Brit. J. Nutr. (1960), 14, 239

Haematopoiesis in the rat in riboflavin deficiency*

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(*Received* 25 *July* 1959—*Revised* 25 *January* 1960)

Waisman (1944) observed that marked anaemia developed in monkeys in riboflavin deficiency. Wintrobe, Buschke, Follis & Humphreys (1944) observed anaemia in riboflavin deficiency in the pig, but Terrill, Ammerman, Walker, Edwards, Norton & Becker (1955) did not. Axelrod, Lipton & Elvehjem (1941) found no anaemia in dogs in riboflavin deficiency, whereas Spector, Maass, Michaud, Elvehjem & Hart (1943) observed mild anaemia, which became severe if the animals were subjected to slight haemorrhage. György, Robscheit-Robbins & Whipple (1938) studied the regeneration of haemoglobin in dogs made anaemic by an inadequate diet, when repletion was stimulated by haemorrhage, and found that the production of haemoglobin was increased by the addition of more riboflavin to the diet.

Working with rats, Carpenter & Kodicek (1952) found only insignificant anaemia in riboflavin deficiency. Endicott, Kornberg & Ott (1947), Shukers & Day (1943) and Kornberg, Tabor & Sebrell (1945–6) found that anaemia developed only occasionally in the rat in riboflavin deficiency. Kornberg *et al.* (1945–6) carried their investigation further by studying the haematopoietic process stimulated by haemorrhage. As the result of repeated haemorrhage the haemoglobin and haematocrit values fell, the former to a greater extent than the latter, and consequently there was a decrease in the mean corpuscular haemoglobin concentration. These values eventually levelled off in the control animals, but continued to fall in those deficient in riboflavin. Only haematocrit values were measured during recovery from repeated haemorrhage. In the riboflavin-deficient animals they fell lower and did not rise to the same extent as in the controls. The authors interpreted the results of their experiments as indicating that riboflavin deficiency impaired the process of regeneration of red cells and haemoglobin. Their results, however, do not fully support this conclusion because red-cell counts were not done, but they do indicate some type of impairment in the haematopoietic process.

There is evidence, therefore, that in the rat riboflavin is involved in haematopoiesis, which becomes evident only when a stress is put upon the system, as by severe haemor-

* Issued as N.R.C. no. 5680. Presented in part at the 21st Annual Meeting of the Canadian Physiological Society in Ottawa in June 1957, and at the 21st International Congress of the Physiological Sciences in Buenos Aires in August 1959.

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rhage. This matter became of interest to us when we found that in riboflavin deficiency in the rat the levels of several important protein fractions of the body, including haemoglobin, were not adversely affected by riboflavin deficiency, and their regeneration was not impaired after dietary protein depletion and mild haemorrhage (Mookerjea & Hawkins, 1960). We therefore did some further haematological investigations. We studied the erythrocyte system and the effect upon it of haemorrhage of more severe degree than we had previously used. We also studied the effect upon the haemoglobin level of the administration of cobalt, which under normal conditions produces polycythaemia (Waltner & Waltner, 1929; Orten, Underhill, Mugrage & Lewis, 1932). We compared the regeneration of plasma protein after repeated haemorrhage with that of haemoglobin.

EXPERIMENTAL

The type and weights of the rats, and the experimental regimen were the same as described in a previous paper (Mookerjea & Hawkins, 1960), but only the diet containing 16% protein was used. The rats were arranged in experiments in the same way, i.e. those in groups A and B were given riboflavin in their vitamin supplement, and those in group C were not. Those of group A were used as food controls, and those of group B as weight controls, for group C.

Five experiments were done, in which twenty-four, thirty-six, sixty, or seventy-two rats were used. After 56–84 days, depending upon the experiment, the mean body-weight in group A differed from that in groups B and C by 10–15%.

Expt 1. This experiment with sixty rats was terminated at this stage. Haemoglobin and haematocrit values, and red-cell counts were obtained on the blood over a final period of 15 days.

Expt 2. After 78 days on the experimental regimen each of the twenty-four rats was subjected to haemorrhage, blood equivalent to 2% of the body-weight being taken on each of two alternate days. Haemoglobin values were determined just before each haemorrhage, and at intervals of 3 days for 26 days thereafter.

Expt 3. After 84 days on the experimental regimen each of the thirty-six rats was given daily intraperitoneally 0·3 mg cobalt as 1 ml of a saline solution of cobalt chloride. This treatment was continued for 49 days, during which haemoglobin values were determined at intervals of 4–14 days.

Expt 4. There were seventy-two rats in this experiment. After 74 days on the experimental regimen one-third of the animals from each group were killed, and the blood-haemoglobin and plasma-protein values determined. The remaining rats were subjected to haemorrhage as in Expt 2. Half were killed 2 days after and the other half 7 days after the last haemorrhage, and blood was obtained for determining the haemoglobin and plasma-protein values.

Expt 5. After 56 days on the experimental regimen the twenty-four rats were subjected to haemorrhage as in Expt 2. Haemoglobin and haematocrit values and red-cell counts were obtained just before each haemorrhage, and 3 and 6 days after the last.

Procedure. Blood taken directly into appropriate pipettes from the cut tail was used

for obtaining the values in all experiments except Expt 4, in which the plasma protein was measured, and the animals were killed to obtain blood from the heart. Haemorrhage was accomplished, haemoglobin and plasma protein were determined, and *P* values were calculated as in preceding experiments (Mookerjea & Hawkins, 1960). Red-cell counts were done by the standard method, with pipettes calibrated within the tolerance required by the U.S. Bureau of Standards. Haematocrit readings were made by the method of Van Allen (1925). From the haematological measurements, values for mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration were calculated in Expts 1 and 5.

RESULTS

The results of Expt 1 are shown in Table 1. They do not indicate any important differences in the haematological picture between the riboflavin-deficient rats and their controls, with the exception that the mean corpuscular volume was somewhat smaller in the former. In most of the experiments, however (see Figs. 1–4), the mean

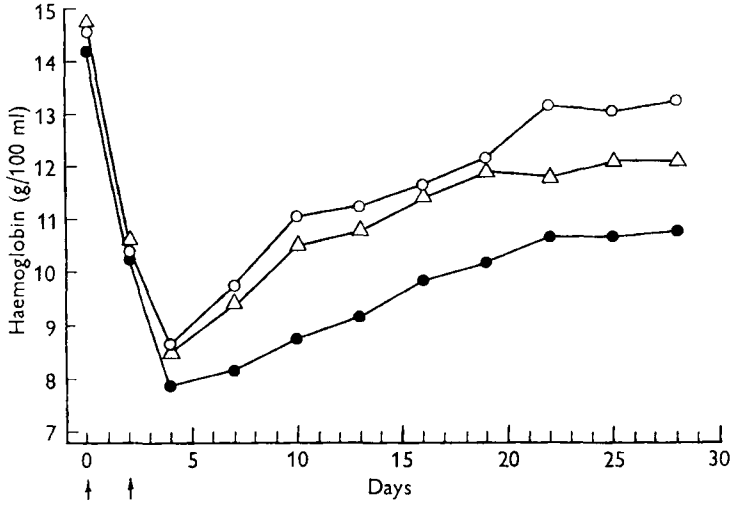

Fig. 1. Expt 2. Regeneration of haemoglobin after severe haemorrhage in the rat after 78 days on experimental regimen. ○—○, group A, food controls; △—△, group B, weight controls; ●—●, group C, riboflavin-deficient rats. Each point represents the mean value for eight rats. Arrows denote haemorrhage equivalent to 2% of the body-weight. *P* values between B and C were < 0·01 in the 2nd week and < 0·05 in the 3rd and 4th weeks after the last haemorrhage.

haemoglobin content of the blood of the riboflavin-deficient animals was somewhat lower than of those in the two control groups. The other haematological values obtained in Expt 5 (see Fig. 4), where this difference was greatest ($P < 0{\cdot}05$ between B and C), indicated that the lower haemoglobin content was a reflection of a lower mean red-cell count. These findings are in agreement with those of previous workers in demonstrating that mild anaemia occasionally occurs in riboflavin-deficient rats, but is not a characteristic or important result of the deficiency.

Expts 2–5 (see Figs. 1–4) offered substantial evidence that in riboflavin deficiency the haematopoietic system of the rat cannot respond normally to an extra stimulus imposed by severe haemorrhage or by the administration of cobalt.

Figs. 1, 3 and 4 show that as a result of severe haemorrhage in the animals deficient in riboflavin the haemoglobin concentration fell to a lower level and afterwards was not restored to the same extent as in control animals. This effect did not happen with plasma protein (Fig. 3). The results in Fig. 4 show that it was a reflection of parallel changes in the haematocrit level rather than in the red-cell count. As would be expected under those conditions, the new cells that were liberated into the circulation after haemorrhage were larger, but apparently their supply did not continue for so long in the riboflavin-deficient animals. In them the mean corpuscular volume and the mean corpuscular haemoglobin eventually became significantly lower than among the

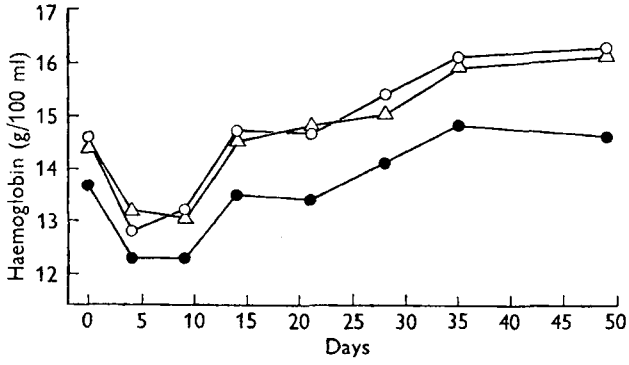

Fig. 2. Expt 3. Effect of the intraperitoneal administration of 0·3 mg of cobalt/day on the haemoglobin level of the rat after 84 days on experimental regimen. ○—○, group A, food controls; △—△, group B, weight controls; ●—●, group C, riboflavin-deficient rats. Each point represents the mean value for twelve rats. *P* values between B and C were < 0·05 in the 1st and 2nd weeks and < 0·01 in the 3rd week. Mean haemoglobin value at the end was 107% of the initial for C and 112% for A and B.

Table 1. *Expt 1. Mean values, with standard deviations and ranges, for haemoglobin and red-cell characteristics of rats after 65–79 days on a diet containing 16% protein*

Nutritional state	No. of rats	Red cells (10^{-6}/mm^3)	Haematocrit value (volume %)	Haemoglobin (g/100 ml)	Mean corpuscular volume (μ^3)	Mean corpuscular haemoglobin ($\mu\mu$g)	Mean corpuscular haemoglobin concentration (%)
A (food controls)	20	8·13 ± 0·6 (7·15–9·54)	44 ± 2·3 (38–48)	14·7 ± 0·8 (12·7–15·8)	55 ± 3·5 (47–60)	18 ± 1·4 (16–20)	33 ± 1·2 (30–35)
B (weight controls)	20	8·28 ± 0·5 (7·44–9·12)	44 ± 2·0 (41–49)	14·7 ± 0·7 (13·2–16·0)	53 ± 3·4 (49–65)	18 ± 1·1 (16–19)	33 ± 1·5 (30–35)
C (riboflavin-deficient)	20	8·50 ± 0·7 (7·33–9·76)	43 ± 2·4 (37–47)	14·5 ± 0·6 (13·1–15·6)	51 ± 5·2 (42–63)	17 ± 1·5 (15–20)	34 ± 1·4 (31–37)
Significance of difference between A and C		—	—	—	$P < 0·02$	$P < 0·05$	—

control animals, whereas the mean corpuscular haemoglobin concentration did not. Their haematological picture thus developed the characteristics of that in microcytic anaemia.

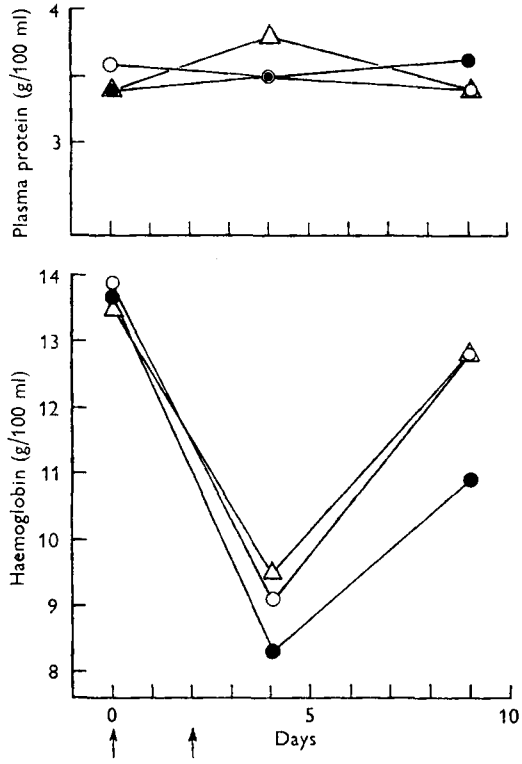

Fig. 3. Expt 4. Regeneration of haemoglobin and plasma protein after severe haemorrhage in the rat after 74 days on experimental regimen. ○—○, group A, food controls; △—△, group B, weight controls; ●—●, group C, riboflavin-deficient rats. Each point represents the mean value for eight rats. Arrows denote loss of blood equivalent to 2% of the body-weight. *P* value between B and C was < 0·05 2 days after, and < 0·03 7 days after, the last haemorrhage.

DISCUSSION

We have shown previously that in riboflavin-deficient rats the regeneration of several important protein fractions of the body, including haemoglobin, is not impaired after dietary protein depletion with or without mild haemorrhage (Mookerjea & Hawkins, 1960). The present experiments have provided evidence that there is in this vitamin deficiency in the rat an occult impairment in the haematopoietic system, which becomes apparent when the system is markedly stimulated, as by severe haemorrhage or repeated injections of cobalt. Such haemorrhage does not affect the regeneration of plasma protein in the riboflavin-deficient animals.

Under these conditions the changes in the haemoglobin level are essentially proportional to those in the haematocrit value (the mean corpuscular haemoglobin concentration remains essentially the same), and the number of red cells is not materially altered, and consequently the defect is reflected in the size of the cells. Whether or not

this effect is characteristic of cobalt intoxication we do not know, because we studied only the haemoglobin level in that experiment, but cobalt typically produces polycythaemia. Kornberg *et al.* (1945–6) found that after severe haemorrhage in the riboflavin-deficient rat the resulting anaemia was accompanied by low mean corpuscular haemoglobin concentration. To that extent their observations differed from ours.

It is important to consider that this defect in riboflavin-deficient rats subjected to severe stimulation of the haematopoietic system may not be a defect in the process of

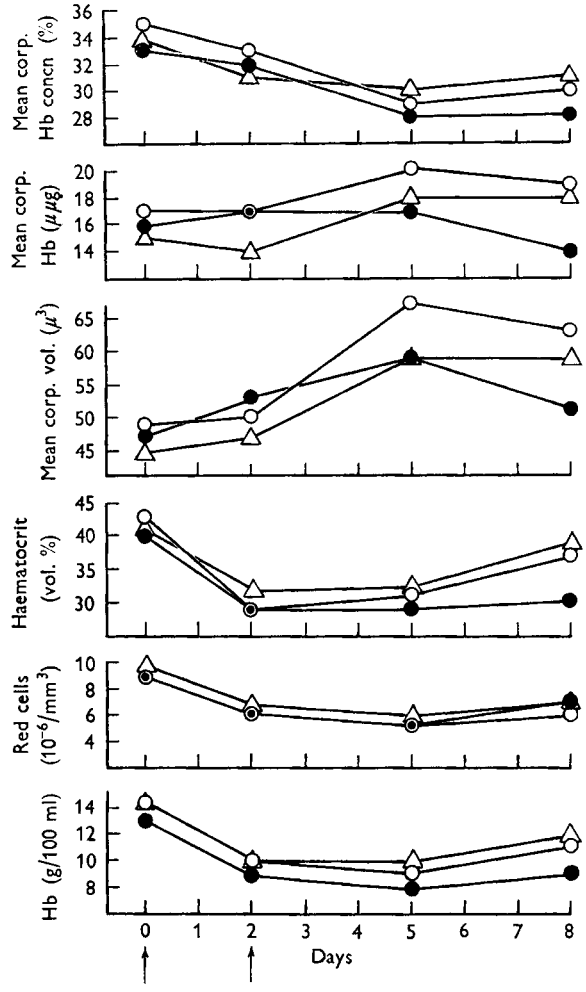

Fig. 4. Expt 5. Regeneration of erythrocytes and haemoglobin after severe haemorrhage in the rat after 56 days on experimental regimen. ○—○, group A, food controls; △—△, group B, weight controls; ●—●, group C, riboflavin-deficient rats. Each point represents the mean value for eight rats. Arrows indicate loss of blood equivalent to 2% of the body-weight. *P* values at the end were < 0·01 between C and A or B for haematocrit value and mean corpuscular haemoglobin, and < 0·05 for haemoglobin and mean corpuscular volume. Mean corpuscular volume at the end was 106% of the initial for C, and 128% and 131% for A and B.

haemoglobin synthesis, or if it is it is most likely to be in a process peculiar to that synthesis. It is suggested that the defect is more likely to be in some stage of the development of the erythrocyte.

SUMMARY

1. In five experiments young albino rats were divided into three groups with the same mean body-weight. Those of one group were deprived of riboflavin. Those of another were given the same amount of food, and those of the third group were given less food to keep their body-weights within the same range as of those deprived of riboflavin. Depending upon the experiment, the animals were maintained on the regimen for 56–84 days, at which time the body-weights of the animals deprived of riboflavin were 10–15% lower than of those that had received the same amount of food, with riboflavin. At this stage haematological examinations were made. Depending upon the experiment, the animals were then subjected to severe haemorrhage or repeatedly injected with cobalt, and further haematological examinations were made.

2. Mild anaemia appeared occasionally in the riboflavin-deficient rats, but it was not a characteristic or an important result of the deficiency. After severe haemorrhage, however, the haemoglobin level in these animals fell lower and was not restored to the same extent as in the controls. Also in these animals the increase in the haemoglobin value induced by repeated injections of cobalt was not as great as in the control animals.

3. In response to severe haemorrhage the change in the haemoglobin value was similar to that in the haematocrit value, whereas the changes in the red-cell count were not essentially different from those in the control animals. In the riboflavin-deficient animals the liberation of larger cells into the blood after severe haemorrhage did not continue for so long a time as in the controls. In them the mean corpuscular volume and the mean corpuscular haemoglobin eventually became smaller, and the mean corpuscular haemoglobin concentration remained essentially the same as in the controls. After the typical initial response to haemorrhage, therefore, the red-cell picture in the riboflavin-deficient animals resembled that in microcytic anaemia.

4. These experiments provided evidence that in the rat there is an occult defect in the haematopoietic system in riboflavin deficiency, and it suggests that the defect is in the formation or development of the erythrocyte.

We are indebted to Miss Verna G. Leonard, Mrs R. C. Dicks and Mr M. W. McLeod for assistance with these experiments.

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Printed in Great Britain