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Influence of Cobalt on Reproduction of Mice and Rats

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Reproduction studies with mice and rats kept on whole plant rations have been in progress in this laboratory for several years. In the first experiments, poor reproduction performance was observed (*1*), but later much more satisfactory results were obtained with a slightly modified diet (*2*). The only difference in the composition of the diets was that the salt mixture supplement used originally did not contain cobalt, whereas in the later experiments 0.2% $CoCl_2$ was added to the salt complement. Because of the apparent influence of this small amount of dietary Co on reproduction, the present study was undertaken.

The rats used were of the Sprague-Dawley strain, raised in our laboratory; the mice were of the same albino strain used in the previous experiments. The rats had been kept on a whole plant ration for at least 3 generations prior to the start of the present experiments, and the mice for at least 5 generations. The animals were kept on the basal ration in common cages until females became pregnant. These were put in single screen-bottomed cages and given the respective experimental diets and water *ad lib*. Litters were reduced by random selection to 6 and weaned at the age of 28 days.

The composition of the basal diet was: solvent-extracted soybean meal, 46; cornmeal, 46; sesame oil containing 0.2% percomorphum oil and 0.2% wheat germ oil, 5; salt mixture II USP, 2; thiamine, 0.3 mg; riboflavin, 0.3 mg; calcium pantothenate, 2 mg; pyridoxin, 0.2 mg; choline hydrocloride, 100 mg; nicotinic acid, 2 mg; folic acid, 0.025 mg; biotin, 0.01 mg; inositol, 10 mg; PABA, 25 mg. The diet has been analyzed by microbiological methods and found to contain about 0.5 μg/100 g of vitamin B_{12} activity (*2*). Cobalt was supplied in the diet by adding 0.2% $CoCl_2$ to the salt mixture, or in the drinking water in a concentration of 0.2 mg% of $CoCl_2$; when cobalt was injected, a 0.1% solution of $CoCl_2$ in physiological saline solution was used, of which 0.2 ml was injected in each female 2–6 days prior to giving birth. A total of 130 litters of mice and 64 litters of rats was used in the present study.

The effect of cobalt on the reproduction of mice was studied in 4 series (Table 1). In the control group receiving the soybean-corn ration without added $CoCl_2$, the number of young weaned/litter born was 3.4. With the addition of 4 mg% $CoCl_2$ to the diet, the ratio of young weaned/litter born was 4.7. When the drinking water contained 0.2 mg% $CoCl_2$, the survival of the young was practically equal to the group receiving the Co supplement in the diet. Intraperitoneal injection of a single dose of 0.2 mg of $CoCl_2$ into the mother before giving birth did not significantly improve the survival of the young in comparison with the control group. Performance of the group receiving vitamin B_{12} in the diet was markedly better than that observed with the Co-supplemented diets.

The results obtained with rats (Table 2) were similar to those observed with mice. Only 1.4 young survived the 4-weeks weaning age out of each litter born on the diet without added Co, whereas on the Co-supplemented diet 1.75 survived, on the average. When Co was added to the drinking water, the corresponding value was 2.9. The mean weights of the young at the age of 28 days were 35, 54, and 55 g, respectively. The group receiving the $CoCl_2$ in the drinking water did as well as a group receiving the corn-soybean diet supplemented with 40 μg/kg of vitamin B_{12}. The supplementation also caused a significant weight gain of the mothers during the suckling period.

The difference between the numbers of litters dead in the control series and in those receiving a supplemented diet was never significant. This may be due to the fact that the feeding of the supplement was started only a few days before the birth of the litters.

The results of these experiments indicate that cobalt is of definite benefit for the reproduction of both mice and rats when they are kept on a whole plant ration low in vitamin B_{12}. The amount of Co present in the diet was not determined. The daily amount of Co de-

TABLE 1

REPRODUCTION OF MICE, KEPT ON A SOYBEAN-CORN RATION FOR SEVERAL GENERATIONS WITH AND WITHOUT COBALT OR VITAMIN B_{12} SUPPLEMENTS

Group	Supplements	No. litters born	No. litters dead	No. young weaned/ litters born	Wt of young at 28 days (± S.E.$_m$) *	Wt change of mothers (± S.E.$_m$)
1 (Control)	None	42	11	3.4	11.5 ± 0.377	− 1.3 ± 0.341
2	4 mg% $CoCl_2$	20	1	4.7†	11.9 ± 0.482	+ 0.7 ± 0.459†
3	0.2 mg% $CoCl_2$ in the drinking water	28	4	4.5†	11.7 ± 0.327	0 ± 0.408†
4	0.2 mg $CoCl_2$ injected i.p.	21	5	3.0	11.4 ± 0.375	− 0.4 ± 0.650
5	4 μg% Vitamin B_{12}	19	2	5.0†	15.2 ± 0.364†	− 0.5 ± 0.534

* S.E.$_m$ = Standard error of mean.

† Difference from control lot significant at 5% level.

TABLE 2

REPRODUCTION OF RATS, KEPT ON A SOYBEAN-CORN RATION FOR SEVERAL GENERATIONS WITH AND WITHOUT COBALT OR VITAMIN B_{12} SUPPLEMENTS

Group	Supplements	No. litters born	No. litters dead	No. young weaned/ litters born	Wt of young at 28 days ($\pm$ S.E.$_m$)*	Wt change of mothers ($\pm$ S.E.$_m$)
1 (Control)	None	18	11	1.4	35 ± 1.776	0 ± 6.99
2	4 mg% $CoCl_2$	12	8	1.75	54 ± 4.350†	16 ± 2.35†
3	0.2 mg% $CoCl_2$ in the drinking water	21	9	2.9†	55 ± 3.580†	11 ± 2.40
4	4 µg% Vitamin B_{12}	13	4	3.2†	59 ± 5.233†	24 ± 4.67†

* S.E.$_m$ = Standard error of mean.

† Difference from control lot significant at 5% level.

rived from the supplement would be approximately 0.4 mg in the diet and 0.02 mg in the drinking water, respectively, for the adult female rats. Underwood (*3*) observed no improved growth in rats ingesting only 0.4 µg/day of Co when the diet was supplemented with this mineral. It has been concluded, therefore, that the normal need of the rat, if any, would not be higher than 0.4 µg/day of Co for growth. Our unsupplemented diet probably provided not less than this amount.

Lately it has been shown that Co forms part of the molecule of vitamin B_{12} (*4*) and that the addition of its salts to the culture medium stimulates the microbiological synthesis of this vitamin (*5*). It therefore seems possible that a stimulation of the intestinal synthesis of vitamin B_{12} is a likely explanation for the results described in this study. The fact that Co administered parenterally had no significant effect lends weight to this explanation, although the effect of prolonged and repeated administration of smaller doses of parenterally administered Co should be studied. It is known that ruminants have a dietary need for cobalt, the relation of which to the internal microbiological synthesis of vitamin B_{12} is still not clear (*6*). Klosterman *et al.* (*7*) have found that Co stimulates the growth of pigs fed a soybean-corn ration, whereas in a recent report (*8*) Co has been stated to be ineffective in speeding the growth of rats fed a diet low in vitamin B_{12} and containing iodized casein. The different techniques used in this study and in our experiments make a comparison of the results difficult, but it seems possible that the effectiveness of Co supplements depends on the special microbiological flora prevalent in the experimental animals.

It should be kept in mind that a considerable part of the human population subsists on diets very poor in animal products and therefore proba low in vitamin B_{12}. In these people the possible dietary need for cobalt should be considered.

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