ANEMIA IN THE FLEXED TAILED MOUSE, MUS MUSCULUS

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HUNT, MIXTER, and PERMAR (1933) have described the mode of inheritance of flexed tail in the house mouse, a mutation discovered by HUNT in the rodent colony of the Department of Zoology at the MICHIGAN STATE COLLEGE. Flexed tailed mice are characterized by fusions between adjacent caudal vertebrae, and as a rule by angular or spiral bends in the tail. The character is a Mendelian recessive, but there is a deficiency of flexed individuals in F_2 and backcross populations because the mutants have a higher death rate than their normal siblings. Newborn flexed mice are anemic, and the purpose of this paper is to describe the inheritance and characteristics of this anemia.

To avoid the reprinting of tabular data from the first paper, the tables of the two publications are numbered as one series, tables 1 to 10 being in the first, and 11 to 17 in this contribution.

THE INHERITANCE OF ANEMIA

Early in the experiments the writer (H. R. HUNT) noticed that in litters bred by matings between flexed and F_1 animals, the flexed young were considerably lighter in color than their normal tailed siblings. The hairless condition of newborn mice gives them a reddish color, for the blood shows through the skin. The lighter pink of the flexed mouse immediately after birth is frequently more noticeable on some parts of the body than others. The hips and top of the head are particularly favorable places for detecting the paleness. It seemed to the writer that the lightness of the flexed young was probably due to an anemia of some kind, so Mr. RUSSELL MIXTER undertook a study of the numbers of erythrocytes and the hemoglobin contents of blood from flexed and normal tailed animals at different ages.

The anemic appearance of young flexed mice varies, in some animals the condition being very obvious, while in others careful scrutiny is necessary to detect any difference from the normal siblings. The litters should be examined for classification on the day of birth and by good daylight. Occasionally one can not decide definitely whether the young mouse is anemic or normal, and such cases have been designated as doubtful. Flexed young are usually anemic at birth, but straight tailed newborn mice frequently show this condition, and on the other hand a flexed ani-

mal is occasionally as red as its normal newborn siblings. These matters will be considered when discussing whether the flexed and anemic conditions are due to the same gene.

This section is intended to throw light on the mode of inheritance of anemia, which was discovered only after the work on the inheritance of flexed tail was under way. Thus the experiments were not designed for its study, so they did not definitely establish the way in which it is inherited. For example, we do not know whether this anemia breeds true, nor are we certain that our P_1 flexed animals were anemic at birth. However the writer's experiments accumulated considerable data all of which are consistent with the view that anemia, like flexed, is a Mendelian recessive, so the facts are presented as a contribution to the question and not as a final solution.

Table 11 summarizes the evidence concerning the inheritance of anemia. It includes the F_2 generation, the offspring of $F_1 \times$ flexed matings, and the progeny of flexed males paired with normal females which, in turn, were bred by crosses between F_1 and flexed animals. Unfortunately no special effort was made to study anemia in the F_1 generation, but the records show that four litters containing 20 animals were observed, and that all were normal.

The F_2 generation will be considered first. Every doubtful case was excluded from the F_2 data presented in table 11. All the dead have been eliminated, for the identification of a dead individual as anemic or normal is hazardous. Also, if the classification of one or more animals in a litter was uncertain, the whole litter was excluded from the table. The small number of such doubtful cases may actually have been selected at random from the normal and anemic classes, but there is ground for the suspicion that they had mild cases of anemia. To have eliminated such cases without disposing of their normal siblings would have unjustifiably reduced the percentage of anemics. There were 1373 F_2's, of which 1076 were normal and 297 clearly anemic. Thus $21.63 \pm .75$ percent were anemic.

This is 3.37 percent less than the 25 percent expected on the assumption that anemia is recessive, yet it approximates 25 percent closely enough to arouse the suspicion that anemia is actually such a character. It has already been shown that flexed animals probably have a higher prenatal death rate than normals, so the deficiency in anemic young may be due to the same cause. This difficulty may be partly overcome by considering only large litters. In table 11 the data are given for litters of 7 or more, and 9 or more young. A population consisting of litters of nine or over on the day of birth must have been selected very strongly against a prenatal death rate, and therefore against any differential rate as between normals and anemics. This technique has been discussed already in con-

TABLE 11
The inheritance of anemia.

CLASSES OF NEWBORN MICE	FREQUENCY OF ANEMIA AMONG NEWBORN MICE								
	IN ALL LITTERS			IN LITTERS OF 7 OR MORE YOUNG			IN LITTERS OF 9 OR MORE YOUNG		
	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	ANEMICS	PERCENT ANEMIC
F_2 generation	1076	297	21.63± .75	755	216	22.25± .90	363	108	22.93±1.31
Progeny of F_1×flexed	490	424	46.39±1.11	360	322	47.21±1.29			
Progeny of normal females (bred by the cross F_1×flexed)×flexed males	241	195	44.72±1.61	105	89	45.88±2.41			

nection with the inheritance of flexed (HUNT, MIXTER, and PERMAR 1933). There were 971 young in litters of 7 or more per litter. Of these, 216 were anemic, or $22.25 \pm .90$ percent. Litters including dead animals were excluded, not only on account of the possibility of classifying the dead erroneously, but also because death may have occurred before birth, and the aim was to reduce the effect of the prenatal death rate as much as possible. Where the condition of the blood was doubtful in one or more animals, the whole litter was thrown out of the computation. If from the above litters of 7 or more young, those containing 9 and over are considered, there were 108 anemics and 363 normals, or 22.93 ± 1.31 percent of anemics. Partial correction for a prenatal mortality thus raises the percentage of flexed animals to within about 2 percent of the 25 percent that should be approximated if anemia is due to a single recessive factor. This justifies the suspicion that the type of anemia with which we are dealing may be caused by such a gene.

The fact should not be overlooked, however, that to secure even such a high percentage of anemic young as 22.93 percent in F_2, the prenatal death rate among the anemic young might be quite high. The following computations illustrate this point, assuming that anemia is a simple recessive character.

Assumed prenatal death rates of normals.	Computed prenatal death rates of anemics.
0 percent	10.7 percent
3 percent	13.6 percent
5 percent	15.0 percent

The prenatal death rates would have to be not far from 10 percent higher among anemics than normals, even in the litters of 9 or more, to give 22.93 percent of anemics. The anemia may, conceivably, so handicap the fetus that such a differential mortality actually exists. A conclusive study of prenatal death rates, using comparable normals as controls, is highly desirable.

Table 11 shows the results of backcrossing F_1's with flexed animals. Let us first consider all litters, regardless of their sizes. Here again all dead young were discarded in the computations, and in addition all litters were eliminated which contained one or more animals whose blood status was doubtful. The reasons for these omissions have already been discussed in connection with the F_2 generation. There were 914 young mice of which 46.39 ± 1.11 percent were anemic. This is 3.61 percent less than the 50 percent to be expected in a backcross generation if the character is a simple Mendelian recessive. The table gives the data, also, for litters of 7 or more. Litters containing dead young, or young which could not with certainty

be classified either as normal or anemic, have been omitted. This selection of litters on the basis of large size would be expected to increase the percentage of anemic animals if they have more than the normal prenatal mortality. 47.21 ± 1.29 percent were anemic, which was a small and statistically unreliable increase over 46.39 ± 1.11 percent. Again the facts, as far as they go, justify the idea that the anemia may be the expression of a recessive gene, for the percentages are suspiciously close to 50 percent.

The last horizontal column of table 11 deals with the results of crossing flexed males with normal females which were bred by crosses of F_1 with flexed animals. The litters used in computing the percentages were selected in exactly the same ways as in the two preceding experiments. If the anemia is due to the flexed gene itself, or to a recessive gene very closely linked with flexed, then one would expect an approximation to 50 percent among the offspring from such matings. The percentages of anemics actually found were 44.72 ± 1.61 percent for all the litters, and 45.88 ± 2.41 percent in litters of 7 or more. These percentages, again, are suggestive.

STUDY OF THE BLOOD OF THE FLEXED TAILED MOUSE

Mr. RUSSELL MIXTER was assigned the task of determining the numbers of erythrocytes and leucocytes in the bloods of flexed and normal mice at birth and subsequent ages, and of measuring the hemoglobin percentages in the same strains. These studies, together with the material on inbreeding already reported, were submitted as a graduate thesis in the Department of Zoology at the MICHIGAN STATE COLLEGE. No attempt will be made to review the voluminous literature on anemia in man and animals, but Mr. MIXTER'S findings will be presented together with some comments on the genetic significance of his observations.

It was necessary, of course, to compare the cell counts and hemoglobin percentages of flexed mice with corresponding data from normal controls to determine whether the flexed animals were actually anemic. Two types of controls were used: normals carrying flexed (heterozygotes), and homozygous normals. Flexed and heterozygous normal tailed young were secured by mating heterozygotes with flexed. Seven heterozygous (F_1) normal males were mated with 14 flexed females. In addition, 1 flexed male was bred with 6 heterozygous normal females. The mixed litters produced by these matings provided material for reliable comparisons between normal and flexed young. As a rule, when observations were made on a flexed animal the same procedure was carried out on a normal litter mate. Thus such factors as health of the mother, temperature of the animal house, chance fluctuations in the quality of the food, external and internal parasites, et cetera, which may have affected the erythrocyte and hemoglobin content of the flexed animals, would produce, presumably, similar effects

on the heterozygous controls, so that the differences between the groups were due in the main to the normal gene of the heterozygous controls.

The other type of control was the homozygous normal. These mice were produced by 1 homozygous normal male mated with 8 homozygous normal tailed females. The offspring of these crosses lived under approximately the same conditions as the flexed animals, so differences between them could not have been due, in any considerable degree, to the environment. But the heterozygous siblings of the flexed young were doubtless better controls than the unrelated homozygotes, because the former more than the latter must have resembled the flexed young in the distribution of genes other than the flexed gene. It is important to remember these distinctions, for, as will appear later, the two control series differed from one another as well as from the flexed mice.

Blood cell counts and hemoglobin determinations were made at birth, at one, two, three, and four weeks after birth, and further at six, eight, ten, and twelve weeks. Adults also were investigated. These were mainly the parents of the young on which observations were made. Four of the flexed adults were not parents of the mice used in the blood problem, but of those recorded in the inbreeding experiment. The ages of the adults ranged from six months to nearly two years.

The blood could not be secured by the same method throughout the whole series. Newborn animals are so small and have such a limited supply of blood that the sample was taken from the severed neck after decapitation. Thus no counts at later dates were ever made on animals counted at birth. Sometimes the blood of week-old young was secured in this manner, but more often it was from the femoral vein. Wherever possible a mouse yielded samples at the end of the first, second, third, et cetera, weeks. From two weeks on, blood was usually obtained from the severed tip of the tail, several millimeters being cut off each time a sample was secured. Occasionally the blood came from the leg if the tail would not bleed. The flexed and their normal controls were treated alike; if the sample was taken from the leg of one, it was secured from the same place on the other. The blood from the decapitated newborn mice was probably slightly diluted with lymph, but such dilution occurred both in the flexed and their normal controls, so that the method of securing the sample did not invalidate the comparison.

Mr. MIXTER used a Will certified pipette in securing samples when both erythrocytes and leucocytes were to be counted, and a Trenner pipette when the number of leucocytes only was to be determined. A 1 percent solution of sodium chloride tinged with a small quantity of Gentian Violet was used when both types of cells were counted, while a $1\frac{1}{2}$ percent acetic acid solution tinged with Gentian Violet was utilized for the white cell

counts alone. The blood was diluted 200 times with the salt solution and only 20 times with the acid solution. The first determinations were made with a Levy-Hauser single counting chamber, the average of two successive counts being taken as the record for the animal. The Levy-Hauser double counting chamber was used in most cases, for two samples could then be obtained at one filling of the chamber. The percentage of hemoglobin was estimated by using a Tallquist color scale, which is adequate for an approximate, but not for a highly accurate determination. This scale gives the concentration (percentage) of hemoglobin in a blood sample as compared with normal human blood whose hemoglobin content is taken as 100 percent.

It should be understood clearly that Mr. MIXTER'S object was to determine the cause of the pale color in most of the newborn flexed mice. It might, conceivably, have been due to an abnormally thick skin. He selected animals for the study of the blood because they were flexed tailed and not on the basis of color at birth. Consequently this is a report on the blood of flexed tailed mice and not an extensive investigation of anemia. The genetic relation of flexed tail to anemia, the histology of this type of blood, and the fetal history of anemia are problems yet to be attacked.

The erythrocytes of the flexed animals and the two types of controls will be considered first, after which the hemoglobin and leucocyte content will be discussed. Table 12 gives the average numbers of red cells per cubic millimeter of blood in the flexed and normal mice, beginning at birth, then at weekly and fortnightly intervals up to twelve weeks, and finally at the adult stage. The number of individuals used in making each computation is indicated. The data of table 12 are plotted in figure 1. Table 13 gives the frequency distribution of red blood cells at birth in the three categories of animals. The purpose of the table is to show the extent to which the flexed and their controls overlap.

Table 12 and figure 1 reveal that there was a rapid increase of erythrocytes from birth to about the fourth week in the controls and the flexed. For some reason the red cells of the homozygous normals remained nearly constant from the second to the third week, but the fourth week brought an augmentation which compensated for this lag. After about the fourth week there was a slower growth in erythrocyte content until the eighth or tenth week, when the adult level was reached. The erythrocytes of the flexed mice increased from 3,550,000 per cubic millimeter at birth to 11,360,000 at eight weeks; this latter number remained about the same at ten and twelve weeks, and at the adult stage. The heterozygous normals started at birth with a larger number of red cells (4,850,000 per cubic millimeter) than the flexed, the number increasing relatively rapidly to the fourth week, but not so rapidly as in the flexed animals, as is shown by the

TABLE 12

Red blood cells of flexed and normal mice.

AGE OF THE MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED
Birth	3,550,000	27	4,850,000	22	4,020,000	15
1 week	5,130,000	26	5,450,000	31	5,090,000	15
2 weeks	6,810,000	23	6,840,000	22	6,600,000	15
3 weeks	8,120,000	20	7,710,000	20	6,750,000	15
4 weeks	10,050,000	17	9,510,000	17	9,200,000	12
6 weeks	10,510,000	15	10,090,000	15	9,870,000	4
8 weeks	11,360,000	13	10,320,000	13	10,000,000	2
10 weeks	11,170,000	15	10,690,000	14		
12 weeks	11,500,000	14	10,790,000	14		
Adults	11,210,000	16	11,040,000	15	9,690,000	12

fact that the normal curve crossed the flexed at the second week. Thereafter the heterozygous normals had a lower concentration of erythrocytes than the flexed. These normals reached a level at about the tenth week which was but little lower than for the twelfth and the adult stage. The homozygous normal controls showed a birth count of 4,020,000 red cells, which was intermediate between the flexed and heterozygotes, but closer to the former than the latter. The homozygous normal and the flexed curves intersected at one week, and thereafter the homozygotes had fewer erythrocytes than either the heterozygotes or the flexed.

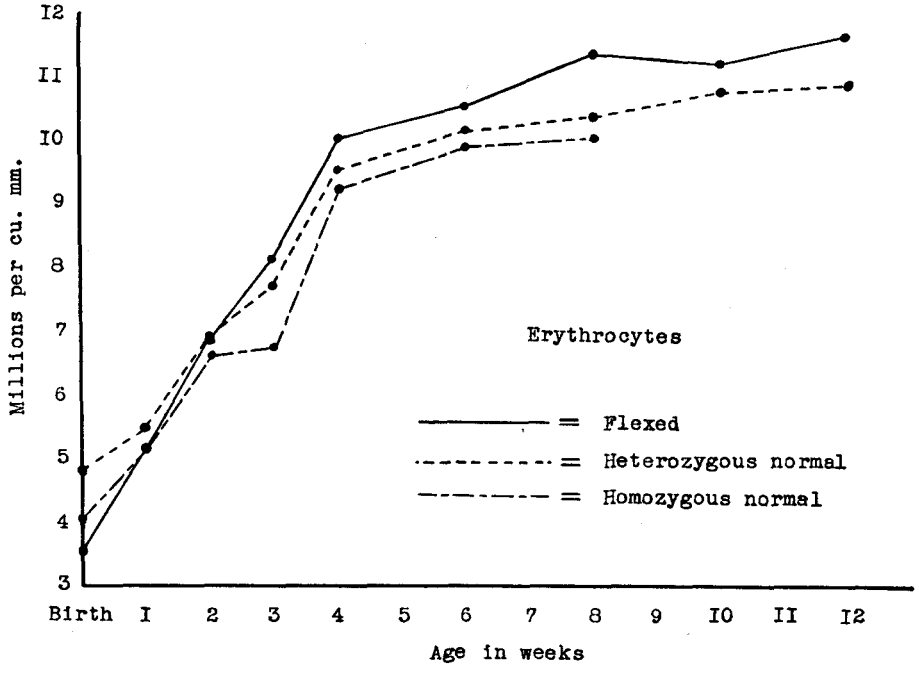

FIGURE 1.

The lack of erythrocytes which on the average afflicts the flexed animals is characteristic of early infancy, the group as a whole rapidly recovering from the disability. Let us examine critically the evidence for this statement. Table 13 shows the frequency distribution of red cell counts in the flexed and controls at birth. There was much overlapping among the three types, nearly all the homozygous normals falling within the range of variation of the flexed. The probable causes of these overlaps will be discussed later. We are not concerned for the present with the blood counts after the second week, though the apparent superiority of the flexed animals after this age may be worth further study. The average numbers of erythrocytes at birth were as follows:

Flexed: 3,550,000 ± 99,900
Homozygous normals: 4,020,000 ± 112,700
Heterozygous normals: 4,850,000 ± 111,400

TABLE 13

Frequency distribution of red blood cells in flexed and normal mice at birth.

CLASSES (NUMBER OF RED CELLS PER CUBIC MILLIMETER)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
2,400,000–2,490,000	2	..	..
2,500,000–2,590,000	..	..	..
2,600,000–2,690,000	4	..	..
2,700,000–2,790,000	..	..	..
2,800,000–2,890,000	2	..	..
2,900,000–2,990,000	1	..	1
3,000,000–3,090,000	1	..	1
3,100,000–3,190,000	1	..	..
3,200,000–3,290,000	1	..	1
3,300,000–3,390,000	2	..	..
3,400,000–3,490,000	..	..	..
3,500,000–3,590,000	..	1	1
3,600,000–3,690,000	..	..	2
3,700,000–3,790,000	..	..	..
3,800,000–3,890,000	3	..	..
3,900,000–3,990,000	1	2	1
4,000,000–4,090,000	1	2	2
4,100,000–4,190,000	..	1	..
4,200,000–4,290,000	1	1	..
4,300,000–4,390,000	3	1	2
4,400,000–4,490,000	..	..	..
4,500,000–4,590,000	1	1	1
4,600,000–4,690,000	1	2	..
4,700,000–4,790,000	1	..	..
4,800,000–4,890,000	1	..	..
4,900,000–4,990,000	..	..	3
5,000,000–5,090,000	..	2	..
5,100,000–5,190,000	..	2	..
5,200,000–5,290,000	..	1	..
5,300,000–5,390,000	..	1	..
5,400,000–5,490,000	..	1	..
5,500,000–5,590,000	..	..	..
5,600,000–5,690,000	..	..	..
5,700,000–5,790,000	..	1	..
5,800,000–5,890,000	..	..	..
5,900,000–5,990,000	..	1	..
6,000,000–6,090,000	..	1	..
6,100,000–6,190,000	..	..	..
6,200,000–6,290,000	..	..	..
6,300,000–6,390,000	..	..	..
6,400,000–6,490,000	..	..	..
6,500,000–6,590,000	..	1	..

The difference between the flexed and the homozygous normals was 470,000 ± 151,000 erythrocytes per cubic millimeter. This difference was only 3.1 times as large as its probable error, so it was just barely significant statistically according to conventional standards. The difference between the flexed and the heterozygotes was 1,300,000 ± 150,000, and since this difference was 8.7 times its probable error it can scarcely have been due to chance. Thus the flexed animals are definitely deficient in erythrocytes at birth.

The evaluation of these differences should take into account the nature of the controls. The heterozygous normals were, theoretically, better controls than the homozygotes. This was particularly true of the newborn young. Usually the blood count of a flexed animal was paired with the count for a normal litter mate. Thus a flexed animal at birth had been subjected, as a rule, to about the same prenatal environmental influences as the normal heterozygous mouse which was selected to serve as its control, for both had developed in the same uterus at the same time. The homozygous controls, of course, were always developed in uteri which were carrying no flexed mice. Their uterine environment may have been quite different, on the whole, from that of the flexed animals with which they were compared within a few hours after parturition. Also, the heterozygous normal controls were probably, on the whole, genetically more like the flexed animals than were the homozygotes, for the heterozygotes came from the same group of parents as the flexed. So if there are factors other than the one with which we are now concerned that affect the number of erythrocytes and the amount of hemoglobin, the heterozygotes would be expected to resemble the flexed in the possession of these factors more closely than the homozygotes.

Thus it is conceivable, though of course not proved, that some of the homozygous normal controls may have possessed factors, other than the main factor for anemia, which depressed the erythrocyte content at birth, and that such factors were less frequent in the flexed animals and the closely related heterozygous controls. This might account for the relatively small difference between the flexed and homozygotes at birth. Such an explanation of the higher counts in the heterozygous normals at birth in contrast with the homozygotes and the flexed is, I think, more plausible than an assumption to the effect that heterozygosity with respect to the flexed gene raises the erythrocyte count over the normal level in some mysterious manner.

To conclude, flexed animals as a group are lacking in red blood cells at birth, but on the average they reach the normal level when one to two weeks old. This anemia is not a permanent or fatal defect.

The facts with regard to hemoglobin are presented in tables 14, 15, and

TABLE 14

Hemoglobin of flexed and normal mice.

AGE OF MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED
Birth	38.2	44	60.3	41	56.9	26
1 week	49.0	43	57.9	46	53.3	15
2 weeks	54.0	36	56.3	31	56.0	15
3 weeks	58.2	36	57.0	33	52.0	15
4 weeks	58.2	31	61.5	30	60.8	12
6 weeks	60.7	15	61.7	15	61.3	4
8 weeks	65.4	13	66.5	13	60.0	2
10 weeks	65.0	15	64.3	14	....	..
12 weeks	67.1	14	67.5	14	....	..
Adults	68.8	16	64.3	15	65.8	12

TABLE 15

Frequency distribution of hemoglobin in flexed and normal mice at birth.

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
30	13	..	..
35	7	..	..
40	10	2	..
45	12	..	2
50	1	1	2
55	1	3	8
60	..	25	13
65	..	6	..
70	..	3	1
75	..	..	..
80	..	1	..

TABLE 16

Frequency distribution of hemoglobin in flexed and normal mice at one week of age.

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
35	1	..	..
40	5	..	..
45	14	..	..
50	11	9	7
55	6	11	6
60	6	19	2
65	..	6	..
70	..	..	..
75	..	..	..
80	..	1	..

16, and in the curves of figure 2. As previously mentioned, the Tallquist method was used in determining the relative concentrations of hemoglobin. Table 15 shows the frequency distribution for the flexed and normal mice at birth, while table 16 gives such data at the age of one week. Hemoglobin is the substance which gives the blood its characteristic red color, so if the paleness of the infant mice is due to an anemia, one would expect to find less hemoglobin in the blood of the newborn flexed than in the two groups of controls. Such was the case.

The flexed animals had at birth an average hemoglobin concentration of 38.2 percent of the normal human standard. This increased rapidly during the first three weeks, reaching the level of the controls during the third

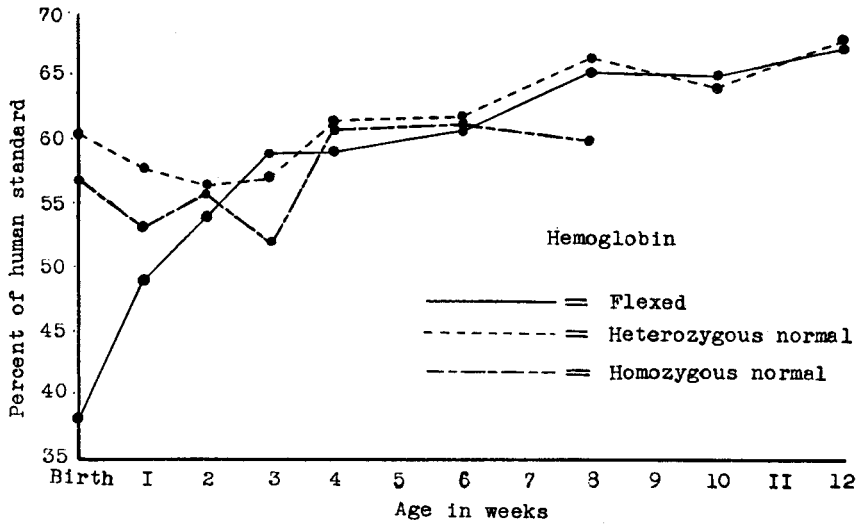

FIGURE 2.

week, thereafter slowly increasing until an adult percentage of 68.8 percent was attained. The heterozygous normal mice began postnatal life with an average hemoglobin percentage of 60.3 .This decreased slightly during the first and second weeks, then came a gradual but irregular rise which carried the hemoglobin percentage to 64.3 percent in the adult. The curve for these heterozygous controls followed closely the curve for the flexed from the third to the twelfth week, though the flexed adults had 4.5 percent more hemoglobin than the adult controls. The flexed animals increased their hemoglobin by over 30 percent from birth to adulthood, while the corresponding increase for the heterozygous normals was only 4 percent. The homozygous normals had 56.9 percent of hemoglobin at birth; the percentage declined irregularly until the third week, after which came a rise which carried the hemoglobin content to 65.8 percent in the adult. No observations were made at the end of the tenth and twelfth weeks. The

more irregular course of the curve for the homozygous controls may have been due to the relatively small numbers of individuals observed. The hemoglobin content of the homozygotes at birth was, as with the erythrocyte counts, intermediate between the flexed and the heterozygous controls. It is worthy of note, also, that though the flexed mice enter the world markedly deficient in hemoglobin, on the average, yet the survivors not only speedily overtake the normals when about two weeks old, but on the whole slightly exceed them at maturity. Thus the anemia from which the flexed mutants suffer automatically disappears during infancy, in this respect differing decidedly from the severe lethal anemia found by DE ABERLE in homozygous dominant white animals.

Table 15 shows that there was some overlapping in the distributions of flexed and control groups at birth, but this was not as pronounced as with the distributions of erythrocyte counts presented in table 13, which shows that the homozygous normals fell almost completely within the range of variation of the flexed, and that half the heterozygous normals did the same. In table 15 only 15 percent of the heterozygous and 46 percent of the homozygous normals come within the limits of the flexed distribution. Moreover, the differences between the flexed animals and the controls at birth were statistically significant, as the following computations show.

Average percentage of hemoglobin for the flexed: $38.2 \pm .68$ percent
Average percentage of hemoglobin for the homozygous normal controls: $56.9 \pm .69$ percent
Average percentage of hemoglobin for the heterozygous normal controls: $60.3 \pm .70$ percent

The difference between the flexed and homozygous normal controls was $18.7 \pm .97$ percent, and since this difference was 19.3 times as large as its probable error it was highly significant statistically. The percentage for the heterozygous straights exceeded that for the flexed by $22.1 \pm .98$ percent. This difference was likewise of great significance because it was 22.6 times the size of its probable error. Thus the hemoglobin percentages emphatically testify that, on the average, the flexed group was anemic when born.

The flexed and anemic animals were more nearly alike at one week of age than at birth, but the differences were still significant, as the following data show.

Average percentage of hemoglobin for the flexed: $49.0 \pm .66$ percent
Average percentage of hemoglobin for the homozygous normal controls: $53.3 \pm .61$ percent
Average percentage of hemoglobin for the heterozygous normal controls: $57.9 \pm .57$ percent

The percentage of hemoglobin in the heterozygous controls was $8.9 \pm .87$ percent higher than in the flexed animals. This difference was over ten

times the magnitude of its probable error. The homozygous normals had $4.3 \pm .90$ percent more hemoglobin than the flexed, and this difference too was significant because it was 4.8 times as large as its probable error.

Thus the flexed animals attain the hemoglobin level of the normals at about the second week, while the number of erythrocytes reaches the normal level perhaps a little earlier (fig. 1), but all the data show that the flexed animals are on the average deficient in erythrocytes and hemoglobin at birth, but that these defects are rapidly remedied.

An examination of figures 1 and 2 shows at a glance that the individual erythrocytes of both types of controls were presumably, on the average, more richly endowed with hemoglobin at the time of the mouse's birth than were the erythrocytes of the flexed individuals. The red cell curves of all three groups follow much the same course, a rapid infantile increase being followed by a lower rate. This was not the case with the hemoglobin percentages. The hemoglobin and erythrocytic curves of the flexed animals resemble one another, but the hemoglobin percentages of the two groups of controls did not show the marked infantile increase which was found in the flexed group. Whereas the flexed animals were 30.6 percent below their adult level, the controls had only 4.0 percent and 8.9 percent less hemoglobin at birth than when full grown.

Quantitative estimates of the relative average amounts of hemoglobin in each erythrocyte of the control types and flexed at birth, and of the adults will be of interest. However, the reader is cautioned not to take these estimates too seriously, for the numbers of mice used were not large; also, as will be pointed out shortly, the controls may possibly have included a few anemic animals and the flexed group a number of normal blooded individuals.

Suppose we define a unit of hemoglobin as the average amount carried by an erythrocyte of a flexed animal at birth. Suppose, further, that we use throughout the computations the volume of blood which in a newborn flexed mouse contains 100 erythrocytes. We may now find, by using the data in tables 12 and 14, how many units of hemoglobin, on the average, an erythrocyte of a flexed animal carries when adult. The hemoglobin of flexed mice increased from birth to adulthood by 80.1 percent of the amount present at birth. Thus there would be 180.1 units of hemoglobin ($100+80.1$) in the volume of adult blood used as our standard volume. The number of erythrocytes, during this period, increased by 215.8 percent of the number present at birth, so that there would be 315.8 erythrocytes per unit volume of blood in the adult ($100+215.8$). The average hemoglobin quota of the adult's erythrocytes in flexed mice would therefore be, 180.1 units $\div$ 315.8 erythrocytes $=$ .57 units. Thus the hemoglobin load of each erythrocyte decreased from 1.00 unit at birth to .57.

The hemoglobin per red cell at birth in the heterozygous normal controls is computed as follows. These controls had 1.579 as much hemoglobin per unit volume of blood at birth as did the flexed (60.3 percent, hemoglobin at birth of the controls ÷ 38.2 percent, hemoglobin at birth of the flexed). The heterozygous controls had also 1.366 as many erythrocytes per unit volume of blood at birth as the flexed mice (4,850,000, erythrocytes at birth of the controls ÷ 3,550,000, erythrocytes at birth of the flexed). The number of units of hemoglobin per erythrocyte for the heterozygous normals when born would be, therefore, $1.579 \times 100 \div 1.366 \times 100 = 1.16$ units.

To determine, next, the units of hemoglobin per red blood cell of the adult heterozygous controls, we make use of the facts that the hemoglobin of these increased over the content at birth by 6.6 percent of the amount at birth, and that the increment for the erythrocytes was 127.6 percent of the number at parturition. Thus the average hemoglobin quota in the red blood cell of the adults was $157.9 + (157.9 \times .066) \div 136.6 + (136.6 \times 1.276) = .54$ units.

The following summary brings together all the estimates from the available data.

Units of hemoglobin per erythrocyte for

	Flexed	Heterozygous normal controls	Homozygous normal controls
At birth	1.00	1.16	1.32
In adults	.57	.54	.63

The adult erythrocytes in all three types of animals are thus seen to have carried about the same quantities of hemoglobin, the flexed being intermediate between the controls. On the other hand, the controls' red blood cells were endowed at birth with from 16 percent to 32 percent more hemoglobin per cell than the flexed animals. Thus the blood of the newborn mutants was not only deficient in total hemoglobin content, and in erythrocytes, but each erythrocyte carried less than the normal quantity of hemoglobin. These estimates are probably of slight quantitative value, yet they seem to bring out another important fact about our flexed mutation.

Mr. MIXTER studied the leucocyte content also of his animals. His findings are summarized in table 17, which gives the average numbers of leucocytes per cubic millimeter at the different ages indicated from birth to maturity. All three types showed an irregular increase with advancing age, but the flexed animals did not differ significantly from the controls. The blood deficiencies of the flexed did not therefore involve the white cell content as a whole. Mr. MIXTER did not enumerate separately the different types of leucocytes, however, so it is conceivable that a deficiency of one type of leucocyte in the flexed may have been offset by an increase in another type.

TABLE 17

Average numbers of leucocytes per cubic millimeter.

AGE	FLEXED		HETEROZYGOUS NORMAL		HOMOZYGOUS NORMAL	
	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS
Birth	4297± 202	16	4129± 238	16	3993± 229	26
1 week	4192± 139	15	4481± 201	15	5000± 326	14
2 weeks	5086± 354	15	6167± 586	11	5000± 360	15
3 weeks	5805± 385	15	6502± 323	15	5600± 547	17
4 weeks	7435± 434	12	5340± 477	12	5600± 657	12
6 weeks	7700± 486	8	8000± 759	8	4500± 370	4
8 weeks	11200±1255	9	8700± 595	10	.. ..	..
10 weeks	8700± 690	12	10600±1106	11	.. ..	..
12 weeks	12700± 846	12	11800± 668	12	.. ..	..
Adult	10000± 834	15	9600± 748	13	11000± 738	12

ARE THE FLEXED AND ANEMIC CHARACTERS DUE TO THE SAME GENE?

We may now inquire whether the characters flexure and anemia are the results of a single recessive gene or of two recessive genes which are closely linked. This question can not be answered from the data at hand, but there are certain suggestive facts. If the flexed gene causes the anemia, then anemia and flexed should always occur together (unless some agencies, genetic or environmental, can suppress one of these characters without eliminating the other), and a normal tail would always be associated with the absence of this type of anemia. In other words there would be no cross-overs. But there is enough evidence at hand to shake one's faith in such a theory. Individuals that may possibly be the results of crossing over between genes for anemia and flexed were found.

Tables 2 and 4 contain such cases (see HUNT, MIXTER, and PERMAR, 1933). Among the 963 newborn backcross progeny reported in table 2, 78 are recorded as anemic but normal tailed. There are 1478 newborn F_2 mice in table 4, and of these 81 were normal tailed and anemic. Such a large number of exceptional cases would be good evidence that crossing over between genes for flexed and anemia had occurred if the classification of the newborn young could be depended on, but it can not. The identification of flexures in the tails of very young mice is sometimes difficult or impossible. Flexed is a highly variable character, as has been shown already. It grades into normality so perfectly that no absolutely defined boundary can be designated. Mr. MIXTER reports that among 415 offspring from flexed×flexed crosses, 3 anemic young were observed which at the age of 21 days showed no caudal stiffness or flexure. If such conditions are encountered in weaned flexed mice, it is obvious that the

absence of a bend in the very pliable tail of a newborn mouse does not prove it to be normal tailed.

The uncertainty in classifying the tails at birth is brought out by a study of the following 100 F_2 young which were branded with a hot needle on the day of birth, then observed when they reached the age at which the animals were usually given their final rating.

Normally red at birth, normal tailed at birth, and normal tailed at the final count:	75
Normally red at birth, doubtfully normal tailed at birth, and normal tailed at the final count:	12
Normally red at birth, straight tailed at birth, and doubtfully normal tailed at the final count:	1
Probably anemic, straight tailed at birth, and normal tailed at the final count:	1
Doubtfully anemic, straight tailed at birth, and doubtfully flexed at the final count:	1
Anemic, flexed at birth, and flexed tailed at the final count:	5
Anemic, doubtfully straight tailed at birth, and flexed tailed at the final count:	2
Anemic, doubtfully flexed at birth, and flexed tailed at the final count:	2
Anemic, straight tailed at birth, and the tail form transitional at the final count:	1

It will be seen that in five cases the tail was described as straight or doubtfully straight at birth, and that this verdict was reversed or rendered doubtful when the final count was made. Thus at least some of my newborn mice which were described as straight tailed anemics at birth may actually have been flexed, so the evidence from such cases does not prove that crossovers have occurred between genes for flexure and anemia.

Tables 2 and 4 record 5 flexed tailed newborn individuals (3 of them backcross young and 2 F_2's) which appeared to show the normal redness of non-anemic animals. Two of these turned out to be normal tailed, for no deaths occurred in the litters to which they belonged, and at the final counts after weaning, all the animals in their litters were found to have normal tails. This left three cases of supposedly flexed young which seemed normal blooded when the newborn were examined. In view of the fact that an ordinary muscular bend in the tail of an infant mouse might occasionally be taken for a flexure, no conclusions can be drawn from these three cases.

The writer found two newborn mice in a litter, not elsewhere recorded in this paper, both of which had flexed tails and a normal red color. Mr. MIXTER'S examination of the bloods showed one to have 5,890,000 red cells per cubic millimeter with 55 percent hemoglobin, and the other 65 percent hemoglobin and 5,300,000 red cells. Reference to tables 13 and 15 shows that the first animal had a normal erythrocyte count and a hemoglobin percentage which was at the extreme upper limit of the flexed distribution, while the second one was normal as regards hemoglobin percentage and red cell content, being entirely outside the range of flexed animals in both respects. To sum up the matter, the data are too scant and in many respects too uncertain to demonstrate whether or not the flexed and anemic traits are due to separate genes.

If recovery from anemia begins before birth, an occasional newborn flexed animal might have normal blood.

It should be kept in mind that Mr. MIXTER studied the blood of flexed animals rather than of anemics, so that his quantitative findings are not accurately descriptive of anemia. When newborn young were selected for blood counts and hemoglobin determinations, they were classified as flexed or straight tailed. Reference to tables 2 and 4 will show that there were quite a number of "intermediate or doubtful" cases where the writer was unable to classify the tails in newborn young. Considerable experience is required for this work. The tail may be bent as a consequence of muscular contraction on one side, or it may have a slight flexure which can be identified only after a rather close scrutiny. One of the main characteristics which the writer has assumed to identify newborn flexed tailed animals is a rather distinct angularity in the region of the flexion, usually quite different in appearance from the curvilinear aspect of a normal tail whose muscles are bending it. But there are undoubtedly cases where even experienced observers would not agree on the classification at birth. That is why it is so important to defer, as we have, final judgment as to the number of flexed animals in a litter until it is three to four weeks old, when the flexed condition can usually be identified positively.

Thus it is conceivable that Mr. MIXTER may have classified as flexed some newborn mice that were actually normal tailed. If the same gene causes both the flexed and anemic characters, such classification would place some normal blooded animals in the flexed category, thus causing the distributions of erythrocytes and hemoglobin percentages for flexed animals to overlap the ranges of both controls. Tables 13 and 15 show that such overlappings occur. Likewise, he may have classified as normal some animals that were homozygous flexed. This could happen wherever the genetically flexed animal would have shown only stiffness when full grown. Such events would extend the distributions of blood counts and hemoglo-

bin percentages of the heterozygous normal controls over into the ranges of the flexed animals. The above theory might account for the overlapping of the flexed and normal distributions of hemoglobin percentages, but it is difficult to believe that it explains the overlaps in the erythrocyte counts for flexed and homozygous normals, because their distributions cover almost the same range. Further study of the blood of newborn mice is needed to establish accurately the relationship between blood content and the paleness or redness of the mouse at birth.

MORTALITY OF FLEXED ANIMALS

One of the by products of Mr. MIXTER's study of inbreeding was considerable information about deaths among infants in the second generation of inbred flexed animals. Unfortunately, normal controls bred and reared at the same time and under strictly comparable environmental conditions were not available, so that positive conclusions can not be drawn. The facts will be given, however. The record of deaths is as follows:

TOTAL NUMBER OF 2ND GENERATION MICE AT BIRTH (ONE ESCAPED)	NUMBERS DYING AT					TOTAL DYING	PERCENTAGE DYING
	1–3 DAYS	4–7 DAYS	8–14 DAYS	15–21 DAYS	22–28 DAYS		
625	79	60	32	14	17	202	32.3

About one-third of these flexed infant mice died before they were 28 days old. This seems like an exceedingly high mortality, presumably higher than the normal rate. The deaths were most numerous during the first three days, then sharply declined. It is worth noting that 84.7 percent of these deaths occurred during the first two weeks, the period of anemia, suggesting that this may have been an important contributory cause. Whatever the reasons are for this high mortality, it appears probable that young flexed mice are lacking in vitality, and this view is consistent with the fact that the writer has had some difficulty in maintaining the flexed stock.

The flexed mutation presents some interesting similarities to, and contrasts with, the hereditary anemia described by DE ABERLE (1927) in homozygous dominant white mice. DE ABERLE's animals weighed less than normals, as did ours also (Mr. ALEXANDER A. ANDREWS' unpublished data on flexed), and both were much lighter in color than newborn normal mice. The anemia in the homozygous dominant whites was very severe, for all the afflicted young died within ten days after birth. Our flexed animals probably have a higher death rate than normals during infancy, but they recover from the anemia while still young. We can not accurately compare the blood of DE ABERLE's and our anemics because, as has been

said already, the blood of flexed, rather than of anemic, mice was studied by Mr. MIXTER. DE ABERLE's newborn normal mice had an average of $4,740,020 \pm 117,932$ red cells per cubic millimeter, while the newborn anemics' average was $663,009 \pm 15,714$. Her normal controls had 89.78 ± 1.59 percent of hemoglobin, as judged by human standards, while the percentage for the anemics was $22.13 \pm .56$ percent.

SUMMARY

1. The anemia in the flexed tailed strain of mice is probably a recessive character.

2. Flexed animals are on the average deficient at birth both in hemoglobin and erythrocytes, but this defect disappears, on the whole, at about the age of two weeks.

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