STUDIES IN MINERAL METABOLISM WITH THE AID OF ARTIFICIAL RADIOACTIVE ISOTOPES. VI. COBALT*

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The induced radioactive isotopes have provided new and valuable tools for the study of mineral metabolism.[1] This communication is the report of a test study to demonstrate the applicability of radioactive cobalt in biological investigations.

Cobalt metabolism holds considerable interest for comparative biochemistry because of the proximity and chemical association of this element to iron and manganese in the periodic table. The relation of cobalt deficiency to "coast disease"[2] "pine disease"[3] and "bush sickness"[4] in sheep, and to the "salt sickness"[5] of cattle in Florida, has indicated that cobalt is an essential for the maintenance of health in these animals.

A supplement of 1 mg. of cobalt per day for 14 days was sufficient to cure or prevent the appearance of cobalt deficiency in sheep for a period of 6 months.[3] The basal cobalt requirement of cows has been estimated as 1.0 mg. per day.[5] Ahmad and McCollum[6] have analyzed a number of foodstuffs from various sources, and found a cobalt content varying from 6 to 47 micro-gm. per 100 gm. of dry weight. No data are available on the cobalt requirement of the rat. From the analysis of Ahmad and McCollum it may be estimated that rats thrive on diets that supply less than 1 micro-gm. per day.

Larger doses of cobalt have been found to produce polycythemia in many species of animals.[7, 8, 9] With an improved nitroso-R-salt colorimetric method, capable of estimating cobalt down to amounts of 0.05 micro-gm. of cobalt,[10] it has been found that the bodies of rats on a normal diet contain about 5 micro-gm. of cobalt, and that polycythemia develops when the cobalt content increases to 40 or 50 micro-gm.[11]

Experimental Procedure.—The isotope employed was Co^{56}, with a half life of 270 days.[12] It was prepared in the Radiation Laboratory of the University of California by bombardment of iron with deuterons, according to the nuclear reaction:

$$_{26}Fe^{56} + {}_{1}H^{2} \longrightarrow {}_{27}Co^{56} + 2{}_{0}n^{1}$$

After preliminary extraction of the iron, the radioactive cobalt was separated from the residue as potassium cobaltinitrite. This was dissolved in concentrated nitric acid, evaporated to dryness, then redissolved in distilled water and 1 mg. each of inert iron and manganese added as carriers. By addition of 1 ml. of 1 per cent silver nitrate in 6 *N* acetic acid,

TABLE 1

DISTRIBUTION OF LABELED COBALT

(In micro-grams, 96 Hours after Administration)

TISSUE	ORAL ADMINISTRATION			INTRAPERITONEAL INJECTION		
	CONTENTS IN WHOLE TISSUE MICRO-GM.	CONTENTS PER 100 GM. FRESH WEIGHT MICRO-GM.	CONTENTS PER 100 GM. DRY WEIGHT MICRO-GM.	CONTENTS IN WHOLE TISSUE MICRO-GM.	CONTENTS PER 100 GM. FRESH WEIGHT MICRO-GM.	CONTENTS PER 100 GM. DRY WEIGHT MICRO-GM.
Whole blood	*	*		0.04 ± 0.004	0.78 ± 0.07	
Brain	*	*		*	*	
Lung	*	*		*	*	
Liver	0.020 ± 0.003**	0.21 ± 0.03	0.67 ± 0.10	0.077 ± 0.005	0.67 ± 0.04	2.2 ± 0.13
Kidney	0.013 ± 0.003	0.60 ± 0.14	1.89 ± 0.45	0.044 ± 0.004	2.14 ± 0.19	8.2 ± 0.73
Pancreas	0.011 ± 0.003	1.50 ± 0.42	2.50 ± 0.70	0.022 ± 0.004	2.34 ± 0.37	4.3 ± 0.68
Spleen	0.013 ± 0.003	1.70 ± 0.42	5.60 ± 1.40	0.018 ± 0.003	1.96 ± 0.35	7.8 ± 1.39
Stomach	0.015 ± 0.003	0.60 ± 0.13	*	*	*	
Small intestine	0.012 ± 0.003	0.16 ± 0.04		0.030 ± 0.004	0.33 ± 0.04	
Large intestine	0.020 ± 0.003	0.43 ± 0.06		0.033 ± 0.004	0.64 ± 0.069	
Bone	0.029 ± 0.004		0.42 ± 0.06	0.024 ± 0.004		0.33 ± 0.06
Skin	*			0.013 ± 0.003	0.02 ± 0.01	0.04 ± 0.01
Residual muscle and carcass	0.139 ± 0.007	0.12 ± 0.01		0.157 ± 0.007	0.12 ± 0.01	
Total in body	0.27			0.46		

* Radioactivity measurements made, but amounts found were not statistically significant.

** $\frac{m}{100}\sqrt{\left(100\,\frac{\sqrt{n/t}}{n}\right)^2+\left(\frac{100\,\sqrt{n_0/t_0}}{n_0}\right)^2}$, where m is the mass, n is the number of counts in t minutes in the sample and n_0 the number of counts in t_0 minutes in the standard.

and 2 ml. of 50 per cent potassium nitrite. the cobalt was again precipitated as silver potassium cobaltinitrite. This salt is much less soluble than the simple potassium salt, so that the loss in purification was minimized. The cobalt was precipitated four times by this procedure, and after the final precipitation, no radioactive contaminant was detectable in the filtrate. The amount of cobalt was determined colorimetrically, using alcoholic ammonium thiocyanate. It was made up as a cobalt chloride solution containing 0.1 mg. Co per ml. No non-radioactive cobalt was added at any time, so that the specific radioactivity was high, of the order of 5 microcuries per mg. Co. Radioactivity measurements were made with a thin

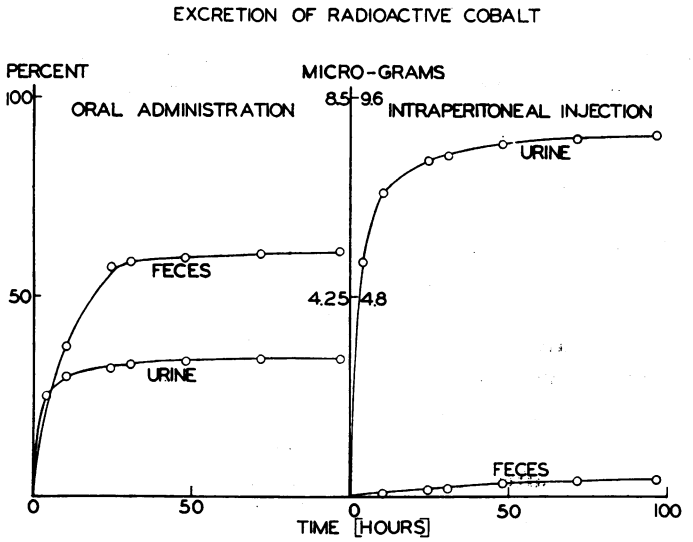

FIGURE 1

The routes of excretion of radioactive cobalt administered orally and by intraperitoneal injection.

copper wall Geiger-Müller counter, with a scale of eight circuit. Measurements were sensitive to 0.01 micro-gm. cobalt.

Two twelve-weeks-old male rats, weighing about 250 gm., were used for the experiments. After being fasted twenty hours, they were each given 10 micro-gm. of radioactive cobalt; one by intraperitoneal injection, the other by stomach tube. They were then placed on the stock laboratory diet, and the urine and feces were collected separately at regular intervals. The animals were sacrificed after 96 hours by anesthetizing with ether, and draining the blood by cardiac puncture. The various organs were removed and the bone separated from muscle and residual carcass by heating with 3 *M* ammonium hydroxide for several days.

To each sample, 2 mg. of inert cobalt were added as carrier, and the tissue

was dried and ashed at 600° for several hours. The ash was dissolved in a small amount of dilute hydrochloric acid, filtered if necessary, evaporated to dryness and redissolved in distilled water. The cobalt was precipitated as the sulfide by addition of excess freshly prepared ammonium sulfide. The precipitate was washed with a small amount of dilute hydrochloric acid, dried and the radioactivity measured with the Geiger-Müller counter. The total labeled cobalt recovered was 8.5 micro-gm. (85%) after oral administration, and 9.6 micro-gm. (96%) after intraperitoneal injection. An aliquot containing 0.5 micro-gm. Co*, or 5 per cent of the administered dose, was treated in the same manner as the analytical samples and was used as a standard.

Results and Discussion. (*a*) *Excretion.*—The graphs of excretion plotted in figure 1 show that even this minute quantity of cobalt was eliminated very rapidly, about 70 per cent being excreted within the first 10 hours, and over 90 per cent within two days. When parenterally administered, the chief path of excretion was the urine, although there was a small but continuous elimination in the feces. This is in agreement with the work of LeGoff,[13] who found that the urine was an important path of cobalt excretion in the rabbit. In contrast to cobalt, when radioactive manganese was parenterally administered,[14] excretion took place almost exclusively in the feces. The initial elimination of radioactive iron,[15] when given intravenously, takes place in both urine and feces.

When the cobalt was given by stomach tube, over 60 per cent was recovered in the feces. The difference between this value and the amount excreted in the feces after injection demonstrates that only about half of the orally administered cobalt was absorbed. Most of the absorbed cobalt was rapidly excreted in the urine.

After 96 hours, the body retained only 0.46 micro-gm. of the cobalt given parenterally, and 0.27 micro-gm. of the cobalt given by stomach tube. In the experiments with the "trace" elements, cobalt and manganese, over 90 per cent of even small doses was eliminated in the first few days, while when radioactive iron was given parenterally, only 2 to 8 per cent was excreted in a comparable period. Evidently the body retains very little of the administered cobalt and manganese, and the requirements for these elements must be very small.

(*b*) *Tissue Distribution.*—The distribution of radioactive cobalt in the various tissues is shown in table 1. The amounts present are very small, and, in general, conform to values found when large doses of cobalt are fed.[11] The glandular organs, particularly the pancreas, liver, spleen and kidneys, show relatively high concentrations. The pancreas is of particular interest because of a possible relation of cobalt to insulin[16] in this organ. The concentration in bone may have some bearing on the polycythemic effect

of cobalt. However, the concentrations are so low that the requirements of the tissues for cobalt, if any, must be very small.

Summary.—1. Radioactive cobalt provides an extremely useful tool for biological studies of cobalt. The isotope may be prepared with a high specific radioactivity, so that doses comparable to the amounts present in the diet may be used.

2. The chief path of cobalt excretion is the urine, in contrast to manganese, where excretion is exclusively in the feces, and iron, where excretion may take place by both paths.

3. Even after administering a small dose (10 micro-gm.), most of the cobalt was quickly eliminated, and less than 5 per cent was recovered in the body after four days, indicating that the requirement of the body for cobalt is exceedingly small.

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