metals and porphyrin derivatives, and cobalt was reported to be inserted into deuteroporphyrin as rapidly as iron, while cobalt was incorporated into protoporphyrin at approximately one fourth the rate of iron [4].

The *in vivo* incorporation of radioactive manganese into human erythrocytes as evidence for the biosynthesis of manganese porphyrins has been reported [5]. In this paper, gel filtration, atomic absorption spectroscopy along with radiochemical techniques were employed and data is presented to suggest the *in vivo* synthesis of cobalt porphyrins in rats.

2. Materials and methods

2.1. *Animals*

In addition to standard laboratory diets and water, adult male rats (weight approximately 300 g) were given one ml daily intraperitoneal injections of 1 mM $CoCl_2$ in saline tagged with cobalt-58. This dose (59 μg cobalt per day) was given to all animals; however rats 1–3 (see fig. 1) were removed from the cobalt schedule three weeks before they were sacrificed after having received the daily injections for 10 weeks. Animals No. 4–6 (fig. 1) received the the injections continuously for 12 weeks and until the day they were sacrificed. In other experiments injections were continued for longer periods. Blood samples were collected by cardiac punctures.

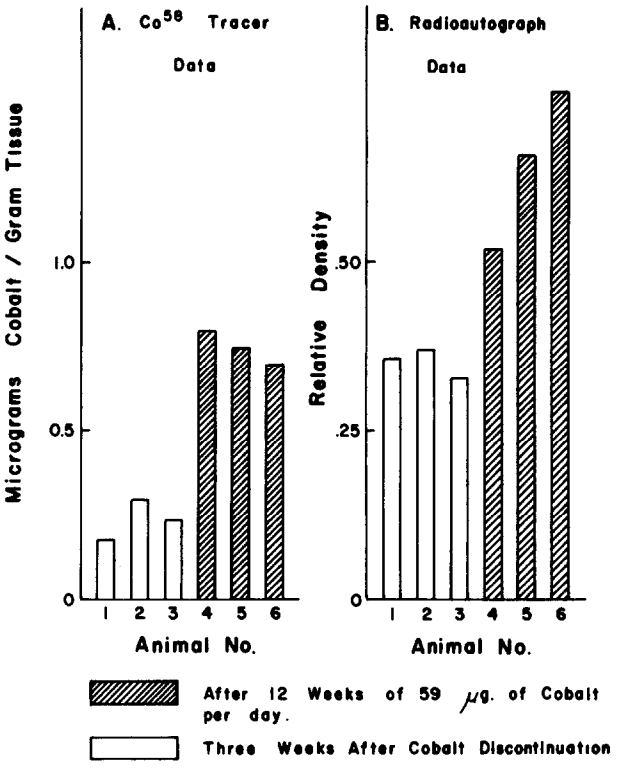

Fig. 1. Radiochemical data for cobalt deposition in rat cardic tissue.

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2.2. *Radioautography*

Heart tissues were removed, quick frozen, thin sliced, placed on X-ray type photographic paper and radioautography carried out at 5°. The radioautographs were further analyzed with a densitometer and the mean-density readings per unit area is given in fig. 1B. The entire hearts were wet-ashed after the radioautograph process and total cobalt was determined by liquid scintillation counting. In fig. 1A the deposition of cobalt per gram of wet tissue is computed.

2.3. *Preparation of red cell hemolysate and hemoglobin*

Plasma was removed by centrifugation and red blood cells washed three times with 1.2% sodium chloride solution and the supernatant liquid aspirated. In some experiments, packed cells were hemolyzed according to the method of Hinson and McMeekin [6] in which case hemoglobin crystals were obtained. In other experiments, the cells were hemolyzed by the addition of 1–2 volumes of water and 0.4 volumes of toluene and stroma and cell debris separated from the hemoglobin solution by centrifugation. This stroma free hemoglobin solution was then filtered through absorbent cotton. Hemoglobin was also isolated from hearts by homogenizing minced tissues in a Potter Elvehjem type homogenizer. The aqueous hemoglobin extract was then treated in a manner similar to that removed from red blood cells.

2.4. *Cobalt analysis*

A Techtron AA-5 Atomic Absorption Spectrometer was used in experiments where the total cobalt concentration was determined.

3. Results

Blood cells collected at designated intervals following injection of cobalt chloride were hemolized and assayed for ^{58}Co. The fact that activity increased with time up to approximately 5 to 6 weeks before reaching a plateau suggests the saturation of a cobalt "pool" which could correspond to a threshold concentration of a specific metabolite such as a blood porphyrin. This ^{58}Co activity could not be removed by dialysis.

After animals had received ^{58}Co injections for two months, an aliquot of cell hemolysate was prepared and was passed through a Sephadex G-50 column. Fractions under the hemoglobin peak were counted and the ^{58}Co peak concided with the hemoglobin peak to again suggest a "cobalt porphyrin" protein.

Radioautographic techniques were used to demonstrate the accumulation of ^{58}Co in cardiac tissue. Radioautographs included exposures through three section planes of the hearts: tranverse; frontal and sagittal. ^{58}Co appeared to be uniformly dispersed through these sections in a homogeneous fashion showing little or no evidence of site localization and approximately fifty per cent remained after animals were removed from the cobalt schedule. Results from densitometer tracings of the radioautographs are given in fig. 1B, and the total cobalt content of the hearts is given in fig. 1A. In another experiment, cobalt levels in heart extracts from animals receiving cobalt injections for several months ranged from 1×10^{-5} to 5×10^{-5} M. No cobalt was detectable in tissue extracts from control animals.

4. Discussion

The results show that a non-dialysible cobalt compound appears in erythrocytes and accumulates in cardiac tissue following 2–3 months daily injections of cobalt chloride. The deposition of cobalt in heart tissue could be due to its role as a "substituted" constitutent in certain hemoproteins, since hemoglobin extracts of these tissues contained the cobalt compound as was demonstrated by atomic absorption, radiochemical and gel filtration experiments. The time course of the appearance of ^{58}Co in erythrocytes resemble that which followed the incorporation of M (II) into human erythrocytes [5]. *In vitro* experiments of Johnson and Jones [4] showed that the rate of formation of metalloporphyrins was sensitive to the type of porphyrin; that is, both cobalt and iron were rapidly inserted into deuteroporphyrins while cobalt was less readily inserted into protoporphyrins than iron. Thus, it would seem that metalloporphyrin forming enzymes may show greater specificity toward the porphyrin than toward the metal.

In nature the important porphyrins occurring free (uncomplexed with metals) are protoporphyrin and the isomers of uro- and coproporphyrins [7] and the

hems (iron complexes of porphyrins) are unquestionably the most important biologically active hemoprotein. However, minute amounts of other porphyrins could be present, in which case, cobalt-porphyrins could perhaps be synthesized in very small, yet detectable quantities.

These observations are of special interest in view of the fact that coprotoporphyrin is used in cancer chemotherapy. Its cytotoxic activity is assumed to be attributable to its affinity to lipoproteins involved in membraneous structures of tumor cells [7, 8]. However, the mechanisms of these compounds with regard to direct effects on various types of tumor cells is not fully understood.

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