

THE LIFE-HISTORY OF THE FORMED ELEMENTS OF THE BLOOD, ESPECIALLY THE RED BLOOD CORPUSCLES.

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I. THE RED BLOOD CORPUSCLES.

THE origin and the fate of the mammalian red corpuscles have been the subjects of an extraordinary number of scientific papers from workers in various fields of biological research. Contributions have been made from the side of pathology, of normal histology, and of embryology, so that to discuss the subject in all its aspects becomes a difficult undertaking. The results of investigation along these different lines are not at all in agreement, so that many theories radically different from one another have been proposed. Indeed, the embryologist, the pathologist, or the histologist often works at the subject without any reference to the results made known by the investigations of the other, inasmuch as the journals in which these results appear are likely to be read only by the specialists in whose interest they are published. To one who reads over the literature even incompletely, the conviction comes, I think, with a good deal of force, that the various phenomena which have been observed and described, and which have served as a basis for the divergent theories, might all find a simpler and better explanation under some one theory. One cannot help believing, in other words, that in the mammalia the method of production of the red corpuscles is essentially the same in disease as in health and in the adult as in the foetus; and furthermore that the formation of these elements takes place not in a number of different ways, but according to some one scheme of reproduction, as in the development of tissue elements in general. Many authors, on the contrary, have advanced one theory of the formation of the red corpuscles as the result of their own work, but have admitted at the same time that the different views advocated by others might hold good under certain

conditions of health or age. On *à priori* grounds it seems to me that most persons will incline rather to the other view, that the production of the red corpuscles takes place in one way under all conditions of life, though in some cases the process may be accelerated or abbreviated, and in others retarded. Inasmuch, then, as decisive proof that the red corpuscles are formed by two or more different methods is wanting, it is allowable to examine critically the different theories which have been proposed, and to endeavor to discover whether the phenomena they are intended to explain cannot be grouped under a common theory. My own investigations have extended over a period of two years; and though they are in many respects incomplete, yet in a number of points satisfactory conclusions have been reached, and it seems to me, as I shall endeavor to show, that the phenomena which I have observed help to some degree at least in reconciling different theories, and indicate that one general plan of formation holds good in all cases.

Since 1838 it has been known that the red corpuscles of the foetal animal are at one period nucleated cells. This was first shown by Rudolf Wagner (1) for the embryo bat and by E. H. Weber (2) in a human foetus of twelve weeks. Most of the observations made upon these nucleated red corpuscles of foetal blood, their occurrence and their relative numbers at different periods of foetal life, we owe to Kölliker (3) and to Paget. Kölliker found that in a sheep's embryo of three and one-half lines the red corpuscles are all nucleated, and Paget makes the same statement from the human embryo of four lines (fourth week). In a human embryo of three months the nucleated corpuscles in the circulating blood make up from one-sixth to one-eighth of the total number of corpuscles, while at five months they are still quite numerous. In a human foetus of this age (five months) which came under my own observation, and which had been born about five or six hours, and was brought to me still enclosed in the amniotic sac, I found that the majority of the red corpuscles were not nucleated, though nucleated forms were still very abundant. Among the nucleated corpuscles some were found with the nucleus fragmented incompletely into a number of pieces, forming what has been

described by Kölliker and by Neumann as the first stage in the disappearance of the nucleus. In foetal cats I have found nucleated red corpuscles in the blood even at birth, though they were few in number. In cat foetuses of an earlier age they are proportionally more numerous. The youngest cat embryo which I examined was one measuring 2.5 cms. from the crown of the head to the root of the tail. A drop of its blood taken from the heart and stained in methyl green (a 1 per cent solution in 0.6 per cent NaCl) showed a number of interesting things which, so far as I know, have not been noticed, or at least not dwelt upon before. In the first place, there were two distinct forms of red corpuscles present in the blood: one, large, oval, and nucleated, resembling somewhat the corpuscles of the reptiles or amphibia. In shape, they were biconcave, irregular, or apparently, in some cases, biconvex, and were so extremely plastic as to appear semi-liquid. When treated with staining reagents, they took on an oval biconvex form. These corpuscles were distinguished, moreover, by the deeper tint of the hæmoglobin which they contained. Their nuclei were variable in size, were usually placed eccentrically, and were characterized by the fact that without exception in the embryo of this age they stained a homogeneous bluish color with the methyl green, showing no trace of nucleoli or intranuclear network. The size of these corpuscles in their long diameter varied from about two to four times the diameter of the red corpuscle of the adult mammal. The second form was circular in outline and of the usual size of the cat corpuscles — some of them were nucleated, and some had lost their nuclei (see Fig. 1). The nuclei of the nucleated forms were in some cases stained a uniform green blue color from the methyl green, like the oval corpuscles just described; but in other cases the nuclei showed an intra-nuclear network or granulation. It is worthy of special emphasis that all the red corpuscles which were non-nucleated belonged to this class. Diligent search through a number of preparations failed to reveal a single large oval red corpuscle which did not have a nucleus.¹

¹ After this article was in the hands of the printer, Hayem's extensive work on the blood, "*Du Sang et de ses Altérations Anatomiques*, 1839," came into the author's possession. In it is found a reference to these large corpuscles. He speaks of them as giant nucleated corpuscles, discoid and concave in shape, though occasionally irregular and flat or subglobular.

It is permissible, perhaps, to suppose that the large oval corpuscles represent the form of the red corpuscle characteristic of the ancestors of the mammalia, and to speak of them therefore as ancestral corpuscles, while the smaller circular corpuscles of the usual size of the nucleated red corpuscles of the mammalia exhibit a modification of this ancestral form which has become characteristic of the blood of most of the mammalia. These latter corpuscles under normal conditions lose their nuclei and become changed to the biconcave red corpuscles of the circulating blood, the transition, in the young embryo, taking place in the blood itself. It is not probable that there is any essential difference in the way in which the two forms of red corpuscles are produced; but it is possible that the large (ancestral) form represents an entire embryonic cell, in which hæmoglobin has become developed, while the true mammalian form arises from similar cells after they have broken up by karyokinesis into smaller daughter-cells, in each of which the nucleus is larger relatively to the size of the whole cell. A similar difference in the red corpuscles of the very young embryo has been recorded by Erb (4). He states that, in the blood of two young pig embryos (1 in. long) he found some nucleated red corpuscles of great size and elliptical form, having a superficial resemblance to the red corpuscles of the frog. Most of the red corpuscles had nuclei, and those that had not were always the smaller variety, similar in outline to the corpuscles of the adult animal.

The nuclei of the nucleated red corpuscles of the young embryo (except the larger variety) are lost while in the circulation, and the presumption is that this fate is met by all the truly mammalian red corpuscles. As the embryo grows older and the production of new corpuscles becomes localized in different organs, — liver, spleen, and marrow, — more and more of the early history of the corpuscle is passed over while still in the blood-forming organ, and more and more of the red corpuscles are sent into the blood stream in the non-nucleated stage. After birth, and throughout adult life under normal conditions, we find only non-nucleated red corpuscles in the circulating blood. The nucleated period in their life-history has passed while they were in the blood-forming organ (the red marrow).

Under certain conditions, however, as I shall endeavor to show later, such as severe hemorrhage or anæmia from pathological causes, some of the nucleated red corpuscles escape from the blood-forming organ before the loss of the nucleus has taken place. Neumann (5*h*) states that, in the pig, — unlike other mammals, — one can always find in the normal adult animal some few nucleated red corpuscles in the blood. I have met with a similar exception in the opossum. In several animals which I have examined I was able in each case to find a few nucleated red corpuscles in the blood.

The loss of the nucleus is one of the most important events in the life-history of the red corpuscle, and has naturally been the subject of much discussion. It is usually believed, as taught by Kölliker (3), for the embryo, and by Neumann (5*h*), for extra-uterine life, that the nucleus disappears within the corpuscle by absorption, which may be preceded by fragmentation. I desire to come back to this subject under another heading, and will not discuss it fully now. It is my belief that the nucleus is lost not by absorption within the corpuscle, but by migration or extrusion from the corpuscle, as shown in Fig. 2. I mention this view at this time to call attention to some evidence in favor of it found in the examination of the blood of the young cat embryos of 2.5 cms. In the blood of this embryo I obtained a number of specimens, such as are shown in Fig. 2, in which the nucleus was seen in the act of passing out of the corpuscle. This appearance has been seen under different conditions by a number of observers, but has usually been explained as a post-mortem change or as the effect of mechanical pressure, action of reagents, or some similar cause. Now, it seems to me that these explanations will not hold in this case, because none of the nuclei of the large oval corpuscles were found extruding, though they were submitted to the same treatment exactly; and, furthermore, amongst the smaller true mammalian corpuscles, only those were found with the nucleus extruding in which the nucleus stained a homogeneous tint with the methyl green. No nucleus showing an intra-nuclear network was ever found escaping from the cell, though there were many such cells in the preparation, and they had passed through the same treatment as the corpuscles with the other kind of nucleus. This last fact, as I shall show later,

holds good for the nucleated red blood corpuscles in the blood-forming organs of cats of all ages, and presumably for other mammals also. In face of these two facts it is hard to believe that the extrusion of the nucleus is in any sense an accidental occurrence. On the contrary, it is the normal means by which the nucleated corpuscle passes into its non-nucleated form.

The place and manner of origin of the first red corpuscles of the embryo have given rise to a number of different theories. Reichert (6) taught that the first corpuscles are formed from the mass of cells from which the heart is developed, the central cells of the mass becoming the blood corpuscles, while an intra-cellular liquid which forms represents the blood plasma. Kölliker (3) afterwards extended this theory so as to take in the great blood-vessels as well as the heart. He believed that the first blood corpuscles of the embryo are colorless nucleated cells like the other embryonic cells, and at first are found in the solid cords or masses from which the heart and first blood-vessels are developed. The peripheral cells form the walls of the blood-vascular organs, while the central cells are floated away in the plasma which forms between the cells. The red corpuscles are spherical nucleated cells which multiply by division in the blood stream. Within recent years a somewhat similar view has been proposed by Ziegler and by Wenckebach. Wenckebach's (7) first observations were made upon embryos of *Perca fluviatilis*. These embryos are so transparent that they can be examined entire under the microscope. Ziegler (8) worked chiefly with the salmon. Both state that the heart begins to beat and forces into circulation a colorless plasma before the red corpuscles appear. These are seen later, and are formed first, according to Wenckebach, from a mass of cells lying in cross-section between the aorta and intestine, and outlining the position of the future posterior vertebral or cardinal vein. The blood plasma, percolating through this mass of cells, washes off the central ones, to form the first red corpuscles, while the peripheral cells form the walls of the future vein.

Ziegler also describes a mass of cells found in cross-section between the notochord and intestine, which he calls the intermediate cell mass. The central cells of this mass become the first blood corpuscles in the way described, while the peripheral

cells form the walls of the single median cardinal vein, which anteriorly splits into the two posterior cardinal veins. Moreover, from this central mass of embryonic corpuscles cords of similar cells branch off toward the yolk on either side, forming the outlines of new blood-vessels (veins), and serving as centres of origin for new red corpuscles. Both authors believe that these embryonic blood corpuscles are capable of multiplication, since karyokinetic figures are not unfrequently seen. The first red corpuscles, according to this view, are true mesoblastic cells, set apart for the formation of certain veins as well as red corpuscles. Most English embryologists, on the contrary, adhere to the view proposed by Klein (9), Balfour (10), etc., a general account of which is given in Foster and Balfour's *Embryology of the Chick*. According to this theory, the blood-corpuscles are formed endogenously within large mesoblastic cells found chiefly in the area vasculosa and area pellucida. These cells send out processes which unite, forming thus a protoplasmic network. At the nodal points of this network the nuclei of the original cells multiply, to form groups. The protoplasm around each of these assumes a red color from the development of hæmoglobin, and the groups eventually break up to form nucleated red corpuscles. By a similar method, blood corpuscles are formed in the connecting processes, while some of the protoplasm remains granular and uncolored, forming the walls of the future vessels. The nuclei scattered along the walls are also derived, like the corpuscles, from the nuclei of the original mesoblastic cells. The plasma in which the corpuscles float is a secretion from the protoplasmic walls of the newly formed vessels.

Gensch (11) in a communication giving the results of some work done under Kupffer upon the teleosts (*Esox lucius* and *Zoarces viviparus*) states that the first blood corpuscles develop out upon the yolk beyond the boundary of the mesoblast. In this region there is a layer of large plasmodium like cells lying beneath the epiblast, but not forming a continuous epithelium. The cells become united to one another by processes, and the blood corpuscles are constricted off from them, forming the blood islands seen in the blastoderm. This layer of formative cells is called by Kupffer the "secondary endoderm." The theory differs from those previously mentioned not only in the

way in which the first corpuscles are formed, but also in the fact that the original cells are not derived from the mesoblast. In this last respect Kupffer's theory bears some resemblance to the well-known parablasic theory of His (12). His believes that the mesoderm in the sense of the term used by Remak can be separated into an axial and a peripheral portion. The axial portion is formed in the higher animals in the neighborhood of the primitive streak; in the lower animals, in the groove of the blastopore. From it are derived the muscles, the chorda, the generative epithelium, the duct of the pronephros, etc. It falls into two layers, which taper off laterally, but do not extend beyond the body proper of the embryo. The peripheral portion of the mesoderm forms what His calls the parblast; and, when first formed, it lies outside of the body of the embryo, arising in fact from the white yolk. The parblast gives rise to the blood corpuscles and blood-vessels as well as the general connective tissues. Though arising outside of the body, it afterwards grows in from the periphery, penetrating the germ layers, so that the parablasic cells become inextricably mixed with the cells of the germ layers.

To which of these various theories the balance of evidence tends it is impossible to say. In my own work I have not attempted at all to follow the development of the first corpuscles in the germ layers of the embryo. If we suppose that the method of formation of the first red corpuscles in the germ layers is similar to that which prevails in later embryonic life and in extra-uterine life, then it seems to me highly probable, for reasons which I will give presently, that the general method described by Reichert and Kölliker, and afterwards extended and modified by Wenckebach and Ziegler, is most worthy of belief. According to this view, the primitive blood corpuscles form one variety of mesoblastic cells, which become arranged in masses or strings that mark the position of future blood-vessels (veins). The central cells become red blood corpuscles; the peripheral cells form the walls of the vessels.

Development of the Red Corpuscles in the Later Stages of Embryonic Life.

Kölliker (3) first proved that the liver, as soon as it is formed, becomes the seat of production of new red corpuscles. This

fact has been abundantly confirmed by all observers since Kölliker's time, and is capable of easy demonstration. The way in which the red corpuscles develop in the liver has not, as far as I know, been described in any detail. Kölliker held simply that the liver contained certain nucleated white corpuscles which become transformed to red corpuscles by the development of hæmoglobin, and which subsequently lose their nuclei. Neumann (5*d*) states that he finds in the liver nucleated red corpuscles in greater numbers than can be accounted for by supposing that they are carried there by the splenic veins and other vessels opening into the liver. They must be formed in the liver then *de novo*, and he suggests at least two methods by which they are produced. First by endogenous formation in certain large cells. A number of nuclei arise in these cells by a process of endogenous division, and a homogeneous yellow substance collects round each nucleus. Each nucleus with its surrounding colored protoplasm constitutes a red corpuscle, and this is afterwards liberated and undergoes its further development. In addition he finds in the embryonic liver a number of free nuclei which are undoubtedly the same in structure as the nuclei of the nucleated red corpuscles. How these free nuclei arise, and what becomes of them, he leaves undescribed, but supposes that they represent one step in a second method of production of nucleated red corpuscles. He seems to suggest, indeed, that the free nuclei form round themselves a protoplasmic envelope in which hæmoglobin is afterwards developed, and in this way they are converted to nucleated red corpuscles,— a view which, as we will see, has been proposed by others to account for the formation of new corpuscles in the marrow in post-natal life. Neumann says, moreover, that a new development of capillary blood-vessels is taking place in the liver throughout almost the whole of embryonic life, and in some way the formation of the new red corpuscles is connected with the existence of these newly forming blood-vessels. Foa and Salvioli (13) believe that the nucleated red corpuscles found in the liver are derived from colorless corpuscles,— “hyaline cells,”— which in turn arise by constriction or segmentation from the large giant cells found in the liver. It is undoubtedly true that in the embryonic liver the nucleated red corpuscles are formed from colorless cells. Whether one studies

the liver from sections or from teased specimens, he finds abundant proof for this in the transitional forms, which occur in considerable numbers. With reference, however, to the origin of these colorless cells, I cannot agree either with Neumann or with Foa and Salvioli. In sections of liver in the later periods of embryonic life one finds the nucleated red corpuscles and their colorless predecessors lying between the rows of liver cells, scattered irregularly, and without any very apparent relationship to the other elements of the liver. But in the earlier periods of embryonic life, — in the embryo, for instance, 2.5 cms. long, — sections of the liver show the origin of the blood corpuscles quite distinctly. One sees in such sections that the blood-forming cells are not scattered without order, but are grouped into cords or strings lying between the columns of true liver cells which are just beginning to show a typical structure and arrangement. The cords of blood-forming cells resemble those described by Wenckebach, Ziegler, etc., in the germ layers of the young fish embryo, and here also undoubtedly mark out future blood-vessels. One often sees the solid mass of cells stop more or less suddenly while the channel becomes filled with coagulated plasma containing here and there fully developed red corpuscles with or without nuclei. A drawing showing the appearance described is given in Figs. 13 and 14. I have obtained similar cords of blood-forming cells, evidently developing blood-vessels, in longitudinal sections through the posterior limb of the same embryo, as shown in Fig. 15, which indicates that though the production of red corpuscles at this time is most active in the liver, it is also going on in other parts of the body, probably wherever new blood-vessels (veins) are forming. If we accept the theory proposed by Kölliker, Wenckebach, and others, as to the method of formation of the first blood corpuscles in the embryo, then their production in later embryonic life is seen to follow essentially the same plan. One might suppose, indeed, that the cords of blood-forming cells in the young liver are directly or indirectly derived from the original median mass of blood-forming cells which first appears in the embryo, though I have no observations at all which can be taken as evidence for such an hypothesis. A similar method of origin of the red corpuscles in the marrow of birds during extra-uterine life has

recently been described by Denys (14), as I shall have occasion to mention later on.

During the second half of intra-uterine life the spleen also takes part in the formation of red corpuscles. This was first made known by Kölliker, and has been confirmed by a number of observers since his time. In the foetal cat the sequence in which the blood-forming organs enter upon their function is as follows. First, the liver; then, as the production of new red corpuscles in the liver becomes diminished, the function is taken up by the spleen. So that at the time of the maximum activity of the spleen the liver takes but little part in the process. Still later in embryonic life, after the long bones of the limbs have been formed, one finds that the young marrow has begun to produce new red corpuscles, while the activity of the spleen in this respect has suffered a decided diminution. Shortly before birth it is easy to prove that at least three organs are taking part in the formation of red corpuscles, — namely, the liver, the spleen, and the bone marrow, — and even after birth for a certain short time the same is true; in the cat, for as long as three or four weeks. Later than this, however, nucleated red corpuscles showing signs of active multiplication are found only in the red marrow of the bone. The liver certainly takes no part at all after this time in the formation of red corpuscles; and in the spleen one finds under normal conditions no indication of the presence of nucleated red corpuscles. Whether or not the spleen plays any part in the formation of the colorless cells from which the nucleated red corpuscles are afterwards produced will be discussed later.

While it must be accepted that during embryonic life red corpuscles are made in the three organs mentioned, it seems to me quite certain, also, that they are formed during this period in other parts or organs of the body, — wherever, in fact, developing blood-vessels (veins) are found, — though I have no evidence for this other than the section already described, which passed through the long axis of the posterior limb and showed a developing blood-vessel with its young corpuscles lying in the muscular tissue. In describing the development of the red corpuscles at this period in the life of the animal, — that is, just before and shortly after birth, — mention should be made of the discovery of the vaso-formative cells by Ranvier

(15) and by Schäfer (16). Both of these observers have found in the foetus, or in the new-born mammal (rat), large connective tissue cells, within which red corpuscles are produced endogenously. The cells become elongated, and connect with one another to form capillary blood-vessels. The newly formed corpuscles are never nucleated, and in this respect differ from the corpuscles produced endogenously in the germ layers of the embryo according to Balfour and others. In his recent book, Hayem confirms the work of Ranvier, and states in addition that blood plates as well as red corpuscles can be seen in the vaso-formative cells. The hæmatopoietic value of these cells cannot be very great, as they have not been found at any other period in the animal's life, except at birth or shortly afterward. It seems to me that more extended observations are needed before we can accept such a peculiar method of production as one of the normal means by which new red corpuscles are formed. Most of the recent work has shown that the red corpuscles pass through a nucleated stage, and are not formed endogenously within larger cells, so that the isolated observations even of such distinguished histologists cannot be weighed against the combined work of so many other investigators. Possibly the appearances upon which the theory is based may be capable of another explanation.

The White Corpuscles and Blood Plates during Embryonic Life.

I have little that is new to add to our knowledge of these two elements of the blood during embryonic life ; but the little I have is worthy, perhaps, of being placed upon record, especially as it is a subject which seems to have attracted very little attention and about which our information is deficient. In the youngest embryo which I examined (cat, 2.5 cms. long), no ordinary white corpuscles could be found, though the blood was thickly crowded, of course, with nucleated and non-nucleated red corpuscles. Occasionally a colorless corpuscle was found ; but these differed so much from the usual white corpuscle of the circulating blood, from both the uninucleated and multinucleated form, that it seemed probable that they did not belong to the class of leucocytes, but were embryonic cells which had got into the blood accidentally, either in opening the

heart or in some other way. In any case, they were extremely few in number, and did not resemble the white corpuscles of the grown animal. The blood plates also were entirely absent from the blood of this embryo. Not a single specimen could be found, though a number of preparations were examined. It seems to me that this fact has a bearing upon the theories of the origin of this element, and I shall refer to it again when discussing that subject. In a human embryo of five months, both white corpuscles and blood plates were found, though both were present in small numbers. The white corpuscles were of two kinds, as in the adult, — one variety of small size, with a single vesicular nucleus resembling the lymphocytes; and the other of larger size, faintly granular, with several nuclei, or, more correctly, with a fragmented nucleus. At this age in the human embryo, the great majority of the red corpuscles have lost their nuclei. In a cat embryo of 9 cm. length the leucocytes and blood plates were both found, though the former were present in small numbers. I have not been able to find any special reference to the occurrence of these elements in the foetal blood, except in a paper by Neumann (5*d*). In the examination of human foetuses made by Neumann, he states that generally the white corpuscles were very few in number, but makes no reference to the variations with the age of the foetus. The fact that the white corpuscles are so late in appearing is important, not only in its bearing upon the old theory that they become changed into red corpuscles, but also in the fact that it furnishes a means of determining their influence upon the chemical composition of the plasma.

II. FORMATION OF RED CORPUSCLES DURING EXTRA-UTERINE LIFE.

Historical Review.

The greater portion of the literature of the red blood corpuscles bears upon this side of the subject. Very many different views have been proposed; and a brief presentation of the most important of them may be of value, both as an indication of the drift of opinion upon the subject, and also to enable me to present my own work afterwards in the briefest possible way, without detailed reference to or comparison with other

views. I shall not attempt to make the review as complete as the material which I have accumulated might enable me to do, since some of the work published does not need special mention. I have added, however, an appendix, giving the complete list of articles which I have been able to consult.

Before 1869 it was quite generally believed that the red corpuscles are formed from the white corpuscles, most probably while in the circulation. This theory found its way into the text-books, and, to a certain extent is still advocated by some histologists. In fact, some of the most recent investigations favor this view, although the evidence is so overwhelmingly against it.

Feuerstack (17) in a recent series of observations, made upon animals with nucleated red corpuscles, describes in the circulation transitional forms between the white and the red corpuscles. The colorless cell from which the other forms are derived has a relatively large nucleus and small cell body. The cell substance increases while the nucleus becomes smaller, and not unfrequently takes a peripheral position. Hæmoglobin develops in the cell, which gradually changes in shape from a spherical to an oval form. Most of the transitional stages are found in the bone marrow and spleen, though in these organs they occur not in the parenchyma, but within the blood-vessels. Feuerstack gives no sections to show how these developing corpuscles are placed within the blood-vessels of the marrow and spleen. When he says that the red corpuscles are derived from white corpuscles, he differs somewhat from the older observers, who thought that the white corpuscles might change to red anywhere in the circulation, — were, indeed, continually undergoing such a change, — while Feuerstack limits it to the blood-vessels of the marrow and spleen. On the other hand, his conclusions, if not taken too literally, agree very well with some new views of Denys upon the formation of red corpuscles in the marrow of birds. While the older observers accepted this view of the origin of the red corpuscles without much question, no one was able to obtain satisfactorily the transitional forms, so that Kölliker (3) was forced to say that the question was still undecided. Erb (4) asserted, however, that he was able to get transitional forms in the circulation by means of a certain method of treatment. Blood after treat-

ment with acetic or picric acid gave him peculiar red corpuscles which contained fragments or granules of what appeared to be nuclear matter. These granules might be many or few in number and varied greatly in size. He believed that the cells represent the transitional forms between the white and red corpuscles, and thought that they were more numerous after a severe hemorrhage, during the period of regeneration of the blood. In rabbits, moreover, after a starvation of seven to nine days, the transitional forms could no longer be found. His complete theory of the origin of the red corpuscles is as follows. White corpuscles arise in the spleen and lymph stream and get into the blood first as small uninucleated cells, with a scanty cell substance. While circulating in the blood, these leucocytes increase in size, the increase affecting both the nucleus and the cytoplasm. The nucleus then begins to fragment, and finally breaks up into granules, while hæmoglobin develops in the cell, making thus one of his transitional forms. The fragments of nuclear matter gradually disappear; the cell becomes smaller and takes the shape of a normal red corpuscle. The transitional forms of Erb can undoubtedly be found in the circulation under certain conditions, but Erb was in error in believing them to form an intermediate stage between the ordinary white corpuscles of the blood and the red corpuscles. Their real significance I shall describe later. A number of other theories proposed during this period found but little support. For instance, Wharton Jones (18) thought that the red corpuscles are the liberated nuclei of the white corpuscles, but seems to have had no stronger reason for this belief than an alleged agreement in size. Gerlach, Funke, Schaffner, *et al.*, taught that the red corpuscles are formed endogenously within certain large colorless cells found in the spleen. But the cells containing red corpuscles, which they found, and upon which the theory was based, were afterwards shown by Kölliker to be not the mother-cells of the red corpuscles, but, on the contrary, their destroyers. At present, there can be no doubt that the white corpuscles of the blood are never transformed into red corpuscles, though it must be borne in mind that this does not mean that the red corpuscles of the blood are not derived from white or colorless cells. On the contrary, as we shall see, it is now the general belief that the red cor-

puscles spring from colorless cells found in the blood-forming organs ; but these colorless cells are not the white corpuscles of the blood, indeed never under normal conditions get into the circulation.

The most important discovery of the century with reference to the development of the red corpuscles was made simultaneously and apparently independently by Neumann (5*a*) and by Bizzozero (19*d*). In 1868 these observers found that in the red marrow of the bone nucleated red corpuscles occur, which are similar to those found in the embryo, and that they are present throughout the life of the animal. A nucleated red corpuscle can only be interpreted as the predecessor of a non-nucleated red corpuscle, and the discovery therefore meant that the red marrow of the bones is an organ for the production of new red corpuscles throughout extra-uterine life. In his first papers Neumann spoke of the nucleated red corpuscle as being derived from colorless lymphoid cells, and described transitional forms ; but in his later papers he does not lay so much stress upon the transitional forms, while still believing without doubt that the red cells are derived from colorless ones. With reference to the change from the nucleated red corpuscle to the ordinary form, he agrees practically with Kölliker's view of the nature of this change in the embryo. The loss of the nucleus takes place by a process of absorption within the cell, and may be preceded by fragmentation. Neumann (5*b*) believes that in the adult the bone marrow is the sole organ for the production of new red corpuscles, and gives a number of experiments to prove that the spleen takes no part in their formation, either under normal conditions or after severe hemorrhage. Bizzozero also found the nucleated red corpuscles in the marrow, and afterwards showed that these cells are capable of multiplication by indirect division. This latter observation has been confirmed abundantly by later investigations, and makes a second important step in our knowledge of the origin of the red corpuscles. Bizzozero placed too much weight apparently upon this growth of the nucleated red corpuscles, and overlooked the importance of the colorless cells from which the red corpuscles arise. The nucleated red corpuscles of the marrow are derived, he thinks, from the similar embryonic cells occurring in the liver and spleen during foetal life. When

these organs begin to lose their hæmatopoietic function, some of the nucleated red corpuscles found in them are carried in some way to the marrow, where they form centres of growth for similar cells throughout life. Bizzozero, like Neumann, thinks that the red marrow alone possesses this function during extra-uterine life, but, unlike Neumann, he believes that the spleen may temporarily resume its blood-forming functions after severe hemorrhage when the marrow alone is unable to regenerate new corpuscles with sufficient rapidity. As evidence for this statement he publishes experiments made by himself (19c) and also in connection with Salvioli, in which it was shown that, with dogs and guinea-pigs, nucleated red corpuscles can be found in the spleen of the adult if the animal has been subjected previously to a severe bleeding, or, better, to a number of successive bleedings: under such conditions, not only were the simple nucleated forms found, but nucleated cells in process of division by karyokinesis. In man, also, after death from anæmia there are several cases recorded in which nucleated red corpuscles have been found in the spleen (Foa [22], Pellacani). On the other hand, Neumann (5h) contends that even after severe hemorrhage nucleated red corpuscles are not found in the spleen, or are found in such small numbers that their presence may be accounted for by the fact that they occur also in the circulating blood, especially in the vena azygos, which brings back blood from the red marrow of the ribs. With reference to this last point, it is undoubtedly true that in the blood of animals after severe and repeated hemorrhages, nucleated red corpuscles may be found, and similarly in the human subject it is known that in pernicious anæmia, leukæmia, etc. (Osler and Gardner [23], Laache [25]), nucleated red corpuscles may be found in the circulation. But it must be borne in mind that in the spleen of animals after strong hemorrhage one may find nucleated red corpuscles in cases when they are absent from the general circulation; and furthermore they may occur in the spleen in large numbers, and showing every sign of an active multiplication. Neumann (5h) himself admits that in one case in which the animal (dog) had been bled a number of times, and in which septic infection had developed, he could find nucleated red corpuscles in the spleen, but not in the circulating blood. He

accounts for this exception by attributing it to the septicæmia; but this does not seem to be a satisfactory explanation. Several experiments of my own on this point I will describe in the proper place: it is sufficient to say here that they confirm the view of Bizzozero and others that the spleen may be made to resume its hæmatopoietic activity.

While the fundamental discovery of Neumann and Bizzozero has been generally accepted so far as it fixes the function of producing red corpuscles in the marrow, a number of observers have differed from them and from one another as to the method by which the red corpuscles are formed in that organ. Rindfleisch (26) describes the nucleated red corpuscles, but differs from all others in believing that these cells lose their nuclei not by a gradual absorption, but by an extrusion. Rindfleisch is generally quoted as saying that the nucleus is extended naked from the corpuscle; but this is an error. He says that "the nucleus, surrounded by some colorless protoplasm, leaves the cell, which remains as a bell-shaped body of a reddish yellow color." The further fate of the extruded nucleus, with its envelope of protoplasm, he leaves undiscussed. He describes and figures the nucleus in the act of escaping, but was not able to watch the process in a living cell, though he used all sorts of means—heat, electricity, reagents of different kinds—to act upon the corpuscles. After the extrusion of the nucleus, the red corpuscle has first a bell shape, and is afterward moulded into a biconcave disc by the movement of the circulating blood. Malassez (27) believes that the nucleated red corpuscle is derived from an undifferentiated marrow cell, which contains little or no hæmoglobin, and in which the nucleus is diffuse. He describes three intermediate stages in the transformation which he is able to recognize constantly in the marrow: 1. Spherical cells of large size, which stain very feebly with eosin or hæmatoxylin, and contain no hæmoglobin, or only a trace. The nucleus in these is not a distinct morphological structure, the nuclear matter being diffused throughout the cell. He designates these cells as protohæmatoblasts.

2. Cells of the same size, with a granular protoplasm, and still containing little or no protoplasm. A nucleus is now present, and is spherical, large, and uniformly granular.

3. Cells of smaller size, containing hæmoglobin. The nucleus, also, is smaller, and shows a reticular structure. The next stage is the nucleated red corpuscle proper, which differs from (3) in the deeper tint of the hæmoglobin and the smaller size of the nucleus. The nucleus is distinguished further by the fact that it stains more deeply with hæmatoxylin. Malassez differs from other histologists in his explanation of the way in which the ordinary non-nucleated red corpuscle is derived from the nucleated form. According to him, the latter do not lose their nuclei at all, but give rise to the ordinary red corpuscles by a process of budding. The buds are constricted off, and are first spherical, but afterward become biconcave, partly from the mechanical action of the circulating blood, partly because of an unequal diminution in bulk. Foa and Salvioli (13) also derive the nucleated red corpuscle from a colorless or "hyaline cell." This latter cell originates both in the embryo and the adult from the large giant cells found in the marrow during extra-uterine life, and in the liver and the spleen of the foetus during the period when these organs are producing red corpuscles. The giant cells, myeloplques, of Robin are of two kinds, at least, in the red marrow. One variety is large, finely granular, and contains a number of oval separate nuclei, which correspond to the myeloplques as usually described. The second variety is not multinucleated, but has a very large coiled, or twisted, nucleus, made up, apparently, of a number of smaller nuclei, which are, however, still in connection with one another. This variety Bizzozero described as the "giant cell with budding nuclei"; and Foa and Salvioli believe that they give rise to the hyaline cells, from which the nucleated red corpuscles are afterwards formed. They give to this kind of giant cell, therefore, the name of hæmatoblast. The hæmatoblasts separate into a number of smaller hyaline cells, the large nucleus breaking up into separate "buds," each of which becomes the nucleus of a hyaline cell. The hyaline cells change to nucleated red corpuscles by the development of hæmoglobin within the cell substance; and these latter pass to the non-nucleated form in consequence either of an absorption or an extrusion of the nucleus. In a later paper, Foa (22) expresses his belief that the nucleus disappears within the cell by absorption. Osler

(28), in his Cartwright lectures, describes in the adult marrow seven different kinds of cells. He derives the nucleated red corpuscles, in the first place, from a colorless cell 9 to 12 μ . in diameter, with a smooth, homogeneous protoplasm and a finely granular nucleus. This cell shows, moreover, a peculiar flexibility. These cells, in turn, are derived from what he calls "protoleucocytes," which are solid-looking lymphoid elements, 2.5 to 5 μ . in diameter, resembling free nuclei, though some of them may have a narrow rim of protoplasm. The nucleated red corpuscle is transformed to the non-nucleated corpuscle by the gradual disappearance (absorption) of the nucleus, after which the corpuscle becomes condensed to the flattened disc shape.

The most elaborate, and probably the most important, contribution to our knowledge of the development of the red and the white corpuscles which has been made recently is found in a series of papers by Löwit (29). The most important conclusions at which he arrives are as follows: the blood-forming organs of the adult, among the cold-blooded as well as the warm-blooded animals, are the bone marrow, the spleen, and the lymph glands. In all of these organs we meet with two kinds of colorless cells. One of these he calls "leucoblasts"; and they are destined to form the leucocytes of the blood and lymph. To the second he gives the name of "erythroblasts": from these the red corpuscles are developed. These two sorts of cells are distinguished from each other by differences in the structure of the nucleus, in the method of multiplication, and in the properties of the cell protoplasm. The leucoblasts have a nucleus which is relatively quite large. It contains one or more small heaps of chromatin, sometimes irregular in shape, from which a system of delicate lines and bands radiates toward the nuclear membrane. This latter consists of a distinct, often doubly contoured, band of chromatin substance, on the inner side of which one frequently finds irregular projections connected with the intra-nuclear network. The leucoblasts multiply by a process less complicated than ordinary karyokinesis and more complicated than simple direct division. The chromatin granules during division show some movement, though of an irregular character, from the equator toward the poles. He proposes to call this *divisio per granula*. The erythro-

blasts have a nucleus which shows always a chromatin reticulum, but no true nucleolus. They never make amoeboid movements nor ingest foreign particles, and finally they develop hæmoglobin in the cell substance, passing thus into nucleated red corpuscles. Cell division takes place with the formation of the usual karyokinetic figures. Löwit designates this method of multiplication as *divisio per fila*, in contradistinction to the method found in the leucoblasts. He states that he has never been able to find transitional forms between the two kinds of cells, though in the organs in which they occur they are found freely intermingled with each other. The leucoblasts enter the lymph stream, and eventually reach the blood as uninucleated leucocytes. These are rather small, and are devoid of the power of making amoeboid movements,—a fact which was pointed out long ago by Schultze. In the blood stream, they increase in size, the nuclei become elongated and constricted, and finally fragment to form the so-called multinuclear leucocytes. He believes, then, with many others that the multinuclear leucocytes are not cells in the act of multiplication, but, on the contrary, are disintegrating; and the multinuclear stage so-called is probably followed by a complete dissolution of the cell.

In the veins coming from the blood-forming organs the uninucleated leucocytes predominate greatly in number. In the right heart the number of uninucleated forms is still relatively large, while in the left heart they become less numerous, and in the peripheral arteries they show a striking diminution. In other words, the transition from the uninucleated to the multinucleated forms takes place chiefly in the venous system during the brief interval of time required for the blood in the veins to pass from the lymphoid organs to the left heart. The erythroblasts, after the development of hæmoglobin, become nucleated red corpuscles. In the marrow of the bone all the intermediate stages may be obtained without difficulty. So in the liver and spleen of the fœtus and in the spleen of the adult in some cases after severe hemorrhage similar transitional forms are found. But in the lymph glands transitional forms between erythroblasts and nucleated red corpuscles cannot be obtained. Hence he concludes that the transition in this case takes place in the lymph stream or the blood or both,

or, as a third possibility, the erythroblasts are carried to the marrow and there undergo the final changes. The nucleated red corpuscles pass into the usual red corpuscles by a loss of the nucleus. This, he thinks, occurs in the way described by Kölliker and by Neumann; that is, by disintegration and absorption within the cell. In his latest paper Löwit describes some new and rather remarkable observations upon the erythroblasts. He finds that he can obtain erythroblasts easily from the veins which bring back blood from the blood-forming organs; while in the superior vena cava they occur rarely, and in the left heart and arterial system they are entirely wanting. Though few erythroblasts are found in the superior cava and right heart, nevertheless blood from these portions of the vascular system, when treated for a number of hours with a modified Pacini's liquid, shows a considerable number of red corpuscles which contain a granular body of the shape and general appearance of a nucleus. The granules may be few or many, and in some cases they are connected by a sort of nuclear network. Löwit's description corresponds very well to the "transitional forms" of Erb which have already been mentioned. He interprets these structures as erythroblasts in which the nucleus is disappearing. Apparently, then, he believes that an erythroblast may develop its hæmoglobin and lose its nucleus by absorption while in the venous blood and during the time required for that blood to flow from the blood-forming organ to the left heart. The blood that flows from the lungs to the left heart must contain, therefore, a number of newly formed corpuscles. Nevertheless, comparisons made between the blood of the left and the right heart showed that the former contained fewer corpuscles and less hæmoglobin than the latter. Hence, during the passage of the blood through the lungs there must occur also a more or less important destruction of red corpuscles.

It has been generally believed that in the marrow the nucleated red corpuscles are not arranged in any definite way, but are mingled indiscriminately with the other elements of the marrow. Denys (14) in a recent very interesting paper states that this is not the case, at least not in the marrow of the bird. He accepts the terms erythroblast and leucoblast proposed by Löwit, and states that in sections of the marrow

which have been treated with a double stain of fuchsin and methyl green the nuclei of the erythroblasts stain green, while those of the leucoblasts stain red. Moreover, this method of staining shows that the two kinds of cells are not intermixed without order; but on the contrary they are sharply separated, the erythroblasts lying in cords or strings which are clearly marked off from the masses of leucoblasts. These cords of erythroblasts form in reality a part of the vascular system of the marrow in the following way. Between the well-defined arteries and veins of the marrow there are two capillary plexuses. One, a system of arterial capillaries comparatively few in number, is connected with the larger arteries, and is composed of long, narrow vessels with distinct, doubly-contoured walls. These open suddenly into large venous capillaries which are nearly filled with erythroblasts, and form, in fact, the cords of erythroblasts found in the marrow. The blood stream flows through these imperfectly formed vessels in a central channel which is more or less open, while the plasma probably percolates through the whole mass of erythroblastic cells. These capillaries have a very delicate endothelial wall which marks them off from the leucoblasts, and the erythroblasts filling them are so arranged that the youngest lie next to the wall and the most matured next to the central channel, where they can be floated off by the blood current. The similarity of these cords of erythroblasts or developing veins to the developing veins found in the germ layers of the embryo by Wenckebach (7) and Ziegler (8), and described and figured in the liver and posterior limb of the embryo by me, will be apparent at once. It would seem that the manner of development of the red corpuscles is the same in the adult as in the foetus. Unfortunately, Denys has not as yet shown that the same arrangement is found in the marrow of the mammal, while others positively state that no regular grouping of the blood-forming cells occurs: so that this point remains to be investigated. I shall have occasion to refer to it again. Feuerstack (17), it will be remembered, held that in the birds and other animals with nucleated red corpuscles the development of the corpuscles takes place in the blood-vessels of the marrow; but he gives no definite description of how this occurs. So, more recently, Geelmuyden describes for the marrow of the frog

what seems to be an arrangement similar to that given by Denys for the pigeon. He says that in the injected marrow of frogs the blood corpuscles do not lie free in the marrow, but are contained in definite vessels. Within the lumen of these vessels there are a great number of narrow cells which lie along the walls of the vessels, while the blood corpuscles of the circulating blood pass through the middle. Hayem (31) holds an entirely different view of the origin of the red corpuscles. Hayem, as is well known, deserves the credit of giving the first elaborate description of the blood plates. Although these elements had been mentioned, and to a certain extent studied, before his time, Hayem's investigations into their structure and meaning seem to have given the impulse to the great amount of work which has been directed to them within recent years. He attributed to the blood plates the very important function of forming the new red corpuscles. The blood plates, in fact, are in his opinion only young red corpuscles possessing the shape of the red corpuscles, — biconcave discs, — and in many cases having a greenish tint from the hæmoglobin which has begun to form in them. He speaks of the blood plates, therefore, as "hæmatoblasts." As proof for this view, he states that intermediate forms can be found between the typical blood plate and the ordinary red corpuscles, and these intermediate forms are especially numerous after severe hemorrhages when we should expect a rapid regeneration of new corpuscles. These statements, however, have not met with confirmation from the work of others. Most of those who have studied the blood plates agree in the conclusion that they do not develop into red corpuscles, however much they differ on other points. It is rather interesting that Zimmermann (32), who was one of the first to notice the blood plates, to which he gave the name of "elementary particles," also thought that they develop into red corpuscles.

Gibson (33) believes with Löwit that the spleen and the lymph glands as well as the marrow take part in the production of red corpuscles throughout extra-uterine life. To establish the fact that the spleen makes red corpuscles he removed that organ from three dogs. In two of them he was able to demonstrate a slight diminution in the number of red corpuscles, while the effect upon the number of white corpuscles was not

constant. His results were not striking, but were sufficient to convince him that the spleen has a distinct though subordinate part to play in the production of red corpuscles. As proof that the lymph glands also produce red corpuscles, he cites an experiment in which the thoracic duct was ligated for thirty-seven days before the animal was killed. Post-mortem examination showed that some of the lymph glands, especially those of the mesentery, had a reddish appearance, and contained a number of nucleated red corpuscles. Moreover, enumeration of the red corpuscles of the blood of this animal proved that a diminution of about 13 per cent. had taken place. Gibson's theoretical views of the way in which the red corpuscles are formed are as follows: In some of the colorless marrow cells the nucleus begins to increase in size, while hæmoglobin develops in the body of the cell. Later, as the hæmoglobin becomes fully formed, the cell shows a diminution in size which affects the nucleus also, so that finally one of the small typical nucleated red corpuscles is produced. Just how this becomes changed to the non-nucleated corpuscle is not stated very clearly. In one place he seems to agree with the view of Kölliker and Neumann that the nucleus fragments and is absorbed, while in other places he speaks of the nucleus becoming a blood plate. He describes the blood plates under the name of "colorless microcytes," and thinks that they are formed in part from the fragmented nuclei of the white corpuscles and in part from the fragmented nuclei of the nucleated red corpuscles. In addition to the "colorless microcytes," he describes in the blood what he calls "colored microcytes," which he believes to be the same as the "hæmatoblasts" of Hayem. These he considers to be simply fragments of red corpuscles formed in some way or other in the circulating blood. Gibson seems to be describing here the microcyte of pathological literature, small, spherical, deeply colored corpuscles very common in the blood in progressive pernicious anæmia, leukæmia, chlorosis, etc. [See Osler (24), Laache (25), Eichorst (34), *et al.*]

Obrastzow's (35) theory bears some resemblance to that of Osler already described. The nucleated red corpuscles are derived from colorless cells, which in turn are formed from free nuclei, or little spheres of nuclear matter (protocleucocytes), each of which develops round itself a layer of protoplasm. The

colorless cell thus produced may change either into a nucleated red corpuscle or into an ordinary marrow cell. According to Obratzow, the nucleated red corpuscles of most authors—hæmatoblasts, according to his nomenclature—possess in the living state no nucleus, the nuclear matter being diffused throughout the cell. After the death of the cell, the nuclear material becomes condensed to form a typical nucleus such as is always described for the cell. The process of condensation or separation of the nuclear matter resembles very much the coagulation of blood, nuclear substance having properties similar to though not identical with those of fibrin. The transformation of the hæmatoblasts to red corpuscles consists chiefly in the disappearance and absorption of the nuclear matter. Obratzow has seen in his preparations nucleated red corpuscles, or hæmatoblasts, with the nuclei partially or completely extruded from the cell in the way described by Rindfleisch. He explains this, in accordance with his theory, as the result of post-mortem changes brought about by the condensation of the protoplasm after death. Arndt also believes that the nucleus of the nucleated red corpuscle does not exist in the living cell, but is formed in consequence of post-mortem changes. Indeed, he goes further than this and denies that any nucleus is present in the living red corpuscles of the lower vertebrates,—birds, reptiles, amphibia, etc. The apparent nucleus so easily seen in these cells is caused by the action of reagents or by post-mortem changes. The nucleus seen in the nucleated red corpuscles after the death of the cell consists histologically of a gelatinous ground substance containing a number of granules. He speaks of these granules as “elementary corpuscles,” and thinks that they are of the same nature as the granules found in protoplasm generally.

Afonassiew (36) concludes that red corpuscles may be regenerated in three different ways: 1. Nucleated red corpuscles multiply by division and are finally changed to non-nucleated red corpuscles. 2. The blood plates increase in size; each forms round itself an envelope of protoplasm in which hæmoglobin becomes developed, making a nucleated red corpuscle. This loses its nucleus by extrusion and becomes an ordinary red corpuscle. Under normal conditions this series of changes takes place only in the marrow. He seems to think that the

extruded nucleus in this case again becomes a blood plate and may enter upon a similar course of development. 3. In cases of strong anæmia one finds occasionally that certain of the red corpuscles (the poikilocytes, apparently, of the pathologist) constrict off small bits of their substance to form small red corpuscles (microcytes?) somewhat larger than the blood plates which afterwards develop into normal red corpuscles while in the circulation. Boettcher (37) contends that the red corpuscle of the blood in man and the mammalia generally is nucleated, though the nucleus under ordinary conditions is not visible. His evidence for this belief is not at all conclusive: it seems to rest chiefly upon the fact that reagents which dissolve the hæmoglobin out of the corpuscles, especially chloroform, leave behind a colorless sphere, considerably smaller than the original corpuscle, which he takes to be the nucleus. When the action of chloroform upon a red corpuscle is watched, it can be seen, he says, that the reagent dissolves off the peripheral colored portion of the corpuscle, leaving behind the colorless nucleus. Efforts to bring out this nucleus by the action of ordinary staining reagents failed except in two cases, once from the blood of a person who had died from leukæmia, and once from the blood of a tuberculous woman. It is fair to suppose that in both of these cases he was dealing with nucleated red corpuscles which had passed into the circulation.

Sappey (38) also asserts that the mammalian red corpuscle is nucleated, and that to bring out the nucleus one must treat the blood with some reagent which will make the corpuscles spherical. He recommends the following liquid: water, 500 grms.; sodium sulphate, 40 grms. Add to this solution acetic acid in the proportion of 1 to 49. Quite recently, Cuenot (39) has advanced a theory of the development of the red corpuscles which in some respects is more fanciful than any yet described. He believes that the red corpuscles are formed in the spleen, and in mammals that the whole development is carried on in this organ, while in the lower vertebrates a certain portion of the development takes place in the circulation. The spleen, according to Cuenot, contains two kinds of colorless corpuscles,—some of large size and but little refractive, which are destined to form the white corpuscles; and some of smaller size, which are very refractive, and become the nuclei of

future nucleated red corpuscles. These are not naked nuclei, but are surrounded by a very thin envelope of colorless protoplasm. The protoplasmic layer becomes enlarged, and small granules are constricted off from the nucleus, and set free in the cell. In some way these nuclear granules start the formation of hæmoglobin, either because they contain the necessary iron or because they act as a sort of hæmoglobin ferment. As the hæmoglobin develops, the granules disappear, and the nucleus becomes smaller. In the mammals the nucleus becomes entirely absorbed in the process, so that the fully formed mammalian corpuscle is non-nucleated.

If we attempt to sum up the facts with reference to the development of the red corpuscles which seem to be fairly well established, we will be obliged, as one can readily see from the foregoing review, to confine ourselves to a few fundamental points. In the first place, it is perfectly well proved that during extra-uterine life the red corpuscles are developed in the red marrow of the bones. Whether or not the spleen and the lymph glands participate in this function is not definitely determined. In the second place, it is generally admitted that the red corpuscle is first a nucleated cell, and that it loses its nucleus in the marrow or other blood-forming organ. Whether the nucleus is lost by extrusion or disappears within the cell by absorption is not settled; but the majority of writers certainly favor the latter view. In the third place, it is pretty conclusively shown that the nucleated red corpuscle is derived from a colorless cell — erythroblast, to use Löwit's term — which is formed in the marrow. The origin of this cell is the point about which, perhaps, there is least agreement. Finally, none of the recent work supports the theory that the red corpuscles are derived from the white corpuscles (leucocytes) of the circulating blood, so that this time-honored theory must be definitely abandoned.

Experimental Work.

My own work has been confined almost entirely to one mammal, the cat, partly because there was not sufficient time to make a complete series of parallel experiments and observations upon other animals, and partly because, by confining the work to a single mammal, a thorough familiarity with the

different kinds of cells was obtained, and observations made upon different individuals were capable of a closer comparison. It cannot be doubted that in its essential features, certainly, and in all probability in most of the minor details, the genesis of the blood corpuscles in the cat is the same as in man or in any of the higher mammalia.

In the course of the work I have made use of many different methods of treatment; but the methods which I have used most, and which have given me the best results, are these. When studying fresh specimens of liver blood, marrow, etc., the reagent invariably used was a 1 per cent solution of methyl green made up with 0.6 per cent solution of sodium chloride. The tissue was teased either in normal salt solution or in its own plasma, and then further teased in a drop of the methyl green. I did not use acetic acid in combination with the methyl green, as this reagent quickly dissolves out the hæmoglobin from the nucleated red corpuscles, while with the methyl green alone this does not happen unless the quantity used is too great relatively to the amount of tissue teased. The blue-green color given by the methyl green to the nucleus of the nucleated red corpuscles served to make the hæmoglobin in the cell protoplasm more distinct by contrast. The fresh tissue was examined also without the addition of any staining reagent after teasing in its own liquid, in normal salt solution, or, best of all, in blood serum which had been previously prepared from the same animal.

The marrow, spleen, and liver of the fœtus as well as the adult were studied in section, and specimens were taken from normal animals, from animals which had been bled, starved, injected, etc.

The tissue was usually hardened in a cold saturated solution of mercuric chloride according to the directions given by Gaule. Sections were cut in paraffin, and were stuck to the cover slip by the alcohol method, using 70 per cent alcohol. The sections were then stained by two or more different methods. The stains usually employed were: first, a triple stain of hæmatoxylin, eosin, and saffranin, used successively according to Gaule's method; second, alum carmine; third, Biondi's triple stain of acid fuchsin, methyl green, and orange used in mixture; fourth, the Shakespeare-Norris stain for hæmoglobin,

consisting of a mixture of borax carmine and indigo carmine. This stain was subsequently abandoned, as it was found not to work as a differential stain for hæmoglobin after mercuric chloride hardening. In several cases where sections were made of a foetal femur, with its contained marrow, the tissue was fixed in Flemming's solution, and afterwards decalcified in saturated picric acid solution. These sections treated with the indigo-carmine solution gave very beautifully the apple green stain to the hæmoglobin in the red corpuscles. Another method which I used frequently, both for the blood itself and the blood-forming tissues, is one recommended by Flemming, as follows: the fresh tissue is quickly teased upon a slide in its own liquid, and a large drop of diluted Flemming solution is dropped upon it, and the specimen then kept for twenty-four hours in the moist chamber. By that time a number of the cells have become firmly adherent to the slide, so that it can be washed in water. It is then covered with saffranin for twenty-four hours, being kept in the moist chamber. The saffranin is washed off with absolute alcohol, with or without acid, according to the depth of the stain, and the specimen treated successively with oil of cloves, xylol, and balsam. This method gave excellent results.

Development of the Red Corpuscles during Extra-uterine Life.

The importance and even the existence of the nucleated red corpuscles has been denied by some authors, as I have attempted to show in the historical review of the subject. But that these cells are found in the red marrow of the bones throughout healthy life, and that they give rise to the red corpuscles of the circulating blood, has been proved beyond any reasonable doubt, and upon the whole is as well accepted as most of the facts of physiology. What we desire, then, is a complete knowledge of the life-history of the nucleated red corpuscle, its origin, its method of growth or reproduction, and the way in which it is changed to the non-nucleated corpuscle. These corpuscles are found chiefly, if not exclusively, in the adult in the red marrow. Hence most of the work has been done upon that tissue.

Origin of the Nucleated Red Corpuscle.

Most authors agree that the nucleated red corpuscle is derived from a colorless cell existing in the marrow, but there is considerable difference of opinion as to the characteristics and origin of this cell. Löwit (29), it will be remembered, gives to it the name of erythroblast, and describes certain histological characteristics which enable him to recognize the cell wherever seen. Others derive the nucleated red corpuscles from what are known as the ordinary marrow cells, and others still, as Osler (28), describe a peculiar kind of cell in the marrow from which the nucleated red corpuscles are derived, and which correspond more or less closely to the erythroblasts of Löwit. Before speaking of my own view, it will be necessary to describe briefly the different sorts of cells found in the red marrow of the cat. In teased specimens of the marrow we meet, in the first place, with the morphological elements of the blood, the red corpuscles, white corpuscles, both uninucleated and multinucleated, and the blood plates. Of the marrow elements proper, we have, first, the nucleated red corpuscle. By this term is meant a nucleated cell colored with hæmoglobin. The size of these cells is quite variable, and they are frequently found in different stages of cell division, as described by Bizzozero (19*d*), the most common figure being the diaster. But the most marked peculiarity in the structure of the nucleated red corpuscles is found in the nucleus. In some of these cells, which for the sake of clearness I will speak of as the immature nucleated red corpuscles, the nucleus is characterized by an intra-nuclear network of chromatin, at the nodal points of which are found conspicuous granules of a similar material, which stain, however, more deeply than the reticulum. In badly preserved specimens, therefore, the nucleus seems to be composed of a number of fine or coarse granules imbedded in a clear or slightly colored matrix. The cell protoplasm of these immature forms is, as a rule, only slightly tinged with hæmoglobin, and makes a relatively thin envelope round the nucleus (see Fig. 8). Others of the nucleated red corpuscles, which may be distinguished as the mature forms, have a nucleus which shows no sign of a reticulum when stained with methyl green, hæmatoxylin, saffranin, etc. The nucleus, when stained,

shows usually, indeed, no structure whatever, but takes a deep uniform tint, as though the chromatin material were evenly diffused throughout (see Fig. 8). The nucleus of this form is generally smaller, both relatively and absolutely, than that of the immature cells; and the cell protoplasm is more deeply tinged with hæmoglobin. It is very common to find these cells with the nucleus either placed eccentrically or partially extruded, while in the immature cells no such appearance is ever seen. As the names I have chosen indicate, I consider these two forms the two extremes in the life of the nucleated red corpuscle. Intermediate stages between the extremes are, of course, of frequent occurrence; for instance, corpuscles with a nucleus which stains deeply and nearly uniformly, but shows large or small irregular clumps of a deeper staining material, like the granules of the nucleus in the younger forms, or others in which the nucleus contains smaller granules staining deeply and some indication of a reticulum between the granules; while the material between the granules and reticulum, the nuclear liquid, also takes the stain to a certain extent. The morphological difference between the two extreme types of nucleus is associated with a difference in chemical structure, as far as this can be determined by staining reagents. When sections of the marrow are treated with the triple stain, — hæmatoxylin, eosin, saffranin, — the nucleus of the immature forms takes the hæmatoxylin, while that of the mature forms stains a brilliant red with the saffranin; and the nucleus of the intermediate stages shows a combination tint of some shade of purple (see Fig. 9). The distinctness with which this difference in staining comes out depends, of course, upon the time of exposure to the different dyes. If the section has lain too long in the hæmatoxylin, all the nuclei of the preparation may be stained a dark blue or purple; while, if the exposure to the hæmatoxylin has been too short, the saffranin color predominates to the exclusion of the others. In some degree, however, the difference between the nuclei may be discovered in all cases; and when the staining has been properly regulated, it comes out with great distinctness. The time for the action of each dye varies naturally with the thickness or character of the sections; but usually a minute to a minute and a half was found to be the proper time of immersion in each of the staining reagents. It is worthy of

mention that the nucleoli of the marrow cells and giant cells, as well as the nuclei of cells during karyokinesis, when treated with the triple stain, take the saffranin in preference to the hæmatoxylin, like the nuclei of the mature nucleated red corpuscles; whereas the reticulum of the resting nucleus of most cells, unlike the nucleolus, stains most easily with the hæmatoxylin. A similar difference in the behavior of the nucleolus and the dividing nucleus has been noticed before by Steinhaus (48) for epithelial cells, and by Hodge (40) for nerve ganglion cells. With the triple stain of Biondi, the nucleus of the mature nucleated red corpuscles stains an even solid green, and in the nucleus of the immature forms the reticulum and granules at the nodal points stain a light green, while the nuclear material between the meshes of the reticulum remains unstained.

2. The next most important element of the marrow from our standpoint is a colorless cell, similar in structure to the immature form of nucleated red corpuscle, from which it differs in fact only in the absence of hæmoglobin from the cell protoplasm. The nucleus is granular without anything like a definite nucleolus. In well-preserved specimens the granules are connected by an intra-nuclear reticulum, which stains less deeply than the granules. This form of cell has been described by Osler (28), and also by Löwit (29) and others, as the progenitor of the nucleated red corpuscle. Löwit has given to the cell the name of erythroblast. It seems to me that the name is a convenient one, and I shall make use of it hereafter. At the same time, I wish to say that I do not accept Löwit's theory of the origin and permanent histological characters of these cells, which has been described in the historical review. On the contrary, my investigations have brought me to quite different conclusions, as I shall show in the proper place. Drawings of this form of cell are shown in Fig. 8.

3. The ordinary marrow cell is a large, colorless cell, with a characteristic nucleus and a faintly granular protoplasm. The nucleus is of a vesicular character, having an oval shape, a doubly contoured nuclear membrane, and one or more conspicuous nucleoli. From the nucleolus a scanty reticulum stretches out toward the peripheral membrane (see Fig 12, *a* and *b*).

4. Wandering cells. These are like 3 in structure, except that the nucleus, instead of being oval, is pulled out to an

elongated strap shape, and may be bent into a horseshoe, or may be coiled upon itself one or more times, like the leucocytes found so abundantly in the cat's blood. These cells, are, however, larger than leucocytes; and it is probable that they belong to the same class as the ordinary marrow cells (Fig. 12, *c* and *d*).

4. Some of the ordinary marrow cells have their protoplasm loaded down with coarse granules which stain readily with eosin, methyl green, etc. (Fig. 12, *e* and *f*). Sometimes these cells are very numerous: they evidently play some important part in the metabolic changes going on in the marrow. They do not appear to be confined to the marrow, since Heidentain has described what seems to be the same cell in the lymphoid tissue of the intestine, though he was unable to arrive at any satisfactory conclusions as to its function.

6. The so-called giant cells. In the red marrow of grown animals these are always of the kind described by Bizzozero as giant cells with budding nuclei to distinguish them from the multinucleated giant cell, or myeloplague, found in developing bone, in pathological formations, etc. A more detailed description of these cells with a discussion of their functions is given in an accompanying paper.

7. Free nuclei are found sometimes in considerable numbers. In size and in the way in which they stain, they resemble exactly the nuclei of the matured nucleated red corpuscles, and there can be but little doubt that they arise from these cells.

With reference now to the origin of the nucleated red corpuscles, there seems to be little doubt that they are derived in the first place from the colorless cells (No. 2) known as erythroblasts. There has been some difference in the description of these cells as given by various observers; but there is enough agreement to justify one in believing that the same cell is meant by all, and that the erythroblast is converted to the nucleated red corpuscle by the development of hæmoglobin in the cell protoplasm. This point might be regarded as generally accepted. The real difference of opinion lies in the theories as to the derivation of the erythroblast. While Löwit (29), Denys (14), and others believe that it constitutes a distinct variety of cell found in the marrow and other blood-

forming organs, that it multiplies by indirect division, — *divisio per fila*, — and is not derived from any other element of the marrow, Osler (28) and Osbratzow (35) think that it develops from naked nuclei found in the marrow, and Foa and Salvioli believe that it is constricted off from the giant cells. The theory of Löwit is the best supported by observations and experiments, and has met with most corroboration. While I with others before and after Löwit have satisfied myself of the existence of the erythroblasts, I cannot agree with him that they are not derived from other simpler cells found in the marrow.

In sections and teased specimens of the liver of the embryo and of the marrow of the embryo and adult, I have obtained evidence to show that the erythroblasts are derived from cells of the marrow similar in structure to the ordinary marrow cells; that is, large cells with oval vesicular nucleus and a faintly granular protoplasm. Drawings intended to illustrate the way in which these cells give rise to the erythroblasts are given in Fig. 11. The marrow cells themselves have the characteristics of embryonic cells; and those from which the erythroblasts are derived are undoubtedly descendants, but little if any changed, of the original mesoblastic cells from which the marrow is formed. In the embryonic liver, as well as in the embryonic marrow, these cells are found, together with the transitional stages to the typical erythroblast. This derivation is particularly well marked in the developing blood-vessels of the liver of the young embryo. As I have already said, these vessels consist of a mass of cells destined to become red corpuscles; and some of them are typical erythroblast, while others are of the character of the marrow cells or correspond to what Löwit calls leucoblasts, and others still represent intermediate stages. None of these cells can be regarded as leucoblasts, according to the definition of Löwit, since at this time no typical leucocytes are found in the circulating blood. The embryonic cell from which the erythroblast is derived is found in the marrow of the adult as an ordinary marrow cell. In fact, the marrow cells seem to be undifferentiated cells, like the cells of the original mesoblast; and, while some may change to erythroblasts, others become loaded with coarse granules or develop into the fat cells of the yellow

marrow. Of course, there may be a difference in structure in these apparently similar cells, according to the fate which befalls them; but, if so, it is not apparent as a morphological characteristic. The ordinary marrow cell, as has been described, is characterized histologically by its vesicular nucleus, which has one or more prominent nucleoli and a scanty reticulum. In the forms intermediate between this and the erythroblast we find that the nucleoli, or nucleolar matter, becomes scattered throughout the nucleus in the form of smaller granules; while the reticulum becomes more pronounced, and unites with the granules to give the characteristic nucleus of the erythroblast. While in the latter cell, therefore, we have no distinct nucleoli, we do have a number of small granules of nucleolar material situated at the nodal points of the reticulum. This transformation from a marrow cell to an erythroblast does not take place by gradual changes going on in one cell, but makes its appearance more or less gradually in successive generations. The original marrow or embryonic cell multiplies by indirect division; and the daughter-cells, instead of having a single large nucleolus, have several smaller ones scattered throughout the nucleus and connected with its reticulum, showing thus an approximation to the structure of the erythroblast, the cells also being of a smaller size. These cells in turn multiply; and their offspring either become erythroblasts or at least resemble them more closely. One cannot say how many generations—one or more—are necessary for the change. All that can be observed is that between the large embryonic cells and the smaller erythroblasts there are found cells intermediate in size and in the structure of the nucleus; and it seems more reasonable to suppose that these changes take place after successive divisions during the re-formation of the nucleus from the chromatin filaments rather than from a process of condensation and alteration going on in each cell. Denys (14) has found in the marrow of birds that the erythroblasts are separated from the other elements of the marrow, and lie in cords, which are in reality a part of the vascular system of the marrow. I have described a similar arrangement in the liver of the young embryo cat. But if such an arrangement of the erythroblasts exists in the marrow of the cat, it is certainly very much obscured, as repeated examina-

tions of sections of the marrows of cats of all ages has not revealed a separation of this character. On the contrary, the erythroblasts seem to be scattered among the other elements of the marrow without any apparent regularity. It is possible that careful injection of the marrow will throw more light upon the subject. On *à priori* ground, I should think that in the mammalian marrow there must be some such arrangement as that described for the bird and the embryo, as it would furnish the simplest explanation of the way in which the newly formed red corpuscles develop and gain entrance into the circulation, and would prove that the process of formation in the adult and foetus and among the chief classes of vertebrates is essentially the same. The embryonic cells from which the erythroblasts are formed must also, of course, lie in the unformed vessels with the erythroblasts, as is the case in the embryo.

Growth and Reproduction of the Nucleated Red Corpuscles.

Since the observations of Bizzozero (19) it has been known that the nucleated red corpuscles multiply by indirect division (karyokinesis) like most of the other cells of the body. Though his observations have not been disputed, other writers have described different methods of growth, some of which have been mentioned already. Foa and Salvioli (13) believe that the nucleated red corpuscles are recruited continually from the giant cells, Löwit (29) that they are developed from the erythroblasts, and Malassez (27), Osler (28), and others take a similar view. None of them, except Bizzozero, seem to lay much stress upon the independent reproduction of the nucleated red corpuscles themselves. It is quite easy to show, nevertheless, that Bizzozero's observations are perfectly correct, and that not only the erythroblasts, but the nucleated red corpuscles also, multiply by indirect division. Simple examination of teased specimens of the marrow, especially of kittens which have been bled severely, gives usually a number of corpuscles undergoing division, such as are shown in Figs. 5 and 10. Specimens teased in methyl green solution show sometimes a portion of the spindle, as indicated in the figure; but the chromatin filaments are not well preserved. The reagent seems to swell the filaments into a mass, but, in spite of this, it is

not difficult to recognize the chief stages of karyokinetic division. When the marrow is preserved in Flemming's solution, and the sections are stained in saffranin, the nuclear figures are very well preserved, and undoubted nucleated red corpuscles, showing the skein, monaster and diaster, can be obtained without trouble, as shown in Fig. 10. Nucleated red corpuscles with two nuclei and the cell partially constricted between, — that is, the last step in the process of division, — are especially common. We must admit, then, that the nucleated red corpuscles have the power of independent multiplication. But this power of reproduction is not unlimited; and this, it seems to me, is an important fact which has hitherto been overlooked. It is not difficult to determine when the cell has lost its power of reproduction: it is indicated plainly by the appearance of the nucleus. The changes in the structure of the nucleus of the nucleated red corpuscle have been described already in detail, especially the two extremes designated as the mature and immature form of the nucleus. The immature nucleated red corpuscles have a nucleus like that of the erythroblast, preserving a definite reticulum, and, like the erythroblast, it is capable of karyokinetic division. But the offspring or daughter-cells of this form have nuclei belonging to the intermediate class, in which the reticulum is less marked, and the whole nucleus shows a tendency to diffuse staining. These cells are very common in the marrow, and it is probable that they also are capable of multiplication. But sooner or later the offspring of these cells show nuclei with no reticulum at all, and staining diffusely and deeply with the different dyes. This is the mature form, and is further characterized by the deeper color of the hæmoglobin in the cell substance. This cell is now ready to lose its nucleus, and become an ordinary red corpuscle; and, as far as I can determine, nucleated red corpuscles which have reached this stage are incapable of any further multiplication. The mature corpuscles are usually smaller than the immature forms, as the successive offspring show a gradual diminution in size both of the nucleus and the cell substance. It is impossible to say how many generations intervene between the youngest nucleated red corpuscle, in which hæmoglobin has just appeared, and the mature form, with its peculiar nucleus and greater hæmoglobin contents.

The number, of course, may not be constant, at least not for different conditions of life. All that one can actually observe, and this point I wish to emphasize, is that the cells which I have described as the mature and immature forms of the nucleated red corpuscle really exist in the marrow at all times, that the latter undoubtedly multiply by karyokinesis, and that the former bear every indication of being nearer the condition of the non-nucleated red corpuscle, both in size and depth of color, and in the fact that they are no longer capable of reproduction. The theory which I have suggested offers a simple explanation of these phenomena. One other hypothesis which might be suggested, and which has in fact been proposed, is that the nucleated red corpuscle, after it has been formed from the erythroblast by the development of hæmoglobin, begins to undergo a process of condensation which results in making both the cell and the nucleus smaller. But this theory does not take into consideration the fact that what I have called the younger forms of the nucleated red corpuscle are without doubt capable of active multiplication, and that the offspring seem to show in general a diminution in size and a definite change in the character of the nucleus.

The Transformation of the Nucleated Red Corpuscle to the Red Corpuscle of the Blood.

The essential factor in the transformation is the loss of the nucleus. After it was known that in the foetus the nucleated red corpuscle loses its nucleus and changes to the non-nucleated form, Kölliker (3) proposed the theory that the nucleus is destroyed by absorption within the cell. The absorption may be preceded by a fragmentation of the nucleus more or less complete, such as one often sees in examining the blood of a young embryo. Kölliker does not seem to have given any microscopic proof for his view other than the partial disintegration of the nucleus. Neumann (5*b*), after he had clearly shown that the nucleated red corpuscle exists also in post-natal life as the precursor of the non-nucleated form, adopted the theory of Kölliker to explain the disappearance of the nucleus. He was able to follow the process best in the human foetus (five months), and describes the nucleus as becoming smaller, more homogeneous, and finally notched or indented. In addition, he describes red

corpuscles with only one or two small granules of nuclear matter, which he takes to represent the last step in the disappearance. There is very little satisfactory proof, then, for the theory, since no one, of course, has been able to follow the process through all its changes, and the appearances described above might easily be explained in other ways. Nevertheless, the theory has been generally adopted by those who believe in the nucleated red corpuscle and its functions. Malassez, of course, upon his theory of budding, is not obliged to explain the loss of the nucleus, nor are those who believe in an endogenous formation of the red corpuscles; but, outside of these theories, which cannot be said to have a strong support at present, the general belief among histologists is that the nucleated red corpuscle loses its nucleus by absorption in the way described by Kölliker and Neumann. There seems to be, indeed, only one other alternative: if the nucleated red corpuscle changes to the non-nucleated form, the nucleus either disappears by absorption within the cell or by extrusion from the cell. This latter view has been seriously supported only by Rindfleisch (26). As I have stated in the historical review, Rindfleisch believes that the nucleus escapes from the nucleated red corpuscle surrounded by a small layer of colorless protoplasm, and leaves behind a bell-shaped corpuscle which eventually becomes a biconcave disc. He figures corpuscles in which the nucleus was seen in the act of escaping from the cell. Others have seen similar examples of extruding nuclei, but have concluded that it was an accidental and not a normal phenomenon. The chief result of my own work has been to obtain what seems to me indisputable evidence that the extrusion of the nucleus is the normal method by which the nucleated red corpuscle loses its nucleus and passes into the non-nucleated form. Unlike Rindfleisch, I have never been able to discover with the highest objectives (Zeiss Hom. im. $\frac{1}{8}$ and apochromatic im.) that the escaping nucleus has an envelope of protoplasm round it. On the contrary, it goes out of the corpuscle entirely naked, and can be found as a free nucleus in sections and teased specimens of the marrow, and also in the embryonic liver, as has been previously described by Neumann (see Fig. 2). In many cases in the marrow, and especially in the foetal liver, I have seen the homogeneous nucleus partially

segmented or notched in the way described by Kolliker (?) and Neumann, and interpreted by them as an indication that the process of absorption had begun. Nevertheless, I have seen nuclei of this character already partially extruded from the cell, showing that the partial fragmentation of the nucleus is not conclusive proof that it is in process of absorption. To show that the escape of the nucleus is a normal and constant phenomenon we have the following facts :

In specimens of the marrow of kittens and adult cats, especially after repeated bleedings, and also in the blood-forming organs of the embryo when teased out in their own serum and stained with methyl green, one can easily find very many examples of nucleated red corpuscles in the act of losing their nuclei. In some animals the number of examples is striking — a dozen or more may be seen in a single specimen ; while at other times, especially in unbled animals, it may be difficult to find a single example. But in bled animals, especially bled kittens, in which it is fair to suppose that the process of blood formation is greatly accelerated, no difficulty will be found in obtaining a number of examples showing all the steps in the act of extrusion, from the time when the nucleus has only an eccentric position up to the period when it lies completely outside the cell, as shown in Fig. 2. The frequency with which this phenomenon occurs, especially when the production of red corpuscles is increased, requires that it should be explained. Now it must be a normal occurrence, or else it comes from the action of the reagents, or possibly it is the result of post-mortem changes taking place in the cell after removal from its normal environments.

There are a number of facts which may be adduced to show that the phenomenon is not an accidental or post-mortem change, but a normal occurrence. In the first place, most of the specimens were obtained from pieces of the marrow (or liver in the embryo) which were taken as quickly as possible from the animal after killing, and treated with methyl green, so that only a few minutes intervened between the death of the animal and the action of the methyl green. This reagent, as is well known, is an excellent fixative. It preserves fairly well the nuclear figures of karyokinesis, and fixes the blood plates quite as well as osmic acid. It is not likely, then, that such a re-

agent would cause in one of the cells of the marrow an expulsion of the entire nucleus, and in others preserve the delicate karyokinetic figures; and, on the other hand, the fact that the marrow was submitted to the action of the reagent so quickly after the death of the animal, probably before the death of the marrow cells, precludes the possibility of post-mortem changes of the nature required to expel the nucleus from a cell. So in several cases, both in the adult and the kitten, after severe bleeding, and also in the foetus, I have found examples of extruding nuclei in the circulating blood. In these cases, the drop of blood was taken from the living animal and mixed at once with the methyl green, so that there was no opportunity for post-mortem changes (see Fig. 2). Moreover, I have obtained cases of extrusion frequently in sections of marrow which had been taken from the animal as quickly as possible after bleeding, and hardened in mercuric chloride. Here, again, we have an excellent fixative quickly applied, which ought to have prevented post-mortem changes on the one hand, and on the other should not have acted with such violence upon one of the kinds of cells found in the marrow as to force out the nucleus. To adopt either one of these hypotheses to explain the extrusion is not permissible in the light of our knowledge of the action of this reagent on cells in general.

In the second place, all the red corpuscles which I have seen with the nuclei extruding belong to the class of mature nucleated red corpuscles. Never have I seen a nucleus extruding from a nucleated red corpuscle which showed a nuclear network. This indicates that the escape of the nucleus is not owing to any accidental or post-mortem changes, since there is no reason under such conditions why all kinds of nucleated red corpuscles should not have been affected in the same way. It shows, also, that the extrusion of the nucleus is the normal end to the life history of the nucleated red corpuscle, since it is found only among those which seemed to have reached full maturity and are prepared, as far as size, color, etc., are concerned, to become ordinary red corpuscles. It seems to me that this fact is a very important one in its bearing upon the question under discussion, and, so far as I know, it has not been noticed before. I have been impressed with this pecu-

liarity of the extruding nucleus, not only from the study of teased specimens stained in methyl green, but also from an examination of sections of marrow stained with hæmatoxylin, eosin, and saffranin. It is not difficult to find in these sections a nucleus in the act of extruding, and in all cases such nuclei belonged to the mature nucleated red corpuscles as shown by the fact that they stain with saffranin in preference to the hæmatoxylin in the way that I have described. Osler (28), who has figured and described the extruding nuclei, but does not think they occur normally in the living tissue, states that they are more abundant in the marrow twenty-four hours after death than in the fresh cadaver. This may well be, even if the phenomenon is a normal occurrence, since the marrow cells probably survive some hours after somatic death, and the mature nucleated corpuscles may lose their nuclei partially or completely as in life, and the stoppage of the circulation would lead to an accumulation of such examples in the marrow. However, in the cat, at least, under the conditions mentioned, they can be found in abundance immediately after death. Whether or not with this animal the number is increased twenty-four hours after death I have never determined. The presence of granules within a newly formed red corpuscle has been taken as a proof that the nucleus is absorbed within the cell, the granules being looked upon as remnants of a former nucleus. The existence of such cells cannot be questioned; but, taken alone, they cannot be considered as strong proof for the theory of absorption nor as any objection to the theory of extrusion; for I have in a number of cases found red corpuscles containing these granules in which, nevertheless, the nucleus was in the act of extruding, as shown in Fig. 2. The granules in such cases evidently did not mean that the nucleus had been absorbed. Erb (4), it will be remembered, described such corpuscles in the circulating blood; they form his transitional stage between the white and red corpuscle. Löwit (29*d*) has newly discovered them, especially in the blood of certain veins after treatment with a modified Pacini's liquid, and has laid great stress upon them as transitional forms between the erythroblasts and red corpuscles. Foa (41) also has recently described granulations of this character as part of the normal structure of every red corpuscle and easily brought out

by appropriate treatment with methyl blue and chromic acid. I have met with corpuscles containing granulations very frequently, particularly in the blood-forming organs. In sections or teased specimens of the blood-forming organs, the newly formed red corpuscles are often characterized by the ease with which they lose their hæmoglobin. Under such conditions the granulations come out very distinctly. Sometimes the granules — which stain, by the way, like nuclear chromatin — are so arranged as to represent the outline of the nucleus, and I have obtained such cells in which the nucleus at the same time was fixed in the act of extrusion (see Fig. 7). It is an interesting fact with reference to the corpuscles containing granules that they are usually newly formed corpuscles, and on that account occur most abundantly in the fœtal blood or in the blood-forming organ (marrow) of the adult. There is no evidence to show that the granules are the last remaining fragments of an absorbed nucleus. On the contrary, all that we know about them is opposed to such a view. They must be looked upon, it seems to me, as bits of the nuclear chromatin (membrane) left behind when the nucleus leaves the cell. What their fate is, whether finally absorbed or whether they last throughout the life of the corpuscle, is not known.

In this connection I may refer to a curious phenomenon which has come under my notice and upon which I am now working. On one occasion, after bleeding a medium-sized cat very severely (a loss of 90 cc. of blood), it was found upon examining the blood twenty-four hours afterward that the majority of the corpuscles in the animal contained a single good-sized piece of nuclear matter, too large to be called a granule, but having the shape and appearance of a large nucleolus. This fragment stained readily with methyl green just like the nucleus: it could be seen also in the unstained corpuscles as a refractive particle (see Fig. 4). I cannot recall ever having seen anything corresponding to this described, except, perhaps, the first stage of the malarial germ as pictured by Marchiafava, with which, indeed, the appearance seen by me seemed to be identical. Closer examination of the corpuscles showed that the fragment of nuclear matter, as I shall call it, always lay imbedded in the periphery of the spherical corpuscle after treatment with the methyl green. When care was taken to

make the corpuscle rotate in the liquid, I found no exceptions to this position of the fragment. A remarkable thing about the phenomenon was its persistence. Even two weeks after bleeding, a drop of the blood taken from the ear showed a number of these corpuscles. I was successful afterwards in getting the same result from other cats, though I had many failures. The necessary condition seems to be that the animal should be bled quickly and severely. At first, I supposed that the objects in question were simply large granules floating in the blood which had adhered to the corpuscles; but I was soon convinced that this was not the case. The fragments could not be detached from the corpuscles either by shaking or by the addition of water, acetic acid, and other reagents, which dissolve out the hæmoglobin from the corpuscles. Moreover, a number of corpuscles were without the fragments, and in normal cats no such appearance could be obtained. The only satisfactory explanation of the phenomenon which has occurred to me is that the fragment is a bit of the nucleus left adhering to the corpuscle at the time that the nucleus escaped. Under the conditions necessary for the appearance of the phenomenon, we may suppose that the process of production of new red corpuscles was vastly accelerated, and that therefore the extrusion of the nucleus was not as perfect as under normal conditions. The portion remaining in the corpuscle is not absorbed at all, but probably remains with the corpuscle up to the time of its dissolution. Whether or not my view as to the origin of the fragment is correct, there can be no doubt that it is not absorbed in the corpuscle while in the blood, but remains with it up to the time of its destruction. At the suggestion of Dr. Bowditch, I had hoped to use the phenomenon to measure the average length of life of the red corpuscle of circulating blood, but have hitherto met with certain difficulties which I hope soon to overcome.

After I was convinced from a study of teased specimens and sections that the nucleated red corpuscle loses its nucleus by extrusion, it seemed to me that it might be possible to watch the process taking place in the living cell. The experiments that I made for this purpose were not very numerous, for reasons that will be given below; but they were successful in a measure, at least. The method employed was to use the

marrow of very young kittens, about a week old, which had been bled rather severely from the jugular vein some twenty-four hours previously so as to increase the processes of blood formation. The marrow was teased out quickly in an indifferent solution of some kind upon a slide, the edges of the cover slip were sealed with paraffin, and the slide was kept at a temperature of 37-38° C., by means of a warm stage. Various indifferent solutions were tried, such as normal salt solution, amniotic liquid, aqueous humor, and blood serum; but successful experiments were obtained only when the serum of the same animal was used as the teasing liquid. The other liquids were given only one or two trials; but as far as the experiments went, they indicated that even such liquids as normal salt solution and amniotic liquid are sufficiently abnormal to cause a suspension of the living activities of the nucleated red corpuscles. Two experiments were made with the animal's own serum as the teasing liquid. In the first I saw two cases of extrusion, in the second only one, in which I was able to follow the process in part at least. In picking out the corpuscle to be observed I found it was necessary to choose one in which the nucleus already showed signs of extrusion, for otherwise it would be impossible except by accident to select a cell which had reached the proper stage. It was not difficult to find a number of corpuscles with the nucleus beginning to extrude. Many of them showed no further change, though watched for some time; but in three cases I was able to follow the last stages of extrusion until the nucleus lay completely outside of the cell. Sketches were made of one of these successful cases, though unfortunately it was the most incomplete of the three. The drawings are given in Fig. 2. The experiments were discontinued because of the improbability of obtaining a cell in which the process could be watched from the beginning to the end. The results, as far as they went, were still further proof to me that the extrusion of the nucleus is a normal phenomenon, since it was obtained only when the conditions were most favorable for preserving the life of the cell. I have spoken of the escape of the nucleus as an extrusion, but it is quite possible that migration would be a more accurate term. I was not able to convince myself that the escaping nucleus in the living cell showed definite amoeboid movements,

though the sketches made (see Fig.) seem to indicate that such movements occur. The figure shows, indeed, that the corpuscle as well as the nucleus undergoes changes in shape; but this was caused in part at least by the rolling of the cell so as to present different surfaces in successive drawings. *A priori*, it seems much more likely that the extrusion should result from some active movement on the part of the nucleus rather than from contractile changes in the cell substance. For it seems to be generally admitted now that in certain cells — lymph cells especially (Arnold) — not only movements of the nucleus may take place, but movements of the granules and filaments in the nucleus. After the escape of the nucleus, the spherical red corpuscle eventually becomes a biconcave disc. I have not attempted to follow this change, though I feel convinced that the bell shape which Rindfleisch ascribes to the corpuscles which have just lost their nuclei is a mistake. The red corpuscles even of the circulation, as is well known, frequently take this shape when treated with reagents of any kind, or even when examined without the addition of any liquid. It seems to me very natural to suppose that the biconcavity of the mammalian corpuscle is directly caused by the loss of the nucleus from its interior. Certainly as long as the corpuscles in the fœtus and the adult retain their nuclei, they remain more or less spherical, and after they lose their nuclei they become biconcave. The mechanical conditions of the circulation undoubtedly have some influence upon this change, but the initial cause lies apparently in the migration of the nuclear mass from the middle of the cell, so that the viscous material of the corpuscle is permitted to sink in. The biconcavity is of course a decided physiological advantage, as the absorptive surface is thereby considerably increased, so that upon the doctrine of natural selection, one can readily understand why such a variation should have become permanently established. Among the Camellidæ, it is true, we have biconvex non-nucleated corpuscles. So far as I know, no one has investigated the hæmatopoietic function in these animals, but it is possible that small spherical erythroblasts are not formed in them as in the other mammalia.

If we grant that the nucleated red corpuscle loses its nucleus by extrusion when it passes to the non-nucleated form, then

we are in a position to explain the budding corpuscles of Malassez. In several instances, when examining the marrow, I have met with appearances which seemed to justify Malassez's theory. Nucleated red corpuscles were seen with one or more non-nucleated corpuscles apparently budding out from them. Sketches of such cells are given in Fig. 3. They seem to me, indeed, to be better examples, as far as the drawings go, of the process of budding than those figured in Malassez's (27) own paper. I cannot say that these examples of budding are common; on the contrary, I obtained them clearly only in two cases, in both of which the notes of the experiments record that the animal had been bled so severely that it did not make a good recovery, but remained weak and anæmic; and it is possible that this is sufficient to explain their occurrence. I was at first inclined to believe that we must admit that, under certain conditions at least, new red corpuscles may be produced by budding in the way described by Malassez. But a simpler explanation of these forms suggested itself. What seem to be examples of budding are most probably cases of multiplication of nucleated red corpuscles by division, in which the process was not carried out to the complete separation of the newly formed corpuscles, though from one or more of the new cells formed the nucleus has escaped, leaving the non-nucleated corpuscle as an apparent bud on its sister-cell. As evidence for this explanation, one may find in the apparent buds granules of nuclear matter staining blue with the methyl green, such as I have described as occurring sometimes in the newly formed red corpuscle after the extrusion of its nucleus. Moreover, one frequently meets with two, three, or more mature nucleated red corpuscles joined in a cluster or chain as the result of recent division, and such as would produce apparent examples of budding if one or more of the cells lost their nuclei. This would be more likely to happen, of course, in animals in which too severe a bleeding had impaired the processes of cell development in the marrow as in the other tissues of the body. The explanation that I have adopted seems to me to be preferable to supposing that in the marrow new blood corpuscles are formed from the same cells by two entirely different methods of reproduction.

Fate of the Extruded Nucleus.

If the nucleus of the nucleated red corpuscle is extruded, the next point to be determined is what becomes of it. Naked nuclei, similar in all respects to the nuclei of the mature nucleated red corpuscles, can be found easily in the marrow, where, indeed, several observers have called attention to them, and also in the foetal liver at the time of its hæmatopoietic activity, where they have been noticed before by one writer, at least, — Neuman (5*d*), — who has described them very carefully and attributed to them some function in connection with the production of new corpuscles. It is fair to assume that the free nuclei are turned out into the blood stream along with the new red corpuscles. In that case, one of two fates awaits them. Either they persist as a morphological element of the blood, or they are dissolved in the blood plasma. Upon the first hypothesis, we can only suppose that the free nuclei become the blood plates, as no other element of the blood resembles them in size or structure. This theory has, in fact, been proposed by Gibson, though as far as I can see, he gives no proofs in its favor. I was also at first impressed with this idea; but the only experiment which suggested itself to me to test the hypothesis gave me unfavorable results. The nuclei of mature nucleated red corpuscles, when treated with the triple stain of hæmatoxylin, eosin, and saffranin, show a preference for the saffranin, while other nuclei take the hæmatoxylin. If the blood plates are derived from these nuclei, they ought to show something of the same behavior toward the triple stain. On the contrary, specimens of blood plates treated with the triple stain always take the hæmatoxylin, though they do not stain deeply. The method of preparing and staining the blood plates was as follows. A drop of blood was placed upon a slide, a cover slip was dropped upon it, and moved round once or twice. The slip was then taken off, and by this time a number of blood plates had adhered to its under side. It was next immersed in Hayem's liquid, to fix the blood plates and wash off the excess of blood plasma, and was then hardened like a piece of marrow in mercuric chloride, followed by alcohol, and afterwards stained. I obtained in this way good specimens of blood plates, somewhat deformed, of course, in

consequence of the time which elapsed before getting the slip into Hayem's liquid. The method also gave beautiful permanent specimens of fibrin reticulum and of red corpuscles, which retained their normal shape and stained deeply with eosin. The negative result of this experiment, together with certain other facts which will be given later in speaking of the blood plates, convinced me that there is no connection between the blood plates and the nuclei of the mature nucleated red corpuscles. There remains, then, only the theory that the liberated nuclei are dissolved in the blood plasma, and go to form in all probability one of the proteids of the blood.

It is, perhaps, unwise to speculate further upon the fate of the dissolved nucleus without some experimental basis to reason upon. However, my idea is that the free nuclei are dissolved in the blood plasma while still in the blood-forming organ. I have seen appearances in the marrow in sections which may represent this process of dissolution; that is, one meets occasionally with what seem to be globules of varying size from tiny drops to spheres larger than a white corpuscle which, like the free nuclei, stain deeply with saffranin, though of a different tint. Usually these are found in clusters of different sizes, and possibly they represent the free nuclei, undergoing changes preparatory to solution, though I have not found intermediate stages. These globules are evidently not a fat of any sort, as one might suppose from their general appearance, since otherwise they would have been dissolved during the process of imbedding. With reference to the material produced by the nuclei after solution, there seemed to me certain reasons for believing that the fibrinogen of the plasma is the product formed. Influenced chiefly by this idea, I asked Mr. Dreyer of the Johns Hopkins University, and formerly assistant in physiology, to investigate the changes in the blood plasma caused by severe bleeding. His results, which are very interesting in a number of ways, have not yet been published. It may be said, however, that with reference to the fibrinogen, he found that its percentage in the plasma was always increased, sometimes nearly as much as 100 per cent., over what it had been in the same animal before bleeding, the analysis in all cases having been made twenty-four hours after the bleeding. This striking increase in the fibrinogen is more remarkable because

at the same time there was usually a diminution in the total proteids of the blood. As far as it goes, this result is in accord with the hypothesis that the fibrinogen is formed from the liberated nuclei of the nucleated red corpuscles. I have in progress other experiments for the purpose of further testing the hypothesis.

The Hematopoietic Function of the Spleen.

All the facts bearing upon this question have already been stated in various parts of this paper. It may be convenient, however, to bring them together in the form of a brief statement of the different views held. It is well known and universally admitted that for a certain period during embryonic life, the spleen takes part in the formation of red corpuscles, as is shown by the fact that numerous nucleated red corpuscles, some of them in the act of multiplication, may be found in it. Shortly after birth, the spleen no longer contains nucleated red corpuscles and for this reason the majority of writers who believe that these cells are the predecessors of the ordinary red corpuscles, have concluded that under normal conditions the spleen during extra-uterine life takes no further part in the production of new red corpuscles. This function is relegated entirely to the red marrow. On the other side, a number of investigators, while admitting the absence of nucleated red corpuscles from the spleen under ordinary conditions, have nevertheless classed it with the lymph glands under the head of the hæmatopoietic organs, because they hold that the colorless corpuscles from which the nucleated red corpuscles are formed are produced in this organ. The most elaborate form of this theory is found in the works of Löwit (29) upon the origin of the erythroblasts, an account of which is given in the historical review. For my own part, I have not been able to convince myself that erythroblasts are continually forming in the spleen or lymph glands, as I have not been able to get any intermediate stages between them and the nucleated red corpuscles, and therefore take sides with those who think that the red marrow is the only organ as yet discovered, in which new red corpuscles are produced during post-natal life. This statement applies, however, only to the spleen under ordinary conditions of life. Bizzozero (19)

was the first to discover that in a number of animals, after severe and repeated bleedings, the spleen again might contain nucleated red corpuscles showing signs of active multiplication. This was denied by Neumann, who held that after such an operation, the nucleated red corpuscles found in the spleen were not more numerous than those present in the circulating blood. But Bizzozero's observations have met with confirmation at the hand of others, — Gibson (33), Foa (22), *et al.*; and I also in several cases have been able to show without any difficulty that in the cat, after severe and repeated bleedings, and in some cases after a single strong hemorrhage, nucleated red corpuscles can be found in the spleen with every indication that they are multiplying there. The balance of evidence is strongly in favor of this power of the spleen to resume its embryonic function when the demand for new red corpuscles is very urgent. In what way severe anæmia stimulates the spleen to a renewal of its hæmatopoietic activity is not known. It is very interesting in this connection to find that, when the spleen of the adult is partially excised, it is regenerated, and during the regeneration not only nucleated red corpuscles, but giant cells are found just as in the developing spleen of the embryo (Foa [42], Tizzoni [43], Griffini [44]). It may be that in the adult spleen a number of undifferentiated or erythroblastic cells are contained which become aroused to activity in consequence of severe anæmia, for the same reason, whatever it may be, that the cells of the marrow are stimulated to increased growth and multiplication by the same conditions.

Life-History of the White Corpuscles and Blood Plates.

It is quite generally agreed that the origin of the white corpuscles of the blood is to be found in the lymph leucocytes, or lymphocytes, to borrow a convenient term, which in turn are formed in the lymphoid tissues of the body, and especially in the so-called compound lymphatic glands. The lymphocytes are characterized by a vesicular nucleus, usually with a nucleolus and a scanty reticulum, and by a very small protoplasmic envelope. In the blood we meet with two chief varieties of leucocytes, — uninucleated and multinucleated. The uninucleated forms do not all have the same structure: some of them

resemble exactly the lymphocytes, and may be regarded as lymphocytes newly arrived in the circulation and as yet unchanged in structure (Erb, Löwit). These are characterized physiologically, as was pointed out some years ago by Schultze (45), by not possessing the power of making amœboid movements. A second form of uninucleated leucocyte is characterized by its large, finely granular, protoplasmic envelope. This form is amœboid, and it seems most reasonable to suppose that it is derived from the first form, or lymphocyte, since this latter cell is the only or chief form in which the leucocytes of the lymph enter the blood. The first variety of uninucleated leucocyte passes into the second in consequence of a growth in the cell protoplasm while in the blood current, the protoplasm meanwhile acquiring the power of contractility. A third variety of uninucleated leucocyte, and what seems to represent a third stage of development, is like the last, except that the nucleus is no longer oval or spherical, but is drawn out to an elongated strap shape, and may take either a horse-shoe form or may be more or less coiled into a spiral. This form of cell is especially abundant in the cat's blood, and possesses the most active amœboid properties. The origin and meaning of the multinucleated forms has been for some time a subject of dispute among histologists. Formerly it was generally thought that they represented cells in process of multiplication by direct division; and this view is still warmly supported by Arnold and others. The normal fate of the multinucleated cell, according to this view, is to divide into a number of cells corresponding to the number of nuclei. Others, and especially Löwit (29), have urged that the multinucleated forms are cells on the way to disintegration, and the so-called nuclei are made simply by the fragmentation of the nucleus of a uninucleated leucocyte, and represent the first step in the process of destruction. As far as my observations go, they support Löwit's view. I have never seen any indication of the multinucleated cells segmenting to form new cells. On the contrary, there is every reason to believe that they are undergoing a course of retrograde changes, the normal termination of which will be the disintegration and dissolution of the cell. With reference to the derivation of the multinucleated forms from the uninucleated by fragmentation of the nucleus, I have been able

to find all intermediate stages in the process as shown in Fig. 16. They are derived always from the third variety or third stage in the life of the uninucleated leucocyte, the elongated nucleus breaking up into the smaller fragments; and it is not difficult to find cells such as are shown in the figure in which the fragmentation is going on. According to this view, the different varieties of leucocytes found in the blood are in reality different stages in the life-history of the white corpuscle, and pass one into the other. To complete the life-history, one other stage must be described,—that of the disintegration of the multinucleated form. A close examination of the multinucleated cells, especially when in the act of disintegrating, has impressed me with the belief that the fragmented nuclei persist for a certain time in the circulation as the blood plates, though doubtless the blood plates also, sooner or later, go into solution.

This view of the origin of the blood plates is not new. Gibson (33) supports it, and gives some evidence in its favor; and Hlava (47) especially has given a number of arguments—none of which, however, are very conclusive—to prove this derivation. One is led, at first, to such a theory by noticing the very striking resemblance between well-preserved blood-plates and the fragmented nuclei as far as size, shape, and general appearance are concerned. This resemblance is still further increased when the blood plates are examined in the blood of an animal which has been repeatedly bled. Under such conditions, one gets, or may get, blood plates which have one or more granules within them staining more deeply than the rest of the plate, and resembling very closely the chromatin granules found in the fragmented nuclei of the leucocytes, as shown in Fig. 6. Something similar to this seems to have been obtained by Afonassiew. We may suppose in this case that the increased activity in the processes going on in the blood in connection with the regeneration, not only of its formed elements, but of its characteristic proteids, have led to a more rapid breaking down of the leucocytes, and that some of the fragmented nuclei are liberated as blood plates before reaching the usual degree of maturity. There is, moreover, a very close similarity in the way in which the fragmented nuclei and the blood plates stain. As far as I have been able to test them,

they stain alike, except that the blood plates take the stain more feebly. In the case already mentioned, in which the preserved blood was treated with a differential stain, successive staining in hæmatoxylin, eosin, and saffranin, the blood plates, like the nuclei of the leucocytes, took the hæmatoxylin. The same is true of methyl violet (Gibson) and methyl green. If this view of the life-history of the leucocytes of the blood is correct, it seems probable that they play an important part in the formation of the blood proteids. The young lymphocytes increase in size by the formation of new protoplasm; and in the end this again passes into solution in the plasma. Schmidt long ago stated that the paraglobulin of the blood is derived from disintegrated leucocytes. In fact, if I understand him correctly, he believes that the paraglobulin is all formed in this way after the blood is shed. Later investigations of the serum and plasma have shown that this latter statement is not correct, though there is apparently an increase in the amount of paraglobulin in the serum over that in the plasma. Still, it may be considered probable that the paraglobulin of the blood is derived wholly from the breaking down of the leucocytes, and that the constant supply of paraglobulin in the blood is derived from the continual disintegration of the multinucleated leucocytes. The fibrinogen, on the other hand, is possibly derived from the liberated and dissolved nuclei of the mature nucleated red corpuscles, and perhaps of the blood plates also, if they, too, represent nuclear material. We know little or nothing at present of the genesis and relationship of the blood proteids or of the nutritive value and significance of each. The fact that their percentage amounts in the plasma remain practically constant under many different conditions of nutrition indicates that they are regenerated continually in proportion as they are used up; but how this happens is one of the darkest as well as one of the most interesting points in the physiology of the blood. It seems to me that the question must be studied, in part at least, upon the hypothesis of their derivation from the formed elements of the blood in the manner here suggested, somewhat as we look upon the ground substance, or matrix, of the connective tissues as having its origin from the cellular elements.

SUMMARY.

The chief conclusions to which the investigation has led may be briefly summarized in the order in which they are presented in the paper as follows:—

1. In the very young embryo two forms of red corpuscles are found,—one large, oval, and always nucleated, resembling the corpuscles of the lower vertebrates, and one small, biconcave, circular in outline, and found both nucleated and non-nucleated. The latter are the true mammalian corpuscles; the former represent possibly ancestral corpuscles. The true mammalian corpuscles lose their nuclei by extrusion.

2. In the first part of embryonic life new red corpuscles are produced in the liver from groups of mesoblastic cells outlining the position of future blood-vessels (veins). The central cells of these cords become red corpuscles, while the peripheral ones form the walls of the veins. Similar developing blood-vessels are found in the embryonic muscular tissue of the posterior limb. It is probable that new red corpuscles are formed in all parts of the body where blood-vessels are being developed.

3. In the second half of the embryonic life red corpuscles are formed in the liver, the spleen, and the marrow of the bones, the function being most active first in the liver, then in the spleen, and finally in the red marrow. In the cat the liver and spleen lose this function three or four weeks after birth, and henceforward the red marrow alone produces new red corpuscles.

4. The white corpuscles (leucocytes) and blood plates do not occur in the circulating blood of young embryos, but make their appearance in later embryonic life. In the human foetus of five months both are present.

5. In the healthy animal during extra-uterine life the red corpuscles are produced only in the red marrow. They occur first as nucleated cells, the nucleated red corpuscles, found only in the red marrow of the bones. These cells differ in structure with their age, and two extreme types may be distinguished,—one mature and ready to be converted to a non-nucleated corpuscle, and one immature, as shown by the char-

acter of the nucleus and the amount of hæmoglobin. This latter form multiplies by karyokinesis, and the daughter-cells sooner or later appear as mature nucleated red corpuscles, which then lose their nuclei by extrusion, and become non-nucleated red corpuscles. The biconcavity of the red corpuscles is probably caused in the first place by the removal of the nucleus from the middle of the spherical cell. The liberated nuclei go into solution in the blood plasma, and probably form or help to form the fibrinogen of the plasma. The immature or young nucleated red corpuscles are derived from spherical colorless cells, erythroblasts, having a definite histological structure and found in the marrow. These cells multiply actively by karyokinesis. The erythroblasts in turn are derived from larger embryonic cells, usually described in the adult as ordinary marrow cells. The structure of the nucleus differs from that of the erythroblast. The erythroblasts are not derived each from one of these larger cells by a process of condensation, but the embryonic cells multiply by karyokinesis, and the daughter-cells of the first or following generations acquire the structure of erythroblasts. The chief point in the paper is the proof that the mature nucleated red corpuscles lose their nuclei by extrusion, and not by absorption, in changing to the ordinary red corpuscle of the circulation. The act of extrusion can be observed in part in the living cells.

6. Very severe and sudden bleeding (in cats) is followed by the appearance in the circulation of red corpuscles containing a large fragment of nuclear material. This fragment persists until the corpuscle disappears. Apparently the greatly accelerated production of new corpuscles causes a too rapid extrusion of the nuclei, so that a portion remains entrapped in the corpuscle.

7. The apparent gemmation of non-nucleated red corpuscles from the nucleated forms, as observed by Malassez, is probably owing to the multiplication of the nucleated cell and the subsequent loss of a nucleus from one or more of the daughter-cells before the complete separation of the cells has been effected.

8. While the spleen of the adult mammal does not take part in the production of new red corpuscles under normal conditions, it may be made to resume this function in consequence

of prolonged or extreme anæmia produced by repeated bleedings.

9. The leucocytes of the blood are derived from the lymph leucocytes (lymphocytes). The latter enter the circulation as small corpuscles with vesicular nuclei and scanty protoplasm, and are not amœboid. They develop into larger cells, with finely granular protoplasm which possess amœboid movements. These have at first an oval vesicular nucleus, which afterwards becomes elongated and assumes a horseshoe or spiral shape. From this last form the multinucleated cells are derived by fragmentation of the nucleus. The fragmentation of the nucleus is probably followed by the disintegration of the whole cell.

10. The fragmented nuclei after the disintegration of the cell persist for a time in the circulation as the blood plates.

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EXPLANATION OF PLATE.

FIG. 1. Blood from the heart of a foetal cat, 2.7 cms. long, stained with methyl green, shows the large nucleated corpuscles (ancestral form) and the ordinary circular biconcave mammalian corpuscles. One of the latter is shown with its nucleus escaping.

FIG. 2. Shows the way in which the nucleus escapes from the nucleated red corpuscle. 1, 2, 3, 4, represent different stages of the extrusion noticed upon the living corpuscles; the drawings are colored to correspond with the rest of the figure. *a*. Specimen from the circulating blood of an adult cat bled four times. *b*. Specimens from the circulating blood of a kitten forty days old, bled twice. *c*. Specimens from the blood of a foetal cat 9 cms. long. Others from the marrow of adult cat, two of the figures showing the granules present in the corpuscle which have been interpreted erroneously as a sign of the disintegration of the nucleus. All the specimens stained with methyl green.

FIG. 3. Examples of apparent budding of the nucleated corpuscles, resulting from the extrusion of a nucleus from one of the cells after division. From the marrow of a cat. Stained with methyl green.

FIG. 4. Examples of the large nuclear granules found in the newly formed red blood corpuscles (cat) after severe and sudden bleeding.

FIG. 5. Multiplication of the nucleated red corpuscles. Methyl green. Marrow of young kitten after bleeding.

FIG. 6. White corpuscles and blood plates, stained with methyl green, from the blood of an adult cat, bled once to 90 cc., and treated with methyl green and acetic acid. To show the origin of the blood plates from the nuclei of the multinucleated leucocytes.

FIG. 7. Newly formed red corpuscles from section of marrow of femur in a foetal cat 9 cms. Shakespeare-Norris stain of indigo carmine. To show the granules with outline of nucleus seen in the newly formed corpuscles after extrusion of the nucleus and the dissolution of the hæmoglobin.

FIG. 8. Nucleated red corpuscles stained with methyl green, to show the mature and immature forms and the intermediate stages and the colorless erythroblasts.

FIG. 9. Nucleated red corpuscles from sections of the marrow, stained in hæmatoxylin, eosin, and saffronin, to show the preference of the nucleus of the mature form for saffranin, and of the immature form for hæmatoxylin.

FIG. 10. Karyokinetic figures of the nucleated red corpuscle, from a specimen of young marrow teased in Flemming's solution, and afterwards stained in saffranin.

FIG. 11. To show the origin of the erythroblasts and nucleated red corpuscles from the embryonic cells (marrow corpuscles). From the liver of a foetal cat 2.7 cms., teased in Flemming and stained in saffranin.

FIG. 12. To show the marrow corpuscles. *a* and *b* with oval nuclei, *c* and *d* with coiled nuclei, and *e*, *f*, with the protoplasm loaded with coarse granules. Specimens teased in Flemming and stained with saffranin.

FIG. 13. From a section of the liver of a foetal cat 2.7 cms., showing the development of the liver vessels and the nucleated red corpuscles. To the right of the figure the newly formed vessel contains a number of non-nucleated red corpuscles, surrounded in the section by the coagulated plasma.

FIG. 14. A second section from the same liver.

FIG. 15. White corpuscles from the blood of a young kitten bled once. Treated with methyl green and acetic acid, to show the origin of the multinucleated from the uninucleated forms.

