Study of Hematopoiesis in Riboflavin Deficient Rats with ^{59}Fe as Tracer

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(Received September 14, 1967)

Rats were deprived of riboflavin for 2 months by feeding the diets lacking in this vitamin. Radioactive iron was injected to those rats intravenously to study the half time for its disappearance from plasma (PDT $\frac{1}{2}$), plasma iron turnover, red cell iron utilization and its turnover. Similar experiments were performed in normal pair-fed rats and the results obtained were compared with riboflavin deficient animals. These studies suggest that there is a disturbance in the iron utilization and this leads to defective hematopoiesis under riboflavin deficiency.

Many workers have studied the role of riboflavin in hematopoiesis by producing experimental riboflavin deficiency in different animals (1-4). Working with rats Carpenter *et al.* (5) found only insignificant anemia in riboflavin deficiency. Endicott and coworkers (6) and Shukers and Day (7) found that anemia developed only occasionally in the rat in riboflavin deficiency. Kornberg *et al.* (8) carried their investigations further by studying the hematopoietic process stimulated by hemorrhage. They interpreted their results as indicating that riboflavin deficiency impaired the process of regeneration of red cells and hemoglobin. Mookerjea and Hawkins (9) provided evidence that in riboflavin deficiency the hematopoietic system did not respond normally to an extrastimulus imposed by severe hemorrhage or by the administration of cobalt. They suggested that there was a defect in the formation or development of erythrocytes in riboflavin deficient rats.

Since the early work of Hahn *et al.* (10), radioactive iron has been widely used to study the hematopoiesis and the state of iron nutrition in various hematological disorders. Study with ^{59}Fe is today a standard technique for evaluating bone marrow function. To gather more information on the state of hematopoiesis in riboflavin deficiency the radioactive iron technique was employed in this investigation.

EXPRRIMENTAL

Young male albino rats weighing 80-90 g were divided into two groups with the same average body weights. Group A comprised of normal rats and group B of riboflavin deficient animals. The animals were pair-fed on 16 % protein diet.

Particulars regarding the diet were the same as reported earlier (11). After 60 days on experimental regimen rats were injected with ^{59}Fe as $^{59}\text{FeCl}_3$ ($1 \mu\text{Ci}/100 \text{ g}$ body weight) through the caudal vein. Some animals of comparable body weights were selected from each group and 0.02 ml of blood was drawn out from each at the intervals of 2, 6, 8, 12, 15 and 21 days after the injection of ^{59}Fe by a supraorbital technique due to Riley (12). Curve was obtained for the appearance of radioactive iron in the red blood cells by plotting the activities of blood cells against the time as percentage of administered dose; the maxima of the curves give the percentage of the iron utilization by the erythrocytes (Fig. 1).

FIG. 1 Appearance of ^{59}Fe in Red Blood Cells

Remaining animals from both normal and deficient groups were used for the plasma iron clearance study. Plasma samples were prepared from the blood taken at the intervals of 5, 30, 60, 90 and 120 minutes after the injection of ^{59}Fe . The plasma clearance curves and the half time for plasma disappearance ($\text{PDT}_{1/2}$) of ^{59}Fe were obtained. The rats were then sacrificed to collect blood through hepatic vein, which was used for the determination of hemoglobin, packed red cell volume, total erythrocyte and reticulocyte counts by the standard techniques (13). The pooled plasma samples were used for the determination of total iron concentration (14). Radioactive measurements were done in a Geiger-Müller Counter. Sufficient counts were recorded to keep the statistical error below 5%. Blood volume, plasma iron clearance rate, red cell iron utilization and its turnover rate were calculated according to Veall and Vetter (15) and Bothwell *et al.* (16). Erythropoietic indices were also calculated (16).

$$\begin{aligned} \text{(A) Erythropoietic index} &= \frac{\text{Whole blood plasma turnover of riboflavin deficient rat} \times 100}{\text{Whole blood plasma turnover of normal rats}} \\ \text{(B) Erythropoietic index} &= \frac{\text{Reticulocyte number in blood of riboflavin deficient rat} \times 100}{\text{Reticulocyte number in blood of normal rats.}} \end{aligned}$$

$$\text{Reticulocyte number in blood} = \% \text{ reticulocyte} \times \text{erythrocyte per } \mu\text{i.}$$

RESULTS

Results are shown in Table I and Fig. 1.

TABLE 1
Blood Characteristics of Normal and Riboflavin Deficient Rats
(Averages and standard deviations)

Blood characteristics	Normal rats ^a	Riboflavin deficient rats
1. Erythrocytes (10 ⁶ /μl)	6.12 ± 0.32	6.03 ± 0.13
2. Reticulocytes in blood (in per cent)	2.09 ± 1.1	3.71 ± 1.6
3. Packed cell volume (PCV)	41.05 ± 3.86	36.19 ± 4.1
4. Mean corpuscular volume (MCV, μ ³)	67.00 ± 11.9	60.0 ± 12.6
5. Blood volume (ml/100 g body wt)	6.41 ± 1.7	6.42 ± 1.48
6. Half time of plasma clearance (PDT ½) (in minutes)	66.80 ± 12.5	77.90 ± 10.6
7. Circulating plasma iron (μg/100 g body weight)	12.61 ± 2.2	14.15 ± 2.7
8. Plasma iron turnover rate (mg/day per 100 ml of whole blood)	0.167 ± 0.07	0.147 ± 0.09
9. Erythropoietic index		
(A) From plasma turnover	—	88
(B) From reticulocyte number	—	176
10. Red cell iron utilization as percentage of injected doses	77.00 ± 13.0	71.3 ± 9.0

^a 10-12 rats were examined for each group.

Riboflavin deficient rats showed decreased packed cell volume, mean corpuscular volume and increased plasma iron clearance time (PDT_½) and circulatory plasma iron concentration. Erythropoietic index, calculated from reticulocyte number was increased. However, it was decreased when obtained from whole blood plasma turnover in riboflavin deficient rats. Red cell iron utilization was slightly depressed under riboflavin deficiency.

DISCUSSION

Mookerjee and Hawkins (9) observed a microcytic and hypochromic anemia in riboflavin deficient rats with no change in red cell number compared to normal rats. In our experiments also this has been shown by riboflavin deficient animals.

Bothwell and coworkers (16) have considered the whole blood plasma iron turnover as an index of erythropoietic activity of the marrow under the various hematological disorders. In riboflavin deficiency whole blood plasma iron turnover is depressed, possibly indicating a hypofunction of the marrow in deficient animals. The marrow hypoactivity under this condition is also apparent from the decreased ⁵⁹Fe uptake by the red cells (Fig. I).

The erythropoietic index as calculated from the whole blood plasma turnover is depressed but it is more when obtained from reticulocyte number in riboflavin deficient rats. This type of discrepancy has also been observed by Bothwell *et al.* (16) in their studies. They stated that it was due to earlier release of young reticulocytes from the marrow which require longer time for the maturation in

blood. This may be the plausible explanation for the higher reticulocyte count in blood of riboflavin deficient rats.

In riboflavin deficient rats, the circulating plasma showed increased iron concentration compared to normal rats. This may be due to (a) increased iron absorption or (b) decreased iron utilization or (c) increased red cell destruction. The gradual fall in the red cell activity after reaching the maximum in deficient rats (Fig. 1) supports the third possibility, *i.e.* increased red cell destruction in the present study. Bothwell *et al.* (16) also interpreted increased plasma iron concentration as an indication of increased red cell destruction.

Riboflavin deficiency possibly interferes with the hemoglobin formation due to defect in the synthesis of porphyrin (17-19) and thus causes a microcytic and hypochromic anemia. In spite of the presence of iron, its utilization is depressed in riboflavin deficiency, virtually reflecting the bone marrow hypoplasia. On the other hand, due to defect in the formation or development of cells (9), the average life span of red cells possibly decreases (as evident from the earlier fall in the activity of red cells in deficient rats). This causes a kind of hemolytic anemia and produces a stress on the hematopoietic system and finally it leads to reticulocytosis in riboflavin deficiency.

ADKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. B. D. Nagchaudhuri, Director, Saha Institut of Nuclear Physics for advice and encouragement in carrying out this investigation. They also wish to express their thanks to Dr. F. Hosain and Dr. S. Mukherji for their help and advice in the preparation of the manuscript. One of the authors (K. B. U.) expresses his gratitude to the Department of Atomic Energy, Government of India for financial assistance.

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