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IS COBALT OF ANY SIGNIFICANCE IN THE TREATMENT OF MILK ANEMIA WITH IRON AND COPPER?*

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Cobalt has been found by Underwood (1) to be a very general contaminant of iron compounds. In view of recent work demonstrating that cobalt is active in animal metabolism in exceedingly minute quantities (2–5) and of the well known effect of comparatively large doses of this element in inducing polycythemia, the possibility that cobalt might be playing some part in the treatment of milk anemia of the rat with common iron salts plus copper could not be overlooked. The iron compounds used in such studies have frequently been purified with respect to copper but never, so far as is known, freed of cobalt and fed under conditions designed to minimize extraneous sources of this element. It was decided, therefore, to make a comparison of a cobalt-free iron salt with the same salt plus cobalt for growth promotion and hemoglobin regeneration in the anemic rat when fed adequate copper.

EXPERIMENTAL

Rats from four litters were rendered anemic on whole cow's milk in the usual manner. The milk was taken directly into enamel containers and fed in cobalt-free dishes. The rats were kept in wire mesh cages coated repeatedly with aluminum paint and kept scrupulously clean. At weaning they were divided into three groups for treatment. Group I consisted of six rats which were given milk *ad libitum* plus a daily supplement of 0.05 mg. of copper

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and 0.02 mg. of manganese together with 0.5 mg. of iron in the form of cobalt-free ferric chloride. The ferric chloride was freed of cobalt by extraction with ether in the manner described by Underwood (1). Group II consisted of three rats which received the same treatment as Group I except that the iron salt was unpurified. This salt contained 41 parts per million of cobalt or 119 parts of cobalt per million of iron. Group III was made up of five rats which received the same treatment as Group I together with an addition of 0.1 mg. of cobalt per day in the form of cobalt chloride.

The animals received the above treatments for a period of 3 months during which time frequent weighings and hemoglobin determinations were made. All were then killed except four females which were continued on their treatments for a further period of 6 months.

Cobalt determinations were carried out on the whole bodies of a large number of rats at birth and at weaning, on the livers of several of the experimental animals, and on occasional samples of the milk as fed. The method of Kidson, Askew, and Dixon (6), with slight modifications, was used. Recovery tests with added cobalt showed this method to be satisfactory, though amounts below 0.5 microgram of cobalt could not be determined with any certainty on the above types of material.

Results

A growth of 2 to 3 gm. per day took place in all rats. No consistent differences in live weight between the three groups were evident at any stage of the experiment.

The rate of increase of hemoglobin levels may also be considered optimum in all three groups. The mean results are given in Table I. No lowering of the rate of hemoglobin regeneration has followed the use of the purified cobalt-free iron nor has the addition of as much as 0.1 mg. of cobalt per day produced any stimulating effect in Group III. 2 weeks prior to the completion of this phase of the experiment the level of cobalt fed was raised to 0.15 mg. daily. A mild but definite polycythemia resulted in four out of five rats so treated.

For the following 6 months three rats of Group I and one of Group II were continued on their respective treatments. All

these rats continued to gain in weight normally during this period, while no fall in the blood hemoglobin levels was evident. The mean level of the three rats receiving the supplement of cobalt-free iron was 14.61 gm. of hemoglobin per 100 cc. of blood on June 21, 1937, and 14.59 on January 3, 1938. The corresponding figures for the rat receiving the unpurified iron salt were 14.73 and 14.55 gm.

TABLE I

Hemoglobin Levels of Rats (Gm. per 100 Cc. of Blood)

	Mar. 22	Mar. 29	Apr. 5	Apr. 12	Apr. 26	May 23	June 7	June 21
Cobalt-free iron supplement								
Mean of 6 rats	3.21	9.85	10.81	10.92	12.61	13.47	14.40	14.85
Range	2.49–4.08	7.57–11.10	10.03–12.40	9.71–12.77	11.48–13.47	11.82–14.20	12.05–16.01	13.61–16.01
Unpurified iron supplement								
Mean of 3 rats	3.64	10.28	10.93	12.04	11.36	11.63	14.96	15.44
Range	2.91–4.58	9.79–10.71	10.52–11.48	11.91–12.63	10.92–12.05	10.20–12.63	14.55–15.25	14.73–16.01
Iron supplement + 0.1 mg. Co* daily								
Mean of 5 rats	3.59	9.39	10.49	11.71	12.47	14.41	14.68	17.63
Range	3.02–4.34	7.61–10.54	9.42–11.52	10.02–13.33	11.10–13.61	12.77–15.78	11.94–16.68	16.01–18.64

* On June 7, 1937, the cobalt level was raised to 0.15 mg. of cobalt daily.

These rats were exposed to a vigorous male at frequent intervals throughout the 6 month period. No successful pregnancies resulted, though this is not an unusual phenomenon with rats fed on mineralized milk.

Samples of milk were analyzed in duplicate for cobalt on five occasions. The results ranged from 8 to 18 micrograms of cobalt per liter with a mean of 11 micrograms. Blanks and recovery tests were made for each analysis with satisfactory results. The levels found are of interest in view of the claims of Wright and

Papish (7) and of Blumberg and Rask (8) that milk is "spectrographically free" of cobalt.

The results of the rat tissue determinations will be considered in the discussion.

DISCUSSION

It is evident that rats on a milk diet require no mineral supplements other than those of iron, copper, and manganese, even for a period as long as 9 months. Three explanations of this finding are possible: (*a*) the rats may be born with large enough stores of cobalt to provide their requirements over this length of time, (*b*) they may obtain sufficient cobalt from the milk, or (*c*) cobalt may not be required by this species. None of these possibilities can be completely eliminated. The first must be considered rather unlikely, at least as a principal factor, as there appears to be no diminution in the cobalt content per unit of dry weight of the whole bodies of rats between birth and weaning. Nor is the level of cobalt in the livers of those rats given the cobalt-free iron supplement significantly lower than that of rats receiving a stock ration. The following figures illustrate these points. The mean cobalt content of nine litters of new born rats was found to be 0.15 part per million of cobalt on the dry matter (range 0.09 to 0.21), while that of seven rats from four litters at weaning was found to be 0.14 part per million of cobalt (range 0.08 to 0.20). The livers of the three rats of Group I (cobalt-free iron) at the end of the 9 month period contained 0.15, 0.26, and 0.28 parts per million of cobalt on the dry matter, while the figures for the livers of three rats on the stock diet were 0.16, 0.28, and 0.43 parts per million of cobalt.

The suggestion that the milk is supplying the rat's requirements for cobalt (if there is one) seems the most logical one. The rats consumed an average of 40 to 60 cc. of milk daily, which would supply them with about 0.6 microgram of cobalt, if the mean of the figures given above for the cobalt content of milk be taken. For a rat weighing 100 gm., *i.e.* about half grown, this represents a daily intake of 6 micrograms of cobalt per kilo of body weight. It is interesting to compare this with the cobalt requirement of the growing sheep. This has been shown by Underwood and Filmer (2, 3) to be close to 0.1 mg. of cobalt per day. For a sheep weighing 25 kilos, *i.e.* about half grown, this represents a requirement of 4 micrograms of cobalt per kilo of body weight. The rats are therefore receiving rather more cobalt per unit of body weight from the milk than sheep making excellent growth increases.

The possibility that rats do not require cobalt at all, though rather unlikely, cannot be overlooked. Its importance to the ruminant is unquestioned, though nothing is yet known as to how it functions within the body. The amounts involved are so infinitesimal, however, that it is not improbable that it is acting in some enzyme system. If it does act in an enzyme system, then species differences are quite conceivable, since one or more species might be able to dispense with a particular enzyme system or at least develop a different chain of events within the system whereby the necessity for the element in question would be avoided or its requirement considerably reduced.

In this connection mention should be made of the finding of Filmer (9) that whole liver cures and prevents the disease of cattle and sheep caused by cobalt deficiency, though it was found by Filmer and Underwood (3) to contain insufficient cobalt to account for its therapeutic properties. It was suggested by these workers (3) that the liver supplies an organic factor which has required cobalt for its formation and through the production of which cobalt normally functions. A diet of whole milk may be supplying enough of this factor to render cobalt unnecessary or at least reduce its requirement to infinitesimal proportions.

SUMMARY

No increase in growth or hemoglobin regeneration was obtained in rats fed whole cow's milk plus copper, manganese, cobalt, and purified cobalt-free iron in comparison to rats on a similar diet without added cobalt. It is concluded that iron, copper, and manganese are the only minerals which must be added to milk under the conditions of our experiment for the normal growth and well being of the rat and that the small amounts of cobalt which contaminate almost all iron salts play no significant part in the treatment of milk anemia in the animal.

The possible cobalt requirement of the rat is discussed and data given which indicate that the requirement of this element must be less than 0.6 microgram per day.

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