Studies in iron metabolism

3.* Cobalt and erythropoiesis in the growing rat

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Numerous factors have been shown to affect the metabolism of iron. Evidence has been obtained from experiments with various species of animal that trace elements, such as copper, zinc and molybdenum, may be essential for the metabolic processes of erythropoiesis. Certain other metals, including cobalt, have been mentioned from time to time as stimulating erythropoiesis and causing polycythaemia. Such alterations in erythropoiesis and in production of haemoglobin afford an opportunity for studying the metabolism of body Fe.

Waltner & Waltner (1929) first reported that Co causes an erythraemia in the rat. Results of subsequent investigations into the effects of this metal on the haematological picture of various species of animal have been reviewed by Schultze (1940), Cartwright (1947 *a*, *b*) and others. The work described here was undertaken to determine the effect of Co given orally on erythropoiesis and the distribution of Fe in the rat.

EXPERIMENTAL AND RESULTS

General. Animals, materials and methods were as previously described (McCall, Newman, O'Brien, Valberg & Witts, 1962; McCall, Newman, O'Brien & Witts, 1962). The effects of small dietary supplements of Co upon weanling female rats fed on diets containing various amounts of Fe were studied in three experiments. Except in their Fe and Co supplements, the semi-synthetic diets used (Table 1) were similar to diet 1 of McCall, Newman, O'Brien, Valberg & Witts, 1962. Diets and water were offered *ad lib.*

Expt 1. Rats in three groups of twelve were fed as follows: group 1 received a standard mixed diet of rat cake (modified diet 41 B, based on diet 41 of Bruce & Parkes, 1949 and Bruce, 1950) supplied by Oxo Ltd, Thames House, London, E.C. 4, group 2 (control group) the semi-synthetic diet supplemented with 34 mg Fe and 0·3 mg Co/kg, and group 3 the semi-synthetic diet supplemented with 34 mg Fe and 3 mg Co/kg. During a period of 3 months the animals were weighed twice weekly, and the concentrations of haemoglobin in the peripheral blood were determined at approximately weekly intervals. The weight increases of these rats are shown in Table 1 and the haemoglobin values in Table 2. Rats fed on the semi-synthetic diet containing

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3 mg Co/kg gained weight more slowly, but had higher blood haemoglobin levels, especially in the last 50 days of the experiment, than rats fed on the diet containing 0·3 mg Co/kg.

Expt 2. This experiment was similar to the first, except that the dietary supplement of Fe was increased. Rats in three groups of twelve were fed for 4 months as follows: groups 1 and 2 (duplicate control groups) on the semi-synthetic diet supplemented

Table 1. *Rate of increase in weight of weanling female rats fed on diets of differing iron and cobalt contents*

Expt no.	No. of rats	Diet Type	Fe content (mg/kg)	Co content (mg/kg)	Days (mean with standard deviation) required for mean live weight of rats to increase from 50 to 150 g
1	12	41 B rat cake	95	?	49 ± 10
	12	Semi-synthetic	34	0·3	47 ± 14
	12		34	3·0	69 ± 10
2	12		50	0·3	46 ± 10
	12		50	0·3	45 ± 10
	12		50	3·0	52 ± 10
3	12		240	0·18	46 ± 11
	12		240	10·0	39 ± 5

Table 2. *Mean values (g/100 ml blood) with standard deviations for concentration of haemoglobin in the peripheral blood of three groups each of twelve female rats fed on 41 B rat cake or on the semi-synthetic diet, supplemented with 34 mg Fe/kg and either 0·3 mg or 3 mg Co/kg*

Days on diet	Rat-cake diet	Semi-synthetic diet (0·3 mg Co/kg)	Semi-synthetic diet (3 mg Co/kg)
0	16·1 ± 0·8	15·7 ± 0·7	15·6 ± 0·8
5	16·1 ± 0·8	15·7 ± 0·7	15·6 ± 0·9
19	16·6 ± 0·7	16·3 ± 1·6	15·1 ± 1·3
26	16·5 ± 1·0	15·4 ± 1·1	15·3 ± 0·7
33	16·3 ± 0·7	15·8 ± 1·4	15·5 ± 0·8
40	16·2 ± 0·8	15·1 ± 1·4	15·5 ± 0·7
47	16·3 ± 0·8	14·7 ± 1·1	15·6 ± 0·7
64	16·6 ± 0·7	14·6 ± 0·8	16·8 ± 1·0
78	16·7 ± 0·3	15·8 ± 0·7	17·2 ± 0·8
89	16·7 ± 0·6	16·0 ± 0·8	18·3 ± 0·8

with 50 mg Fe and 0·3 mg Co/kg, and group 3 on the semi-synthetic diet supplemented with 50 mg Fe and 3 mg Co/kg. Results are shown in Table 1 and Table 3. There was no significant difference in the mean weight increases of the groups, but after 30 days the rats fed on the diet containing 3 mg Co/kg had higher blood haemoglobin levels than those on the diet containing 0·3 mg Co/kg.

Expt 3. Seventy-two weanling female rats were obtained as litters which had been selected to contain even numbers of animals. They were divided between two groups so that each litter was equally represented in each group, and each group contained

thirty-six animals divided equally between three cages. The first group (control) was fed on the semi-synthetic diet supplemented with 240 mg Fe/kg (diet 2 of McCall, Newman, O'Brien, Valberg & Witts, 1962). Except for that present in the cyano-cobalamin (20 μg/kg), no Co was added to this diet 1, whose Co content was 0·18 mg/kg. The second group of rats received the same diet supplemented with 10 mg Co/kg. At the beginning of the experiment the ages of the rats ranged from 21 to 32 days. The

Table 3. *Mean values (g/100 ml blood) with standard deviations for concentration of haemoglobin in the peripheral blood of three groups each of twelve female rats fed on the semi-synthetic diet supplemented with 50 mg Fe/kg and either 0·3 mg or 3 mg Co/kg*

Days on diet	Diet with 50 mg Fe and 0·3 mg Co/kg*		Diet with 50 mg Fe and 3 mg Co/kg
	Group 1	Group 2	Group 3
10	13·9±0·7	13·6±0·7	14·5±0·8
21	14·7±0·5	14·6±0·6	15·2±0·8
35	16·0±0·8	15·6±0·8	16·5±0·8
71	16·8±0·6	16·7±0·8	18·3±0·6
102	15·7±0·5	15·6±0·5	17·4±0·9
112	15·8±0·5		16·5±0·4

* Groups 1 and 2 were in duplicate experiments.

Table 4. *Mean values with standard deviations for results of the various haematological analyses obtained at slaughter in rats fed on the semi-synthetic diet supplemented with 240 mg Fe/kg or 240 mg Fe and 10 mg Co/kg*

Co content of diet (mg/kg)	0·18	10
No. of rats	31	31
Haemoglobin concentration (g/100 ml)	16·6±0·75	17·9
Haematocrit value, micro method (%)	45·2±1·9	49·2
Mean corpuscular haemoglobin concentration (%)	36·7±0·98	36·3
Mean corpuscular volume (μ^3)	61·7±4·4	61·6
Red-cell count (10^{-6}/mm^3)	7·37±0·34	8·03
Reticulocyte count (%)	7·8±5	8·2
Colour index	0·8±0·03	0·77
Plasma Fe concentration (μg/100 ml)	214±64	183

The standard deviation of values in animals given Co is not included because of the slow distribution that resulted from the progressive nature of the erythraemia (Fig. 1).

rats were weighed weekly, and the concentrations of haemoglobin in the peripheral blood were determined at fortnightly intervals. To study the progressive effects of the dietary supplement of 10 mg Co/kg upon some of the Fe compounds in the body, two litter-mates, one from each group, were killed on the day the experiment began and then at intervals of 2, 2 and 3 days in succession for 3 months. To avoid effects due to diurnal variations, animals were killed at the same time of day. At slaughter, the blood haemoglobin concentrations, haematocrit values, red-cell and reticulocyte counts, plasma Fe concentrations and cytochrome *c* contents of the livers and kidneys were determined. The weights of each animal and of its heart, liver, kidneys and spleen and the volume of the caecum were measured, as well as the Fe, fat and water contents of the carcasses of the rats killed after 24, 42 and 63 days on the diet.

The animals in both groups gained weight at similar rates (Table 1). There was no significant difference between the sizes of the livers, kidneys, hearts, spleens and caecums relative to body-weights of the two groups (McCall, 1961), and the figures are not reported here. The results of the haematological analyses made at slaughter are shown in Table 4. Fig. 1 demonstrates the progressive nature of the increases, to
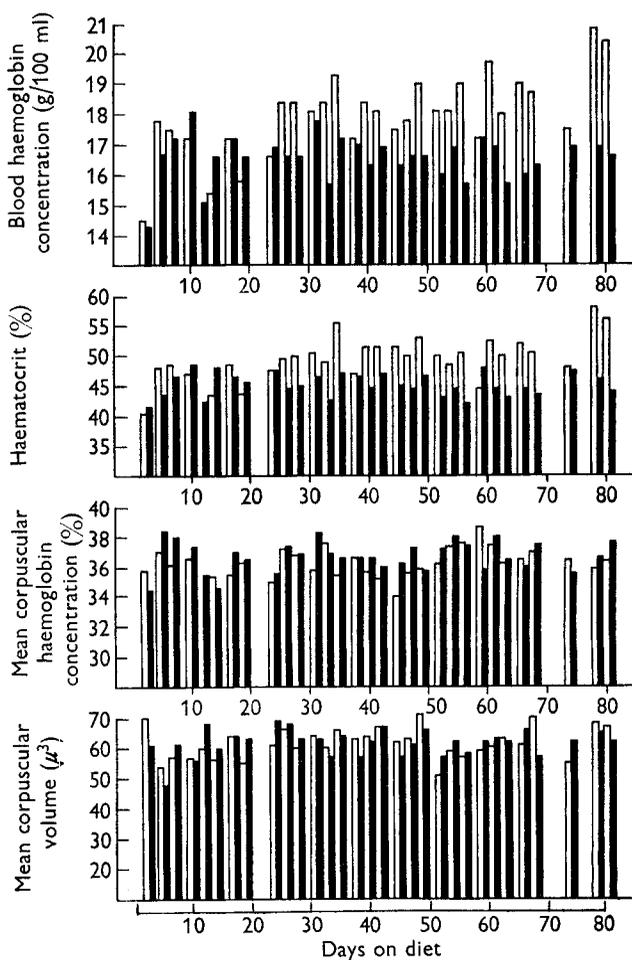

Fig. 1. Development of erythraemia in rats fed on the semi-synthetic diet supplemented with 240 mg Fe and 10 mg Co/kg compared with rats fed on the semi-synthetic diet supplemented with 240 mg Fe alone. ☐, rats fed on semi-synthetic diet supplemented with 240 mg Fe plus 10 mg Co/kg; ■, rats fed on semi-synthetic diet supplemented with 240 mg Fe alone.

levels above those associated with Fe sufficiency, in the blood haemoglobin concentrations and haematocrit values of animals receiving the diet supplemented with 10 mg Co/kg. These increases occurred without significant changes in the mean corpuscular haemoglobin concentrations or mean corpuscular volumes of the red cells.

The cytochrome c contents of the livers of thirty rats from each group are shown in Fig. 2. In both groups the concentrations of cytochrome c first rose, peak concentra-

tions occurring between the 15th and 25th days on the diets. This period coincided with the phase of most rapid growth. The concentration then declined, although the total amount of liver cytochrome *c* increased.
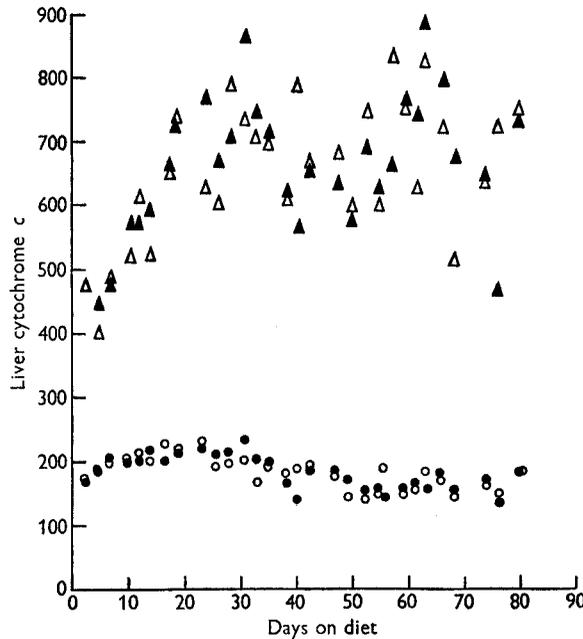

Fig. 2. Cytochrome *c* content of the livers of rats fed on the semi-synthetic diet supplemented with 240 mg Fe/kg or 240 mg Fe and 10 mg Co/kg. △, total cytochrome *c* content (μg) in livers of rats given Fe (240 mg/kg); ▲, total cytochrome *c* content in livers of rats given Fe (240 mg/kg)+Co (10 mg/kg); ○, concentration (μg/g) of cytochrome *c* in livers of rats given Fe (240 mg/kg); ●, concentration of cytochrome *c* in livers of rats given Fe (240 mg/kg)+Co (10 mg/kg).

Table 5. *Iron, water and fat contents of carcasses of six rats fed on the semi-synthetic diet supplemented with 240 mg Fe/kg or 240 mg Fe and 10 mg Co/kg*

Days on diet	24		42		63	
Co content of diet (mg/kg)	0·18	10	0·18	10	0·18	10
Live weight of rat (g)	90	90	128	109	166	196
Weight of carcass (g)	63	69	106	87	138	159
Concentration of Fe in carcass (mg/100 g)	5·60	5·34	5·85	5·47	6·85	5·63
Total Fe content of carcass (mg)	3·53	3·70	6·19	4·73	9·45	8·95
Haemoglobin concentration (g/100 ml blood)	16·9	16·6	16·9	18·1	15·7	18·1
Plasma Fe concentration (μg/100 ml)	204	149	198	136	295	236
Concentration of Fe in the carcass (mg/100 mg) corrected to include Fe lost in the blood removed at slaughter	7·45	7·22	7·33	7·22	8·14	7·20
Total Fe content of carcass (mg) corrected to include Fe lost in the blood removed at slaughter	5·2	5·39	8·09	6·64	11·6	11·4
Calculated Fe content (mg) of the total haemoglobin of the rat	2·74	2·69	3·90	3·56	4·70	6·40
Calculated concentration of Fe in the carcass (mg/100 g) without Fe in blood haemoglobin	4·40	4·23	4·29	3·96	5·31	3·58
Water content of carcass (g/100 g)	66·7	64·8	68·1	65·5	65·6	64·3
Fat content of carcass (g/100 g)	10·0	11·1	11·7	11·7	13·3	14·0

21-2

Because of the possibility of a difference in the amounts of intrarenal fat in the two groups of rats, cytochrome c was determined in the renal cortex during the first part of the experiment. The results were variable, and it was concluded that the inconsistencies depended upon variations in the efficiency of dissection (McCall, Newman, O'Brien & Witts, 1962). Thereafter the cytochrome c content of the whole kidney was measured. Between the 30th and 80th days of the experiment the mean values with standard deviations for the concentrations and total quantities of cytochrome c in the kidneys of seventeen rats from the control group were respectively 285 ± 24 μg/g and 299 ± 40 μg. Values for seventeen rats receiving the semi-synthetic diet supplemented with 240 mg Fe and 10 mg Co/kg were respectively 262 ± 26 μg/g and 264 ± 43 μg.

Results obtained for the six carcasses investigated are summarized in Table 5. The Fe content of the red-cell mass was calculated from the measured haemoglobin concentration and the blood volume calculated from the body-weight of the rat, by means of the relationship observed by Everett, Simmons & Lasher (1956). There was no significant difference between the two groups in the fat and water contents of the carcasses.

DISCUSSION

Before the work described here the lowest level of dietary Co reported as causing erythraemia in the rat was 50 mg/kg diet (Orten, Underhill, Mugrage & Lewis, 1932, 1932–3). Slowing of growth has been reported with levels above 60 mg/kg, and 500 mg/kg was rapidly fatal (Stare & Elvehjem, 1932–3). Results obtained in the three experiments indicate that erythraemia can be caused by as little as 3 mg Co/kg diet and that the effect of Co on weight increase depends to some extent upon the Fe content of the diet. The addition of Co up to 0·3 mg/kg diet did not appear to affect the rate of increase in the rat's weight or the haematological picture. Except for that present as cyanocobalamin, no Co was added to the control diet used in Expt 3 (i.e. diet 2 of McCall, Newman, O'Brien, Valberg & Witts, 1962). It is probable that additional Co is not required by the rat, provided that the vitamin B_{12} content of the diet is adequate (Albritton, 1954).

The concentration of haemoglobin in the blood of rats receiving the semi-synthetic diet containing 3 or 10 mg Co/kg increased to levels higher than those associated with Fe sufficiency (McCall, Newman, O'Brien, Valberg & Witts, 1962; McCall, Newman, O'Brien & Witts, 1962), whether the dietary supplement of Fe was 34, 50 or 240 mg/kg. Judged by the haematological results obtained in Expt 3 (Table 4), this rise in haemoglobin levels was due to an increase in the concentration of circulating red cells and not to any change in their mean volume or haemoglobin content. The rapidity of onset of the erythraemia appeared to depend upon both the Fe and the Co contents of the diet. Haemoglobin levels above 18 g/100 ml blood occurred at approximately 90 days with a dietary supplement of 34 mg Fe and 3 mg Co/kg, 60 days with a supplement of 50 mg Fe and 3 mg Co/kg and 25 days with a supplement of 240 mg Fe and 10 mg Co/kg. In animals receiving 3 mg Co/kg diet, studied for periods longer than 3 months (Expt 2, Table 3), the Co erythraemia appeared to be transitory.

The concentrations of Fe in the plasma of rats receiving Co (Expt 3) were generally

lower than those of the control group. Similarly, in the small number of rats investigated, the carcass Fe contents calculated so as to exclude the Fe in the blood haemoglobin tended to be lower in the animals given Co supplements; when the quantities of Fe in the carcasses were calculated so as to include the Fe contents of the blood, there were no significant differences between the two groups. These calculations demonstrate that, with the development of erythraemia, Fe is distributed preferentially to the red-cell mass.

The mechanism of Co erythraemia is not understood. It has been suggested that it originates from an alteration in cellular respiration (Underwood, 1956). In the experiments described here, 10 mg Co/kg diet caused an erythraemia in rats when the diet contained 240 mg Fe/kg, but did not affect rate of weight increase or the levels of cytochrome *c* in the livers and kidneys.

In those animals receiving low dietary Fe supplements, and additional Co, the rate of weight increase was adversely affected. In Expt 1, rats fed on the diet containing 34 mg Fe/kg and supplemented with 3 mg Co/kg developed erythraemia, and at the same time there was a reduced rate of weight increase. The control rats receiving only 0·3 mg Co/kg diet increased in weight as fast as animals receiving higher Fe supplements (Table 1). However, there was a temporary fall in their blood haemoglobin concentration (Table 2), which coincided with the phase of rapid growth (40–75 days). Presumably in this period the diet contained insufficient Fe (34 mg/kg) to meet the demands of growth and haemoglobin production. The decrease in rate of weight increase during the period of erythraemia, in animals given Co supplements, could have resulted either from 'toxic' effects of Co or from a change in the quantity of Fe available for metabolic processes concerned with growth. Severe Fe deficiency has been shown to cause slowing of growth in rats (McCall, Newman, O'Brien, Valberg & Witts, 1962; McCall, Newman, O'Brien & Witts, 1962). Since the rate of gain in weight in rats receiving 3 or 10 mg Co/kg diet was not affected when the Fe content of the diet was greater than 50 mg/kg presumably the 'toxic' effects of Co can be ruled out.

A possible explanation of the decrease in growth rate follows from the observation by Goldwasser, Jacobson, Fried & Plzak (1958) that rats receiving Co have elevated plasma erythropoietin levels. Erythropoietin is believed to cause marrow hyperplasia, and it is possible than an increase in the number of marrow cells competing for Fe results in the distribution of the limited amount of available Fe preferentially to the red-cell mass at the expense of the other tissues and of growth.

SUMMARY

1. Cobalt was administered to growing rats maintained on a semi-synthetic diet supplemented with varying amounts of iron. The purpose of the experiments was to determine the effects of Co on erythropoiesis and on the distribution of Fe in the rat.

2. The addition of Co at levels of from 3 to 10 mg/kg to the semi-synthetic diet containing 34, 50 or 240 mg Fe/kg and nutritionally adequate in other respects, caused an erythraemia in the rat; its duration and degree appeared to depend upon both the size of the Co supplement and the quantity of Fe in the diet.

3. The detailed haematological findings during the progressive development of the Co erythraemia demonstrated that the increase in blood haemoglobin concentration was due to an increase in the concentration of circulating red cells and not to any change in their size or haemoglobin content, as measured by the mean corpuscular haemoglobin concentration and colour index.

4. No difference was found in the rate of weight increase, the weight of the heart, liver, kidneys or spleen or in the volume of the caecum, between animals receiving diet 2 (240 mg Fe/kg) supplemented with 10 mg Co/kg and control animals receiving diet 2 without added Co.

5. The concentration and total quantity of cytochrome c in the livers and kidneys of erythraemic animals receiving diet 2 supplemented with Co were similar to those for control rats.

6. The determination of the carcass Fe concentrations in three erythraemic and three control animals showed a higher proportion of Fe in the red-cell mass in erythraemic animals, with correspondingly lower concentration of Fe in the rest of the carcass.

7. Co in the diet at levels of up to 10 mg/kg was found to be without effect on the rate of weight increase of rats except when the dietary Fe level was low (34 mg/kg), when Co induced an erythraemia and there was a simultaneous reduction in rate of weight increase. A tentative explanation of this phenomenon is advanced.

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