

HEREDITARY HYPOCHROMIC MICROCYTIC ANEMIA IN THE LABORATORY RAT

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IN a preliminary note on the effect of X rays on rats, an inherited anemia was reported among the offspring of one of the irradiated animals (SLADIC-SIMIC *et al.* 1963). The present paper presents the data of our studies of the genetic transmission of this disease and of the phenotypic characteristics of mutant animals.

MATERIAL AND TECHNIQUES

The group of 17 irradiated rats including the animal that produced anemic progeny (X2) belonged to a stock of rats randomly bred in our laboratory since 1930. The X2 female was one of five females in a litter, which were X-irradiated under conditions reported earlier (SLADIC-SIMIC *et al.* 1963). All irradiated females were mated with normal males from the stock and brother-sister matings for each line were carried through four to eight filial generations.

Rats which had given anemic young in their offspring, were crossed with each other and with unrelated and related anemic animals. Anemic rats of both sexes were crossed with each other and with unrelated and related normal animals.

The concentration of hemoglobin was measured by the method of KHALIFA and SALAH (1951). The osmotic resistance of erythrocytes was determined by Simmel's method. Hemolysis was considered complete at 90 to 100% and absent at 0 to 10% lytic levels. All peripheral blood samples were obtained from the tail. The Price Jones curves for the diameters of erythrocytes were obtained from fixed smears of peripheral blood with the use of an ocular micrometer, and the hematocrit values with the Drummond Microhematocrit. Peripheral blood cells were counted in the standard chambers. Blood smears were stained with the Giemsa technique.

Since it was found in the course of our experiments that anemic rats 30 days of age and older responded to intramuscularly injected iron with an increase in survival time, anemic rats were treated with an iron dextran complex (Myofer, Farbwerke Hoechst AG). The dose was 0.5mg per day, except that pregnant anemic rats received 1.0mg per day.

RESULTS

Pathologic changes in anemic rats: The newborn anemic rats were pale yellow and could easily be distinguished from their normal looking brothers and sisters. The majority of them died a short time after birth, but a few survived for periods up to six months. At birth the anemic rats appeared somewhat retarded in growth compared with their normal looking litter mates. Figure 1 shows the growth curves of anemic rats and their litter mates with weights determined at 10 day intervals. Figure 2 illustrates an extreme case of body wasting as observed in several cases.

In spite of their weakened condition and retarded growth, a few anemic rats

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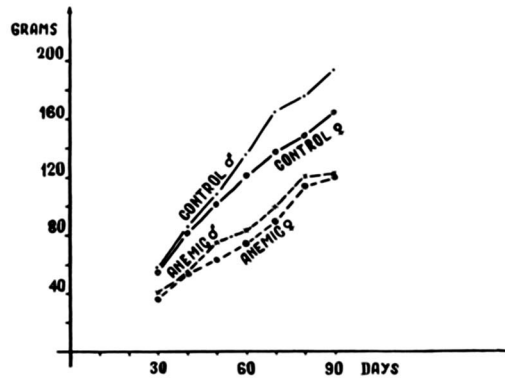


FIGURE 1.—Growth curves of anemic rats and their litter mates.

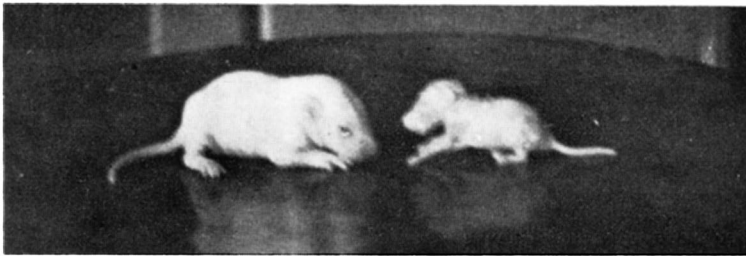


FIGURE 2.—Progeny of X2 inbred line 20 days old. Anemic rat (right), and nonanemic brother (left).

lived long enough to be mated and produce young. Out of a group of 303 anemic males, three reached sexual maturity. Two of them were outcrossed to normal females and the third was backcrossed to his mother. A short time after mating all three males died. Out of 319 anemic females, two lived long enough to be outcrossed to two normal males. One of the females was found dead in an early stage of gestation. The other gave birth to nine stillborn young and died several hours later. Of this litter of nine, one, a female, was of normal size; in seven young the posterior part of the body was underdeveloped and the viscera were exposed (Coelosomy); in the ninth animal hind legs and tail were missing.

The smear of the peripheral blood of the anemic animals was highly characteristic and its morphology was one of the basic criteria for identifying these animals. In some anemics, nearly all erythrocytes were profoundly affected in both form and size. Microcytosis, anisocytosis and poikilocytosis, always present to a slight extent in normal new born rats, were much more marked in anemic animals (Figure 3). It is known that in normal rats microcytic and poikilocytic cells gradually disappear with age; in the anemic rats such cells become more and more numerous to a point where normal looking erythrocytes are difficult to find. Hypochromic leptocytes and target cells were often present in newborn anemic rats, and their number increased with age.

The mean value for erythrocytes in the peripheral blood of 11 anemic rats, at 30 days of age and older, was $4.1 \pm 0.30 \times 10^6$ and for leucocytes $15.7 \pm 1.8 \times 10^3$

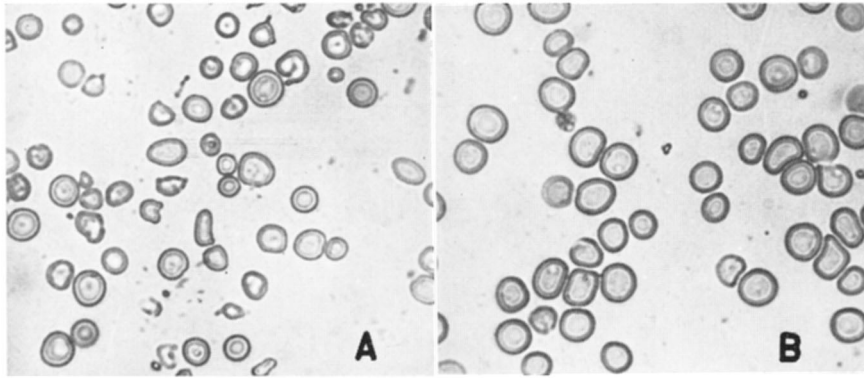


FIGURE 3.—Erythrocytes of a newborn anemic rat (A) and a newborn control (B). Giemsa stain.

per mm^3 ; for the nonanemic control litter mates, the mean value for erythrocytes was $6.2 \pm 0.3 \times 10^6$ and for leucocytes $8.3 \pm 0.9 \times 10^3$ per mm^3 .

In contrast to normal rats where the Hb value (grams of hemoglobin per 100 ml of blood) remained the same throughout postnatal life, that of anemic rats decreased with increasing age from about half of that of the controls at 24 hr *post partum* to a tenth at three months of age (Table 1). In two anemic rats the Hb value was measured twice, and was found to have decreased from 3.0 and 3.1 at 60 days to 1.7 and 1.6 at 87 days of age.

The steady decline of the Hb value with age suggested that iron might play a role in the metabolic processes responsible for the anemia. The intramuscular injection of an iron dextran complex into anemic rats prolonged their life, but in spite of the treatment, the peripheral blood picture remained abnormal (Figure 4). Two groups of rats were subjected to this treatment: newborn and those of 30 days of age and more. Whereas the iron treatment failed to save the life of very young anemic rats, it prolonged the life of rats after weaning and allowed them to reproduce.

Two anemic females mated with two anemic males and iron treated during the gestation period gave birth to six and five young respectively. All the progeny of these two litters survived periods of 76 and 92 days without any treatment. Hemoglobin and hematocrit values and red blood cell counts were obtained, first, at 41 and 57 days of age, and again at 76 and 92 days. The results are presented in

TABLE 1

Concentration of hemoglobin (Hb) in grams per 100 ml of blood

| Age (days) | Controls from stock | | Normal sibs | | Anemic rats | |
|------------|---------------------|----------|-------------------|----------|-------------------|----------|
| | Number of animals | grams Hb | Number of animals | grams Hb | Number of animals | grams Hb |
| 1 | 7 | 11.3 | 7 | 12.1 | 6 | 5.9 |
| 60 | 6 | 14.9 | 6 | 13.5 | 2 | 3.1 |
| 80-90 | 8 | 14.1 | 6 | 13.3 | 8 | 2.4 |

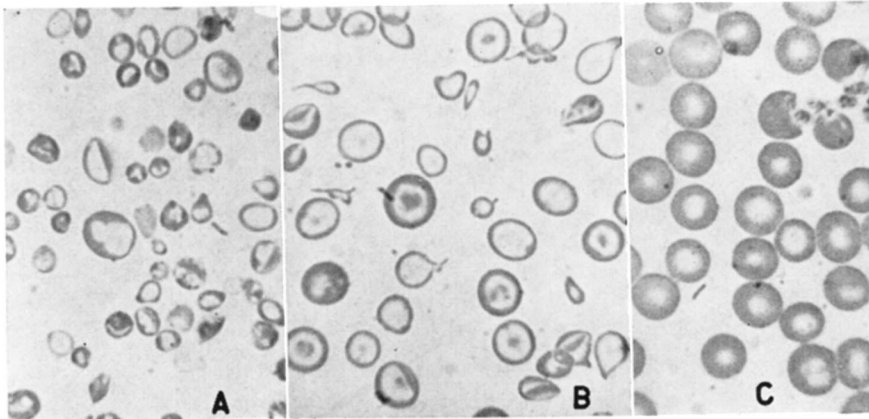


FIGURE 4.—Peripheral blood picture: A. nontreated anemic rat; B. same anemic animal after 8 weeks iron treatment; C. normal control after 8 weeks treatment.

Table 2. As can be seen all three values decreased with age. The calculated mean corpuscular volume decreased also, but the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration remained unchanged.

In 14 anemic rats and 12 normal controls, subjected to continuous iron treatment for 10 weeks, measurements of hemoglobin and red blood cells were made every two weeks. The mean values for red blood cells are presented in Figure 5a. Those of anemic rats are far above the values of the controls except for the first four weeks of iron treatment. In two anemic rats not included in the group of 14 mentioned above, the red blood cell number rose to 18.5 and 21.6×10^6 . In these two animals the iron treatment had to be discontinued because their life seemed to be imperiled.

Although iron treatment raised hemoglobin values too, these remained below the controls for the whole period of treatment (Figure 5b). Nevertheless the calculated mean corpuscular hemoglobin, which is one third that in the controls, did not undergo any change throughout the treatment period (Figure 5c).

TABLE 2

Hematological values of 11 nontreated anemic rats from mothers iron-treated during gestation period

| Age (days) | | RBC ($\times 10^6$) | Ht (percent) | Hb value | MCH $\mu\mu\text{g}$ | MCHC (percent) | MCV (μ^3) |
|------------|-------|--------------------------|-----------------|----------|-------------------------|-------------------|--------------------|
| 41-57 | Mean | 8.5 | 29 | 4.1 | 5 | 14 | 35 |
| | Range | 6.8-11.1 | 20-35 | 3.5-5.4 | 4-6 | 9-18 | 28-47 |
| | sd | 1.4 | 5.3 | 0.6 | 0.6 | 2.2 | 5.8 |
| 76-92 | Mean | 5.6 | 17 | 2.6 | 5 | 16 | 30 |
| | Range | 4.1-6.3 | 12-32 | 2.0-3.6 | 4-6 | 11-22 | 19.39 |
| | sd | 1.1 | 5.1 | 0.4 | 0.6 | 3.4 | 6.1 |

RBC=red blood cells; Hb value=grams hemoglobin per 100 ml of blood; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; MCV=mean corpuscular volume.

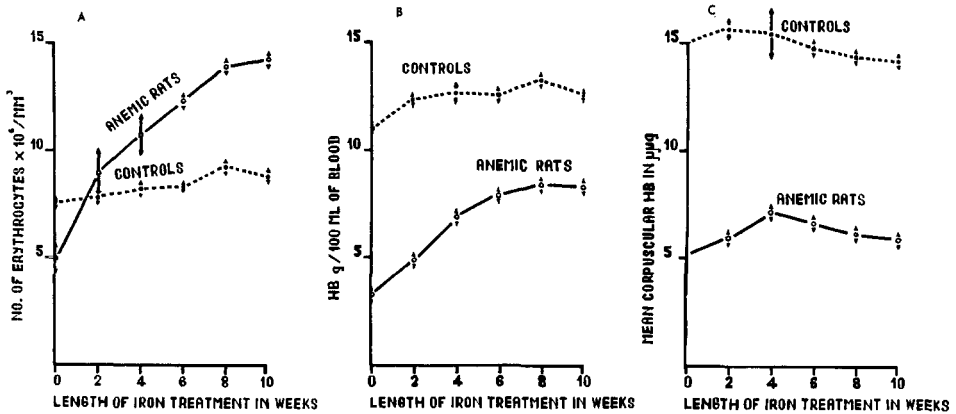


FIGURE 5.—Number of erythrocytes (A), hemoglobin value (B), and mean corpuscular hemoglobin (C), during the period of iron treatment of anemic and control rats.

Four and five weeks after the iron treatment was discontinued, 12 anemic rats and 10 controls of the above two groups were subjected to a second series of measurements for the determination of mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume. Concurrent measurements were made on nontreated anemic and nontreated normal control rats. With the iron treated anemic rats a rise in the mean corpuscular hemoglobin concentration as compared to the nontreated anemic controls was obtained, accompanied by a drop from 29 to 20 μ^3 in the mean corpuscular volume. The mean corpuscular hemoglobin, however, remained unchanged at 5 $\mu\mu\text{g}$ per cell

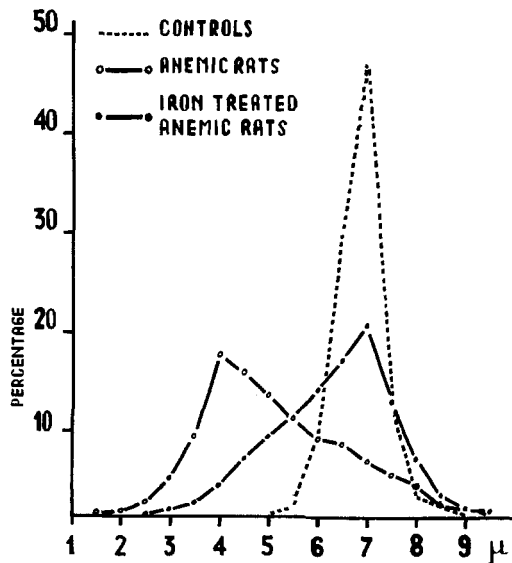


FIGURE 6.—Price Jones curve for red cells of anemic rats before and after iron treatment and of controls.

TABLE 3

*Hematological values of nontreated anemic rats and of controls
and of iron-treated anemic and controls*

| | BRC ($\times 10^6$) | Ht (percent) | Hb value | MCH (μg) | MCHC (percent) | MCV (μ^2) |
|--|--------------------------|-----------------|-------------|--------------------------|-------------------|--------------------|
| Normal rats (14 animals) | | | | | | |
| Mean | 7.8 | 42 | 12.1 | 16 | 29 | 55 |
| Range | 6.2-9.5 | 33-47 | 10.8-14.0 | 14-19 | 26-33 | 44-70 |
| sd | 1.1 | 4 | 0.9 | 1 | 2 | 7 |
| Normal rats, iron-treated (10 animals) | | | | | | |
| Mean | 9.4 | 41 | 12.1 | 13 | 30 | 44 |
| Range | 8.8-10.4 | 34-49 | 10.2-13.4 | 11-14 | 22-34 | 34-53 |
| sd | 0.5 | 3 | 1.0 | 1 | 4 | 7 |
| Anemic rats (9 animals) | | | | | | |
| Mean | 5.7 | 17 | 2.8 | 5 | 17 | 29 |
| Range | 4.3-6.8 | 11-20 | 1.8-3.7 | 4-5 | 13-19 | 25-33 |
| sd | 0.9 | 3 | 0.8 | 0.4 | 2 | 3 |
| Anemic rats, iron-treated (12 animals) | | | | | | |
| Mean | 16.1 | 33 | 8.0 | 5 | 24 | 20 |
| Range | 14.1-17.2 | 23-39 | 4.6-9.4 | 3-6 | 13-32 | 16-26 |
| sd | 1.0 | 6 | 1.3 | 1 | 6 | 4 |

(Table 3). All of these three values are below those obtained in nontreated normal controls. A rise in red blood cell count was also observed in treated controls as compared to the nontreated normal rats.

The Price Jones curves for the diameter of erythrocytes of the iron treated anemic animals as well as for the nontreated controls are presented in Figure 6. Whereas in the nontreated anemic animals the median stands at 4μ , it is shifted to 7 in the treated rats, and occupies the same position as in the controls.

The osmotic resistance of erythrocytes of the iron treated anemic rats was much

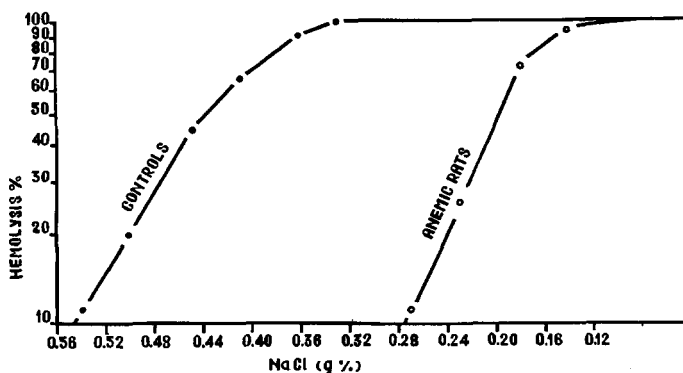


FIGURE 7.—Osmotic resistance of erythrocytes of iron treated anemic and control rats.

of different filial generations of female X2 yielded 460 normal and 131 anemic offspring.

Subsequently, crosses were made in which one or both partners were anemic. In preparation for this an F₂ male which had given anemic offspring before, was mated with four normal females from common stock, in the hope that outcrosses might improve the viability of anemic animals. Anemic rats began to appear in the second filial generation. Two such F₂ anemic males were outcrossed to three unrelated normal females, and gave 17 normal animals which in matings with each other produced 147 young including 34 anemic. Crosses of normal F₂ rats *inter se* gave 330 young of which 24 were anemic. The progeny of one anemic male is presented in Figure 8. Two iron treated anemic males outcrossed to seven normal females from the stock, produced seven litters with 44 young, all of them normal in appearance and with normal looking erythrocytes. The anemic rats in the F₂ generation obtained from the outcrosses have better appearance and longer

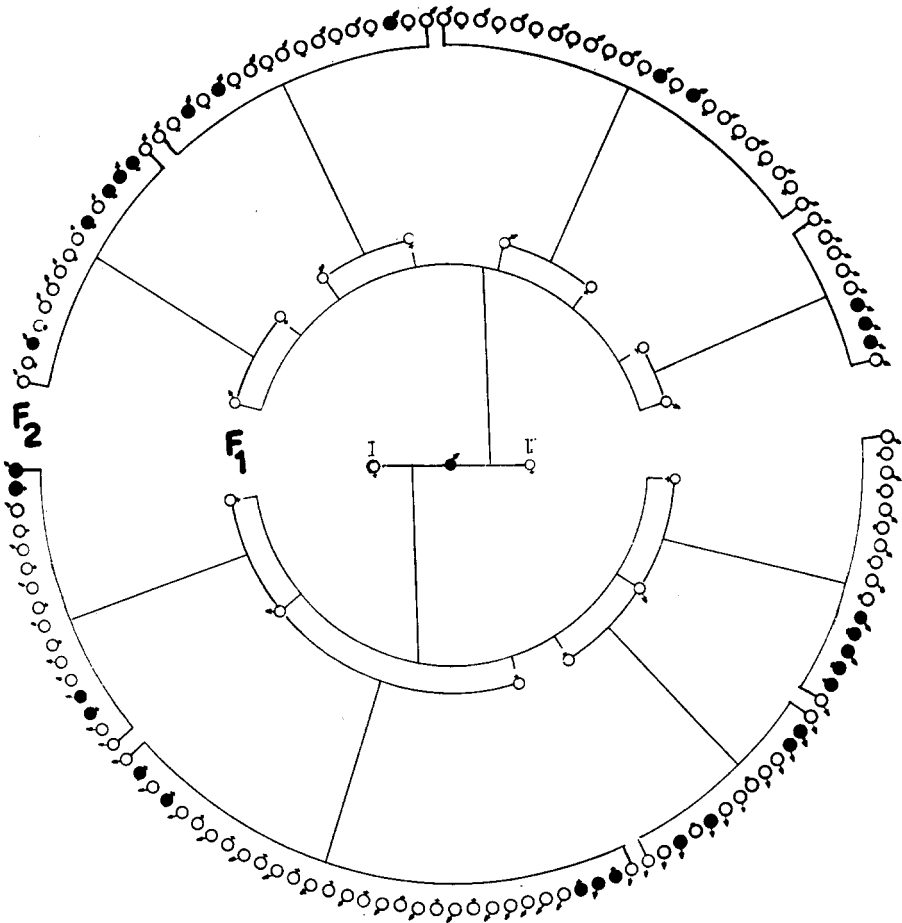


FIGURE 8.—Diagram of the crosses between one anemic male (center of the circle) and two normal females from the stock (No. I and no. II). Anemic rats black circles.

TABLE 5

Summary of crosses involving the mutant gene for anemia (an)

| Matings | Number of offspring | | |
|-----------------------------|---------------------|--------|-------|
| | Normal | Anemic | Total |
| <i>An an</i> × <i>An an</i> | 573 | 165 | 738 |
| <i>an an</i> × <i>An An</i> | 61 | .. | 61 |
| <i>an an</i> × <i>An an</i> | 15 | 12 | 27 |
| <i>an an</i> × <i>an an</i> | .. | 35 | 35* |

* Plus three dead and eaten.

survival time than the anemic animals obtained from brother-sister matings of the X2 female's progeny.

Two iron treated anemic males and one iron treated anemic female mated to animals heterozygous for the anemia, produced five litters with 15 normal and 12 anemic offspring.

Three anemic males and eight anemic females, previously iron treated, were mated and gave nine litters with 38 young, three of which were dead and partially eaten at birth. All remaining 35 rats proved to be anemic. The results from matings of homozygous anemic, heterozygous and normal rats are summarized in Table 5, where the gene responsible for anemia is designated as *an* and its dominant allele as *An*. All the data agree with the assumption that the anemia is inherited as a recessive autosomal mutation.

DISCUSSION

All data obtained in matings of an irradiated female rat (X2) and its progeny indicate that the anemia observed in the offspring is caused by an autosomal recessive mutation. As far as we know no inherited anemia has been described in the laboratory rat (JAY 1963).

The available data do not give a clear answer to the question of whether the mutation was induced by irradiation or whether it was present in the stock prior to radiation treatment. Four of five irradiated sisters were mated with the same male, but anemic offspring appeared in the progeny of only one female. It therefore is unlikely that the male partner was heterozygous for the gene causing anemia. Individual tests of 7 out of 11 F₁ offspring from the mating of X2 by a normal male proved each of them to be heterozygous for the abnormal allele. It would be difficult to assume that the irradiation had produced the same type of change at the same gene locus in seven different germ cells of one irradiated female. This fact rather indicates that the X2 female herself was heterozygous for the mutation.

The steady decline of the hemoglobin value with age in nontreated anemic rats and the rise of this value to about 8 g per 100 ml blood after iron treatment, show that iron deficiency is one of the factors involved in the metabolic processes leading to heavy anemia. The induced polycythemia and the resulting accompanying rise of hematocrit and hemoglobin values explain the visible beneficial effects of

iron treatment upon the recipient animal. Just how polycythemia is brought about is not clear. Its appearance following iron treatment in other types of anemia has not been recorded. We assume that the diet supplied to the general stock of rats was iron deficient, which may explain the rise in red blood cell count in controls after the iron treatment.

Calculated mean corpuscular hemoglobin concentration of iron-treated anemic rats was found to be higher than that of the nontreated anemic animals, whereas the calculated mean corpuscular volume was slightly lower. In both cases the mean corpuscular hemoglobin consistently remains at the level of $5 \mu\mu\text{g}$ per cell. The constancy of mean corpuscular hemoglobin in the anemic rats in spite of aging and iron treatment is intriguing. Before further investigations with, for example erythropoetic stimulants, are undertaken it would be hasty to speculate about the factors responsible for it.

It is known that in iron deficiency peripheral blood contains numerous small red blood cells. This also was the case in our nontreated anemic rats. When the iron deficiency was corrected the diameter of cells and the percentage of target cells increased. The Price Jones method for the determination of cell size diameter involves some cell distortion, but gives valuable information on the distribution of red cell diameters in the peripheral blood of treated and nontreated anemic animals. The microcytosis in the treated animals may, therefore, be explained by the extreme flatness of target cells and leptocytes and the presence of cells with reduced diameters which never completely disappear.

Although iron treatment prolongs the life of anemic rats, it does not affect the basic characteristics of the anemia, i.e., hypochromia and microcytosis. The metabolic pathways by which the young are benefited during their development by iron treatment of the mother requires a special study.

One of the phenotypic characteristics of anemic rats is reticulocytosis, the number of reticulocytes being extremely high in nontreated and much lower in iron treated animals.

The anemia described here may be classified as a hereditary hypochromic, microcytic anemia, associated with reticulocytosis and increased osmotic resistance of erythrocytes. A glance at the parameters which characterize the anemia produced by the series of *W* genes in the mouse described by RUSSELL and FONDAL (1951) shows that this anemia has very little in common with the one described in this paper. The combination of low mean corpuscular hemoglobin with a nearly normal mean corpuscular hemoglobin concentration, and the relatively normal cell diameter together with a low cell volume found in iron treated anemic rats, resemble similar parameters of thalassemia in man. Thalassemia in man is, however, not ameliorated by iron (in the absence of iron deficiency), and indeed large stores of iron are characteristic of thalassemia major. We are aware of the fact that many more hematological data are needed for a more complete correlation between thalassemia in man and hereditary anemia found in the rat.

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SUMMARY

A hereditary severe hypochromic and microcytic anemia in the rat is described. The anemia appeared in the offspring of one of 17 female animals of our random bred rat colony exposed to 50r of total-body X-irradiation at eight days of age. The disease is caused by an autosomal recessive mutation.—The basic phenotypic characteristics of the recessive homozygotes are hypochromia, microcytosis, reticulocytosis, and extreme osmotic resistance of red blood cells. The hemoglobin and hematocrit values and the number of red blood cells which are far below normal undergo further decrease with the process of aging, while the calculated mean corpuscular hemoglobin remains unchanged. The anemia responds favorably to iron treatment. In nontreated animals the anemia usually leads to death at various ages prior to six months. Iron treatment prolongs the life of anemic animals indefinitely, but the pathological forms of erythrocytes remain in large numbers in the peripheral blood throughout and after the treatment.

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