A PRELIMINARY STUDY OF HEMOPOIESIS IN YOUNG FLEXED TAILED MICE

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A PRELIMINARY STUDY OF HEMOPOIESIS IN YOUNG FLEXED TAILED MICE.

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By

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THE HISTORY OF THE FLEXED TAILED MUTATION

The mutation in the mouse, Mus musculus, which has been named "flexed tail" was found in January, 1927, by Dr. H. R. Hunt in his albino stock. The gross appearance of the flexed tailed animal has been well described by Mixter who says of it: "In appearance it varies from a tail which is stiffened by the fusing of a few vertebrae to a stiff tail bent into several angles in different planes. Not only is the tail stiffened and bent but it may also be shorter than the normal length which is about the same as the length of the body.....At birth the flexed mouse is paler and smaller in size than a normal new born mouse".[1]

Breeding experiments by Hunt, Permar, and Mixter have shown that the factor for flexed tailness is probably a simple Mendelian recessive, but that there seem to be modifying factors at work which somewhat affect the expression of this recessive gene. Work on this phase of the genetics of the animal is now in progress.

Environmental conditions such as diet, temperature, litter size, etc., have not been shown, to date, to have any affect on the appearance of the flexed tail. Diet, caging, and temperature have been constant for all mice. Nor has the flexed condition been restricted to any particular color-type of mouse, but is found among albinos, agoutis, blacks, browns, and piebalds.

It was noticed early in the study of flexed tailed animals that at birth they were not only smaller in size than their straight tailed litter mates (in F_2 or backcross litters), but that they were markedly lighter in color. Mice are naked at birth, so the color of the skin and hence of the blood is easily seen. A normal

new-born mouse is a vivid rosy-red, whereas these flexed animals are a pale pink, this being especially noticeable on the top of head and around the thighs and shoulders where the skin is drawn closely over the skeleton. This pale color strongly suggested that an anemic condition was associated with the flexed tailed character. To determine if this supposition were true, Mr. Russell Mixter, in the research already referred to, carried on an extensive study of the circulating blood of these anemic animals. Erythrocytic and leucocytic counts were made, and the hemoglobin content of the blood determined at different age levels beginning at birth and continuing at regular intervals up to adulthood, i.e., six months and on. The same determinations were made on a control group which was made up of two genetic types of normal animals: the heterozygous normal mice which were litter mates of the flexed animals studied, and homozygous normals, the offspring of normal X normal parents. For the reader's convenience I have summarized Mixter's data and included it herein for reference. (See Table I).

This table shows at a glance that the supposed anemia at birth proves to be real. The flexed animals have an average red blood cell count that is less than that of the heterozygous straights by 1,560,000 ± 151,600 cells per cubic millimeter. This difference is 10.29 times the magnitude of its probable error, so it is undoubtedly significant statistically. The count for the homozygous straights exceeds that of the flexed by 690,000 ± 152,000. This difference is 4.5 times the size of its probable error, not as marked a difference as the first one is; but it is statistically significant nevertheless.

The same is true of the hemoglobin content which Mixter expressed as the percentage of the normal hemoglobin content of human blood. The average hemoglobin content for the flexed at birth is 39.4%, and that for the heterozygous straights is 60.7%. The difference is 21.3 ± .93 which is 22.9 times the size of its probable error, so that the difference as between flexed and normal is even more marked in hemoglobin content than is that between the erythrocytic counts of the two. As may be seen from the table the leucocytic count for flexed mice is normal at all ages, so no further consideration will be given it in this discussion.

Incidentally it should perhaps be pointed out here that all three types of animals show a much lower erythrocytic count at birth than at subsequent ages. There is a rapid rise in the number of erythrocytes and leucocytes during the first three postnatal weeks. This increase continues up to about eight weeks, but at a slower rate. Kindred and Cory[2], in a series of studies of the blood of the fetal albino rat, have shown that the number of erythrocytes and leucocytes drops quite markedly just after birth, so that the blood count then is considerably lower than during the last prenatal period. Their work also shows that a rapid rise in the number of blood cells begins about three days after birth and continues in a rather spasmodic fashion up to six weeks, after which the rise continues slowly to $3\frac{1}{2}$ months when the counts are the same as in the adult. They attribute this decrease at birth to three possible causes:

1. Loss of the blood corpuscles circulating in the placenta.
2. Hemorrhage via the umbilical vessels.
3. An increase in the percentage of earlier forms of blood cells at this period.

This condition may then be considered as a so-called "secondary anemia", i.e. an anemia characterized by a reduction in the number of circulating red blood cells attributable to a fairly definite cause.[3] This should not be confused with the anemia associated with the flexed tail, which is entirely different. Since Mixter made no fetal blood counts we do not know definitely if this drop occurs in these mice at birth, but it is quite possible that conditions in the mouse are similar to those in the rat.

One of the interesting features of this particular anemia is that the mice, if they live, recover from it rather rapidly after birth. At the age of one week the erythrocytic count for the flexed animals has practically risen to that for the heterozygous normals and has somewhat surpassed that of the homozygous normals, showing that there is an intense hemopoietic activity in the flexed animal during its first week of postnatal life. At one week, the hemoglobin content of the flexed is still low, although it has improved since birth. At two weeks the hemoglobin content is practically equal to, and at three weeks it surpasses that of the heterozygous straights. It may be said, then, that by three weeks of age all signs of the anemia have vanished. The animal is normal. Once the normal condition is reached, it appears to be maintained for the duration of life, for the comparative counts of the three types at an adult age show no very great differences.

It is interesting to note in this connection that Mixter's data show that the red blood cell counts for the heterozygous straights are higher at all ages at which counts were made than for the homozygous straights. The difference between the erythrocytic counts for these two types at birth is 870,000 $\pm$ 159,000, a difference which is 5.5 times

its probable error. This is not at all what one would expect to find. Any difference between these two types of normals ought, logically, to be in favor of the homozygous straights since they are carrying none of the mutant genes. It has been suggested that the better condition of the heterozygous animals is due to their heterozygosity. This theory has not been tested out however.

In summary then, we seem here to be dealing with two very closely associated genetic conditions, a bone anomaly, and an anemia. These must be caused either by one recessive mutant gene or by two or more very closely linked genes. The present paper will be concerned with a study of this anemia only.

THE PURPOSE OF THE PRESENT STUDY

Anemia is defined as that condition characterized by a deficiency in the oxygen-carrying substance of the blood, hemoglobin. It may be manifested by (1) a diminution of the amount of hemoglobin in each erythrocyte, or (2) a decrease in the number of red blood cells. Anemias are commonly classified as being of two general types: (1) primary - an intrinsic anemic condition of unknown cause, and (2) secondary - those produced by definite causes such as hemorrhage, acute infectious diseases, acute infections, tumors, etc.[3] Evans[4] has pointed out that such a classification is unsatisfactory since probably all anemias are secondary to some definite cause, if that cause could but be discovered. He suggests that although none of the several classifications of anemia in vogue are entirely satisfactory, perhaps the one by Morawitz comes somewhat closer to the root of the matter. Morawitz divides anemias into those due to blood loss or increased destruction of red blood cells, and those due to decreased blood formation, but this does not complete the clinical picture as it does not include changes in hemoglobin content of individual red blood cells, i.e., changes in the color index. These classifications must at best be somewhat arbitrary for clinical use.

It seemed that further studies of this anemia would prove both valuable and interesting. It was hoped that a histological examination of the subject might render, if not proof, at least valuable clues as to the actual causes of this anemia. It will be noticed that no definite diagnosis has been made concerning this anemia. It would be impossible to give it its clinical classification until something was known concerning its cause. If the cause or causes could be

discovered, we might also know more about how the flexed gene actually gets in its work.

As will be shown later this anemia is particularly interesting, since it differs in its characteristics from any other anemia of mice known to date. Mr. Mixter established the fact of its existence, but his data fail to tell the whole story, for they do not show, for instance, whether this is the type of anemia caused by blood loss or destruction of red blood cells, or by decreased blood formation. So with a view to gaining information concerning the histological nature of this anemia, the investigation reported in this paper was begun in January 1931, and has been continued to the present time.

It is to be regretted that more progress has not been made; however, the problem proved to be a genuine Pandora's box out of which sprang an unexpected host of surprising elements the moment the lid was raised. Most of these elements, as will be shown later, have been a hindrance rather than a help to the investigator in her attempt to find out just what else was in the box. However, a start has been made, and it is hoped in the right direction.

NORMAL HEMOPOIESIS

Before the methods used in attacking this problem can be fully understood or the data obtained can be discussed, the salient facts concerning blood formation should be reviewed. Before studying the abnormal condition we must familiarize ourselves with the normal. At this point I shall digress at some length to describe the process of the manufacture of blood tissue.

Blood is not the simple liquid material it would seem to be to the casual observer. Complexity - morphological, chemical, physiological, and genetic - is its most outstanding and constant characteristic. In brief, blood may be considered as a tissue, the constituents of which are several kinds of cellular elements (corpuscles), some small irregular cell-like elements (the platelets), and a liquid medium (plasma). Or as one textbook of histology states it: "It may be considered as a form of connective tissue in which the cells have no fixed spatial relationships and the intercellular substance is a fluid".[5] The corpuscles are of two main types: the erythrocytes or red blood cells, and the leucocytes or white blood cells.

As has been shown in the historical account of this anemia, this problem is apparently concerned only with the erythrocytic elements of the blood. Therefore, throughout this paper attention will be focused on the red blood cells practically to the exclusion of the white blood cells, which though of great importance to the organism do not seem to have any direct bearing on the particular condition with which we are dealing.

As compared with the other tissues of the vertebrate body, the blood cells are very short lived. The exact life span of a human red blood corpuscle is not known exactly, but experimental work has furnished estimates varying from fifteen to forty or more days. Whatever this span may be, we do know that cells are constantly being worn out and destroyed and new ones manufactured to take their place, so that under normal conditions their number is kept at a constant level. This process of the manufacture of blood cells is called hemopoiesis, and the tissues in which it specifically occurs are designated as hemopoietic tissues and in the adult are located outside of the vascular system proper. The hemopoietic tissues may be subdivided into two types not always sharply differentiated from each other: the lymphoid tissue (lymph nodes, spleen, etc.) wherein the lymphocytes are produced, and the myeloid tissue wherein the erythrocytes and granular leucocytes are formed. In the normal adult mammal, the myeloid tissue is more diffuse and is found in several parts of the body, such as the yolk vessels, liver, spleen, and bone marrow.

During foetal life, the first blood cells are formed from the endothelium of the walls of the yolk vessels. These blood cells are nucleated, basophilic cells, and do not much resemble the erythrocyte as we know it. They are very temporary and are soon replaced by blood cells of a more differentiated nature produced within the body of the embryo itself. These new cells, although nucleated, elaborate hemoglobin, and serve to carry oxygen to the tissues of the embryo. After the formation of the liver, the thin strands of mesenchymal cells (which occupy the tiny interstices between the liver cords and their surrounding vascular plexi) begin to differentiate into blood-forming cells.

For the rest of the foetal life and for a very short period after birth, the liver is the center for much blood manufacture. The spleen may also be a center for red cell formation at this period. The bone marrow begins its hemopoietic function in the later stages of foetal life and as birth approaches it more and more takes over the blood-forming functions which are gradually being relinquished by the other organs mentioned. Thus at birth the animal is still in a transitional state showing some of the processes characteristic of the embryo, and some characteristic of the adult. "The transition from the embryonic to postnatal life is not marked by any sudden special changes in the blood or connective tissue. The processes of development and differentiation we have followed in the description .....continue without interruption".[6] Regardless of the place where hemopoiesis occurs, the cells involved are of the same type and the process is the same.

One can scarcely enter any sort of a discussion of this process without at once becoming involved in the more or less conflicting theories and certainly confusing terminology of the hematologists. I shall outline the most outstanding of these theories later on, but to present a relatively uncomplicated picture of this process, I shall at present follow Maximow's description.[6,7]

The red blood cell as found in the circulating blood, is not produced directly from other cells like itself, but is the end product of a series of cell mitoses and accompanying metamorphoses. The first cell entering into the genetic lineage of the erythrocyte is a rather large, free, ameboid, basophilic cell of mesenchymal origin, resembling the large lymphocytes of the adult, and was

named "hemocytoblast" which means "a cell producing blood cells". When they were first observed by Maximow in 1907 he called them "large lymphocytes", and it is his belief that they are "the common stem cells of all the other blood elements in the embryo and in the adult"[7]. These cells are large in size (up to 15μ in humans), have a usually pale basophilic cytoplasm which forms a broad ring around the nucleus. There may be vacuoles present in the cytoplasm. The nucleus is large, sometimes kidney-shaped, and is characterized by clumps of chromatin scattered loosely about, giving it a so-called "basket weave" appearance. The nuclear membrane is coarse and shows up quite conspicuously in stained specimens. The hemocytoblasts may undergo several successive mitoses producing daughter cells like themselves. Finally a significant change occurs. Maximow describes it thus: ".....a peculiar unstable equilibrium of the cell develops at the height of the mitotic process. All of the various potencies of development which are present in the cells are in a latent condition; one of them is suddenly followed and both of the daughter cells which originate from such a mitosis at once show new properties. Their destiny apparently has been fixed during the mitotic processes and they have become either a pair of erythroblasts or a pair of myelocytes of one of the three types".[6] The hemocytoblast is also the stem cell of another cell type, the megakaryocyte which will be referred to further on.

The next several generations in the erythrocytic series are termed collectively erythroblasts. The youngest cell of this group, that is, the cell directly produced from the last hemocytoblastic mitosis, Maximow calls the proerythroblast. The proerythroblasts, or basophil erythroblasts, differ from

the hemocytoblast in several ways. They have not its multipotencies. They can only form erythroblasts. They have lost the ameboid movement of the hemocytoblast, and are fixed. The nucleus is regularly spherical and smaller, and its chromatin shows a more even distribution than does that of the hemocytoblast. The cytoplasm is intensely basic and very homogeneous in appearance. They frequently have the shape of a triangle with well rounded corners.

The next youngest generation of erythroblasts is sometimes called the megaloblast. This cell is always larger than any of its descendents. Maximow does not give its exact dimensions but I assume from his figures[6] that it is somewhat smaller than either the proerythroblast or the hemocytoblast. This rather conflicts with other descriptions of it, e.g., one group of hematologists say: "This (the megaloblast)is representative of the largest red blood-cell we have observed in the erythropoietic series".[8] It shows a more compact nucleus than its predecessors, a less conspicuous nuclear membrane, and cytoplasm containing a small amount of hemoglobin, which, however, may not show up in a stained cell. In cells stained with the Romanowsky mixture (eosin and methylene blue), the cytoplasm may give a polychromatic effect - that is, it may vary in color from purplish-blue, to lilac, to gray.

The succeeding several generations of erythroblasts show progressive tendencies to increase the amount of hemoglobin in the cytoplasm, to condense the nucleus (and thus show a wider ring of cytoplasm around it), and to become somewhat smaller in total size. In contrast, in the earlier generations of this type

the nucleus shows a rather characteristic checkerboard distribution of the chromatin, and a thin ring of protoplasm around it. This means then that a considerable amount of morphological variation is to be observed among the different cells of this group as a whole. The last generation of this erythroblastic series is scarcely to be distinguished from the cells of the next stage, the normoblasts or nucleated erythrocytes.

The normoblast is the smallest cell of the series, with the exception of the erythrocyte. It is either spherical or ovoid in shape and is characterized in a properly stained condition by a clear pink cytoplasm - due to an accumulation of a considerable amount of hemoglobin - and a small, very dense, deep purple nucleus in which scarcely any reticulation can be seen due to the chromatin particles being so closely packed together. This cell still retains the power of undergoing mitosis, and may possibly produce another generation or two of cells like itself. Gradually, however, the nuclei of these cells become pyknotic and more and more hemoglobin collects in the cytoplasm. Eventually the pyknotic nucleus is extruded from the cell. The small, spherical, enucleated red cells then pass through the permeable membranes of the capillaries and are ready to begin their oxygen-carrying function in the blood stream.

Another derivative of the hemocytoblasts which will come under consideration in this paper is the megakaryocyte, or so-called giant cell. Concerning this cell, little was definitely known until rather recently. It is now coming into more prominence, and is believed to play an important role in certain pathological conditions[9]. The theory has been held for some time and is still accepted by some investigators[10,14], that these giant cells form

the blood platelets by fragmentation of their cytoplasm, but Maximow questions the truth of this theory. He says that both the origin and the function of the platelets is unknown[6]. There seems to be no doubt, however, that the megakaryocytes are produced by the hemocytoblasts. In structure, these cells are very large, some of them having a diameter as great as 40μ in humans. They are formed from hemocytoblasts by the growing nucleus of the latter undergoing a hypertrophy, and then becoming constricted in several planes. Following this there are several peculiar mitoses which involve only the nucleus. In these mitoses the daughter nuclei fuse in the telophase stage forming a new larger nucleus. Eventually a polymorphous nucleus is formed, the individual lobes of which resemble the nucleus of the hemocytoblast, i.e., they show the "basket-type" of reticulation due to the irregular grouping of chromatin granules, and the outline of the nuclear membrane is quite conspicuous. The cytoplasm is clear, homogeneous, and faintly basic in its staining reaction. It is very irregular in outline, frequently showing pseudopod-like structures which make the cell somewhat resemble an amoeba. According to Jordan[10], megakaryocytes are found wherever hemopoiesis occurs.

It is rather interesting to note that Jordan attributes to the megakaryocytes the potentiality of forming multiple erythroblasts, at least in the yolk-sac. He describes this transformation thus: "As polykaryocytes (multi-nucleated megakaryocytes) they differentiate erythrocytes intracellularly by a process involving the elaboration of hemoglobin about each nucleus. Each perinuclear halo of hemoglobin-containing

erythrocyte thus differentiated comes to lie free in a vacuole. Subsequently, the giant-cell breaks up and liberates the several erythrocytes.....Such a process of intracellular giant-cell production of erythrocytes has been described also by Foa[11] in bone-marrow. Pugliese[12] and Denys[13] have desdribed both erythrocytes and leucocytes originating in these giant cells".[10]

I can find no reference whatever to such a theory in the work of the outstanding contemporary hematologists, such as Sabin and Maximow, but that does not necessarily disprove it. If it is true that megakaryocytes are hemogenic cells, then they should merit our attention in a study of this kind. For this reason I have included them in my data.

Conflicting Theories of Hemopoiesis: Dr. Florence Sabin makes this statement: "The history of the development of our knowledge of the blood centers around two opposing theories, and nowhere in medicine can there be made a more interesting analysis of the value of constructive theories, whether right or wrong, in the advance of science".[14]

The bones of contention over which the adherents of these two theories have opposed each other are, (1) the immediate origin of the red blood stem cell, and (2) its relationship to the stem cells of the other types of blood cells. The process as I have described it above, showing a basophilic, lymphoid cell (the hemocytoblast) which is the potential source of all types of blood cells, red or white, is the explanation of events as offered by the so-called unitarian, or monophyletic theory or school. It is the most modern theory and much progress in the knowledge of blood has been made under its

influence by such adherents as Dominici, Pappenheim, Weidenreich, Maximow, Danchakoff, and Ferrata.

The opposing theory is called the dualistic, or to be more exact, the polyphyletic theory, and it is associated with the work of Ehrlich, Naegeli, Schridde, Morawitz, and others. It is based on the work of Ehrlich who is attributed with having laid the foundation of our knowledge of blood.[14] According to this theory there is no one cell type which serves as a common stem cell for all blood cells, but instead "there are different stem cells for the red cells, for the leucocytes, and for the lymphocytes, and they are located in specific places in the adult".[14] Ehrlich classified the adult blood-forming organs into the two types already mentioned - the bone marrow or myeloid tissue, giving rise to the erythrocytes and the myelocytes (forerunners of the granulocytes), and the lymphoid tissue giving rise to the lymphocytes. The polyphyletic school claims, furthermore, that the potencies of the cells of one type of tissue are specific for their individual function, and are not interchangeable with those of the other type of tissue. In other words, the lymphoid stem cells cannot produce red blood cells, nor can the red blood stem cells ever produce lymphocytes. This belief is founded upon morphological differences observed by Ehrlich and others in fixed and stained cells. The dualists have interpreted these structural differences as being correlated with physiological and genetic differences in the cells; but the monophyletic school in the work of Maximow and Danchakoff has been able to demonstrate equally well that these morphological differences are not intrinsic properties of the cells but are the

result of the type of technique used, and by means of newer staining methods and extremely intricate technique has shown an essential similarity between the cells of both tissues. Even these claims are being met by the equally modern technique of the modern adherents of the polyphyletic school. For instance, Dr. Sabin, who tends to support this older theory, admits that "the primitive stem cells of the different groups of blood cells cannot be separated in fixed specimens even with the most perfect technique", but she adds that, ".....these stems can be analyzed with the method of vital staining".[14] Thus the battle wages between two equally proficient groups of technicians.

Perhaps the theories of each group are based upon facts which, though essentially the same, have lent themselves to different interpretations. It would seem that the real point of contention is not so much concerned with the similarity between the early cells of the different types of blood cells, as it is with how long a demonstrable similarity exists. To develop this idea further: Both groups believe undifferentiated mesenchymal cells to be the ultimate source of all the blood cells, and recognize, therefore, that blood and connective tissue are very closely allied, both embryologically and morphologically. According to the dualists, some of this mesenchyme early in the embryonic history of the individual, differentiates once and for all into groups of pre-blood cells, sometimes referred to as angioblasts.[15] These angioblasts are of extraembryonic origin (in birds). They proliferate outside the embryo, but later invade it as a consequence of the increase in the cell masses which they form. In the body of the embryo they become the endothelium of the blood vessels, and the erythroblasts; or, erythroblasts may develop from the

endothelium of the blood vessels. So the line of development is: mesenchyme, angioblast, erythroblast; or mesenchyme, angioblast, endothelium of vessels, erythroblast, thus eliminating the hemocytoblasts and proerythroblasts from the picture entirely. The cells derived from this angioblast would then be of two sorts: one, the endothelium and erythroblasts, the other, cells which remain like the undifferentiated cells of the angioblast. This latter group continues, by proliferation, into the postnatal life, and eventually becomes localized in specific places, particularly in the bone marrow. It may then become transformed into erythroblasts in answer to the body's continual need for new erythrocytes, but always a group of undifferentiated cells are held in readiness so that only part of each generation of cells becomes erythroblasts, the other part remaining unchanged for future use. "While this school recognizes the presence of immature, undifferentiated cells in the hematopoietic tissues of the adult, yet they would assign to such cells the power to develop along but a single line predestined from the embryonic period. These cells, then, morphologically indistinguishable from each other, are potentially wide apart".[16]

The monophyletic school likewise names the mesenchyme as the first ancestor of the blood-forming cells, but it postulates that the so-called angioblast and its products are but the temporary blood-forming structures of the early embryo, and are soon superceded by the hemocytoblasts which arise everywhere in the embryonic connective tissues by transformation of the small, undifferentiated, fixed mesenchymal cells. These cells in the adult, although found in the fixed connective tissues throughout the body and believed to be able, theoretically at least, to

transform if necessary into hemocytoblasts, tend to centralize their hemopoietic functions within the red marrow of the bones where they transform into hemoblasts as needed. So, according to this theory, the immediate ancestors of the erythroblast are but recently differentiated mesenchymal cells whose embryonic multipotentialities have been maintained into and through adult live.

Thus, according to one school, the similarity in the adult between the stem cells of red and white blood cells is only morphological, the physiological equality having been lost during embryonic life; and according to the other school, the similarity between them is continued into the adult life and is both morphological and physiological.

MATERIALS AND METHODS USED

Although a wealth of literature is available on blood histogenesis and morphology, and on the many pathological conditions of blood, there is a paucity of material in print which has any direct bearing upon this particular anemic condition or on the proper technique to use in studying it. The only other hereditary anemia so far reported in mice is that in Little's dominant whites. This anemia has been studied and reported by deAberle[17]. Although anemia in these dominant whites is entirely different from that associated with flexed tail, in that it is a severe aplastic anemia which is invariably fatal, Dr. deAberle's work was the primary source of information concerning the method of approach to this problem.

Even this source was of quite limited value, however, since her approach to her problem was of an essentially different nature from mine; so from the first, the methods used were a matter of trial and error in equal proportions.

Materials: It was decided that the first thing to do was to study the general histological picture of the normal animal at birth and to compare this with anemics of the same age. Some time was devoted to raising a group of mice to be used for this work. Homozygous normal females (2 to 6 months of age) were crossed with flexed males. The F_1 female offspring from these crosses were then backcrossed with flexed males. These females were isolated, one to a cage, as soon as pregnancy could be observed, and were then examined daily until the young were born, after which the mother was returned to her mate and bred again. Part of the animals in each litter were selected for this

work and the rest discarded. In order to get as much individual variation as possible only two or four (2 normals and 2 anemics) were used from a litter.

Selection of the new-born young to be used was based entirely on a clear-cut differentiation between anemic (flexed) young, and normal (straight) young. There are undoubtedly borderline cases, both as to amount of tail flexure and as to the anemia, which are hard to differentiate from the normal. In order to avoid errors in classification, only those animals were used which, on the one hand, showed the pale pink color of the anemics and a pronounced grade of tail flexure and, on the other hand, those which had the ruddy color, straight tail and larger size of the normals. Since in all cases (with one possible exception) the pregnant mother had been examined daily, none of the mice used could have been over 24 hours old when they were killed for sectioning. They were, in all cases, old enough to have had at least one feeding, for the stomachs were distended with milk which can be seen clearly through the semi-transparent skin. The exact age in hours was not determined.

Killing: Since it was thought that etherization might have some physiological effect on the blood cells before death occurred and since hemorrhage was to be avoided as far as possible, it was decided to kill these animals by strangulation. A piece of ordinary cotton twine was tied about the neck and suddenly pulled taut. In a very few seconds the body became a purplish-blue and body motions almost ceased. As soon as unconsciousness was assured, a longitudinal incision was made

in the skin about half way between the mid-ventral line and the left side in such a way as to avoid severing the internal mammary arteries and veins which course down the ventral body wall as far as the end of the sternum and on either side of it. All other main vessels were also carefully avoided. This incision extended from the posterior boundary of the abdomen forward to the clavicle, and was ordinarily supplemented by a transverse incision in the region of the axillae. Then, using fine scissors, the underlying muscles, peritoneum, and the ribs were cut along the line of the skin incision. The diaphragm was severed from the ventral body wall with a sharp pointed knife. Thus both the abdominal and thoracic cavities were laid well open, and with practically no loss of blood. The animal was then quickly transferred to a small paraffin-lined dish where it was pinned out (with wooden pins) ventral side up, in such a way that the viscera were thoroughly exposed. Up to this point the heart was still beating. The fixative to be used was squirted into all parts of the body cavities with a small pipette, and the dish was then filled with the fixative, so that the animal was entirely submerged. In this way the fixative was quickly applied to all of the living tissues - a factor greatly to be desired in histological technique. Each fixed animal was assigned a number by which it was designated throughout the entire process which followed. An "A" following a number indicates an anemic animal in all cases, the normals being designated with numbers without letters.

Fixation, Dehydration, Embedding, etc. McClung's "Microscopical Technique"[18] was used quite extensively as a source for histological technique, formulae, etc. The fixative particularly recommended therein for blood work is Helly's Fluid or Zenker-formol, the formula for which is:

Potassium bichromate	25 gm.
Sodium sulphate	10 gm.
Water	100 c.c.
Mercuric chloride	50 gm.
Formalin (added when ready for use)	50 c.c.

This was the first fixative used. It was found that the mice should be left in this solution for about 20 hours to obtain complete fixation. Specimens were left pinned to the dish with wooden pegs throughout fixation.

They were then washed in running water for 24 hours, after which they were put in 35% alcohol, to which had been added a few drops of iodine, for 6 to 12 hours. Then they were moved into 50% alcohol plus iodine until all signs of crystalized sublimate produced by the fixative had been completely removed; this was usually accomplished in 12 hours, during which time there were two or three changes of the alcohol - iodine solution. The head was cut off to reduce the size of the animal to be sectioned. Dehydration was completed in the higher alcohols, the animals remaining in each for four to eight hours. From absolute alcohol they were put into cedar oil for clearing (usually three to four hours) and from this into paraffin oil for 12 to 24 hours, after which they were transferred to paraffin with a melting point of 54° - 58° for 4 to 6 hours, then blocked in paraffin.

Several animals were given this treatment but of these only mouse No.12 and mouse No.13 were used in the final study made. This method did not prove very satisfactory. In the first place, the crystalized sublimate formed by Helly's Fluid is extremely difficult to remove and dangerous to the microtome knife if not entirely dissolved. In the second place, considerable distortion of cells was noticed in some parts of the tissues, especially in the blood cells. In the third place, the tissues were so brittle that it was almost impossible to obtain thin sections that were fit to use. It was decided to use another fixative and a different embedding method.

The second method used was as follows:

1. Killing:- same as above.

2. Fixation in Allen's Fluid, P.F.A.3 - 24 hours.

   Formula:

Picric acid (sat.aqueous sol.)	150 c.c.
Formalin	30 c.c.
Glacial acetic acid	20 c.c.
Urea crystals	2 gm.

3. Wash in running tap water - 24 hours.

4. Dehydration:

   a. 50% alcohol (60 c.c.) plus 20 drops of lithium carbonate - 4 hours.

   b. 70% alcohol - 4 hours.

   c. 80% alcohol - 4 hours.

   d. 95% alcohol - 4 to 8 hours.

   e. Abs. alcohol - 4 to 8 hours.

5. Infiltration and embedding.

   a. Solution of 1.5 gm. celloidin in 100 c.c. methyl benzoate - until clear, 10 to 12 hours.

b. Pure benzine - 3 changes of 3 hours each - total 12 hours.

c. Solution equal parts benzine and 46° - 48° paraffin at 50°C - 12 hours.

d. Pure paraffin (46°-48°) - 4 to 6 hours.

6. Block in paraffin.

This method proved quite satisfactory, so was used for all of the other animals killed.

Sectioning: The animals were sectioned in a sagittal plane, as various trials showed that this was most satisfactory because so much of the general morphology can then be viewed in any one section. Sections were cut 8μ thick, and three to five were mounted on each glass slip. Each slide was then numbered with the number of the mouse from which sections were taken and a number for the slide series.

Staining: Of all the difficulties encountered, perhaps those concerned with the staining technique were the most serious and the most baffling. Some preliminary attempts were made to determine whether Wright's stain for blood smears could be used successfully as a tissue stain. The results were very unsatisfactory. The tissues took the stain beautifully but in the process of dehydrating and clearing (a procedure unnecessary in smears) the delicate stain was so washed out that the resulting slide was useless. This technique was abandoned. A few slides were stained by the ordinary hematoxylin-eosin laboratory method. This is a good process but does not give the delicate differentiation between different blood cell types necessary for this work.

It was decided that perhaps one of the best stains for blood work of this kind would be the hematoxylin-eosin-azure stain, as used by Maximow, and by deAberle in some of her work. The procedure followed was that given by McClung, with certain variations which I found necessary to suit this material. I should add here that all of the directions given in the various sources for blood histological technique are for smears (either for bone marrow, spleen material, or fresh blood) or for living tissue cultures. Nowhere have I found any instructions for the differential staining of blood in sections, except those given by deAberle who studied the general morphology of her animals in this way, so I have had to adapt these other methods to this work as best I could.

The method used for this hematoxylin-eosin-azure stain is as follows:

1. Xylol #1 - 10 minutes
2. Xylol #2 - 10 minutes
3. Alcohol 95% - 10 minutes
4. Alcohol 82% - 10 minutes
5. Alcohol 50% - 10 minutes
6. Water (tap) - 10 minutes
7. Water (distilled) - 10 minutes
8. Delafield's Hematoxylin - 24 hours (5 drops to 60 c.c. distilled water)
9. Water (tap) - 15 minutes
10. Water (distilled) - 24 hours
11. Eosin-azure stain (as follows) - 24 hours

Stock solution A

Eosin, water soluble yellowish 0.5 gm.
Pure distilled water 500 c.c. } 6.0 c.c.

Stock solution B

Azure II-0.5 gm.
Pure distilled water 500 c.c. } 4 to 4.5 c.c.

Pure distilled water 50.0 c.c.

12. Alcohol 95% - 5 to 20 minutes

13. Alcohol, absolute - 5 to 10 minutes

14. Xylol - 5 to 10 minutes

15. Mounted in Damar Balsam.

This staining method proved to be relatively satisfactory. It gave nicely differentiated cytological structure which was of utmost importance.

The main difficulty encountered, and one which was never very successfully surmounted was that of getting the normoblasts, as well as the erythrocytes to show their customary eosin staining reaction. Attempts to remedy this by increasing the amount of eosin in proportion to the azure were not successful. Results were the same as with the weaker solution. A few cells showed the acidic reaction but most of them remained definitely basic. Furthermore, the use of more eosin than indicated above did not give as satisfactory differentiations between the other types of cells. The actual staining results obtained will be discussed more fully later. However, one other type of stain was used in an attempt to reveal the relatively high affinity of normoblast cytoplasm and of erythrocytes for eosin. It was decided to try an acid eosin-hematoxylin stain which is used in the pathological

laboratories at the University of Missouri. The method for treating the sections is as follows.

1. Steps from xylol down to distilled water same as above.
2. Stain in Harris' Hematoxylin - 5 minutes
   (50 c.c. Harris' Hematoxylin plus 2 c.c. glacial acetic acid)
3. Wash in tap water.
4. Decolorize in acid alcohol (50 c.c. of 70% alcohol plus 0.5 c.c. HCL)
5. Neutralize in weak ammonia solution (three or four drops ammonia to 50 c.c. water)
6. Wash in tap water - 3 to 5 minutes.
7. Stain in a 1% alcoholic solution of eosin (1 gr. alcohol-soluble eosin to 100 c.c. 70% alcohol), acidulated in the proportion of 5 drops of a 2% solution of acetic acid to 45 c.c. of the eosin solution - 4½ minutes.
8. Wash in tap water - 5 minutes.
9. 82% alcohol - 5 to 10 minutes.
10. 95% alcohol - 5 to 10 minutes.
11. Abs. alcohol - 5 minutes.
12. Xylol #1 - 2 to 3 minutes.
13. Xylol #2 - 2 to 3 minutes.
14. Mount in Damar Balsam.

This method served to bring out the acid staining reactions of the erythrocytes and of the cytoplasm in some normoblasts, but it was not very effective for the differentiation of types of blood cells other than normoblasts. Only mouse No.12, Mouse No.13,

and some of the sections of mouse No.26 were treated with this stain. All the rest of the slides used were stained with hematoxylin-eosin-azure except three slides of No.18A (one of which entered into this study) which were stained in hematoxylin and eosin only.

From one-half to two dozen slides were made from each animal. If satisfactory results were gained in the first half dozen slides, no more were made. Of these, the most satisfactory slide was selected for making cell counts.

Method of Obtaining the Data: As has been said the first step was to make a survey of the general morphology of the animal at birth. This preliminary examination revealed no very noticeable differences in the histological morphology between the anemics and normals except in the liver. It was seen at once that the liver was still actively functioning as a hemopoietic organ, for in both normals and anemics its peculiar adult morphology, i.e., the cords of parenchymatous cells arranged in radial fashion around central veins, was almost obscured in numerous areas by masses of cells which filled the interstices. (See Plates I to VII and compare with Plate XII). These cells were identified as blood-forming cells of the different types.

The first two animals studied, one an anemic and one a normal, showed quite an obvious difference in the relative numbers of the different types of cells in the erythropoietic series. There seemed to be in the normal animal a great many more normoblasts than any other cell of the series, whereas in the anemics, although there were many normoblasts present, they

appeared to be fewer in number than the erythroblasts, the predecessors of the normoblasts.

It was decided to make a quantitative analysis of these pre-blood cells in the liver and see whether this difference was just a chance occurrence in these animals or a characteristic and constant difference between the two types of mice at birth.

Before doing this, however, the bone marrow was studied to see whether the same picture was presented here. No conclusions could be drawn, however, due to two factors. The first was a technical difficulty. The blood cells in the bone marrow were not clearly stained but appeared undifferentiated dark blue masses of various sizes. Also, these cells seemed to be considerably distorted, often presenting rather geometrical designs. (Plate VIII shows a little of this) The red blood cells present in the capillaries of the marrow were also, as a rule, quite distorted. It was evident that the technique used was not adequate for bone marrow. Probably this distortion was due to poor fixation. It had not been deemed necessary to subject these animals to a decalcification process as the bones seemed very soft and cartilagenous. However, it is quite possible that the dense cartilage prevented the fixative from penetrating thoroughly into the marrow, and poor fixation there resulted. A few slides were obtained later in which staining reactions were more satisfactory (Plate IX shows one of these), but at best the cells were not nearly as well differentiated as in the liver. It was obvious that if any study of the marrow were to be made, a special technique should be used. The second difficulty

encountered in the search for hemopoietic cells in the marrow is the fact that at this stage extensive bone histogenesis is in progress and groups of osteoblasts are present which so complicate the picture that it is difficult to differentiate all of the various bone and blood elements present. For these reasons, it was decided not to attempt a quantitative study of the blood cells in the marrow.

An examination was made of the spleen also to see what possibilities it presented for this study. Although primarily a lymphoid organ later on, it is capable of some red blood cell formation at this early age. Plate X shows why the spleen was not used in this study. It was so densely packed with lymphocytes among which were scattered a few cells of the red cell line, that a quantitative study was next to impossible.

Attention was then centered on the liver. The method of making a quantitative study of the hemopoietic cells of this organ was quite simple. A suitable area was located with the low power of the microscope. This area was sketched roughly on paper and its position noted by means of the scale on the mechanical stage of the microscope. I then counted the hemopoietic cells in sixteen, non-overlapping, high power fields from the liver of each of four normal mice. The same thing was done for each of four anemics. The number of cells belonging to each type in the erythrocytic genetic lineage was observed and recorded for every field. The totals for the sixteen fields constituted the cell count for the animal. Even more accurate information as to the cell counts was desired. So 48 high power

fields were counted in an additional normal mouse. These 48 fields counted were not all on the same slide. Sixteen high power fields were counted on one slide. Then sixteen fields were counted on another slide which had been made from tissue some distance away from that on the first slide. The count of the last sixteen fields of the 48 was made from a third slide made from a still different region of the animal. Thus these 48 counts represented three widely separated areas within the same liver. This same procedure was followed in making 48 counts of an additional normal mouse. Altogether then, counts were made of 5 normal and 5 anemic animals. Two or three different low power areas (indicated in the tables by Roman numerals I, II, III) were necessary to give these sixteen non-overlapping fields. For my own convenience in keeping the data, each field was numbered and is so designated in the tables of the individual counts. (Tables III to XII inclusive - left hand column).

The cells were classified according to their resemblance to Maximow's four types of cells in the erythropoietic series: hemocytoblasts, proerythroblasts, erythroblasts, and normoblasts. Megakaryocytes also were counted. Identification was based upon a comparison with Maximow's color plates[7] and descriptive text.

I was unable to find in the literature any measurements of the hemopoietic cells in mice, so it was decided to determine the average diameter of each type. This was done with a Zeiss eye-piece micrometer which had been calibrated with a stage micrometer. These measurements are tabulated in Table II.

A certain amount of overlapping in the size ranges of the different types is to be noticed, a factor which contributed to the difficulty in differentiating one type from another. Frequently, however, these measurements were an aid in identification.

Difficulties in Cell Identification: Before presenting and discussing the data obtained, there is much to be said regarding the principle difficulties encountered in identifying these cells. Reference has already been made in the section on staining to the fact that the cytoplasm of the erythrocytes and normoblasts had a tendency to take a basic rather than an acid stain, or not to stain at all. This unusual condition could be seen very well in sections of blood vessels. As a rule cut vessels showed a mottled appearance. Some erythrocytes were deeply pink, as they should be. Some were very faintly pink and many were but clear, transparent cells only the outlines of which could be seen. These cells resembled blood cells that had been laked. Plate XI shows this condition very well. The unstained cells show in the picture as thin gray circles, whereas the stained cells are dark. No reason could be found for this reaction, but it must have been due to faulty technique somewhere in the process, for some sections, especially those which received the acid eosin treatment, showed much less of this condition than the rest.

As to the normoblasts, very few of them, except those stained in acid eosin, showed any sign of pink in the cytoplasm. A great number of the cells classed as normoblasts were only extruded normoblast nuclei, but these were counted on the

grounds that such nuclei showed where a normoblast had but recently been formed and transformed into a circulating erythrocyte. When cytoplasm was present it usually showed only as a clear, sometimes faintly bluish halo around the nucleus. This halo was usually hard to see unless the light was greatly reduced and then it was frequently obscured by other cells being packed in close to the normoblast, or partly superimposed on it. Color plate XIII gives fairly accurate representations of the various groups of normoblasts. In the microphotographs (Plates I to VII) the cytoplasm of the normoblasts, if present and in focus, appears either as a white or slightly gray halo. Normoblasts designated on these plates as "nbl*) are examples of this.

A few normoblasts showed a very faint pinkish tint in the cytoplasm, but identification had to be based largely on the size, shape, and staining reaction of the nuclei. However, none of these factors were constant. The size was subject to variation, due to the pyknotic condition of the normoblast nuclei that are about to be extruded, or have been extruded. A "young" normoblast, that is one but recently formed from an erythroblast, would be expected to have an almost spherical, relatively large and somewhat mottled nucleus, whereas in an older normoblast this nucleus has undergone pyknosis which both shrinks and distorts it, and no mottling is visible due to its compactness. Therefore, a certain range had to be allowed in the size of the nucleus. These differences also may be observed in color plate XIII. The normoblasts measured were

those in which the limits of the cytoplasm, whether stained or unstained, could be definitely determined. These measurements showed the cell diameter to be quite constant. (See Table II) No difference was observed in the staining reaction of any of these cell types as between normal and anemic animals, so I do not believe there is any correlation between affinity for stains and the anemia.

Perhaps the most difficult cell of all to identify was the erythroblast. As has already been mentioned in this general description of cells, a relatively large amount of variation is to be observed in the cells of this group even when the staining technique is excellent. To this is to be added the technical difficulties already described, so it is evident that the identification of this cell type constituted a real problem. According to Maximow's color chart, the earliest erythroblasts should be easily differentiated from the proerythroblasts by their noticeably smaller size and lighter cytoplasm. Reference to Table II shows that so far as could be determined there is no sharp line of demarkation between them as to size. Cells which I identified (on the basis of cytological structure plus staining) as proerythroblasts, were frequently as small as 8.4μ in diameter, whereas erythroblasts were frequently as large as 8μ in diameter. The color of the cytoplasm was no sure criterion because the cells were sometimes in closely packed clusters and superimposed on each other.

Erythroblasts at the other end of the series should, theoretically, be easily differentiated from normoblasts by the greatly increased amount of eosin in the cytoplasm of the

latter. Since this increased affinity for eosin was usually not evident, and since here too, the size limits are not sharply marked off, there was only the nucleus to rely upon. Ordinarily the nucleus of the erythroblast showed a marked, if dense, reticulation with blotches of massed chromatin scattered through this reticulation. I have already referred to it as being of a mottled appearance. Such cells were easily identified. Occasionally cells were found in which the nucleus had begun to condense and therefore to stain more deeply and more homogeneously. In such a borderline case it was a choice between calling the cell an erythroblast or a normoblast and some guesswork was inevitable. Color plate XIV shows the different types of erythroblasts observed. Errors in identification were probably about as frequent in the counts of the normal mice as in those for the anemics, so that the distribution of cell types in one kind of mouse can be compared with the distribution for the other.

The smaller proerythroblasts were sometimes hard to tell from erythroblasts, due to similarity in size and in nuclear structure, but ordinarily this type was not hard to identify. (See Plate XV)

Hemocytoblasts were the least numerous of all the cell types. In fact they were rare. According to Maximow they should be unlike any of these other cells. Many hemocytoblasts were observed, however, having an irregular cytoplasmic outline similar to that of the megakaryocytes. See Plate XVII.

Hemocytoblasts can be confused with small mononucleated megakaryocytes, but on the basis of the fact that most of the megakaryocytes have multilobed nuclei and are of a much larger size ordinarily, all cells of this type having but a single nucleus and being under about 14 in diameter were classed as hemocytoblasts.

In summary then, it may be said that:as to diameter there is a continuous series of types which blend into each other, from the normoblasts up to the megakaryocytes; as to staining reactions, all of these cells did not react in a typical fashion and do not clearly demonstrate the progressive changes from the intensely basic hemocytoblasts to the intensely acidic erythrocytes; and as to structure, many deviations from the type descriptions were observed.

PRESENTATION OF THE DATA

The counts for the five normal animals studied are shown in Tables III, IV, Va, Vb, Vc, VI, and VII. Each horizontal column in these tables represents the count in one high power field, and shows the numbers of the five types of cells classified. For example; reference to Table III will show that Field No.1 within low power area I contained 66 normoblasts, 46 erythroblasts, 3 proerythroblasts, no hemocytoblasts, and 1 megakaryocyte - a total of 116 hemopoietic cells. Reading the totals at the bottom of each vertical column, we see that the sixteen fields counted in mouse No.12 yielded 802 normoblasts, 575 erythroblasts, 41 proerythroblasts, 5 hemocytoblasts, and 27 megakaryocytes, or a total of 1450 cells. The last horizontal column shows that of these 1450 cells, 55.31% were normoblasts, 39.65% were erythroblasts, 2.82% were proerythroblasts, 0.34% were hemocytoblasts, and 1.86% were megakaryocytes. The counts of normal animals are summarized in Table XIII. It will be recalled that sixteen fields were used for four of these animals, but that forty-eight fields were counted for mouse No.20. To make the cell numbers for No.20 comparable with those of the other mice, the totals for the forty-eight fields were divided by three and incorporated in Table XIII. The percentage which each class of cells comprised of the total normal count was obtained and its probable error computed. (See last vertical column.)

Tables VIII, IXa, IXb, IXc, X, XI and XII present in exactly the same way the counts made of anemic mice. Table XIV summarizes these counts for the anemics and is comparable to Table XIII.

Table XV shows the differences in the percentages of each type of cell for normals and anemics. The probable error of these differences was computed, and its statistical significance is indicated. (See the last vertical column)

Tables I and II have already been explained.

DISCUSSION OF THE DATA

Table XV shows the final results of this study. The first thing which attracts one's attention is the difference in the relative number of normoblasts present in the anemics and the normals. The normoblasts of the normals comprised 52.51 ± .425% of the total hemopoietic cell content, whereas in the anemics they comprised only 42.37±.418%. The difference is 10.14±.6%. This difference is seventeen times the size of its probable error, which shows that so far as these data go it is not due to chance and is certainly significant.

It is interesting to note further that there is also a significant difference observable in the relative proportions of erythroblasts. This type comprises only 40.89 ±.418% of the total in the normal and 50.31±.425% in the anemics. The difference is 9.42±.6%, which is sixteen times its probable error. This difference is also unquestionably significant so far as these data go.

The difference between the relative proportions of proerythroblasts is not so great. There are 1.15% more proerythroblasts present in anemics than in normals. The difference is 4.2 times its probable error in this case, which is probably significant, yet the difference here is not nearly as marked as it is between the first two cell types.

As to the proportions of hemocytoblasts and megakaryocytes, there was no significant difference as between normals and anemics. The differences between them are probably due to chance. The hemocytoblasts comprised less than 1% and the

megakaryocytes only slightly more than 1% of either the normal or anemic totals.

It would seem then that the deficiency of normoblasts and the excess of erythroblasts and proerythroblasts in these anemic animals at birth are the most significant facts that these data present.

Two possible explanations of the lack of normoblasts in the anemics at once present themselves. First: it might be due to some factor which was destroying the normoblasts in the anemic animals with the result that fewer erythrocytes were formed; or, second: it might be that the development of the blood was retarded in the anemics so that they were born in a more immature condition than the normals, and hence have a lower blood count.

How do the facts support or discredit these theories? Were the normoblasts undergoing destruction? I am inclined to think they were not. In the first place there was no visible evidence of any destructive processes in the liver,- no degenerative changes, pyknosis, or dissolution of tissue. If these cells, manufactured in the liver, were destroyed, it would probably be within the same organ, as very few normoblasts were found in the circulating blood. Most of them become mature erythrocytes before even entering the blood stream. They are incapable of locomotion by themselves, so they remain in the liver sinusoids until carried away as erythrocytes. Furthermore, it is quite possible that any destructive agent would have some effect on other cells in the vicinity, but nothing of this kind was observable histologically, nor was

there any statistical evidence of it, for the total of all cells was about the same for anemics as for normals (6296 to 6240).

It might be suggested that the destruction occurred just after the erythrocytes enter the blood stream, which would then account for the anemia found by Mixter in his work. In that case the hemopoietic centers would contain a full quota of normoblasts and a study of liver tissue only would not show any difference between normals and anemics - only erythrocytic counts would detect the anemia. Such is not the case. The blood showed a deficiency of erythrocytes, and the liver a lack of normoblasts.

Let us consider the second theory. Were these two types of animals equally developed at birth? The greater body size of the new-born normals would indicate a difference in prenatal growth-rate. This theory is supported by the contemporary work being done by A. A. Andrews. If we may postulate a differential growth-rate, then, it would be quite logical to interpret this condition as the result of retarded blood development. Blood is a tissue. Growth in blood tissue would be expressed in terms of the numerical increase in its cells, and would have to be initiated by factors operating on the hemopoietic organs. Let us see what the evidence is in support of this theory. In the first place we know that ordinarily the hemopoietic function of the liver is lost shortly after birth and this role is assumed by the bone marrow. The counts of the normal animals would seem to indicate that hemopoiesis is in process of being concluded, because in these animals the cells of the generations preceding the normoblasts are in a minority. Although a great many

normoblasts are still present, their source (the erythroblasts) is becoming exhausted and no new supply of the earlier stem cells is being proliferated to take their place.

If the normal animals contained just as many erythroblasts and proerythroblasts as did the anemics per unit area, then we might be justified in supposing the deficiency of normoblasts in the anemics to be due to destruction. Since, however, the normals have fewer erythroblasts and proerythroblasts as well as more normoblasts, it seems logical to conclude that a majority of the erythroblasts in these normals has but recently transformed into normoblasts, and the number of erythroblasts is depleted due to their production having practically ceased. If it is proved that development is retarded in these flexed-tailed animals, then the deficiency of normoblasts could very well be interpreted as meaning that the proliferation and differentiation of hemopoietic cells in the liver has not yet reached its postnatal climax in these retarded flexed-tailed animals but is still in the ascendency. In normals of the same age, on the other hand, the process (due to more rapid growth) has reached its climax and is on a descendency leading eventually to complete cessation. If this is the case then the so-called anemia with which we are dealing is not a pathological anemia, i.e., it is not a condition due either to loss of red blood cells or to an inability of the animal to manufacture them. These animals would then be anemic in the sense that a sixteen day embryo would be "anemic" - i.e., have less blood cells and less hemoglobin - if compared with a twenty

day embryo in which blood volumn and hemoglobin **perdentage** has greatly increased.

One more fact of interest should be brought out. Mr. Mixter found (see Table I) that the erythrocytic count in anemias at birth (3,320,000) is approximately 70% of the erythrocytic count in normals at the same age (4,880,000). In other words, there is a deficiency of about 30%. I have shown that according to these data the normoblast count in the anemic's liver at birth (2668) is approximately 80% of the normoblast count in normals (3277) - or is about 20% deficient. This indicates that some relationship undoubtedly exists between the production of normoblasts and the anemia. Perhaps the 30% deficiency he noted would be entirely accounted for were my work based upon counts of more individuals.

The data seem to support our second theory. However, the theory can not be accepted as definitely proved on the basis of these findings alone. Studies should be made of both the normals and anemics at different age levels preceding and succeeding the one I have used. This investigation could be carried out in the manner described in this paper by making quantitative studies of hemopoietic cells in the liver, beginning with the embryonic period at which this process is initiated and continuing into postnatal life as long as any signs of hemopoiesis are visible in the liver.

The only step in this direction which I have taken is the examination of a seventeen-day-old anemic animal. As may be seen from Plate XII, hemopoiesis in the liver has stopped.

Mixter's data also (Table I) show that the anemia has disappeared by that time. This fragmentary evidence, inadequate as it is, supports our theory that hemopoiesis in anemics is merely retarded, but that it runs its course eventually as in normal animals.

However, it must be admitted that the weakest part of this work is the insufficiency in the number of animals studied. A study of Tables Va, Vb, and Vc (normals) and of IXa, IXb, IXc (anemics) shows how much variation may be found in the widely separated areas of the same liver. Also, much variation was encountered among different mice of the same type. Some of this variation is admittedly due to inaccurate classification of some of the cells, but much of it is undoubtedly attributed to intrinsic difference in the animals. Greater accuracy might have been attained had more cells been counted in each animal, as was done in two of the cases. This should have been done to several more animals of each type. Although these data offer clear-cut points of difference between flexed and normal animals, the possibility still exists, due to the small number of animals used, that these differences are due to a chance selection of animals.

Furthermore, fixed sections of tissues, although valuable in any histological study, do not always give a true picture of the cytology of the tissues being studied. Work of this type should undoubtedly be supplemented by observations on the living cells, using the methods of supravital staining and tissue cultures. Such methods might enable one to identify cells of the hemopoietic series with greater certainty.

CONCLUSIONS

1. Hemopoiesis occurs in the liver of mice less than a day old.

2. Although fixed and stained sections of tissue may be used for such a study, other methods (such as supravital and intravital staining, and tissue cultures) should be used to supplement this one.

3. The livers of anemic animals contain only 80% as many normoblasts as do the livers of normals.

4. The livers of normal animals contain only 80% as many erythroblasts and proerythroblasts as do the livers of the anemics.

5. This difference in the percentages of normoblasts, erythroblasts and proerythroblasts in these two types at birth is interpreted as indicating that hemopoiesis is being concluded in the normals but is delayed in the anemics.

6. These data are significant as far as they go, but they are insufficient. More animals should be studied and a more complete analysis of the condition made of the animals at birth, and at preceding and succeeding ages.

Table I

Course of the Anemia From Birth as Shown in Red and White Blood Cell Counts and Hemoglobin Percentages. (Compiled from Mixter's Data)

Age	Average Erythrocyte Count			Average Hemoglobin %*			Average Leucocyte Count		
	Flexed	Hetz. str.	Homo. str.	Flexed	Hetz. str.	Homo. str.	Flexed	Hetz. str.	Homo. str.
Birth	3,320,000	4,880,000	4,010,000	39.4%	60.7%	56.9%	4,297	4,129	3,993
1 week	5,125,300	5,510,000	5,100,000	49.0%	57.8%	53.6%	4,192	4,481	5,000
2 weeks	6,800,000	6,970,000	6,600,000	53.8%	55.6%	56.0%	5,086	6,167	5,000
3 weeks	8,120,000	7,730,000	6,750,000	58.4%	57.0%	52.0%	5,805	6,502	5,600
Adult[1]	11,200,000	11,050,000	9,690,000	68.7%	64.0%	65.8%	10,000	9,600	11,000

*The concentration of hemoglobin is expressed as the percentage of hemoglobin found in normal human blood.

[1]The adults used here were the parents of the back-cross young and varied in age from six months to almost two years at the time the counts were made.

Table II

Distribution of Sizes of the Different Cells
of the Erythrocytic Genetic Lineage Based Upon Absolute Measurements.

Diameter of cell (in micra)	No. of Nbl	No. of Ebl	No. of Pbl	No. of Hbl	No. of Meg
4.5 - 4.9	3				
5.0 - 5.4	10				
5.5 - 5.9	30				
6.0 - 6.4	6	35			
6.5 - 7.4	1	30			
7.5 - 8.4		15*	23*		
8.5 - 9.4			18		
9.5 - 10.4			21		
10.5 - 11.4			18	5	
11.5 - 12.4				7	
12.5 - 13.4				5	
13.5 - 14.4				7	3
14.5 - 15.4					2
15.5 - 20.4					30
20.5 - 25.4					32
25.5 - 30.4					10
30.5 - 35.4					1
35.5 - 40.4					2
Total No. of Cells Measured.	50	80	80	25	80

*All but one of these 23 proerythroblasts measured 8.4μ which was practically the minimum size of this type. None of the erythroblasts measured over 8μ. This overlapping is not as large as it seems to be in the table.

Table III

Count of Blood Cells in Mouse No.12 (Normal)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	66	46	3	0	1	116
	2	57	38	3	0	0	98
	3	67	52	2	0	1	122
	4	35	28	2	0	3	68
	5	64	48	1	0	0	113
	6	32	20	0	0	2	54
	7	34	33	3	1	2	73
	8	51	45	4	2	1	103
II	9	67	39	4	2	2	114
	10	45	45	2	0	1	93
	11	42	43	3	0	0	88
	12	68	36	1	0	3	108
	13	50	38	4	0	1	93
	14	46	27	4	0	4	81
	15	40	20	3	0	4	67
	16	38	17	2	0	2	59
	16	802	575	41	5	27	1450
% of total count		55.31%	39.65%	2.82%	0.34%	1.86%	

*Key to abbreviations used in Tables III to XV inclusive:
Nbl - normoblasts; Ebl - erythroblasts; Pbl - proerythroblasts;
Hbl - hemocytoblasts; Meg - megakaryocytes.

Table IV

Count of Blood Cells in Mouse No.13 (Normal)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	22	32	2	1	3	60
	2	39	40	6	0	3	88
	3	57	37	7	0	1	102
	4	35	32	3	0	3	73
	5	14	32	1	1	1	49
	6	40	26	2	0	1	69
	7	30	35	3	0	0	68
	8	67	45	2	0	0	114
II	9	48	33	3	0	2	86
	10	34	20	0	0	1	55
	11	50	26	3	0	1	80
	12	28	44	7	0	0	79
	13	31	39	5	0	0	75
	14	36	34	2	0	1	73
	15	40	35	5	0	0	80
	16	27	22	5	0	0	54
	16	598	532	56	2	17	1205
% of total count		49.62%	44.14%	4.64%	0.16%	1.41%	

Table Va

Count of Blood Cells in Mouse No.20 (Normal)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	51	17	4	0	0	72
	2	48	15	5	1	1	70
	3	27	26	11	1	0	65
	4	50	55	7	0	2	114
	5	46	25	5	2	3	81
	6	28	51	3	0	0	82
	7	58	36	8	0	5	107
II	8	51	30	4	1	1	87
	9	34	23	4	0	0	61
	10	63	53	5	0	1	122
	11	35	40	7	0	6	88
	12	44	26	4	0	1	75
	13	30	25	0	1	1	57
	14	36	40	2	0	3	81
III	15	50	13	0	1	0	64
	16	32	11	2	0	0	45
	16	683	486	71	7	24	1271
% of total count		53.73%	38.23%	5.50%	0.55%	1.88%	

Table Vb

Count of Blood Cells in Mouse No.20 (Con't.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	50	38	7	0	0	95
	2	43	34	4	0	0	81
	3	60	45	5	0	0	110
	4	65	48	3	0	1	117
	5	30	45	3	0	1	79
	6	40	47	7	0	0	94
	7	14	26	6	0	1	47
	8	65	46	13	0	0	114
	9	34	36	3	2	0	75
	10	25	34	3	0	1	63
II	11	36	36	5	0	1	78
	12	37	32	3	0	1	73
	13	58	41	1	0	2	102
	14	68	35	8	0	2	113
	15	72	40	3	1	1	117
	16	50	50	4	0	1	105
	16	747	633	78	3	12	1473
% of total count		50.71%	42.97%	5.29%	0.20%	0.81%	

Table Vb

Count of Blood Cells in Mouse No.20 (Con't.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	50	38	7	0	0	95
	2	43	34	4	0	0	81
	3	60	45	5	0	0	110
	4	65	48	3	0	1	117
	5	30	45	3	0	1	79
	6	40	47	7	0	0	94
	7	14	26	6	0	1	47
	8	65	46	13	0	0	114
	9	34	36	3	2	0	75
	10	25	34	3	0	1	63
II	11	36	36	5	0	1	78
	12	37	32	3	0	1	73
	13	58	41	1	0	2	102
	14	68	35	8	0	2	113
	15	72	40	3	1	1	117
	16	50	50	4	0	1	105
	16	747	633	78	3	12	1473
% of total count		50.71%	42.97%	5.29%	0.20%	0.81%	

Table Vc

Count of Blood Cells in Mouse No.20 (Con'd.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	60	41	5	0	1	107
	2	62	34	1	1	1	99
	3	12	24	3	0	0	39
	4	47	41	7	0	2	97
	5	42	48	7	0	1	98
	6	43	42	3	0	1	89
	7	48	60	10	2	2	122
	8	73	72	9	0	3	157
II	9	24	40	9	0	2	75
	10	56	38	4	1	1	100
	11	26	40	8	1	0	75
	12	21	45	5	0	0	71
	13	49	53	6	0	0	108
III	14	38	14	1	0	0	53
	15	27	13	0	0	0	40
	16	11	10	1	0	0	22
	16	639	615	79	5	14	1352
% of total count		47.26%	45.48%	5.84%	0.36%	1.03%	

Table VI

Count of Blood Cells in Mouse No.23 (Normal)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	64	45	5	0	3	117
	2	57	52	4	0	1	114
	3	35	26	3	0	0	64
	4	56	43	7	1	1	108
	5	50	23	4	0	2	79
	6	57	36	5	0	3	101
	7	57	44	7	0	1	109
II	8	56	42	2	0	0	100
	9	29	32	2	0	3	66
	10	11	16	2	0	0	29
	11	41	35	3	0	0	79
	12	30	37	3	2	0	72
	13	32	40	4	0	3	79
III	14	30	31	5	0	0	66
	15	37	11	2	0	1	51
	16	52	34	4	0	1	91
	16	694	562	62	3	19	1325
% of total count		52.37%	41.28%	4.68%	0.22%	1.43%	

Table VII

Count of Blood Cells in Mouse No.26 (Normal)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	36	22	6	2	0	66
	2	31	29	3	0	2	65
	3	35	24	2	1	0	62
	4	28	15	8	1	0	52
	5	40	11	4	0	0	55
	6	32	30	9	1	1	73
	7	15	14	3	0	2	34
II	8	37	19	4	0	0	60
	9	25	20	6	1	0	52
	10	27	17	8	0	0	52
	11	28	32	3	0	0	63
	12	39	27	4	0	0	70
	13	33	15	5	0	0	53
	14	26	18	2	0	1	47
III	15	42	15	1	0	0	58
	16	19	12	1	0	0	32
	16	493	320	69	6	6	894
% of total count		55.14%	35.79%	7.71%	0.67%	0.67%	

Table VIII

Counts of Blood Cells in Mouse No.17A (Anemic)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	21	35	5	0	0	61
	2	35	43	9	0	2	89
	3	70	42	7	0	0	119
	4	20	42	7	0	0	69
	5	49	47	8	0	0	104
	6	52	47	5	1	0	105
	7	38	45	8	0	0	91
	8	39	46	9	0	1	95
	9	27	36	7	0	2	72
	10	32	43	5	0	0	80
	11	51	36	4	0	3	94
II	12	28	59	8	0	0	95
	13	32	37	3	0	1	73
	14	29	50	3	0	0	82
	15	63	60	8	0	0	131
	16	37	46	4	0	0	87
	16	623	714	100	1	9	1447
% of total count		43.05%	49.34%	6.91%	0.07%	0.62%	

Table IXa

Count of Blood Cells of Mouse No.18A (Anemic)

	Field No.	Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	14	29	3	0	0	46
	2	44	26	6	0	1	77
	3	27	40	4	0	0	71
	4	11	8	3	0	0	22
	5	8	12	3	0	2	25
	6	6	20	0	0	0	26
	7	47	16	0	0	0	63
II	8	27	30	3	0	2	62
	9	11	37	2	1	2	53
	10	28	32	3	0	1	64
	11	24	37	1	0	2	64
	12	36	14	5	0	0	55
	13	21	39	3	0	1	64
	14	21	33	3	0	1	58
III	15	35	43	1	0	4	83
	16	30	34	6	1	1	72
	16	390	450	46	2	17	905
	% of total count	43.09%	49.72%	5.08%	0.22%	1.87%	

Table IXb

Count of Blood Cells of Mouse No.18A (Con't.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	44	44	5	1	0	94
	2	28	27	7	0	2	64
	3	19	37	8	0	0	64
	4	15	42	6	0	2	65
	5	35	56	4	0	1	96
	6	23	40	8	0	1	72
	7	31	38	9	1	0	79
II	8	21	23	5	0	0	49
	9	25	26	3	0	1	55
	10	40	37	5	1	1	84
	11	17	55	9	0	1	82
	12	30	67	7	0	0	104
	13	15	40	6	0	1	62
	14	16	38	3	0	2	59
	15	29	25	4	0	0	58
III	16	33	35	5	0	0	73
	16	421	630	94	3	12	1160
% of total count		36.29%	54.31%	8.1%	0.25%	1.03%	

Table IXb

Count of Blood Cells of Mouse No.18A (Con't.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	44	44	5	1	0	94
	2	28	27	7	0	2	64
	3	19	37	8	0	0	64
	4	15	42	6	0	2	65
	5	35	56	4	0	1	96
	6	23	40	8	0	1	72
	7	31	38	9	1	0	79
II	8	21	23	5	0	0	49
	9	25	26	3	0	1	55
	10	40	37	5	1	1	84
	11	17	55	9	0	1	82
	12	30	67	7	0	0	104
	13	15	40	6	0	1	62
	14	16	38	3	0	2	59
	15	29	25	4	0	0	58
III	16	33	35	5	0	0	73
	16	421	630	94	3	12	1160
% of total count		36.29%	54.31%	8.1%	0.25%	1.03%	

Table IXc

Count of Blood Cells of Mouse No.18A (Con'd.)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	40	33	4	1	0	78
	2	16	23	6	0	1	46
	3	23	33	4	1	0	61
	4	50	40	4	0	0	94
	5	30	35	3	0	1	69
	6	55	35	1	0	2	93
	7	52	47	5	0	0	104
	8	60	55	4	0	4	123
II	9	43	46	6	0	0	95
	10	46	58	8	0	0	112
	11	42	47	4	0	1	94
	12	31	44	5	0	2	82
	13	49	33	1	1	1	85
	14	32	27	6	0	0	65
	15	23	25	3	0	3	54
	16	34	44	5	0	0	83
	16	626	625	69	3	15	1338
% of total count		46.78%	46.71%	5.15%	0.22%	1.12%	

Table X

Count of Blood Cells of Mouse No.21A (Anemic)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	50	45	0	0	1	96
	2	33	38	1	0	3	75
	3	82	46	0	0	1	129
	4	43	27	2	0	1	73
	5	33	41	3	1	1	79
	6	42	34	2	0	0	78
II	7	18	41	3	0	0	62
	8	19	47	2	0	1	69
	9	28	43	4	0	0	75
	10	34	40	3	0	1	78
	11	39	34	3	0	1	77
	12	28	33	4	0	0	65
	13	35	22	2	1	4	64
	14	38	37	3	0	3	81
	15	25	46	1	0	0	72
	16	19	32	3	0	0	54
	16	566	606	36	2	17	1227
% of total count		46.12%	49.38%	2.93%	0.16%	1.38%	

Table XI

Count of Blood Cells of Mouse 22A (Anemic)

	Field No.	Nbl	EBl	Pbl	Hbl	Meg	Total
I	1	26	43	6	0	1	76
	2	36	32	8	1	0	77
	3	35	48	6	0	1	90
	4	29	37	5	0	1	72
	5	30	40	2	0	0	72
	6	35	37	7	0	3	82
	7	51	30	4	0	1	86
II	8	12	42	7	0	2	63
	9	27	40	8	0	1	76
	10	26	48	6	0	4	84
	11	36	35	5	0	1	77
	12	45	49	7	0	2	103
	13	28	40	6	0	1	75
	14	22	37	5	1	0	65
	15	38	42	6	0	0	86
	16	57	49	5	0	0	111
	16	533	649	93	2	18	1295
	% of total count	41.15%	50.11%	7.18%	0.15%	1.38%	

Table XII

Count of Blood Cells of Mouse No.24A (Anemic)

Field No.		Nbl	Ebl	Pbl	Hbl	Meg	Total
I	1	25	29	3	0	1	58
	2	36	32	3	0	0	71
	3	22	48	6	0	0	76
	4	31	34	5	0	0	70
	5	28	43	8	0	2	81
	6	23	46	5	0	1	75
	7	31	29	9	0	0	69
	8	33	42	3	0	0	78
	9	44	50	4	0	0	98
II	10	35	52	5	0	0	92
	11	31	36	6	0	0	73
	12	9	29	5	0	0	43
	13	19	36	3	1	0	59
	14	54	42	7	0	0	103
	15	26	35	5	1	3	70
	16	20	47	3	1	5	76
	16	467	630	80	3	12	1192
% of total count		39.17%	52.85%	6.71%	0.25%	1.01%	

Table XIII

Summary of all the Counts on Normal Animals (Summary of Tables III - VII inc.)

Mouse		#12	#13	#20 (Average)	#23	#26	Total	P.E. of Percentage
Nbl	No.	802	598	690	694	493	3277	
	%	55.31%	49.62%	50.51%	52.37%	55.14%	52.51%	± .43%
Ebl	No.	575	532	578	547	320	2552	
	%	39.65%	44.14%	42.31%	41.28%	35.79%	40.89%	± .42%
Difference Between % of Nbl & Ebl		15.66%	5.48%	8.20%	11.09%	19.35%	11.62%	
Pbl	No.	41	56	76	62	69	304	
	%	2.82%	4.64%	5.56%	4.68%	7.71%	4.87%	± .18%
Hbl	No.	5	2	5	3	6	21	
	%	0.34%	0.16%	0.36%	0.22%	0.67%	0.33%	± .05%
Meg	No.	27	17	17	19	6	86	
	%	1.86%	1.41%	1.24%	1.43%	0.67%	1.37%	± .09%
Total		1450	1205	1366	1325	894	6240	

Table XIV

Summary of all the Counts of Anemic Animals (Summary of Tables VIII - XII inc.)

Mouse		#17A	#18A (Average)	#21A	#22A	#24A	Total	P.E. of Percentage
Nbl	No.	623	479	566	533	467	2668	
	%	43.05%	42.20%	46.12%	41.15%	39.17%	42.37%	± .42%
Ebl	No.	714	568	606	649	630	3167	
	%	49.34%	50.04%	49.38%	50.11%	52.85%	50.31%	± .43%
Difference between % of Nbl & Ebl		6.29%	7.84%	3.26%	8.96%	13.68%	7.94%	
Pbl	No.	100	70	36	93	80	379	
	%	6.91%	6.16%	2.93%	7.18%	6.71%	6.02%	± .20%
Hbl	No.	1	3	2	2	3	11	
	%	0.07%	0.25%	0.16%	0.15%	0.25%	0.17%	± .03%
Meg	No.	9	15	17	18	12	71	
	%	0.62%	1.31%	1.38%	1.38%	1.01%	1.12%	± .09%
Total		1447	1135	1227	1295	1192	6296	

Table XI

Difference Between Total Percentages of the Different Cell Types for Normals and Anemics (Summary of Tables XIII and XIV)

Cell Type	% of Total Normal Count	% of Total Anemic Count	Difference between Percentages	No. of times difference exceeds P.E.
Nbl	52.51% ±.43%	42.37% ±.42%	10.14% ±.60%	17.0
Ebl	40.89% ±.42%	50.31% ±.43%	9.42% ±.60%	16.0
Pbl	4.87% ±.18%	6.02% ±.20%	1.15% ±.27%	4.3
Hbl	0.33% ±.05%	0.17% ±.03%	0.16% ±.06%	2.6
Meg	1.37% ±.09%	1.12% ±.09%	0.25% ±.13%	2.0

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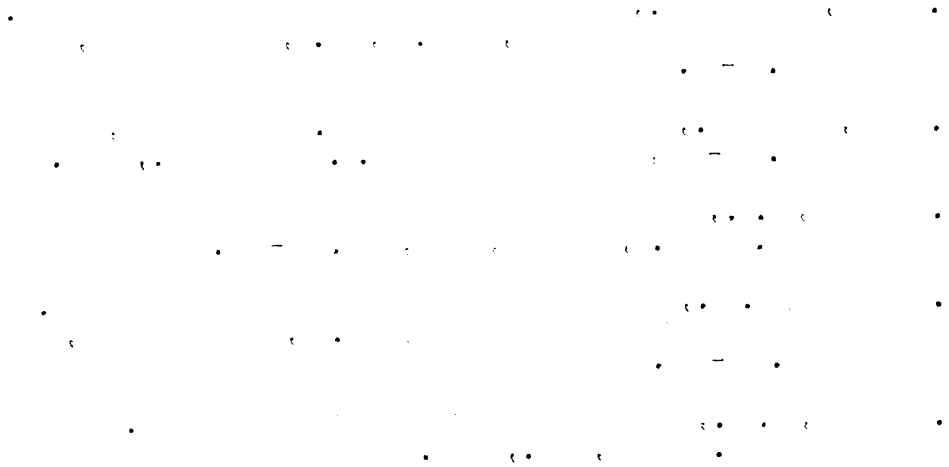


EXPLANATION OF THE FOLLOWING PLATES

The first twelve plates are microphotographs of various tissues and organs of the normal and anemic animals at birth (except Plate XII which is the liver of a 17-day old anemic mouse). These photographs were taken with a Leitz microphotograph camera. Magnification in all cases was x450. Plate VII is an enlargement of the picture used for Plate V, and is intended to bring out the details of cell structure.

Plates XIII to XVI inclusive are free-hand illustrations done with colored drawing pencil by the author. They are drawn approximately to scale (11.0μ to the inch). Magnification was in all cases x600. These plates are to show the various staining reactions observed in the five groups of hemopoietic cells.

KEY TO ABBREVIATIONS USED IN PLATES I TO XII INC.

C - cartilage

c.v. - a central vein

ebl - erythroblasts

ebl' - erythroblasts in mitosos

ecy - erythrocytes

ecy* - unstained or faintly stained erythrocytes

hbl - hemocytoblasts

L - cord of liver cells

lcy - lymphocytes

lcy' - lymphocytes in mitosis

L.n. - nucleus of a liver cell

meg - megakaryocyte

nbl - normoblast

nbl* - normoblast with unstained or faintly stained cytoplasm

obl - osteoblasts

pbl - proerythroblasts

P.l. - polymorphous leucocytes

v - vein

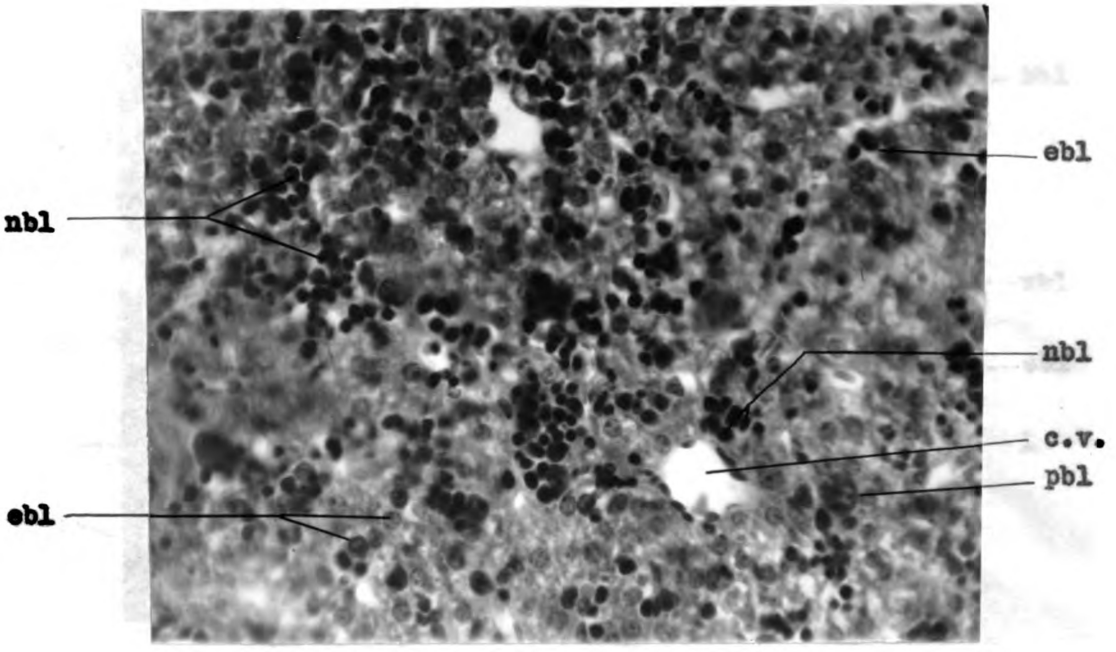


Plate I

Liver of normal mouse (No.12) at birth.

Note the predominance of normoblasts. (Acid eosin-hematoxylin stain)

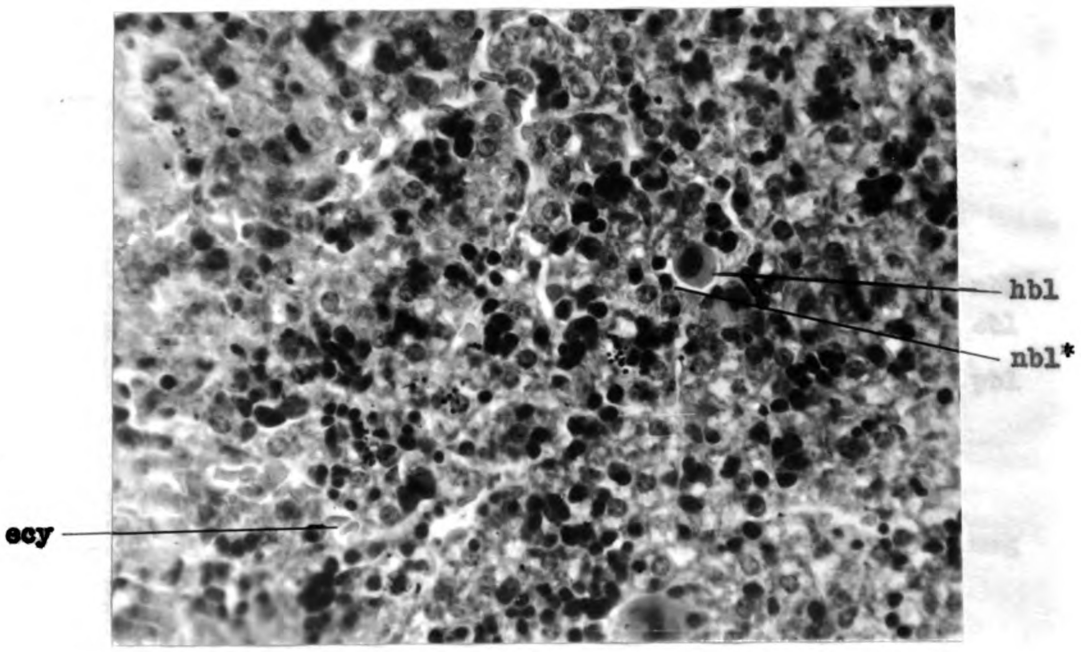


Plate II

Same as above but showing a different area.

Note the difference in size of normoblast nuclei.

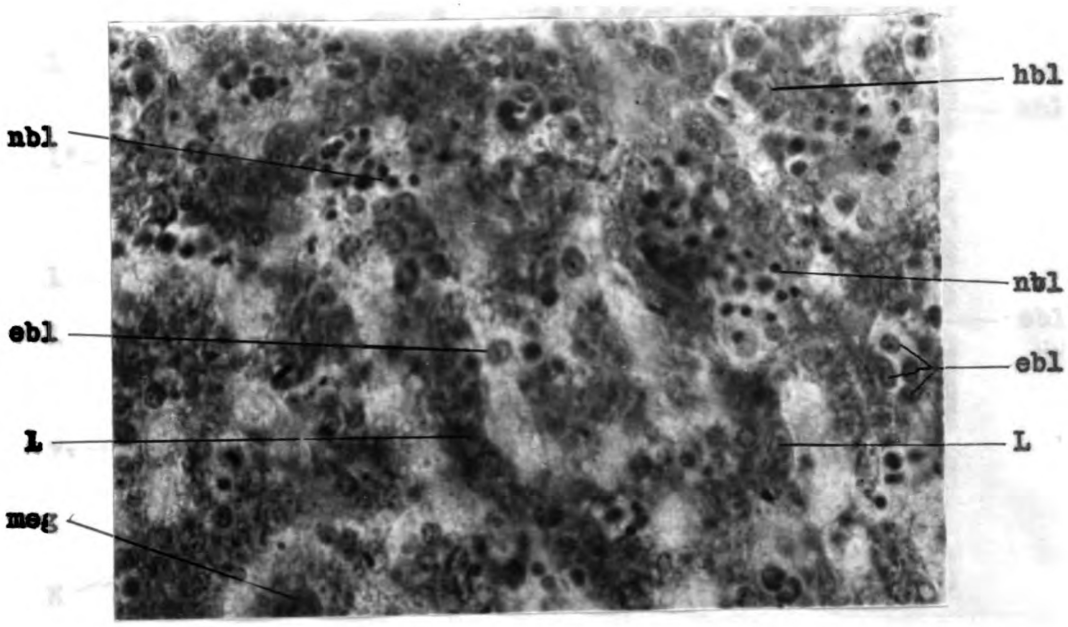


Plate III

Liver of a normal mouse (No.20) at birth.

Note the clusters of normoblasts. (Hematoxylin-eosin-azure stain)

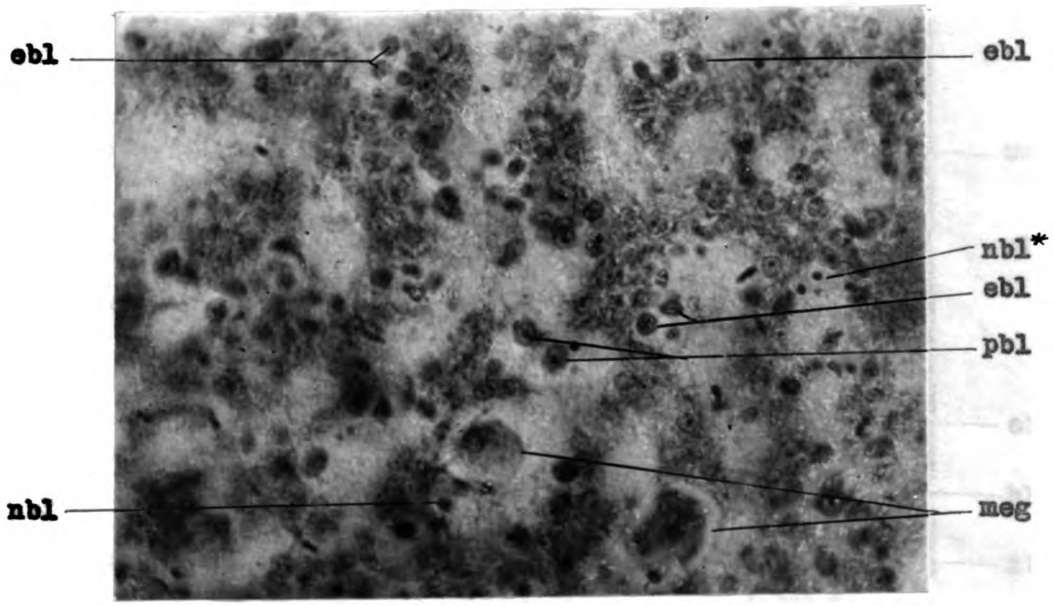


Plate IV

Same as above but a different area.

Note the predominance of erythroblasts. Compare with Plates I,II,III.

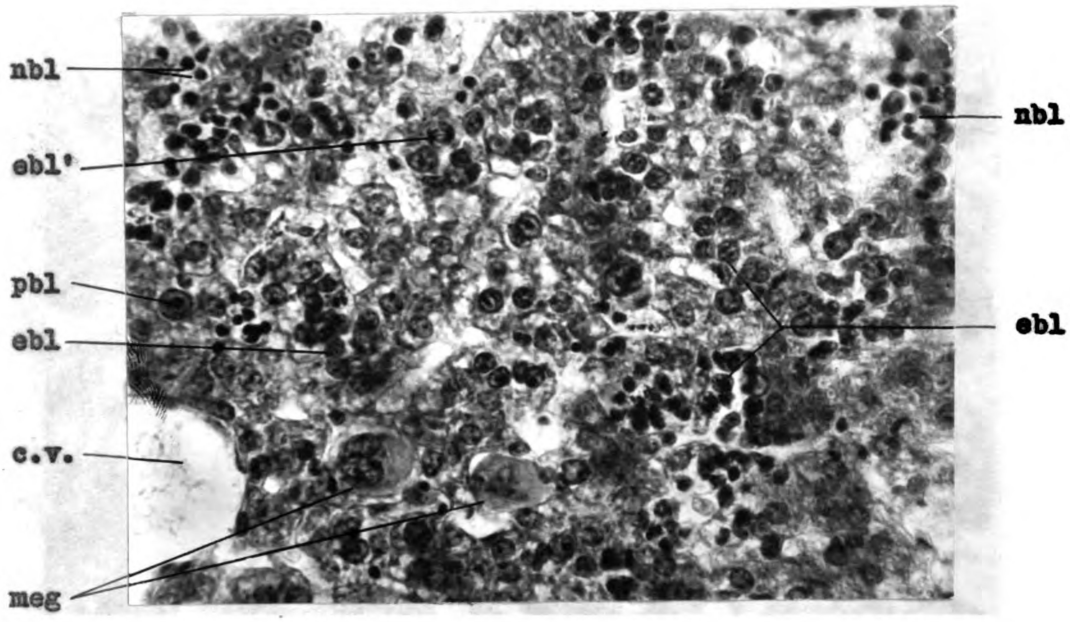


Plate V

Liver of an anemic mouse (No.18A) at birth.

Shows marked predominance of erythrocytes over normoblasts.

Compare with Plates I and II. (Hematoxylin-eosin stain)

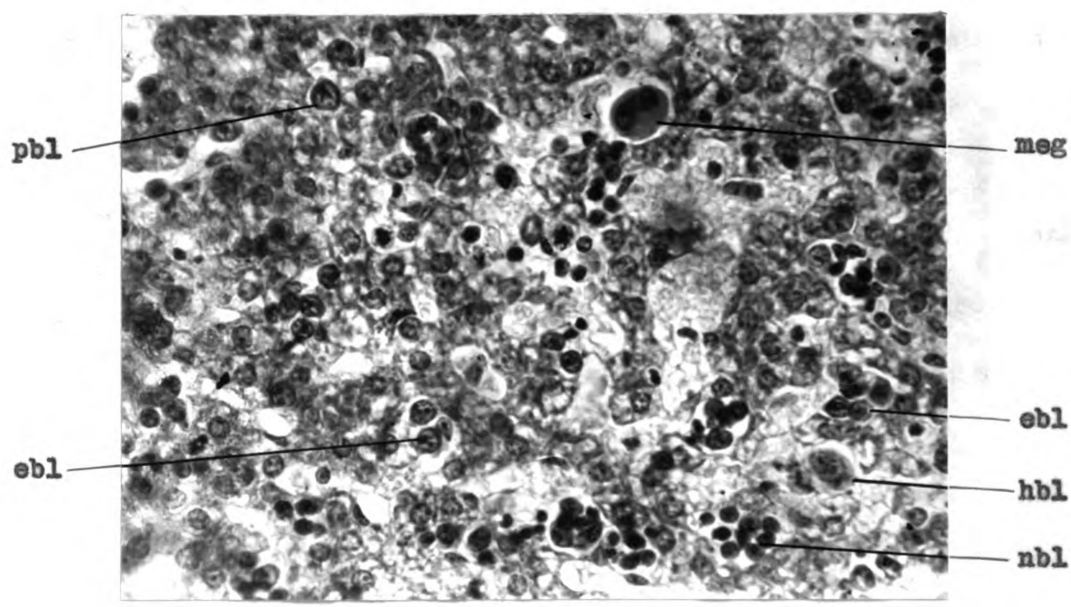


Plate VI

Same as above but a different field.

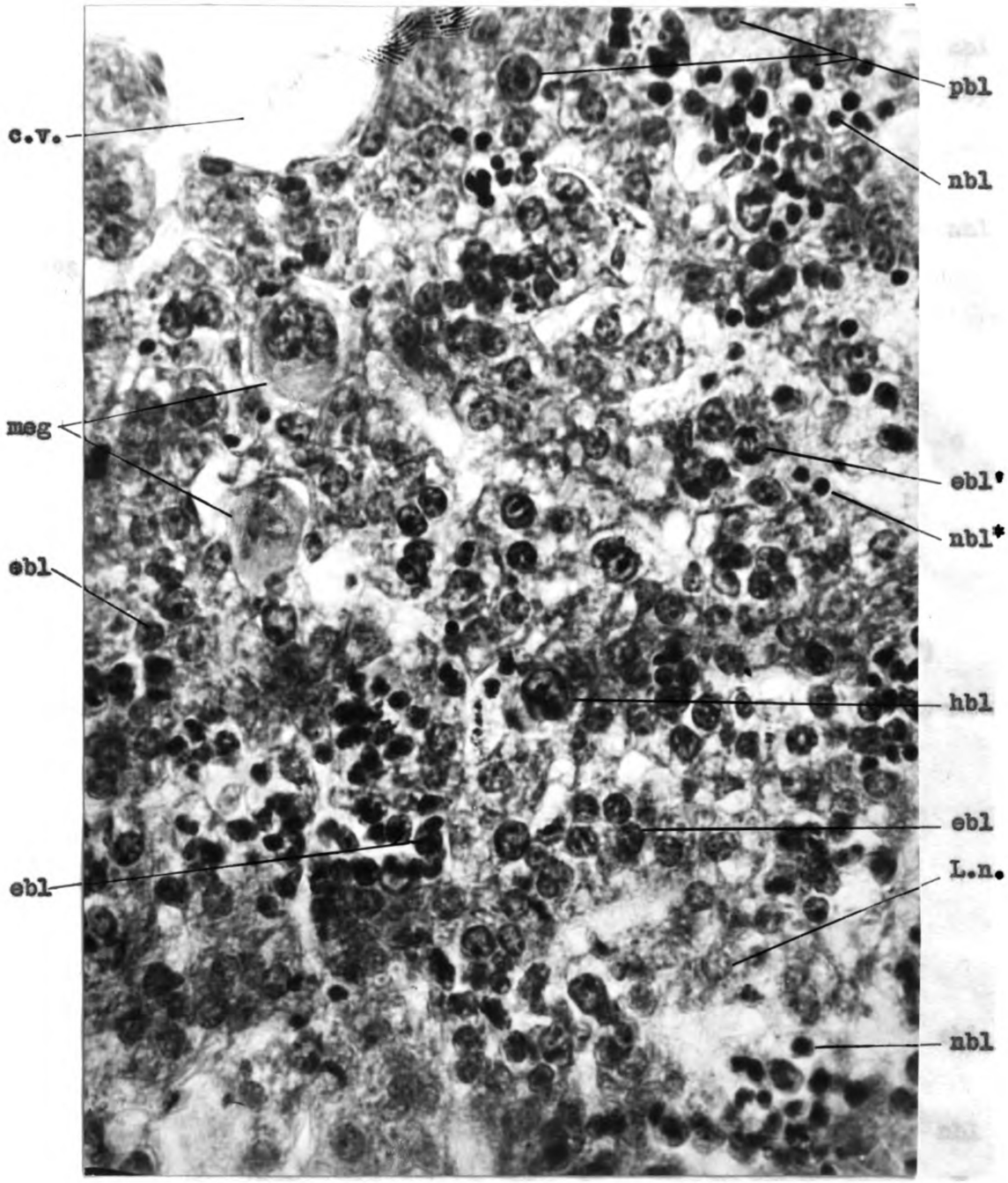


Plate VII

Liver of an anemic mouse (No.18A) at birth.

(An enlargement of Plate V)

Note the morphology of the liver at this age.

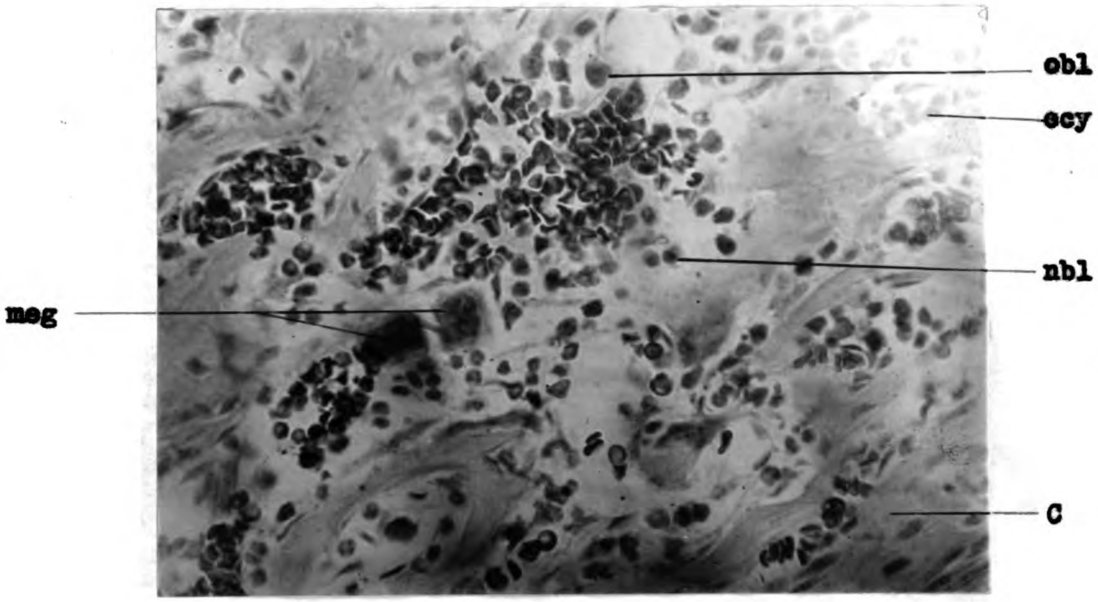


Plate VIII

Bone marrow from the humerus of a normal mouse (No.27)

Note the distorted erythrocytes in top center.(Hematoxylin-eosin-azure).

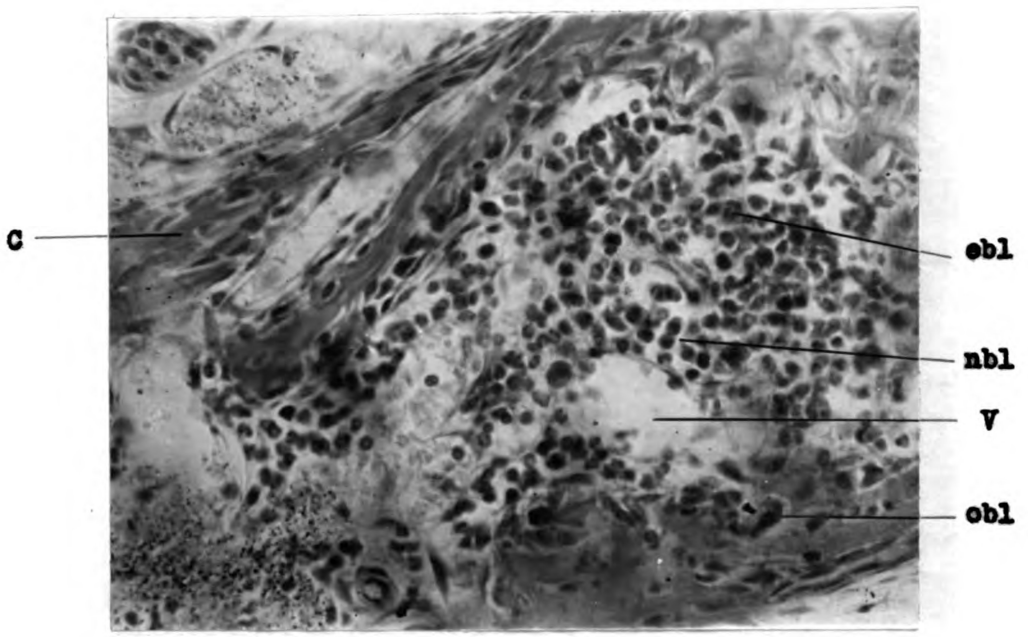


Plate IX

Bone marrow from rib of a normal mouse (No.20).

Note the intensely stained normoblasts and erythroblasts.

(Hematoxylin-eosin-azure stain)

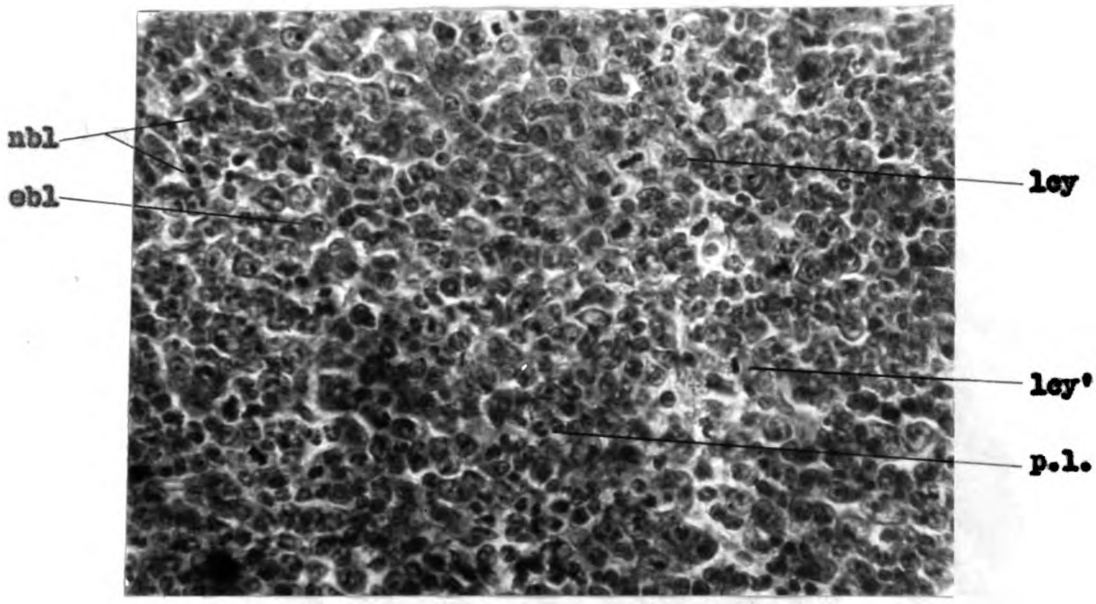


Plate X

Spleen of an anemic mouse (No.18A) at birth.

Note the compactness of the cells, their dense staining, and the numerous mitotic figures. (Hematoxylin-eosin stain)

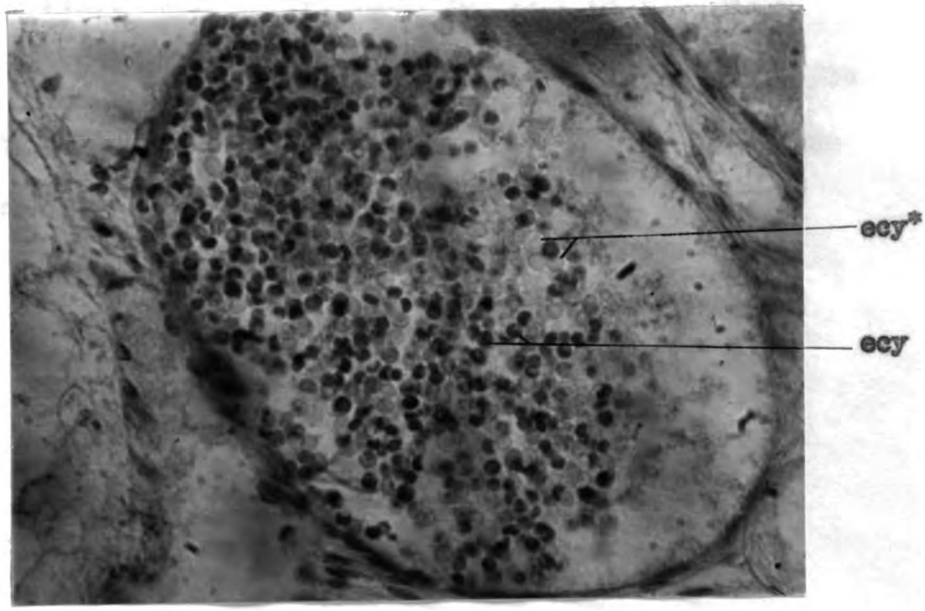


Plate XI

X-section of a blood vessel of a normal mouse (No.20) at birth.

Note the stained and unstained erythrocytes. (Hematoxylin-eosin-azure).

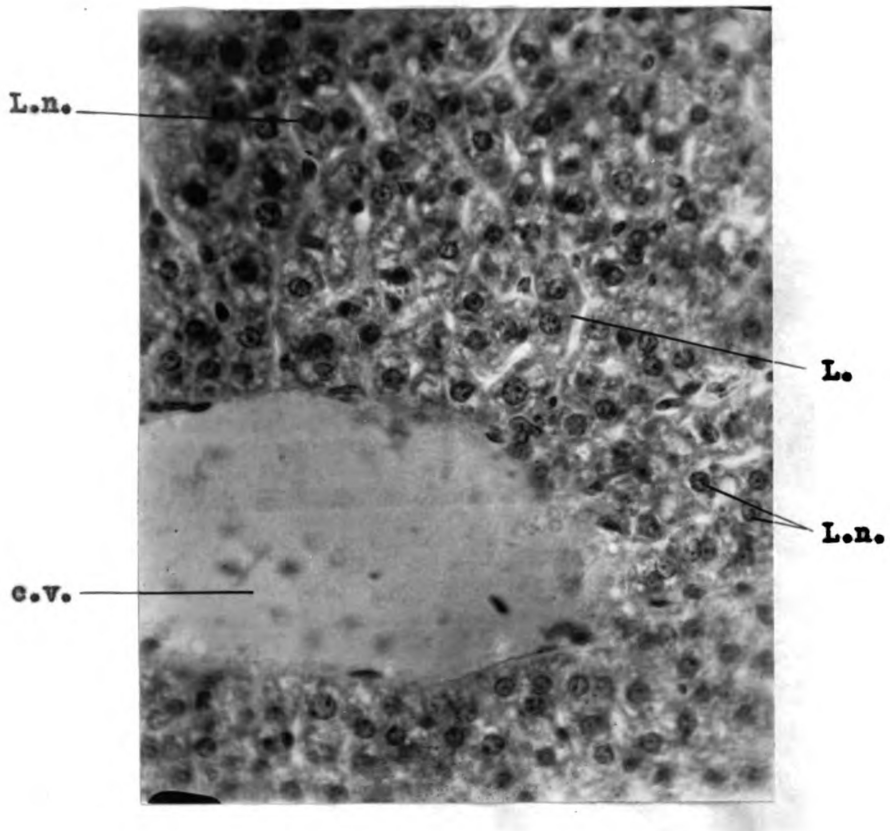


Plate XII

Liver of an anemic mouse (No.26A) 17 days old. Note the characteristic appearance of the liver cords, and the absence of hemopoietic cells. Compare the appearance of this liver with those at birth. (Plates I - VII inc.)

(Acid eosin-hematoxylin stain)

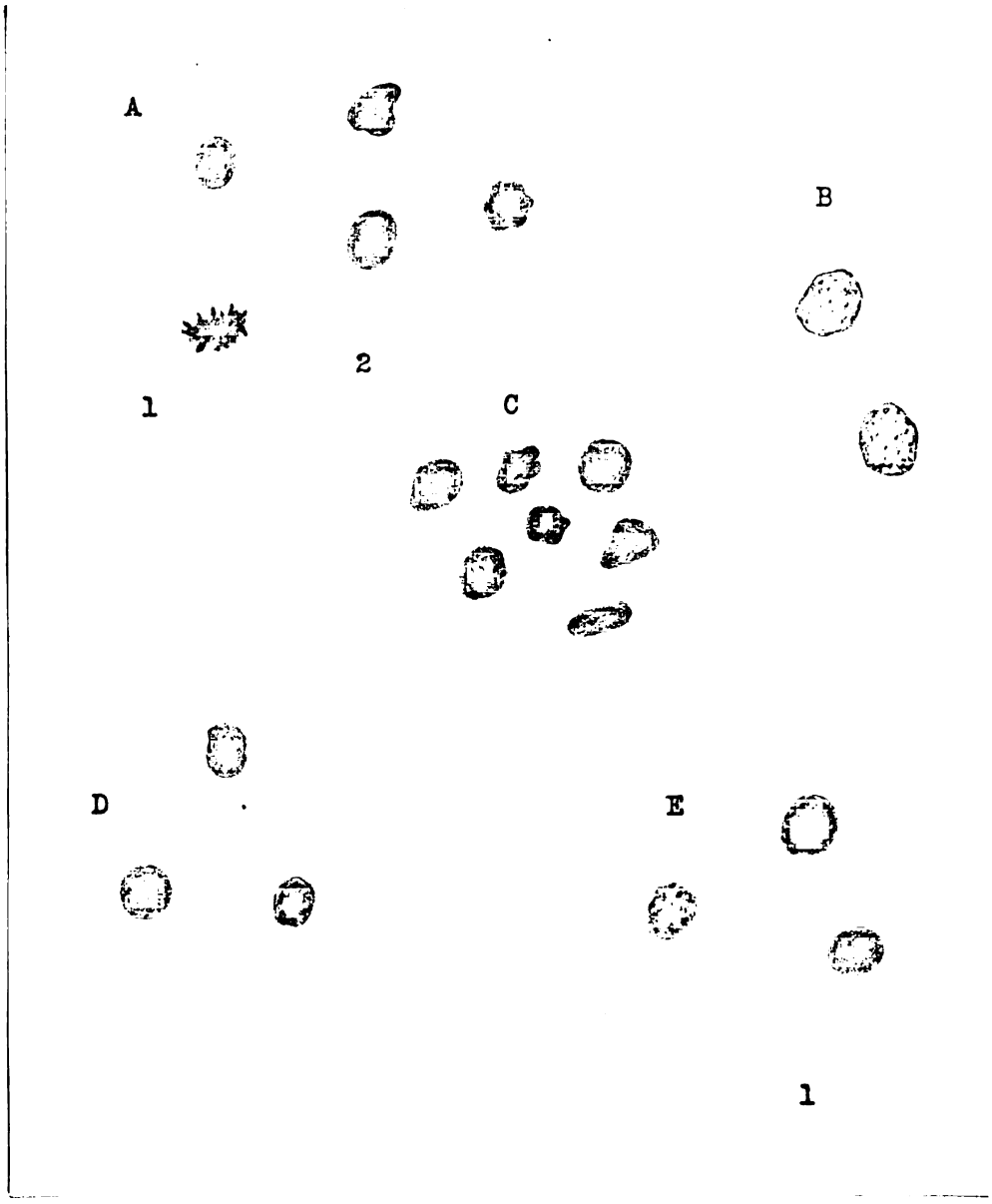


Plate XIII

NORMOBLASTS

Groups A, B, C, and D: Cells stained with hematoxylin-eosin-azure.

Group A: Cells of average size showing the faintly basic-staining cytoplasm, and dense, homogeneous nuclei. No.1 - in mitosis. No.2 - shows a faint trace of pink in the cytoplasm.

Group B: Large sized younger normoblasts; somewhat mottled nuclei.

Group C: Extruded nuclei.

Group D: Cells with unstained cytoplasm.

Group E: Cells stained with acid eosin-hematoxylin. No.1 - shows the nucleus being extruded.

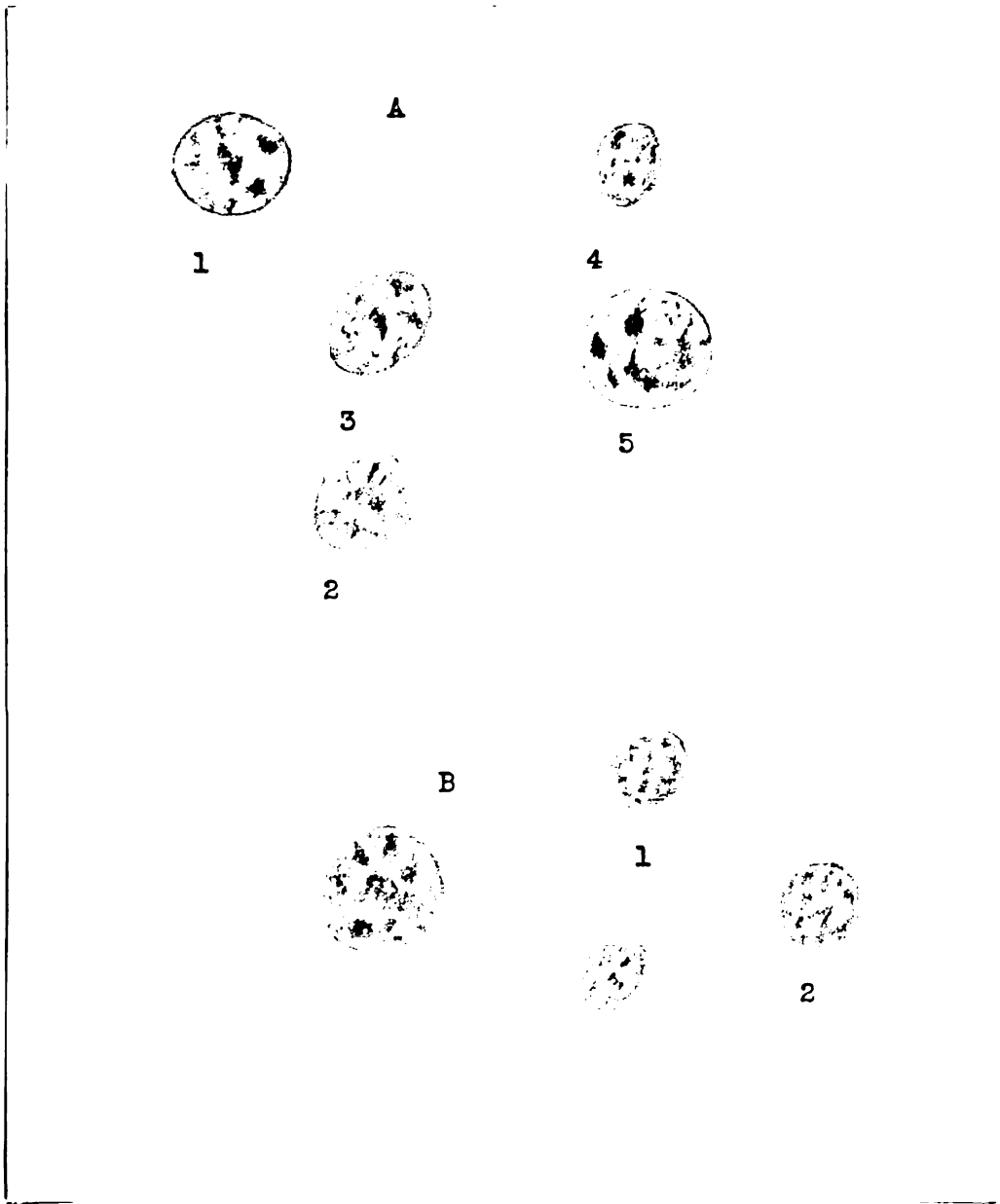
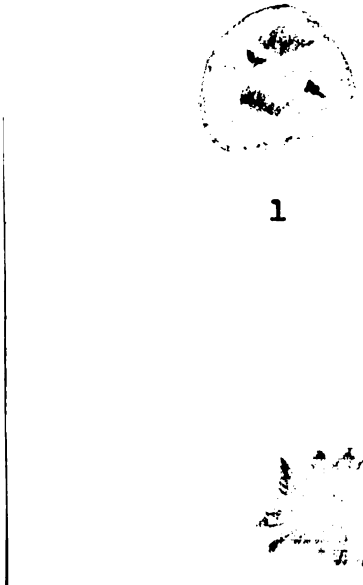


Plate XIV

ERYTHROBLASTS

Group A: Stained with hematoxylin-eosin-azure. Nos. 1,2, and 3 - average sized erythroblasts. No. 3 - shows a pinkish tint in the cytoplasm. No.4 - small sized erythroblast; compare this with normoblasts (Plate XIII). No.5 - large erythroblast.

Group B: Stained with acid eosin-hematoxylin. Compare No.1 and No. 2 with normoblasts.



1

2

3

Plate XV

PROERYTHROBLASTS

No. 1 - Average sized proerythroblast.

No.2 and No. 3 - Large proerythroblasts.

No.3 - In mitosis.

Hematoxylin-eosin-azure stain.

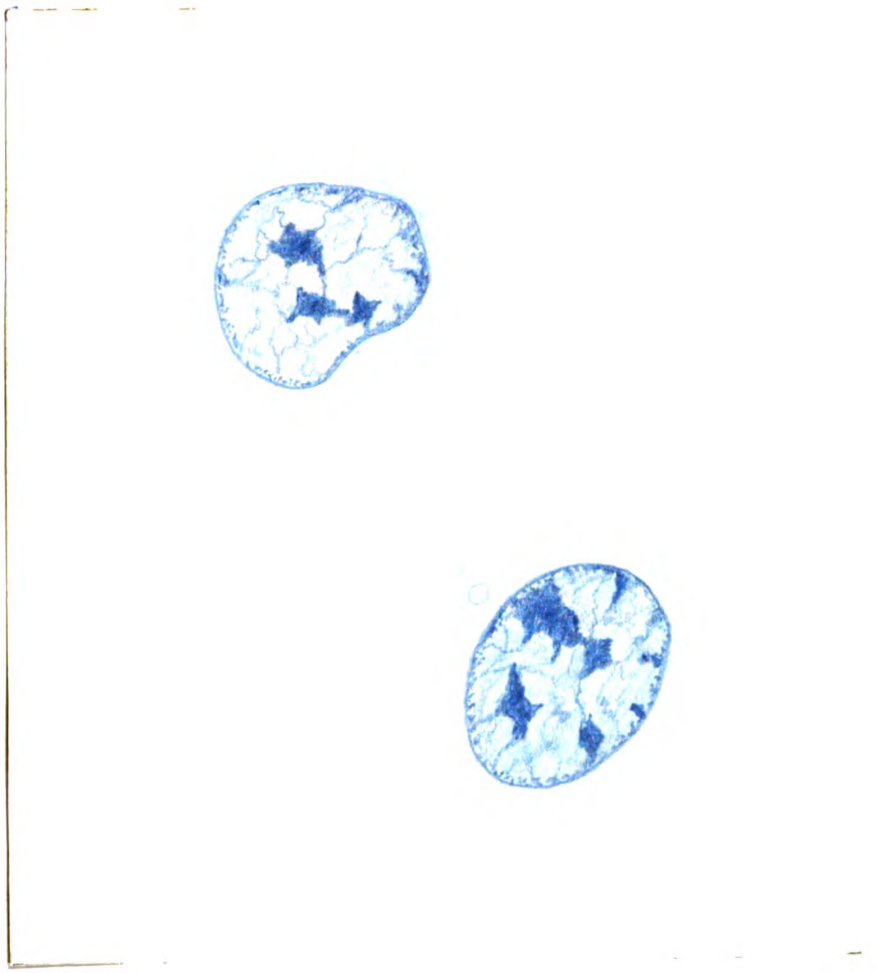


Plate XVI

HEMOCYTOBLASTS

A pair of average sized hemocytoblasts.

Note the irregular extensions of the cytoplasm.

Hematoxylin-eosin-azure stain.

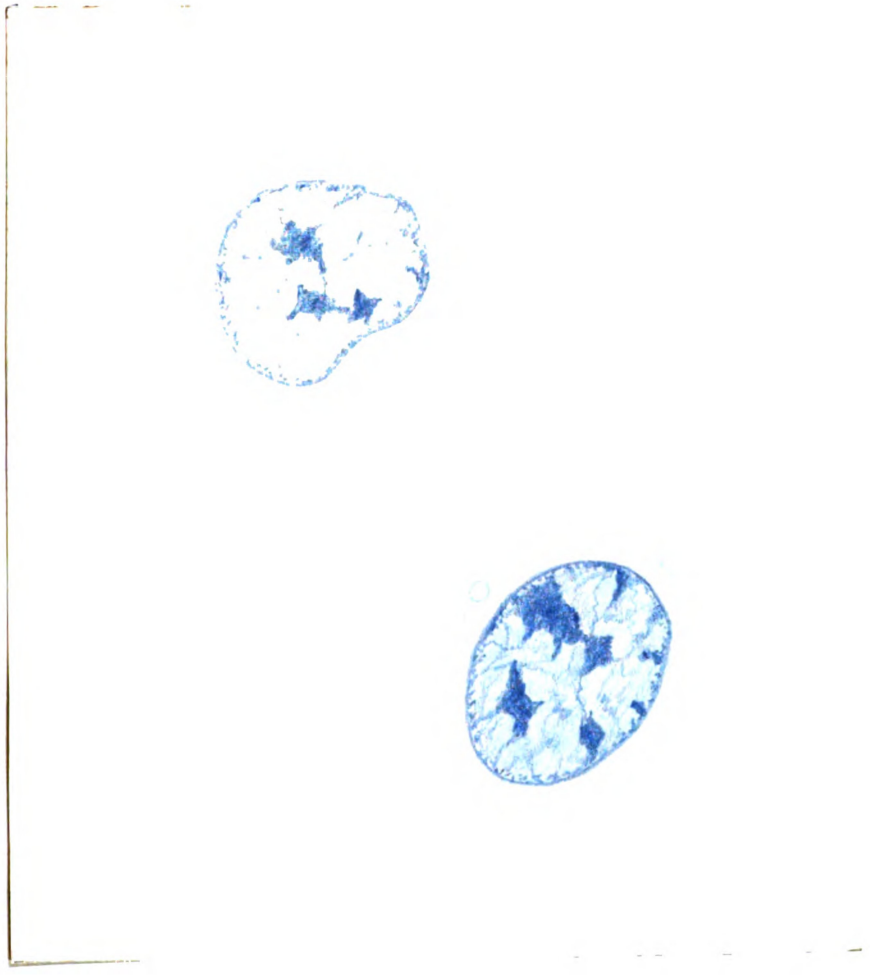


Plate XVI

HEMOCYTOBLASTS

A pair of average sized hemocytoblasts.

Note the irregular extensions of the cytoplasm.

Hematoxylin-eosin-azure stain.

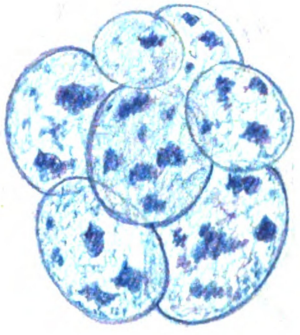


Plate XVII

A MEGAKARYOCYTE

An average sized cell (about 25μ in diameter). Note the multi-lobed nucleus.

Hematoxylin-eosin-azure stain.