THE GENETICS AND PHYSIOLOGY OF LETHAL ANEMIA IN THE RAT[1]

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INTRODUCTION AND LITERATURE

THE "anemic" mutation appeared in the Cornell colony of albino rats which originated from the Osborn and Mendel stock at the Connecticut Agricultural Experiment Station. Since 1919 the Cornell colony has been a closed one, but inbreeding has not been intense. The rats are maintained on an adequate mixed diet (a commercial calf meal) which is supplemented at least once a week with cod liver oil to the extent of three percent of the diet (MAYNARD 1930). The anemia appears spontaneously in the young rats and acts as a complete lethal, since all of the affected animals die, usually within the first two weeks of life. When first observed by the authors the gene was so widespread in the colony that it was impossible to trace it back to any one or two individuals in which it may have first occurred. That the mutation occurred in the Cornell colony is supported by the fact that no anemic animals have been noted in the Osborn and Mendel colony (private communication).

CREW and KON (1933) found a monogenic autosomal lethal in the rat which first manifested itself at about the 9–12th day of life when the young rats began to lose weight. They died usually within five days from inanition. The underlying cause of this failure could not be determined, but the authors stated that neither an anemia nor a physiological leucocytosis was present.

GRÜNEBERG (1938), in describing another simple recessive autosomal lethal in the rat, says, "The most fundamental disturbance so far discovered is an anomaly of the cartilage; this simple mechanism affects several structures of the body in a similar way. Hyperplasia of the ribs and of the cartilage rings in the trachea thus produced leads secondarily to the development of an emphysema of the lungs, and the animals ultimately die from more or less remote consequences of this emphysema." The time of death of these rats varied from 4–39 days of age.

GUNN'S (1937, 1938) case of acholuric jaundice in the rat is another example of a hereditary physiological upset. This is due to an incompletely recessive autosomal gene, the heterozygotes showing some of the characteristic symptoms of the variation, such as increased red cell fragility and reticulocytosis. This mutation is not incompatible with life, for the affected

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animals live and can reproduce. It is characterized by a series of symptoms consisting of increased red cell fragility, bilirubinemia, microcytosis, reticulocytosis, splenomegaly, jaundice and anemia, which are pronounced in the young growing animals.

There are well established cases of hereditary anemias in mice, among which is the anemia associated with the "dominant white spotting" factor (*W*) reported by DE ABERLE (1925a, 1925b, 1927) and DE ABERLE, HOSKINS and BODANSKY (1927). The anemia appears in the homozygous dominant individuals (*WW*) and causes death within a week or ten days. According to DE ABERLE, the anemia is first manifested in the (*WW*) mouse at about the 16th day of intra-uterine life, at which time the hemopoietic cells of the spleen and liver sinuses are deficient in number. The appearance of this anemia thus occurs when the bone marrow is undergoing organization for future hemopoiesis. DE ABERLE stated that the anemia is of an aplastic type and that affected animals have about 25 percent as much hemoglobin and 14 percent as many red cells as the normal mice. These anemic mice tend toward a leucopenia, with about 20 percent as many leucocytes at birth as the normals.

Another case of anemia in mice is that associated with the autosomal recessive flexed-tailed gene first described by HUNT and PERMAR (1928). The associated anemia was first observed by MIXTER and HUNT (1933) and reported on further by KAMENOFF (1935). According to the latter author it appears at about the 14th day after fertilization. It thus precedes the time when the bone marrow begins to take over the hemopoietic function, which in the normal mouse embryo is at the 16th day. The erythrocyte counts in the flexed-tailed mice up to the time of birth are between 50 and 75 percent that of the normals for this period. The anemia persists in the flexed-tailed mice for about two weeks after birth; the blood then becomes normal. ANDERSON (cited by KAMENOFF) has traced the defect to faulty hemopoiesis in the liver of the embryo where the percentage of normoblasts was significantly less and the percentage of erythroblasts was significantly greater than in normal mice.

DUNN (1937) mentions that mice homozygous for the fused gene ($T^t\,T^t$) possess a lowered viability and may have an embryonic anemia.

The occurrence of the anemia described in this paper was reported in a preliminary note by BOGART, SMITH and KIMBALL (1938). This paper greatly extends the observations which were reported at that time.

DESCRIPTION OF THE ANEMIC RATS

This anemia is first noted at about 2–3 days of age at which time the anemic young acquires a pallor that becomes more pronounced so that in a day or two the afflicted rat is easily distinguished from its normal sibs.

These affected animals are usually dead within two weeks. The oldest anemic rat so far recorded survived to only 29 days of age; therefore, this character is a complete lethal. Paralleling the progress of the "anemic appearance" is a jaundice which gives the rat a yellow color that is noted on the exterior and in the internal organs; particularly in the liver and intestinal tract.

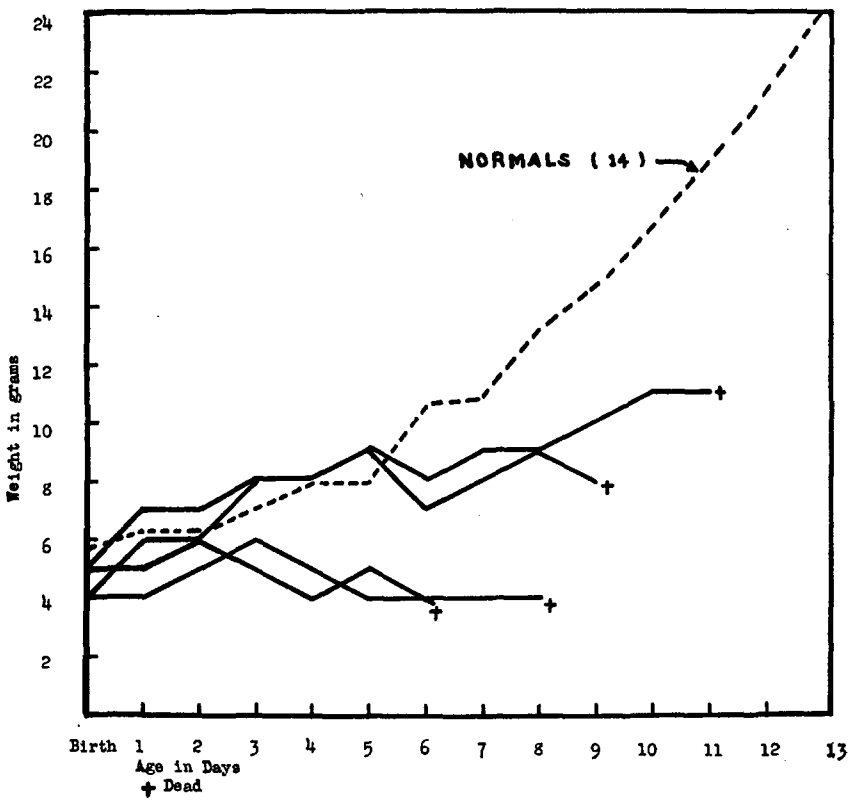


FIGURE 1.—Comparison of growth curves of normal and anemic rats.

The growth of normal and anemic littermates is given in figure 1. The anemic individuals decrease in weight, beginning about 3–5 days after birth and usually continue to lose weight until they die.

Nine anemic and 9 normal young (littermate pairs) ranging from 6–8 days of age were preserved in a 10 percent formalin solution. The hearts, liver, spleen and kidneys were then carefully dissected out, blotted on a filter paper, and weighed to the nearest hundredth of a gram. The difference in the normal and anemic rats did not show a mathematical significance for heart and spleen weights. Although the anemic and normal rats were the same age (littermates), the size in the two groups differed markedly. The anemic young were only a little over half the size of their

normal sibs. The livers and kidneys were actually much smaller, but in relation to live weight of the young rat considerably larger in the anemic rats (table 1). Thus, when the actual weights are considered, the livers and kidneys are significantly larger (odds 26:1 for livers and 400:1 for kidneys) in the normal rats, but when based on percentage of live weight, these organs were significantly larger in the anemic rats. It is difficult to determine whether the difference in sizes of the livers and kidneys is due to the anemia itself or to the interruption of growth which was brought about by the anemia.

TABLE 1

A comparison of organ weights of normal and anemic rats.

	AGE IN DAYS	NO.	LIVE WT. (GMS)	HEART (GMS)	LIVER (GMS)	SPLEEN (GMS)	KIDNEY (GMS)
Normal	6–8	9	11.6	.10±.007	.36±.02	.05±.006	.14±.006
Anemic sibs	6–8	9	6.6	.09±.005	.28±.02	.04±.005	.10±.005

Some attempts have been made to cure the disease, but so far these have all proven fruitless. Liver[3] extract was both fed and injected intraperitoneally in doses as high as .1 ml but without results. A solution of iron sulphate with a trace of copper sulphate was fed to pregnant females and to the young, and still the anemia appeared. To determine whether or not the rat anemia might be due to the infectious *Bartonella muris* organism (ELIOT and FORD 1929), blood of anemic rats was injected into young normal rats, but the results were negative. A study of the blood smears of anemics also failed to show the presence of Bartonella bodies.

GENETICS

The lethal acts as a simple autosomal recessive; the heterozygotes (*An an*) do not present any of the anemic characteristics. No evidence for a sex influence has been obtained.

The results of mating together individuals that had previously produced some anemic offspring were very close to a 3:1 ratio (table 2). This would in itself indicate the recessive nature of the gene. The data given in table 2 show that the sex ratios in both the normal and the anemic offspring do not deviate significantly from the expected 1:1 ratio.

Because of the lethal nature of the gene, it was necessary to use known heterozygous animals for testing the genotype of other individuals. Any animal that produced anemic offspring was considered to be heterozygous, but, if 25 or more normal offspring were produced by an individual that was mated to a known heterozygote, it was assumed that the animal tested was homozygous for the normal condition.

[3] The liver extract was a product of the Eli Lilly Company.

The genotype of the normal offspring resulting from the mating of heterozygous animals *inter se* was tested by mating to one or more known heterozygotes. A total of 85 individuals (19 males and 66 females) was tested. Twenty-eight proved to be homozygous and 57 heterozygous (table 2). These results closely fit the 2:1 ratio, which would be expected if only one pair of alleles is involved. The heterozygotes were evenly distributed between the sexes.

The genotypes of the offspring resulting from the mating of *An An* males to *An an* females and from the reciprocal mating were tested according to the procedure just outlined. A total of 26 individuals—7 females and 19 males—was used. Fourteen were found to be *An An* and 12 *An an* (table 2). There was no discrepancy between the two sexes, although the number of observations was small. The sex of the heterozygous parent did not influence the ratio of homozygous to heterozygous offspring. These results again indicate that only one pair of autosomal alleles is involved.

Because of the possibility that complementary genes might be responsible for the anemic condition, the genotype of ten offspring resulting from the mating of *An An* together was ascertained. In every case these animals proved to be homozygous (table 2). Although the number of individuals tested was not large, the results indicate that complementary genes were not responsible for the anemic condition.

TABLE 2

The results of various matings are shown herein.

	NORMAL				ANEMIC				RATIO	
	TOTAL	MALES	FEMALES	PERCENT	TOTAL	MALES	FEMALES	PERCENT	OBTAINED	EXPECTED
(*An an*) mated together	534	268	266	74.9	179	87	92	25.1	2.98:1	3:1

	An an	*An An*	RATIO	
			OBTAINED	EXPECTED
Genotype of the littermates of the anemics. 85 animals (19♂♂ and 66♀♀) tested	57	28	2.04:1	2:1
Genotype of offspring (*An an*×*An An*)	12	14	1:1.17	1:1
Genotype of offspring (*An An*×*An An*)	0	10	All homozygotes	All homozygotes

In order to determine if *An* is completely dominant over *an*, the hemoglobin was determined in some adult female rats that were of the genotypes *An an* and *An An*. The two groups were not significantly different

and the *An an* females possessed just as much hemoglobin as the *An An* females (table 3).

TABLE 3

A comparison of the hemoglobin of homozygous and heterozygous adult female rats.

GENOTYPE	NUMBER	GRAMS OF HEMOGLOBIN PER 100 ML OF BLOOD
An An	10	14.57±.27*
An an	11	14.42±.38

* Standard errors are used throughout this paper.

More information which indicates that the heterozygous animals are not adversely affected by the presence of *an* is given in table 4. These data were secured from an experiment designed to study the effects of various reproductive regimes on the life span of rats. It was later learned that *an* gene was widely dispersed among the rats in this experiment. Inasmuch as this experiment was not primarily designed to study the genetics of anemia, a complete analysis of the genotypes of the rats involved was not available, but several proved to be heterozygous for anemia. All known heterozygous females were grouped and their performance was compared to the breeding group as a whole, which was thus made up of homozygous and heterozygous females. One observes from the table that, to date, the heterozygous females did not differ in comparison to the total group insofar as span of life and fertility are concerned; in fact, the data indicate that the heterozygous females may be a little superior to the homozygous females in these respects.

TABLE 4

A comparison of the reproductive performance of homozygous and heterozygous normal female rats.

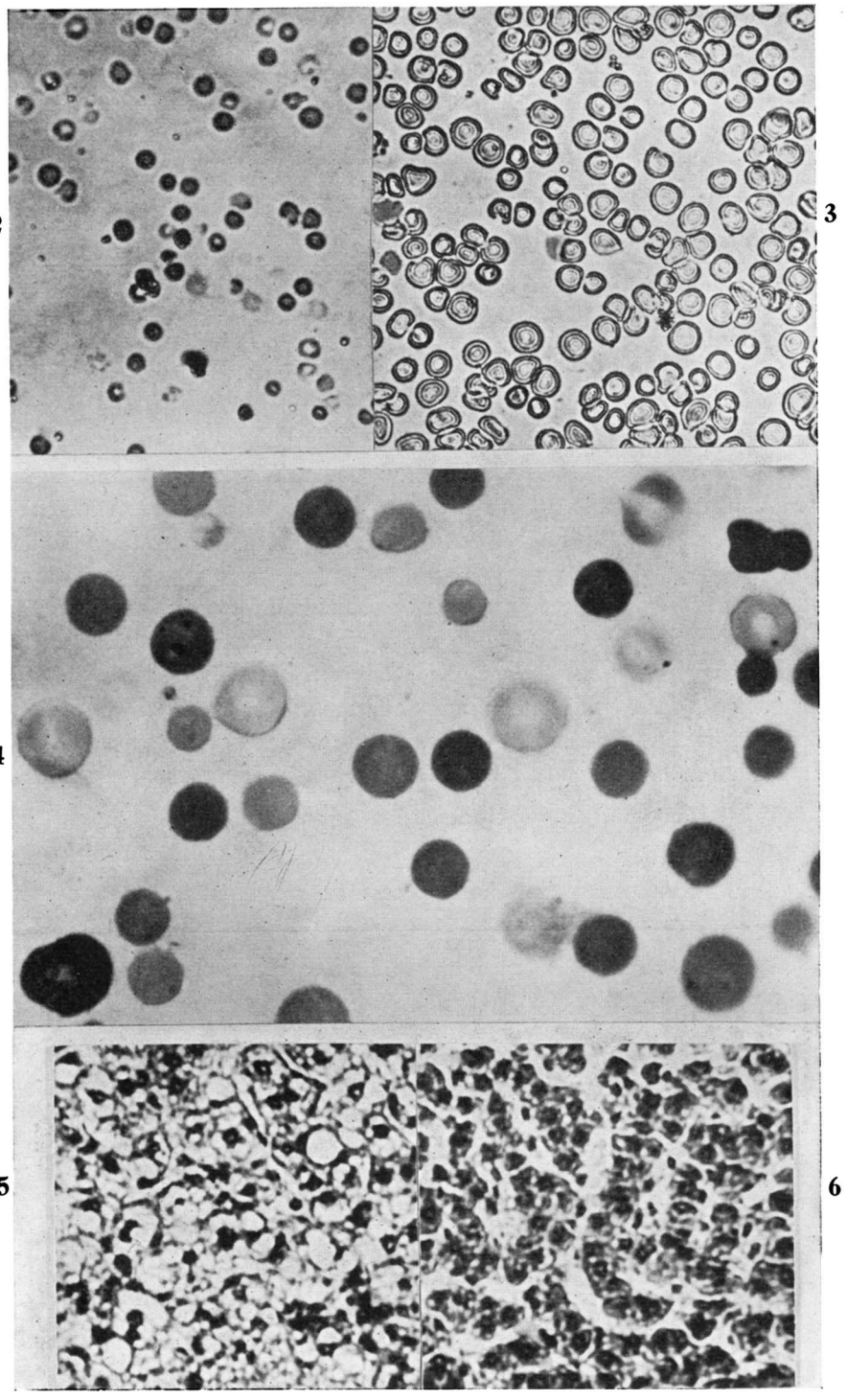
GROUP		NUMBER	PERCENT DEAD TO DATE	AVERAGE LITTERS PER FEMALE	AVERAGE YOUNG PER FEMALE	AVERAGE LITTER SIZE
Bred early	Total	50	26.0	7.9	49.8	6.3
	An an	13	7.7	10.1	63.3	6.3
Bred normal	Total	50	36.0	8.9	53.1	6.0
	An an	12	33.0	8.0	53.0	6.6

Bred early group—delivered the first litter at an average age of 87 days.
Bred normal group—delivered the first litter at an average age of 129 days.

HEMATOLOGY AND HISTOLOGY

1. General morphology of the red cells

Smears were made from blood secured by decapitating the rats and were stained with Wright's alkaline methylene blue and with Giemsa's stain



FIGURES 2–6. (See opposite page for description.)

buffered at pH 6.7 with a phosphate buffer. The erythrocytes of the anemic rats show a marked anisocytosis (variation in red-cell size) due to the presence of microcytes which, microscopically, do not show the concavity of the normal red cells and thus are probably spherocytes (table 5 and figures 2 and 3). The measurements were made by projecting at a magnification of 4,600× and measuring to the closest mm, and the data calculated to micra. There is a slight poikilocytosis and a marked polychromasia (variation in staining ability) of the erythrocytes of the anemic young (figure 4). In addition, the anemic smears contain single and multiple Howell-Jolly bodies and a few nucleated red cells.

TABLE 5

Measurements of red blood cells of anemic and normal 5–9 day old rats.

ANIMAL	NO.	FREQUENCY OF CELLS IN PERCENT							SMALLEST	LARGEST	MEAN
		BELOW 3.3μ	3.3 4.3μ	4.3 5.4μ	5.4 6.5μ	6.5 7.6μ	7.6 8.7μ	8.7μ AND OVER			
Anemics	8	1.7	12.3	37.3	30.9	15.2	2.2	0.4	1.5μ	10.4μ	5.4±.06μ
Normals	7			3.2	18.2	41.2	28.2	9.2	4.3μ	10.4μ	7.2±.06μ

2. Erythrocyte counts and hemoglobin determinations; color index

The red cell counts were made by the usual technics, but instead of using the hematin method the hemoglobin was determined by the iron analysis of the blood. The method of KENNEDY (1927) was used in preference to the easier method of WONG because the former gives truer iron values according to KLEIN (1931). BARCROFT (1928) has pointed out that the hemoglobin of various animals has an iron content of .335 percent. Therefore, the milligrams of iron per 100 ml blood multiplied by the factor .299 gives the grams of hemoglobin per 100 ml of blood. The presence of some non-hemoglobin iron would give an error, but JENKINS and THOMPSON (1937) have shown that in man not more than ten percent of the total iron is of this nature. The error would be very slight in this problem be-

EXPLANATION OF FIGURES 2–6

FIGURE 2.—Blood smear from a 7-day old anemic rat. Compare with fig. 3, which was taken at the same magnification. Microcytes, which are spherocytes, and polychromasia are evident.

FIGURE 3.—Blood smear from a 7-day old normal rat. Note the absence of polychromasia and that these cells are not spherocytes. Compare with figure 2.

FIGURE 4.—Red blood cells from a 7-day old anemic rat, highly magnified. Polychromasia, Howell-Jolly bodies, and spherocytes are striking in this picture.

FIGURE 5.—Section of liver from a 7-day old anemic rat. Bouin, H. and E. technic. Note vacuolar appearance, which is probably due to fat deposits that were washed out.

FIGURE 6.—Sections of liver from a 7-day old normal rat. Bouin, H. and E. technic. Compare with figure 5.

cause the normal and anemic rats are compared. There could be a difference in the percentage of non-hemoglobin iron in the two groups of rats, however. This point has not been investigated. The color index which expresses the amount of hemoglobin per red cell of the anemic relative to the normal is calculated:

$$\frac{\frac{\text{Hb of anemic}}{\text{Hb of normal}} \times 100}{\frac{\text{R.B.C. of anemic}}{\text{R.B.C. of normal}} \times 100} = \text{Color index.}$$

The color indices of some anemic rats are given in table 6. With the exception of two individuals that are high, the indices approximate unity, indicating that the hemoglobin per red cell of the anemic rats is the same as that of the normal.

TABLE 6

Color index of the anemic rats.

AGE IN DAYS	% ERYTHROCYTES OF THE NORMAL	% HEMOGLOBIN OF THE NORMAL	COLOR INDEX
7	37.9	35.8	0.94
7	42.9	38.9	0.91
7	38.8	36.6	0.94
7	41.5	43.5	1.05
7	30.5	32.5	1.07
7	28.8	26.9	0.93
7	25.3	29.6	1.17
7	45.8	59.2	1.29
7	21.3	35.8	1.68
7	33.8	35.8	1.06
7	16.3	25.7	1.58
8	39.5	40.8	1.03
Total 12	Ave. 33.5	36.7	1.10

The anemic rats have only one-third the normal number of red cells (table 7). Our average red cell count of 2,913,000 obtained from 25 normal rats of 7–8 days of age is in agreement with JOLLY (1909) and KINDRED and COREY (1930).

TABLE 7

Erythrocyte counts of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AV. ERYTHROCYTES PER CMM OF BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	7–8	25	2,913,000±47,000	8.1
Anemic	7–8	12	977,000±77,000	27.4

The hemoglobin content of anemic rat blood averages slightly more than one-third that of normal littermates (table 8). The average value of 10.7 gms hemoglobin per 100 ml blood for our normal rats of 5–9 days of age is in agreement with JOLLY (1909) and WILLIAMSON (1926), although these workers used different methods. The average hemoglobin value of 14.5 gms per 100 ml of blood (table 3) for 21 adult rats is in agreement with the figures of KINDRED and COREY (DOWNEY 1938) and WILLIAMSON (1926).

TABLE 8

Hemoglobin content of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AV. GMS HEMOGLOBIN PER 100 ML OF BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	25	10.71 ± .30	14.0
Anemic	7–8	12	3.93 ± .27	23.8

3. Leucocyte and differential white cell counts

There is a fourfold increase in the number of leucocytes in the blood of anemic rats (table 9). When the leucocytosis was analyzed in more

TABLE 9

Leucocyte counts of normal and anemic blood.

GROUP	AGE IN DAYS	NUMBER	AV. LEUCOCYTES PER CMM BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	11	2,700 ± 200	24.0
Anemic	5–9	7	10,000 ± 1,300	34.6

detail by differential counts (table 10), the polymorphonuclear neutrophile was the principal type of cell to show an increase. On a relative basis there was a doubling of the percentage of neutrophiles in the anemics as compared to the normals, and there was a drop of about one-third in the mononuclear cells (lymphocytes and monocytes). However, on an absolute basis, there was an average eightfold increase of the neutrophiles and a twofold increase of the mononuclear cells (table 10).

TABLE 10

Differential white cell counts of anemic and normal rats.

GROUP	NO.	POLYMORPHONUCLEAR CELLS			MONONUCLEAR CELLS
		BASOPHILES	EOSINOPHILES	NEUTROPHILES	(LYMPHOCYTES AND MONOCYTES)
Normal	4	nil	0.2%	26.3%	73.5%
Anemic	3	nil	0.4%	56.3%	43.3%

The average leucocyte count of 2,700 per cmm of blood for normal rats of 5–9 days of age is in agreement with the values reported by KINDRED and COREY (1930) and JOLLY (1909).

4. Reticulocyte enumeration

Reticulocytes were counted from dry smears of supravitally stained blood cells. A drop of blood in one ml of staining solution made up of equal parts of .5 percent sodium chloride and 2.5 percent sodium citrate plus enough brilliant cresyl blue to give a deep blue color was incubated for one hour at 37.5°C. This tube containing the solution was then centrifuged lightly and the supernatant fluid poured off. A smear was made from the residue in the bottom of the tube and the reticulocytes counted under oil. A total of 500 cells was counted to arrive at the percentage of reticulocytes. The anemic rats have only about one-sixth the normal number of reticulocytes. The normal young ranged from 15–43 percent reticulocytes in their blood, whereas the anemics ranged from 1–10 percent (table 11). A high reticulocyte count indicates that the erythropoietic organs (bone marrow and spleen in the rat) are able to respond to an abnormal need for more blood cells. Comparative absence of reticulocytes in man usually indicates an aplasia of the erythropoietic system. The normal young have an anemia which is due to their iron-poor diet of milk. This milk anemia calls forth a reticulocytosis giving the young rat a count of 25–50 percent as compared to the adult count of 2–4 percent.

The clotting time of the anemic blood is normal. This fact, combined with other characteristic symptoms, differentiates this blood abnormality from that caused by a deficiency of vitamin K in the rat (GREAVES and SCHMIDT 1937).

TABLE 11

Reticulocyte counts of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AVERAGE PERCENT RETICULOCYTES	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	17	31.6±2.1	27.4
Anemic	5–9	10	5.3±0.9	53.7

5. Histological studies

Free iron in various tissues. The Prussian blue method was used for this qualitative study (LEE 1937). The livers of every anemic rat studied (9) showed an abundance of free iron primarily in the von Küpffer cells, whereas only traces of free iron could be found in the livers of normal rats. Traces of free iron were found in the splenic capsule of the anemic rats, but there was none in the spleen of the normal rats. Such tissues as skin,

kidneys, intestine, and stomach gave no positive test for free iron in either group of rats.

Fat deposition in liver tissue. The ordinary Bouin's fixed liver material stained with hematoxylin and eosin showed a marked difference between the anemic and normal rats. The liver cells of the anemic rats were vacuolated in appearance following this technic (figures 5 and 6) and indicated that fat material which was originally in these cells had been dissolved out. In an osmic acid fixative, the fat was blackened and preserved. A tremendous fatty deposit was present in the liver cells of the anemic rats, whereas only small and scattered droplets were present in the liver cells of the normal rats (figures 7 and 8). In some of the anemic livers the entire cytoplasm was a fat deposit and the nucleus only did not blacken (figure 9). This tissue differed from adipose tissue in that the nucleus was not displaced by the fat deposit.

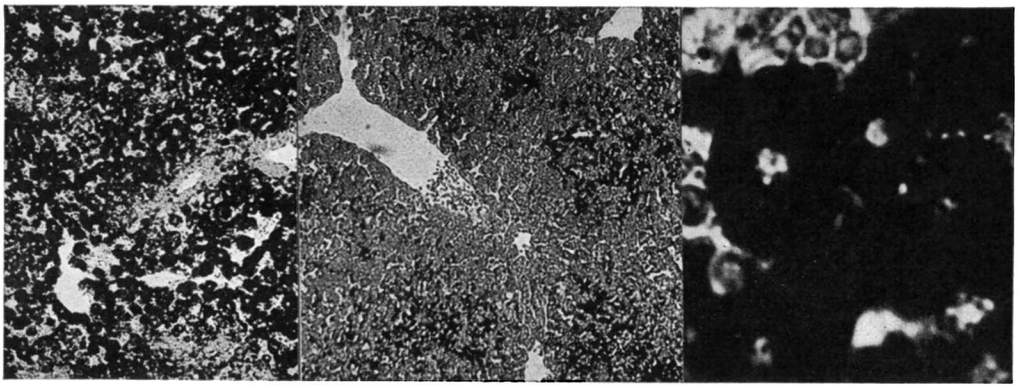
Bone marrow studies. The femora of anemic and normal littermate rats were sectioned longitudinally and stained with hematoxylin and eosin and with Mallory's stain. The bone marrow of the anemic young showed a deficiency of erythropoietic (germ) centers, a much larger proportion of mature red blood cells, and a general looseness (figures 10, 11, 12, 13, 14, and 15). This general looseness in appearance may be due to either the lack of germ centers or to fatty deposits in the bone marrow which were dissolved out by the dehydrating agents. The deficiency of germ centers was a quantitative one in that there was not a complete lack of germ centers but decidedly fewer than in the normal. Observations on the larger proportion of red cells lead us to conclude that there was not an absolute increase in the number of red cells in the bone marrow of the anemic young, but that the higher proportion was due to the marked deficiency of the erythropoietic tissue. If there were an actual increase in number of red cells, it would indicate that there is a failure of the red cells to escape from the place of origin to the blood stream. The proper interpretation, however, seems that there are fewer red cells being produced as a result of the deficiency of germ centers and that the large number of red cells present is a relative rather than absolute difference.

Other tissues. Studies of the spleen revealed no difference between the anemic and normal rat except that more blood cells were present in the spleens of the former. With the exception of the fatty deposits in the liver cells and free iron in the von Küpffer cells of the anemic rats, no histological differences in livers could be detected.

BIOCHEMICAL FINDINGS

1. Bilirubin determinations on blood

The pronounced yellowness (icterus) of the anemic rats led us to suspect a bilirubinemia. A slight modification (ELTON 1931) of the van den Bergh



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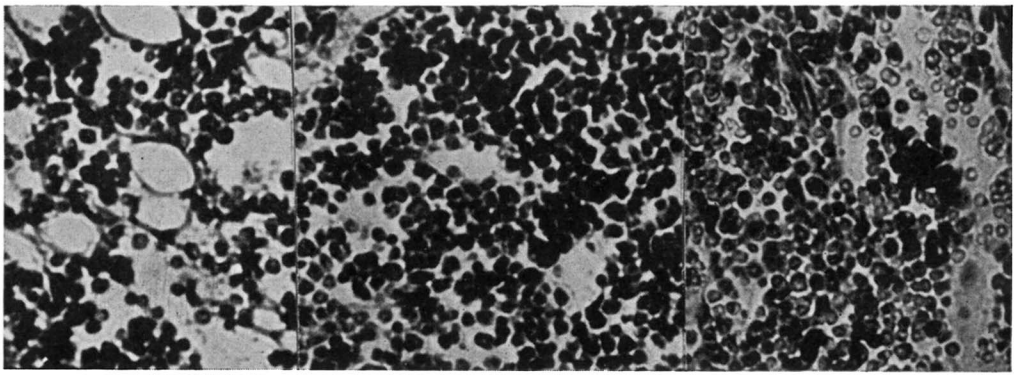

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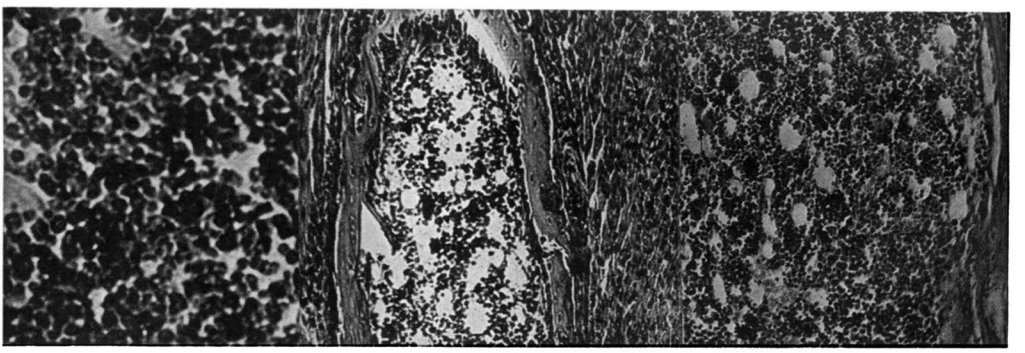

13 14 15

FIGURES 7–15. (See opposite page for description.)

method was used for determining the bilirubin of the blood. The earlier workers thought that the immediate appearance of the pink color at the interface of the plasma and diazo reagent (direct positive reaction) was due to bilirubinemia resulting from an obstruction to the biliary passage. If the pink color is delayed for 3–4 minutes (direct delayed reaction), it was considered an indication of bilirubinemia resulting from hemolysis, but if the pink color was delayed for 10 minutes (direct negative) it indicated a combination of the two types just described. Later work has shown that the type of reaction is dependent, not on the origin, but on the concentration of the bilirubin in the serum, since the rate of reaction is directly correlated with the icterus index (HAWK and BERGEIM 1937). Ten anemic rats 14 to 29 days old each gave a "direct positive" reaction for bilirubin in the blood plasma, whereas the same number of normal sibs gave no detectable response for bilirubin. Because of the small amount of blood available from rats of 6–9 days of age, no attempt was made to obtain quantitative values for bilirubin. The qualitative results, however, have been quite clear cut.

2. Blood uric acid

Inasmuch as there is an increased uric acid output in the urine as a result of rapid blood regeneration in which large numbers of nuclei, with their nucleic acids, are catabolized when the normoblasts mature into erythrocytes (RIDDLE 1930; KRAFKA 1929), the determination of blood

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EXPLANATION OF FIGURES 7–15

FIGURE 7.—Section of liver from a 7-day old anemic rat. Osmic acid fixation. Note the enormous fat deposition.

FIGURE 8.—Section of liver from a 7-day old normal rat. Osmic acid fixation. Only a few scattered fat deposits are present.

FIGURE 9.—Same as figure 7, but highly magnified. The cytoplasm of some liver cells is entirely a fat deposit, the nucleus being the only part of the cell which is not blackened by the osmic acid.

FIGURE 10.—Bone marrow of a 3-day old anemic rat. Note the deficiency of erythropoietic centers and the general open appearance.

FIGURE 11.—Bone marrow of a 3-day old normal rat. Note the abundance of erythropoietic tissue.

FIGURE 12.—Bone marrow of a 9-day old anemic rat. Note the deficiency of erythropoietic tissues and a larger proportion of mature red cells.

FIGURE 13.—Bone marrow of a 9-day old normal rat. Note a greater abundance of erythropoietic tissue and a smaller proportion of mature red cells.

FIGURE 14.—Bone marrow of a 3-day old anemic rat. A marked deficiency of erythrogenic tissue and a general looseness is present.

FIGURE 15.—Bone marrow of a 3-day old normal rat. An abundance of erythrogenic tissue is present.

uric acid might throw some light on the nature of this anemia. Blood uric acid was determined by the direct method of FOLIN (1922) but was proportionately modified to accommodate .2 ml of blood. The blood uric acid of the anemic (1.49 ± .03 mgm per 100 ml) did not differ significantly from that of the normal (1.47 ± .04).

3. Liver fat deposition

Histological studies on the liver of the affected rats showed a high fat content within the liver cells. The chemical findings (table 12) substantiate the histological observations in that the fat content of the anemic rat livers is 2.6 times that of the normals when expressed on a dry weight basis. Even on a "per rat" basis it is 2.7 times higher in the anemic. The liver fat was analyzed from the tissue dried to constant weight at 70°C, 20 mm Hg of pressure and in an atmosphere of carbon dioxide. These dried liver samples, consisting of composites of several rats, were then ground in a mortar and pestle and extracted with 50 ml of anhydrous ethyl ether for 24 hours. The ether was evaporated off on a steam bath; the samples were dried to constant weight in a vacuum at 70°C and weighed.

TABLE 12

Liver fat of anemic and normal rats.

GROUP	AGE IN DAYS	COMPOSITE OF	GMS FAT PER GM DRY LIVER	% FAT PER GM DRY LIVER
Normal	7	10	0.0875	11.33
	5–7	10	0.1391	
Anemic	5–7	13	0.3008	30.08

The fatty infiltration of the anemic rat livers is probably a secondary reaction brought about by a decreased oxidation in the tissues because of the lack of hemoglobin and, thus, the oxygen that it would provide. The high fat content in the livers of some anemic men is similarly interpreted (MACCALLUM 1936) but in the case of hemolytic anemias the fat may be a storage of the excessive lipids obtained as a by-product of red cell destruction.

4. Liver glycogen

Glycogen in the livers was determined essentially according to the method of PFLÜGER (DUEL et al 1934), except that the liver samples were digested for one-half hour instead of three hours in the potassium hydroxide. The glucose obtained upon hydrolysis of the glycogen was determined by the method of SHAFFER, HARTMAN and SOMOGYI, using the reagent 2 given in PETERS and VAN SLYKE (1932).

The livers of the anemic rats contain only about one-third the glycogen of the normal rat livers (table 13). The odds (Student's "t") are more than 25:1 against the view that the difference is due to chance alone. The lower glycogen content of the anemic rat liver is probably another change which is secondary to the primary anemia, although data on this point are lacking.

TABLE 13

Liver glycogen of normal and anemic rats.

GROUP	AGE IN DAYS	COMPOSITE OF	MGM GLYCOGEN PER GM FRESH LIVER	AVERAGE
Normal	5–9	3	17.4	21.0±3.7
		3	24.8	
		3	12.6	
		3	29.2	
Anemia	5–9	3	9.3	8.0±1.1
		3	5.8	
		3	8.9	

DISCUSSION

The genetics of this lethal anemia is clearly indicated, but much further work is required to analyze completely its etiology.

The results of the genetic studies show that the anemic condition is inherited as a simple autosomal recessive. No evidence has been obtained for an embryonic mortality of the homozygous recessive young because the observed ratio of 2.98:1 from 713 offspring fits the expected ratio of 3:1 very closely. The heterozygotes have shown none of the characteristic symptoms of the anemics, which indicates that the normal state is completely dominant to the anemic one. Such a physiological character which is simply inherited is very interesting and worthy of detailed study, inasmuch as most physiological characters are controlled by the more complicated multiple factors.

The possibilities of a nutritional deficiency or of an infectious cause of the anemia are eliminated by the fact that an (*An an*) female produces anemic or non-anemic offspring, depending entirely on whether she is mated to an (*An an*) or an (*An An*) male; and by the fact that with the proper parents (both heterozygous), 25 percent of the offspring are anemic and 75 percent are non-anemic although all offspring had the same "intra and extra-uterine" environment. There might be postulated a hereditary diathesis of the affected young to an invasion of micro-organisms or to an abnormal nutritional requirement for hemoglobin formation, but we have found no evidence for such an hypothesis.

Classification of this anemia in light of the facts known about human

anemias has been very difficult because its course does not parallel that of any of the common human anemias. It may well be that the difficulty resides in the fact that the anemic rats really possess two anemias: (1) the nutritional anemia resulting from the iron-poor milk diet; and (2) the hereditary anemia. It is obvious that to separate the courses of these two is indeed a perplexing task. The combined effects of the two anemias would explain the quick deaths of the affected rats.

Using a simple morphological classification, this anemia is a simple microcytic one. It is simple because the hemoglobin per red cell equals that of the normals; and it is microcytic because of the presence of small cells which decrease the mean red cell diameter. The microcytes of the anemics while decreasing the mean red cell diameter are not as concave as the normal cells; that is, they are spherocytes, and thus possess about the same volume of stroma per red cell as do the normals, and this allows them to carry as much hemoglobin.

Difficulty of classification is encountered when an attempt is made to determine whether the anemia results from faulty formation of erythrocytes (dyshemopoietic anemia) or to an excessive destruction of the red cells (hemolytic anemia). This anemia does not clearly fit into either one of the above classes. However, the present knowledge permits a tentative classification. Taking the picture as a whole, the anemia appears to result from a dyshemopoiesis. The results at hand do not contradict the idea that a faulty bone marrow is generating cells of poor resistance which are easily and prematurely destroyed; that is, dyshemopoiesis and hemolysis are both present.

The theory of dyshemopoiesis is supported by the following facts. The anemic rats possess much fewer reticulocytes than the normals, which means that in spite of the great demand for erythrocytes the erythropoietic organs (bone marrow and spleen) are unable to respond with the immature cells—the reticulocytes. Sections of the anemic bone marrows show that they possess fewer erythrogenic centers than the normals; and a cytological study of the bone marrow smears[4] of anemic young have shown a type of regeneration that is normal qualitatively but deficient quantitatively. The latter two facts can be summed up by saying that the bone marrow is hypoplastic. The dyshemopoiesis is not caused by a complete aplasia of the hemopoietic organs, for signs of some regeneration are present. The polychromophiles and microcytes are taken as evidence of a simple regeneration, whereas the multiple Howell-Jolly bodies are said to result from a pathological regeneration (DOWNEY 1930). The dyshemopoiesis involves the erythrogenic line of cells but not the leucogenic lines

[4] Kindly done by DR. H. R. SEIDENSTEIN.

because the leucocyte counts are not only equal to the normals but are higher.

Bilirubinemia and hemosiderosis are usually taken to indicate an excessive red cell destruction in which hemoglobin is catabolized into bilirubin and hemosiderin too rapidly for the body to eliminate them. This may be the case or it may be that an accumulation of such products results from an inability of the body to reuse them to form new hemoglobin. WITTS (1932) feels that the latter is the case in pernicious anemia of man.

The relationship of polychromophiles to reticulocytes has aroused much discussion. Some workers (TODD and SANFORD 1928) believe that the two are identical and that the first form is found in blood smears stained by Wright's, Giemsa's or the alkaline methylene blue methods, whereas the reticulated form is found when blood is supravitally stained. If this be true, then a contradiction seems to appear in this study where anemic blood shows much polychromasia but has a low reticulocyte count; but in the case of young rat blood, as here studied, the reticulocytes cannot be the polychromophiles, for the normal suckling rat has a very high reticulocyte count and yet blood smears do not show a correspondingly high polychromasia.

This anemia interpreted as a dyshemopoiesis allows an explanation for the time of appearance of the anemia; that is, at two to three days of life. At the time of birth in the normal rat the bone marrow takes over the hemopoietic function which heretofore has been relegated to the liver and spleen. At this time the first erythroblasts appear in the femur which already has been actively producing leucocytes (MAXIMOW 1927). With this in mind it would seem likely that the bone marrow of the anemics is unable to assume fully its function of producing erythrocytes from birth onwards, and the failure is reflected in a sudden appearance of an anemia which runs a quick and lethal course.

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SUMMARY

1. A lethal anemia of rats is described which acts as a simple autosomal recessive.

2. The anemia appears spontaneously at two to four days after birth and causes death usually within two weeks.

3. The blood of anemic young possesses about one-third the number of red blood cells of the normals and about one-third the amount of hemo-

globin. In addition, there is an increased number of leucocytes and a subnormal number of reticulocytes.

4. Anemic blood smears show a marked anisocytosis, slight poikilocytosis, polychromasia and Howell-Jolly bodies. The mean red cell diameter is significantly decreased.

5. Chemical analyses have shown a bilirubinemia, fatty infiltration of the livers and a lowered amount of glycogen in the livers of anemic rats.

6. Histological studies have shown the anemic rats to have a hemosiderosis of the livers and spleens, fatty infiltration of the livers and bone marrows and a subnormal quantity of erythrogenic centers in the bone marrows.

7. The anemia appears to result from an inability of the bone marrow to assume fully its hemopoietic function at the time of birth. Accompanying this dyshemopoiesis may be some hemolysis of defectively formed red cells.

8. The anemia has not responded favorably when liver extracts, iron and copper solution, or normal rat blood have been fed or injected.

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