STUDIES OF LIVING HUMAN BLOOD-CELLS

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The technique of staining smears of blood by the various modifications of Romanovski's method, that is, by mixtures of azur, methylene blue and eosin dissolved in the alcohol which serves as a fixative, has reached so high a degree of perfection and the surveys of blood-cells by this method are so complete, that further progress must now depend on the use of some new method. Such a method is, I think, furnished by the differential staining of living blood-cells, and by this method a whole new range of hematological problems has been opened up.

The method of studying blood-cells with supravital dyes was applied by the writer [1] in 1921 to the study of developing blood-cells in the embryo chick, both within the vessels as seen in the area vasculosa, and in smears of the living cells. During the same year, Miss Miriam E. Simpson [2] made a study of the reactions of living blood-cells to the large group of dyes which Evans and Scott [3] had studied with reference to an analysis of the cells of the connective-tissues. She also used [4] the vital method to separate the group of monocytes, first, in human blood and second, in rabbits under experimental conditions.

The technique of studying living blood-cells is so simple, that it is readily applicable to the study of clinical cases. All that is needed is clean slides and covers, a few drops of a stain and a warm box for the microscope or warm stage incubator, so that the slides can be kept at body temperature. The details of the method are as follows: the coverslips are cleaned as for fixed smears. The slides are also cleaned by the usual technique, that is, they are kept for three or four days in concentrated sulphuric acid to which a few crystals of potassium bichromate have been added. From the acid they are placed in running water, for 2 to 3 hours, care being taken that the water gets between the slides so that all acid is removed. This is essential, since living cells are sensitive to an acid. They are then rinsed 2 to 3 times in distilled water in which they are left over-night, and

finally stored in 80% alcohol. Before using they are dried on a clean towel and given a high polish with jeweler's rouge applied on a piece of clean silk. For the suggestion of this method of polishing I am indebted to Dr. Edward Malone; it is a great advantage because it ensures an even film of the vital dye. To get an even film of the dye, flame the slide; be sure that it is free from dust and then flood it with a dilute solution of any dye dissolved in absolute alcohol, drain off the stain quickly and stand the slide upright until it is dry. The stains can be used over and over but must be filtered frequently to keep them free from dust. The film should be faint but spread evenly on the slide. The slides can then be stored until they are needed, care being taken to keep them free from dust. Slides with any grease will have blotches on the stain and should be discarded. In drying the slides, do not blow on them, for again the stain will be uneven; if the stain is uneven, some of the cells will get too heavy a dose and be killed or damaged.

For stains, I have used neutral red, a combination of neutral red and Janus green, brilliant cresyl blue and azur. For the most part I have used neutral red which is relatively non-toxic. The stock solution of neutral red is made by adding 100 mg. of the dye to 10 c.c. of absolute alcohol. I have used only the dye put up by Grübler before the war, marked *"Zu Injekt. i. vital Gew. n. Ehrlich."* For making the films on the slides, take 10 c.c. of absolute alcohol and add 0.4 c.c. of the stock solution. The strength of the solution can be estimated by the color after one has had a little experience; it should be a clear rose color. The proper strength of the dyes can be readily determined by testing them with normal living blood-cells; the slightest staining of the nucleus will indicate that the stain is too strong. For the neutral red Janus green mixture, take 2 c.c. of the dilute solution of neutral red and add 3 drops of a saturated solution of the Janus green in absolute alcohol. The staining qualities of the mixture last only a few days. This mixture is much more toxic than the neutral red. The eosinophiles are amœboid in it but the neutrophilic leucocytes are not. As Miss Simpson discovered, the Janus green has the very great advantage of being differential for the lymphocytic strain of cells, since all of the lymphocytes show a characteristic arrangement of mitochondria. Azur, which may be obtained by using about 10 to 12 drops of Wright's blood stain in 10 c.c. of absolute alcohol, is a convenient dye but much more toxic than neutral red. The brilliant cresyl blue originally prepared by Grübler is an excellent dye; but the American dye, which I have used, has been mixed with a red dye which is far too toxic for supravital use.

It will be readily recognized that the thickness of these films of dye must vary with the number of cells to be stained in the preparation. For example, in a film of bone-marrow where practically all of the cells take up the dye, the strength of the solution must be greater than for blood where only a small proportion of the cells take any stain. The same is true in studying smears of lymph glands and spleen. For smears of blood a solution of the dye, of such strength that it will stain all of the cells immediately, can be safely and readily used, while for smears of lymph glands, spleen and bone-marrow, better results are obtained with a dye that takes 10 to 15 minutes to react. In a film of embryonic blood, where every single red blood-cell also takes the vital dye, a stronger solution should be used. It should also be noted that the vital staining of the white blood-cells takes a much weaker dose of any dye than the staining of the reticular substance of the red blood-cells, so that some of the white cells may be damaged if all the red cells stain. This fact explains the difficulty of staining the reticular substance of the red cells in marrow, as contrasted with the method used for films of blood, since with the marrow a dilute stain may all be taken up by the white cells.

Films of blood are made on the stained slide exactly as for any fresh film of blood. They must be sealed immediately with a vaseline of high melting point, for which we use Salvoline. Formerly we used melted Salvoline, but have found it better to smear it, while cold, along the edge of the coverslip, lest the heat damage the cells. Vaseline mixed with paraffin can be used; the melting point must be above the temperature of the warm box, otherwise the vaseline will melt, mix with the immersion oil and make the film opaque. After the film has been sealed, it should be put into a warm box; it is not necessary to hurry in getting it into the box, for the cells will show active motility even after having been kept at room temperature for 15 to 20 minutes. This makes it possible to carry the slides from the ward to the laboratory. In case considerable time would have to elapse, an arrangement of carrying the slides on a hot-water bottle could be devised. It will be found that blood from which a considerable amount of fibrin is deposited on the slide tends to have the red cells clump. It is not necessary to have every single red blood-cell distinct as for stained smears, but very much clumping of the red cells makes a film in which the count of the lymphocytes will be too small. The leucocytes will wander from the clumps of red cells but the lymphocytes will not, and may therefore be missed in the count. Of course films with any rouleaux are too thick. As with fixed smears, the cells at the edges must not be counted because an excess of dye collects at the edges so that some of the cells may be killed or damaged.

Since the living cells are sensitive both to acid left on the slide and to the strength of the dye, each new box of slides and each new bottle of the dilute stain should be tested with normal blood to avoid any false inferences concerning abnormal blood. The cells should all stain characteristically without any staining of the nuclei.

We regard any coloring of the nucleus as an evidence of damage to the cell, regardless of the fact that the cytoplasm may continue to move a short time after the nucleus has begun to stain. The amount of motility and the duration of motility are certainly reduced.

The descriptions of the vitally stained cells which follow are limited to human blood; it has been found in the progress of experimental work on animals, which is now in progress, that there are greater variations between the blood-cells of different species than had been established by other methods. The observations on comparative studies will be brought out later. In normal human blood, every type of cell stains so characteristically with a vital dye, that there is rarely a cell that cannot be distinguished with certainty. In abnormal blood, on the other hand, when a given group of cells has been stimulated or damaged, its reactions to the vital dyes may be markedly changed; or when unusual cells have been called into the blood-stream, it may be difficult or even impossible to distinguish them. The difficulties are, I think, not greater than with fixed smears, at any rate they are not always identical with the difficulties in smears; in consequence there are great advantages in a combination of the two methods.

The very marked gain in the use of the supravital method for the study of the white cells is the possibility of distinguishing cells which have been markedly stimulated to activity from cells which are degenerating. The second great advantage is the ability to distinguish the old and dying cells so as to complete the life cycle of each of the three types of white cells.

In making these studies I am very deeply indebted to the physicians of the Johns Hopkins Hospital for the opportunity to study abnormal blood-cells. Through the courtesy of Dr. W. P. Longcope, Dr. H. L. Amoss and Dr. J. G. Huck I have been able to follow cases on the wards and Dr. W. G. MacCallum and Dr. A. R. Rich have allowed me to study material from autopsies in which individual cells were still alive. Without these advantages this work could not have been done.

TERMINOLOGY

There has been considerable discussion regarding the use of the terms, *granule* and *vacuole* in the reactions of living cells to dyes, so I wish to define as exactly as possible the sense in which I have used these terms. By the term *vitally stainable granule,* I mean particles in the cytoplasm of living cells that take a dye in a uniform manner, and do not increase in size during the functional activity. That is to say, by granules, I mean particles that do not change their reaction to vital dyes nor increase in size during different functional states of the cells. As an example of granules, there are the neutrophilic, eosinophilic and basophilic granules of the leucocytes. It is true that with the Romanovski methods there is some variation of the staining of the neutrophilic granules. Beside these, all of the white cells contain mitochondria in the form of rods that react to Janus green. In the use of the term *granule,* I do not intend to indicate any opinion concerning the physico-chemical state of the substance. On the other hand, by the term *vacuole* I mean certain round droplets of fluid within the cytoplasm that take the stain and also change very greatly in size, according to different functional states of the cells. They may change also in color, provided that the dye used is a chemical indicator like neutral red. These are the digestive vacuoles which Shipley [5] has shown contained phagocytized material. He stained clasmatocytes with neutral red after they had stored trypan blue; he could then see particles of the blue dye within the red vacuoles. Evans and Scott [3] have called these vacuoles the "segregation apparatus" of the cell. They are organs of the cell into which phagocytized material is aggregated. It may not be possible, at the present time, to discriminate completely between granules and vacuoles according to these definitions, indeed, as we come to know more about the functions of the blood-cells, these definitions may be shown to be crude; nevertheless, I believe that at the present time these conventions in the use of terms will contribute to clearness. In the specific instances where I do not know whether a given substance is to be classed with the more constant granules or with the functionally changing vacuoles, I shall use the term *stained particle.* Beside the vacuoles which take a vital dye, there are other clear spots in some cells which do not take a vital dye; they occur in cells which evince signs of degeneration and are, I think, to be considered as evidence of cell-death. Thus, there are two types of structures called vacuoles, first, those that take the vital dyes, and vary with the activity of the cell, and second, those that do not take the stain and can be correllated with degenerative processes.

LEUCOCYTES

1. *Polymorphonuclear neutrophilic leucocytes.*

The neutrophilic leucocytes are very characteristic in these preparations both in their reaction to the stain and in their striking motility. In a successful preparation, they are never still except for the brief second when they stop in order to change their direction. On account of their motility the eye picks them up with great ease in making differential counts. In neutral red, azur or brilliant cresyl blue the neutrophilic granules of human blood-cells stain a uniform faint color. The pseudo-eosinophilic granules of other mammalian forms stain differently. The granules are of equal size and are in constant motion, streaming through the cytoplasm. In Janus green, the neutrophilic granules stain a faint green color while the mitochondria stain a brilliant blue. Unless they are stained with Janus green the mitochondria cannot be discriminated in the leucocytes. Besides these two types of granules in the leucocytes, there may also

be one or two vacuoles in the normal leucocyte. These vacuoles react to the dye more slowly, so that it may be 5 to 15 minutes before they are well stained. They are always larger than the neutrophilic granules and may be even as large as the nucleus. These vacuoles vary greatly in size, in number and in color. They also move in the cytoplasm along with the granules. They can, I think, be used as an evidence of how much phagocytic activity is going on in the leucocytes and, if this conclusion is correct, the presence of these vacuoles indicates that the cells perform a physiological function within the blood-stream. In normal blood there may be none of these vacuoles in the leucocytes; in one case of pneumonia, the vacuoles were very large, even as large as the nuclei, and they had the brilliant scarlet color of the acid reaction of neutral red. In this blood there was a great deal of debris in the plasma. This debris cannot be seen within the vacuoles, for it is colorless, but occasionally a little particle of the jeweler's rouge has been left on the slide and can be seen within the cell. In one of our experimental rabbits, I saw one of the pseudo-eosinophilic leucocytes in which there were four vacuoles, three small ones which had the orange color (neutral reaction of neutral red) while the fourth, a very large vacuole, had a brilliant scarlet color (acid reaction to neutral red).

The type of motility of the leucocytes in these preparations is very striking to hematologists who are familiar only with leucocytes in fixed smears. The normal, living leucocyte is never round and never still. The clear blunt pseudopods are constantly being put out from the cell and the granules stream largely in the direction of the flow of the cell, with, however, many counter currents within the cytoplasm. The nucleus is usually in the rear of the cell and, though it does change in form while the cell is moving, strangely enough it seems to be not as amœboid as the nuclei of the lymphocytes. This, however, may be due to the fact that the nucleus is often obscured by the streaming of the cytoplasmic granules. The type of motility which the leucocytes manifest could only exist in a cell in which the cytoplasm is fluid. In the chick of the second and third days of incubation, I have never seen any cells with true amœboid motion, that is, with motion of great speed associated with marked streaming of granules, except in the case of certain wandering endodermal cells loaded with yolk. The young granulocytes which develop outside of the blood-vessels, and can be seen to wander into them, move but slowly and with very little streaming of granules. They require hours to move a distance which a mature leucocyte could accomplish in minutes or seconds. This point seems to me to be very important with reference to the motility of myelocytes. Myelocytes, like the early granulocytes of the living chick, do not have amœboid motion; they progress only slowly, with slight changes of the form of the cell and without streaming of the granules. Hence we may say that the cytoplasm of the young granulocyte of the embryo or of the myelocyte of adult bone-marrow has a cytoplasm which it too much of a gel to permit of rapid motion. This is interesting in connection with the findings of Chambers[6] that a cell undergoing division has its cytoplasm in the form of a gel. The contrast in motility of the myelocyte and leucocyte of circulating blood is very striking in smears of leukemic blood. The point is also evident if one studies a bit of living bone-marrow with the supravital method, because the leucocytes will wander from the central mass and leave the myelocytes in place. This point is probably of importance with regard to the regulation of the supply of leucocytes to the blood-stream, the myelocytes being usually retained in the marrow.

In watching the living leucocytes, it is of course essential to know the normal rate of motility or rather the normal limits of rate before we can make any judgment concerning variations from the normal. So far I have made only qualitative estimates between active and sluggish leucocytes, calling the cells hyperactive when they move in and out of the field while the count is being made so that it is necessary to skip fields in order to avoid counting cells twice. Dr M. McCutcheon,* of the University of Pennsylvania, has an ingenious method for measuring the rate in μ per minute which must be used in studying deviations from the normal.

The leucocytes are in such rapid motion that no drawing could record their form. Often, in their movement, they put out a pseudopod which becomes a very slender filament, stretching across the entire field and ending in a little ball of cytoplasm at the tip. Such a cell is shown in Fig. 1, though many of these filaments are much finer. Such excessively fine strands of cytoplasm show how the leucocytes can get out between endothelial cells or through the tiniest crevices. Often the ball of cytoplasm at the tip of such a process is lost without seeming to damage the cell at all. These fragmented bits of cytoplasm are very characteristic and are never to be confused with platelets because they always show Brownian movement of the discrete particles. As will be described later, the granules of platelets are in clumps which do not show Brownian movement. Within the cytoplasm of the normal leucocyte, there is, I think, no Brownian movement. The granules do not vibrate but either flow with a steady movement or occasionally dart past others in the cytoplasm. As a cell is beginning to die in these films, especially as the power of locomotion begins to diminish and the cell begins to round up, Brownian motion develops which may mean that the cell has begun to take in too much fluid from the surrounding medium.

* McCutcheon:—Studies on the locomotion of leucocytes. 1. The normal rate of locomotion of human leucocytes *in vitro*. Studies on the locomotion of leucocytes. 2. The effect of temperature on the rate of locomotion of human leucocytes. Amer. Jour. Physiol., 1923, vol. 66.

In one clinical case (Med. No. 49329) which I studied, there was Brownian motion in the leucocytes while the cell was actually progressing. It was a case of late general carcinoma in which the bone-marrow was markedly involved in the carcinoma as demonstrated by the X-ray photographs. There was another indication that the blood-cells were damaged in this case, namely, they moved very slowly, showing many fine filaments which were often put out and withdrawn without leading to locomotion. Numerous fine filaments of cytoplasm I had often seen in studying living cells in the chicks in artifical media. In 1920, Miss M. A. Herwerden [7] published a method for demonstrating amœboid movement of leucocytes by keeping them in a warm box for half an hour in Deetjen's solution, and then fixing and staining them. In this treatment the leucocytes become completely covered with similar fine filaments as shown in her Fig. 2 on Plate 1. This is, I think, a very interesting reaction, but in my experience it is a sign, not of locomotion but rather of a paralysis of a leucocyte due to an abnormal surrounding fluid. In a moving cell the pseudopods are few in number and much more blunt. At the last meeting of the American Association of Anatomists, held in Chicago in 1923, E. R. and E. L. Clark [8] described the reaction of living amphibian leucocytes after injections of croton oil into the tadpole's tail. They said that the leucocytes wandered from the vessels, and then became stationary, putting out processes so that they looked like "prickle cells." Subsequently they withdrew the processes and became motile again. I have seen such "prickle cells" in studying mammalian leucocytes from marrow in Locke solution; in the one instance in which I have seen human blood after transfusion, the leucocytes were in the form of prickle cells for about an hour. During this time they did not show any locomotion, then they gradually pulled in the processes and began to move again. It may be, then, that the prickle leucocyte is a cell temporarily paralyzed and the observation may be of great value in studying the effect of transfusion on the activity of the leucocytes.

The vital preparations remain in good condition always for an hour, and usually for four or five hours. When the preparation is first made, the leucocytes may round up for a minute or so, but usually they have started to move almost as soon as the oil immersion lens has been adjusted, provided that the box is warm. It is not necessary to warm the slides before making the film. The length of time the slides last makes it perfectly feasible to make differential counts of the cells, though they can perhaps not be made quite as quickly as with fixed smears. In making the differential counts one should record whether the leucocytes move actively or sluggishly, and whether they show the vacuoles that indicate phagocytic activity. Also a tendency to fragment or any other peculiarity of the leucocytes should be noted. In this way we shall gradually accumulate a knowledge of a new set of criteria of the activity or one might even say of the physiology of the leucocytes.

2. *Non-motile leucocytes.*

All of the white cells of the blood take a vital dye except one small group, and these I have called the non-motile leucocytes. In studying smears of normal blood, it soon appeared that there were peculiar, granular forms which neither took up the dye nor showed any motility. Moreover, in these cells the proportion of the nucleus to cytoplasm is not like that of any other granular form, for the nucleus almost completely fills the cell, the cytoplasm being represented by a rim of irregular, refractile granules around the nucleus. Such a cell is shown in Fig. 2. This particular cell has perhaps a more irregular outline than many non-motile leucocytes, because they tend to be round, but the type of outline will bring out the fact that one may not infer amœboid activity from the mere fact of irregularity of contour of a cell. This cell was described by Schilling [9] in 1908, who discovered it in studying living blood-cells with dark-field illumination. He noted that the granules fill the ectoplasm whch is usually free from granules. He regarded them as dead leucocytes and described similar cells, from sputum, which he thought came from very young leucocytes, the nucleus being smaller and directly in the center of the cell. In this paper Schilling also gave some very interesting observations on following the centrosphere in the amœboid leucocytes. He found that in the moving cell the centrosphere maintained its central position regardless of the position of the nucleus. In these non-motile forms he found no centrosphere whatever and thought it was the loss of the centrosome that allowed the nucleus to take the central position.

By studying living smears, I have seen every transition between the non-motile leucocytes and the actively amœboid neutrophilic leucocytes, indicating that, as a leucocyte ages, it stops moving and becomes round; its membrane then becomes impervious to the vital dyes, the nucleus becomes œdematous and the neutrophilic granules change into large, more refractive particles. Schilling calls this process a coagulation of the granules. As will be seen in Fig. 2, these particles vary in size; the nucleus loses all appearance of structure and appears like ground glass. Streaks across the nucleus indicate where the lobes were before the nucleus became œdematous. They may come from cells which had nuclei with three, four or five lobes, rarely from those with nuclei of two lobes.

I tested these cells by letting a drop of Türk's solution run under the coverslip and found that they are retained and so are probably included in total counts of the white cells. It is a possibility that they may have been included in the differential counts with the Ehrlich technique, their cytoplasm having been fixed by heat. Schilling thinks that they show also in Giemsa stain, appearing as cells with a centrally placed, faintly stained round

nucleus, which had been described as modified lymphocytes by Gött and Weidenreich. I think, however, that they are not retained in the technique which involves a fixation in alcohol. I tested them in 33% alcohol, and the granules are dissolved in 13 minutes, while the neutrophilic granules are unchanged. It is difficult to let stronger alcohol run under a coverslip far enough to reach them, since too much coagulation is produced. If they are retained by the Ehrlich technique, this might account for the fact that the Ehrlich data gave a higher percentage of leucocytes, namely 70 to 72 per cent, as contrasted with the more modern data. In 1922 Bunting [10] pointed out this discrepancy from the Ehrlich data and gave the figure 54.6 as the normal percentage of leucocytes from a large group of students from the University of Wisconsin. Corresponding data from the clinical laboratory of the Johns Hopkins Medical School give 65 as the normal percentage.

In the course of some experimental work on the formation of blood-cells in rabbits which Dr. R. S. Cunningham and I are carrying out, it became evident that, though there may be some rhythm in the occurrence of these non-motile cells, there must be exceedingly careful controls concerning their production. The question must certainly be raised as to whether they actually occur in the circulating blood or not, and wherever direct observation of the circulation can be made, an effort should be made to search for them. In the meantime, lacking such complete proof, it has been only possible to eliminate from the technique all factors which can be shown to increase the number of degenerating forms. Among these factors are excessive heat, pressure from the coverslip when the preparation is too thin, and accidental pressure in focusing the microscope. Notwithstanding these points, which indicate that errors in technique produce and increase the number of these forms, the non-motile leucocyte, even when the coverslip presses too heavily, as in the very thin areas, or when the specimen has been overheated, will appear side by side with perfectly active leucocytes, indicating that there are marked differences in fragility in the different leucocytes in our specimens.

Taking every precaution which we have up to the present time analyzed as affecting the production of these cells, namely, securing free flow of blood, counting only films in which the red cells are thickly spread or even overlapping a little, avoiding undue heat, using a regulator in the warm box and maintaining a uniform temperature of 37.5°C., we still find the non-motile cells in our films. We have been making complete counts of the white cells, with differential counts of fixed smears and of the living cells as often as the vital counts can be made, which proves to be about every 40 minutes throughout the day. We make on an average of 12 counts a day. This experiment we have been making with rabbit's blood and have five such series of counts with normal human blood. We find that there are a few non-motile cells scattered through the different counts, or perhaps none, but at one time, usually about the middle of the day, there appears to be a shower of the non-motile forms, which may reach fairly high percentages, as for example 16 per cent or more. When the fixed smears from blood, taken at the same time as the supravital preparations, were counted, it was found that there were six different structures usually regarded as smudges that had to be correlated with the vital counts. First, there are definite, naked nuclei which are either square or rectangular; they have shadows of granules around them and I think correspond to the non-motile leucocytes. Second, there are very long, naked nuclei also with shadows of granules which correspond with degenerating or damaged transitional cells. Third, in making so many counts from the same individual we find small, round nuclei from the small lymphocytes. Fourth, there are compact masses that are pink in Wright's blood-stain that correspond to masses of debris in the living preparations. In the vital counts, these masses of cellular debris, that are so far disintegrated that they cannot be related to the type of cell from which they came, vary on different days from the same individual. These masses are not included in the total counts. In smears they look more like nuclear material than cytoplasmic; in the vital preparations they take none of the vital dyes, so that there is never any question of confusing them with platelets. These masses are being studied by Dr. David T. Smith in connection with lymphatic leukemias of children, in which they occur in large numbers. Fifth, there are the well known masses of fibrin. These are clearly fibrin in the fresh preparations, and in smears they are large pink masses, looking vacuolated and with delicate fibers on their edges. Sixth, there are the so-called fragile leucocytes, in which the cell membrane ruptures while the covers are being drawn apart. These fragile leucocytes in which the scattered and stained granules make it perfectly clear from what cell they came, whether neutrophilic or eosinophilic, are usually disregarded in making differential counts. There is no doubt but that they are mechanically increased by faulty technique, by dust on the coverslip, or by an uneven sliding apart of the two coverslips. They never occur in the living preparations, where there is no dragging of the cells across the film, nevertheless, in correlating the counts of fixed smears with vital counts they must be included. They can be shown to have been represented in a given double count by actively motile forms in the living state. On the other hand, in one of our series the fragile leucocytes had to be added to the naked nuclei of the fixed smear to give any correlation with the count of the non-motile leucocytes in the fresh specimen. Such preparations show that there is the same difficulty in analyzing the fragile leucocytes in smears as the non-motile forms in the living, to discriminate how much is

due to technique and how much to the condition of the original cell. It may be stated that fragile leucocytes may be produced at any time by rough handling, but that when the leucocytes are about to go into the non-motile phase, they may appear as the fragile forms in the fixed smears.

In the series from human blood, there is also some indication of a period during the day when there is a considerable increase in the proportion of the small lymphocytes which we regard as the old form. I am well aware that this report of the non-motile forms and of the aging of lymphocytes is incomplete, indeed quite fragmentary, and I hope subsequently to give an analysis of the daily cycle of the white cells with a more standardized technique and with more adequate data. It would then be possible to give a curve of the rhythm of the types of white cells. I mention these experiments at this time, however, because in the study of blood in clinical cases, there are often very marked discrepancies which are usually disregarded. For example, there are often smears of blood with great numbers of smudges which cannot be repeated a few hours later. In our experience the actual showers of the non-motile form last only a half hour or so, which would easily account for such cases.

The significance of the non-motile cells, if we can prove that they do occur in the circulating blood, seems to me to be that they give us a chance for the first time to estimate the normal death rate of the leucocytes, indeed to study the normal rhythm of the blood-cells. In the studies made by Arneth [11] on the number of lobes of the nuclei of the leucocytes, there was given an indication of the relative age of the leucocytes to be found in the circulating blood, but we have never had any way of estimating the number that were dying. These non-motile cells seem to me to show that there is a real chemical transformation of the cytoplasm as the cell dies, so that they should be studied from the standpoint of whether they give a substance to the plasma which has to do with maintaining the normal rhythm of the leucocytes. If we can secure such cells, the point might be subjected to experiment. In this connection, the cells of pus must be studied with the vital method. It is obvious that we need to know the mechanism of maintaining the normal rhythm of the different forms of the white cells as a preliminary for studying the causes of variation from the normal and it is certainly a rational idea that the normal death rate might be correlated with this rhythm, that is, that the dying cells might produce the substances that would attract similar cells into the blood-stream. Here it is obvious that there are two factors to be analyzed, the actual production of new leucocytes in the marrow and the calling of them into the blood-stream. The maintenance of the normal rhythm is certainly complex; when large numbers of cells, leucocytes or lymphocytes, have been called from the blood into the tissues, the factors are even more complex, and to analyze this mechanism is now one of the major problems connected with the blood.

3. *Basophilic leucocytes.*

The method of studying blood-cells in the living form disproves conclusively the idea of Weidenreich,[12,13,14] that the human basophilic leucocytes of the blood-stream are degenerating cells. They are unquestionably living cells, as has been maintained by Naegeli [15] and by Bunting.[10] Of course it will now be possible to find the changes in the basophilic leucocytes of the blood-stream that indicate cell death, but so far I have seen only living basophilic cells. Some of them have nuclei that are slightly polymorphic but even the ones with the peculiar rosette-shaped nuclei, such as are shown in Weidenreich's [12,13] figure, are living cells, as proved by the fact that the nuclei do not stain in vital dyes and that the cells themselves are amœboid. In human blood the basophilic granules are intermediate in size between the neutrophilic and the eosinophilic granules and they have the special characteristic of taking the dye unevenly, some being much darker than others. The granules are all round but not all of the same size as are those of the neutrophilic and eosinophilic types. They give a deeper red reaction in neutral red than either of the other forms.

In the motility of the basophilic cells of human blood there is but little streaming of the granules and their rate of motion is much less than that of either of the other two groups of the leucocytes. I have not seen any changes in these cells to be related to function, that is to say, I have not analyzed any of the stained particles as vacuoles. The observations on them have been, of course, limited on account of their small numbers. With the supravital methods the relations of the basophilic cells of the blood to those of the marrow and of the diffuse connective tissues can now be attacked again.

4. *Eosinophilic leucocytes.*

The eosinophilic leucocytes have the large granules which take the dye uniformly and more intensely than the granules of the neutrophilic leucocytes. Like the neutrophilic cells, they show an active amœboid motion with a streaming of the granules. I have never seen them remain motile as long as the neutrophilic cells, but I have seen them move as fast. Usually they move at a slower rate and do not remain in motion in the preparations more than half an hour. I have never made out any vacuoles in the eosinophilic cells. So far I have not studied any cases in which there was a marked eosinophilia. The nuclei are usually two-lobed. When the cell moves, the two lobes may be dragged far apart, a slender thread keeping them from separating. The two lobes change in form as the cell moves, but not as much as the nuclei of the lymphocytes.

5. *Monocytes.*

In studying the living blastoderms of chicks on the third day of incubation, I [16] found that there is one group of the white cells that comes from endothelium. On the

second day of incubation, the origin of the red cells from the endothelium of the vessels can be seen with great clearness. On the second day, all of the single cells or clumps of cells clinging to the inner lining of the vessels of the area vasculosa can be seen to contain hemoglobin by the color of the living cell. The amount of the hemoglobin may be too small to show in the deeply basophilic cytoplasm of the fixed cell, but the yellow tinge is evident in the living state. On the third day, an occasional cell becomes free from the inner wall of the vessel which not only does not show hemoglobin, but does show the stained particles and the stained vacuoles that characterize the monocyte stem. These cells I think are analogous to the monocytes of the adult blood. At the same time, for the next few days of incubation, very large masses of similar cells wander from the outer surface of the endothelium of the vessels of the area vasculosa. These are the clasmatocytes. Aschoff and Kiyono [17] believe that the clasmatocytes of the connective tissues and the monocytes of the blood should be grouped together under the common name of histiocytes.

In studying normal, human blood, it becomes evident that there are two strikingly different types of cells in the blood belonging to the monocytic strain. Indeed they are so different in appearance as to require some proof that they are only two phases in the life of a single cell. The first form corresponds to the large mononuclear form of Ehrlich, a cell entirely different from the large lymphocyte, the second is the transitional cell of the Ehrlich classification.

The large mononuclear cell is shown in Fig. 3. It is a large cell, almost always round, varying in size from a cell slightly larger than a leucocyte to one the size of a myelocyte. The nucleus is usually round, may be slightly indented, and is always eccentric. The most striking characteristic of the cell is that its cytoplasm is completely filled with fine uniform particles that stain slightly darker than the neutrophilic granules in neutral red. The particles of the cells are usually still, or they may be in slight motion. In the very young forms, a spot free from granules in the center of the cell opposite the nucleus indicates the centrosphere. The cell shows no active locomotion, but it may move very slowly so that by watching it 10 to 15 minutes one may perceive a change. After one is familiar with this cell with the vital dye, it can be distinguished without any stain at all, that is, the same granules are very plain in the living cell. It could never be mistaken for the large lymphocyte, as can be seen by comparing Figs. 3 and 7. The cell is to be distinguished from a neutrophilic myelocyte by the fact that the particles are smaller than the neutrophilic granules and have a slightly different shade of color in neutral red. The two cells may be found side by side in bone-marrow and compared. There are, however, but few large mononuclear cells in bone-marrow. It will be noticed that I have used the term "particle," in connection with this cell, advisedly, because so far it has not been possible to determine the nature of the substance in the terms of my definitions.

The transitional cell, on the other hand, varies much more in size than the large mononuclear just described, though the typical ones are very large. They are shown in Figs. 4 and 5. In the living preparations, they are seldom round, in fact they are usually very long, but may be quite irregular, as in Fig. 4. They show considerably more motility than the large mononuclear form and the particles in the cytoplasm are often in quite active motion. The locomotion of the cell is slow and not as correlated with a flowing of granules as is the case of the leucocytes. The cell is especially conspicuous for the amount and the variety of the substances stainable with vital dyes. Its cytoplasm is filled with particles stainable with neutral red, which vary in size all the way from a fine dust to huge vacuoles. At present I do not know the relationship between the particles and the vacuoles, but when the cell is highly stimulated to phagocytic activity so that its cytoplasm is filled with large vacuoles, as in Fig. 5, the fine particles have disappeared. The fine particles also stain in Janus green, giving a greenish tinge to the cell in which can be seen small, scattered mitochondria. Perhaps it is the same fine particles that give the cytoplasm its color in fixed smears. Of all the cells of the circulating blood the transitionals change the most with function, the large mononuclears the least. The only change I have seen in the latter is a slight increase in the motility of its cytoplasmic particles while in the case of the former, if my interpretation of the stainable vacuoles is correct, the transitional is the most phagocytic of all of the cells in the blood stream; that is to say, a single cell can phagocytize a greater amount of substance than any other cell. They vary all the way from a cell filled with fine particles and no vacuoles to a cell whose cytoplasm is so loaded with vacuoles that nothing else is visible.

These two cells are so strikingly different in the living state, that it would be impossible from normal blood to conclude that they both belong to the same group, namely, the monocytes. But I had the opportunity to study one case of abnormal blood in which it became clear that they are merely two phases in the life cycle of the same cell. This very interesting case was one of Malta fever. The patient was admitted to the Johns Hopkins Hospital October 18, 1922, (Med. No. 48513) and showed a fever of a moderate grade correlated with a blood-count in which the total number of the white cells was normal but there were ten per cent of monocytes. The diagnosis of Malta fever was made by Dr. Amoss by finding the organism in the circulating blood. He made an autovaccine and gave it in four doses at intervals of four and five days. The first vital differential count which I made on November 25th was as follows:

Polymorphonuclear neutrophiles	32%
Non-motile leucocytes	6.2
Eosinophiles	1
Basophiles	1
Lymphocytes	39.5
Large mononuclears	.7
Transitionals	18.5

In actual percentage the count did not differ much from this throughout the course of the disease, the monocytes remaining about twenty per cent. After the second dose all of the monocytes were of the transitional form; after the third dose there was a very marked increase in the stainable substance of the transitional cells, the cytoplasm being completely filled with large stained vacuoles. As a sign that they were actively phagocytic, red blood-cells were found engulfed in them. After the fourth dose there was a brilliant demonstration of the fact that the transitionals are merely older, more active large mononuclear forms. There were a few large mononuclear forms of a young stage, that is, the centrosphere was plainer in them than I have ever found it in these cells. From these young forms there was every transition from the mononuclear to the transitional form, of the inactive and active state, on to cells that were degenerating. No sharp line could be drawn between the large mononuclear or young forms and the transitional. Dying cells have very little reaction to the vital dye, show no movement of particles, no locomotion and have clear unstained vacuoles in the cytoplasm. The entire life cycle of the monocytic strain from the youngest to the dying cells could be seen in one preparation.

The subsequent history of the case was also interesting. I had a chance to follow the case long after the acute illness was over. The leucocytes remaining very low, the increase in monocytes being at the expense of the leucocytes, Dr. Amoss gave the patient a series of three doses of typhoid vaccine, in the hope that there might be an increase in the percentage of leucocytes. Instead, there followed a stimulation of the lymphocytic strain of cells. Lymphocytes showed a marked increase in the number and size of the vacuoles in the neutral red and there was a marked variation in their staining reaction in Wright's stain. This gradually subsided but the increased vacuolization of the monocytes continued over a period of some months. Gradually this subsided. The patient was discharged from the hospital with a negative blood culture on December 10, 1922, but it was not until April that the staining reaction of the monocytes became normal. On April 24, 1923, the monocytes were still ten per cent of the white cells, but they were normal in staining reaction. Blood cultures were still negative.

From the study of this case I became entirely convinced that the large mononuclear cell is the younger stage of the transitional form. The transitional form is more active as shown both by increased motility and by increased phagocytic power as indicated by the large numbers of stainable vacuoles.

In connection with these studies of blood, I have been making smears of living cells from lymph glands and from the spleen in different animals. I press out a little fluid from the gland, put it on a slide prepared as for the blood films and then add a small amount of a scraping from a freshly cut surface. In such preparations, as is well known, there are always many clasmatocytes, that is, cells of the transitional type from lymph glands, spleen and bone-marrow, but the cells identical in type with the large mononuclear of the blood stream I have found in any considerable numbers only in scrapings from the spleen. The animals used have been cats and rabbits.

6. *Lymphocytes.*

The lymphocytes have proved to be the most difficult of the white cells to analyze. As a matter of fact we have had no convincing proof as to which is the young form, one group of hematologists thinking that it is the large lymphocyte, the other that it is the small. I am confident that these vital studies offer very considerable evidence toward the view that the large lymphocyte is the young stage. As was shown by Miss Simpson, lymphocytes are characterized by definite clumps of large mitochondria. All of the lymphocytes, with one occasional exception, have a cytoplasm that is strikingly clear in the living form. This is in marked contrast to the fine granulaton of the monocytes. In the cytoplasm of all lymphocytes there are two substances that take vital dyes; first, they all have clumps of mitochondria, and second, two or more small vacuoles reacting to neutral red. In Fig. 7, is the large lymphocyte, showing three vacuoles and the characteristic mitochondria, placed as usual opposite the nucleus. Both of the substances are often in motion and an occasional rod or vacuole is to be found along the rim of the nucleus. The large lymphocyte is almost always rounded up, as shown in this figure; its nucleus is round or oval, seldom irregular and the cell very seldom shows any locomotion. The striking characteristic of the intermediate form is that it is frequently in motion. Also it shows much more variation in the reaction to neutral red. Such a cell is shown in Fig. 6. The intermediate cell is distinctly smaller than the large lymphocyte; it has the same cluster of mitochondria opposite an eccentric nucleus. Among the rods are a few vacuoles of which several are shown in Fig. 6. The cell moves much more slowly than the leucocyte; when the cell is moving, the nucleus is usually in the front end and its form is constantly changing. Both the mitochondria and the vacuoles are in active motion, not in the least correlated with the locomotion of the cell; indeed the particles may be in very active motion when the cell itself is quite still. The particles stainable with neutral red in the lymphocytes vary from one or two up to about ten or even more. The small lymphocytes al-

ways show the same clump of mitochondria, and at least one or two vacuoles. The vacuoles may increase in size and number but less markedly than in the intermediate forms. The small cell seldom moves at all.

In all of the lymphocytes the cytoplasm is much more clear than that of the monocytes; that is to say, the lymphocyte lacks the fine granulation of the monocyte. Indeed it is on this account that the mitochondria are very readily seen in the living cell without being stained at all. This is not true of any of the other cells of the blood. There seems to me to be little doubt but that the substances that stain in neutral red in the lymphocytes belong to the category of vacuoles in my definition; that is to say, they are structures that vary markedly in different functional states of the cell. This can often be made out very clearly in the case of the small lymphocyte where the two or three vacuoles can become markedly larger than usual without causing the least difficulty in discriminating the cell from all other types in the blood. The size of the cell is so distinctive and the massive blotches of chromatin in the nucleus are so plainly seen in the living state that the cell cannot be confused with any other. With the intermediate and the large lymphocyte the case is different. When they are highly stimulated, the neutral red vacuoles may approach in size and number the condition of the monocytes. The mitochondria remain the same and it is then necessary to use the double stain vitally and to correlate the differential counts with studies of fixed smears.

There is one form of the lymphocyte which occurs occasionally in normal blood in which the nucleus is exactly in the center of the cell instead of having the usual eccentric position, and in these cells, which belong to the large and intermediate group, the cytoplasm shows a very fine granulation like ground glass. In the fixed smears these are the cells with markedly basophilic cytoplasm. I think that they will be understood only when we have a complete account of the life cycle of the lymphocytes.

In studying lymphocytes in the living forms, it is striking how seldom the nuclei are as round as in the fixed smears. This is true even of the small forms. In cases of lymphoid leukæmia, I have found marked changes in the nuclei, especially of the small forms. In one case (Med. No. 49241) a large proportion of the small lymphocytes had nuclei which were split in half or fragmented into three parts. While this was very clear in the living specimens, fixed smears did not show these degenerations of the nuclei at all. In this case I saw a nucleus of a large lymphocyte divide by direct division, division of the cell did not follow but there was a division of the mitochondria into two parts opposite the cleft in the nucleus. Subsequently I found two more large lymphocytes with two nuclei and with the same arrangement of mitochondria. All of these observations indicated unusual or pathological conditions of the lymphocytes and it was very striking in this case that there was no increase in phagocytic power, that is to say, there were almost no vacuoles in neutral red in the entire strain of the lymphocytes in this case. Thus there were marked changes in the nuclei of the cells correlated with low functional activity.

It has been striking that the lymphocytes taken directly from the lymph glands have not shown as many or as large vacuoles as those of the blood. I do not know whether there is any correlation to be made between the azurophilic granules and the vacuoles that stain in neutral red, but I think that there is no such change in the size of the azurophilic granules as there is with the vacuoles.

There was a patient in the Johns Hopkins Hospital (Med. No. 489984, Path. No. 7383), whose case was very interesting in connection with the lymphocytes. The condition was exceedingly complex; no definite diagnosis could be made even from the autopsy, but it was probably related to the lymphoid leukemias. The patient was in the hospital during the last month of life. The striking point in the case was the enormous enlargement of spleen and lymph glands. Until the last few days the total count of the white cells was normal and the differential count showed only signs of the so-called pathological lymphocytes. Finally, there was a marked leucocytosis reaching as high as 110,000 cells, during which strangely enough the proportions of the cells were still approximately normal. Myelocytes appeared in the blood-stream during the last four days of life and at autopsy the bone-marrow was found to be active.

I had an opportunity to study smears from the lymph glands, spleen and bone-marrow from this case while the cells were still living. Similar smears from the lymph glands and spleen of cats and rabbits show groups of cells, stuck together in clumps, which are in no sense syncytial masses, since the cells can be separated by pressure. These cells have the following characteristics: the cytoplasm is as basophilic as the nucleus; the nuclei are the least visible of any living nuclei I have yet worked with. In smears with Wright's blood stain, the cytoplasm of these cells is almost as basophilic as the nuclei. In the living state there are no granules of any sort to be made out in these cells. This type of cell may well be the stem cell of the blood-cells that do not come from endothelium, namely, of the leucocytes and lymphocytes. In this case in which the striking changes were in the lymph glands and spleen, the spleen had similar masses except that every cell had a clump of mitochondria like those that characterize lymphocytes. This may represent a differentiation of the primitive cell into a forerunner of the lymphocytic series.

From these studies, it is my opinion that the large lymphocyte is the young form of which only a few occur in the normal blood; that they are comparable to the myelocytes of the granulocytic strain except for the fact

that a few do occur in the blood. The intermediate forms are, I think, more active functionally, first, because they show the greatest amount of motility, and second, the greatest variations in phagocytic power. The small forms are the oldest, but most of them are active cells with power of phagocytosis. They show the greatest number of degenerating forms. The only suggestion which I have concerning the cells with marked increase of the basophilic reaction of the cytoplasm is that they may be near the phase of cell division, in which there is a general increase in basophilic reaction of cytoplasm.

It has already been indicated that there are some difficulties in analyzing the blood-cells in the vital method. The most frequent difficulty is the one just mentioned, that of discriminating lymphocytes, when they are in a state of unusual phagocytic activity, from moderately active monocytes. In such cases comparison must be made with fixed smears and the case must be followed over a period of time. In other instances, as for example in certain cases of infectious mononucleosis, I think that it is possible to say definitely that it is the lymphocytes that cause the increase in mononuclear forms and not the monocytes. Another difficulty, I have found, is the distinguishing of certain very sluggish leucocytes, in which there has been a marked depletion of the neutrophilic granules, from inactive monocytes. As with all other methods, there are from time to time cells which must be regarded as unclassified.

7. Platelets and Megalokaryocytes.

Platelets are very characteristic in the vital dyes. They have two types of granules, particles that occur in clumps and stain with neutral red and discrete particles that stain blue in Janus green. These particles are always still, never in Brownian motion. These characteristics distinguish them from all other forms of debris in vital preparations. In these vital films platelets are more often found in large clumps than they are in fixed smears.

In a case of chronic myeloid leukemia (Med. No. 48856) admitted on November 25, 1922, we were surprised to find very large numbers of megalokaryocytes in the circulating blood. In this case there were 80,240 white cells on November 26th. On November 28th I counted 306 white blood-cells and 19 megalokaryocytes, making 325 cells counted. Besides these there were 18 large masses of platelets without nuclei. Thus the nucleated giant cells made approximately five per cent of the total white cells in the blood. The next total white count was 89,280, made on March 5th. Estimating the number of white cells on November 28th as 80,000, there were then 4000 megalokaryocytes per cu. mm., in the circulating blood. In studying these giant cells on the slide, the granules stainable in neutral red gradually formed in little clumps and the cytoplasm then fragmented into typical platelets on the slide. In Fig. 8 is a typical giant cell as it appeared on November 28th. In Fig. 9 is one with very little cytoplasm but with a nucleus very typical of the megalokaryocyte to be found in the bone-marrow and spleen. On March 4th, the patient was given a dose of X-rays, and two days later the total number of the white cells dropped to 55,350 and all the giant cells had disappeared from the blood.

In Fig. 10 is a cell taken on March 4th, in which I followed the disintegration of the outer mass of cytoplasm into platelets. As will be seen, the nucleus with a very small amount of unfragmented cytoplasm is in the upper left hand corner of the mass, most of the cytoplasm being in the form of platelets. The contents of some of the platelets were not drawn, since the cell became too disintegrated to show their character. These observations seem to me to be a confirmation of Wright's theory that it is the giant cell which gives rise to platelets. Certainly platelets can be definitely separated from the debris of the blood-cells in the vital preparations. Since, according to Minot,[19] the occurrence of giant cells in the blood stream is not as uncommon as had been heretofore supposed, the number of cases identified will I am sure increase where the technique of studying blood supravitally is introduced, since the cells are so much more easily identified in these preparations than in stained smears where the giant cells are almost certainly damaged. Moreover, the occurrence of any very large clumps of platelets in the circulating blood will suggest that giant cells be searched for.

In conclusion, in these studies of white blood cells, it has been shown that there is much evidence to favor the view that the large mononuclear cell is the young form of the monocytic strain and that the large lymphocyte is the young stage of the lymphocytes. The method offers a chance to distinguish cells which have been stimulated from those which are degenerating. I am convinced that the method opens a new phase of the physiology of the white blood-cells. The next step will be to complete the life cycle of each type of white cell. With these data in hand we shall be in position to complete the study of the normal rhythm of the leucocytes and thus have a foundation for analyzing the method for maintaing this rhythm.

LEGENDS

Fig. 1—Polymorphonuclear neutrophilic leucocyte from normal, human blood, drawn from the living cell in which the neutrophilic granules have been supravitally stained with neutral red. This is a free-hand drawing; the motion of the cell was too great for the use of a camera lucida. It shows the general form of an advancing pseudopod becoming a long filament. 1a is a red blood-cell for the magnification.

Fig. 2.—Non-motile leucocyte from normal human blood. The preparation from which this cell was taken was supravitally stained with neutral red, but this cell did not take any of the dye. The large mass in the center is the nucleus, which had four lobes before it became œdematous. The irregular granules which fill both the endoplasm and the ectoplasm were highly refractive. 2a is a red blood-cell for the magnification.

Fig. 3.—Monocyte of the large mononuclear type, from normal human blood, stained supravitally with neutral red. The magnification is not given, but it is the largest type in the circulating blood.

Fig. 4.—Monocyte of the transitional type, from normal human blood, stained supravitally with neutral red. The cell was in motion; in the cytoplasm are two types of substance that reacted to the dye, fine particles and larger, round vacuoles. 4a is a red blood-cell for the magnification.

Fig. 5.—Monocyte of the transitional type, taken from the blood of a patient after an attack of Malta fever (Med. No. 48513), but while the monocytes still showed a marked stimulation to the formation of vacuoles. The vacuoles, which were stained in neutral red, are shown in gray. This is the same type of cell as that shown in Fig. 4, except that it was more active functionally. Drawn with a camera lucida, Leitz Obj. 1/12, Zeiss Comp. Oc. vi, and then enlarged 3×.

Fig. 6.—Lymphocyte from normal human blood, supravitally stained with neutral red. The cell was moving in the direction of the arrow. In the cytoplasm are 21 vacuoles which were stained, and and a clump of mitochondria in the form of rods which were not stained. The nucleus is markedly irregular in consequence of the movement of the cell. 6a is a red blood-cell for the magnification.

Fig. 7.—Lymphocyte of the large type, from normal human blood, supravitally stained with neutral red and Janus green. It shows the characteristic clear cytoplasm of the lymphocytic strain. The nucleus is eccentric; in the main mass of the cytoplasm are four small vacuoles which stained with neutral red and a large clump of mitochondria which stained in Janus green.

Fig. 8.—Megalokaryocyte from human blood, taken from a case of myeloid leukemia (Med. No. 48856). The nucleus is in the upper part of the cell and the cytoplasm shows a marked granulation which was supravitally stained in neutral red. The character of the cytoplasm was accurately copied with the camera lucida. (Leitz Obj. 1/12, Zeiss Comp. Oc. vi.)

Fig. 9.—Megalokaryocyte drawn from the same preparation as the cell of Fig. 8. It shows a cell which has lost most of its cytoplasm; the nucleus is mulberry-shaped. 9a is a red blood-cell which corresponds both to this figure and to Figure 8.

Fig. 10.—Megalokaryocyte from the same case as Figs. 8 and 9. This preparation was taken from the patient on the morning of December 4th, immediately before he received the first dose of X-rays. The slide was given the same exposure to the X-rays as the patient. The giant cells were watched throughout the day for the disintegration of the cytoplasm into platelets. The preparation was kept in the incubator over night and the drawing was begun on the next day, the leucocytes being still alive and moving. On the next day, Dec. 6, an attempt was made to complete the drawing and the dead cell marked 10c was put in. The character of the platelets, however, had changed so they were left with the outlines originally drawn with the camera lucida. On this day all of these cells disappeared from the blood of the patient. 10a is a red blood-cell; 10b is the nucleus of the giant cell. (Leitz Obj. 1/12, Zeiss Comp. Oc. vi.)

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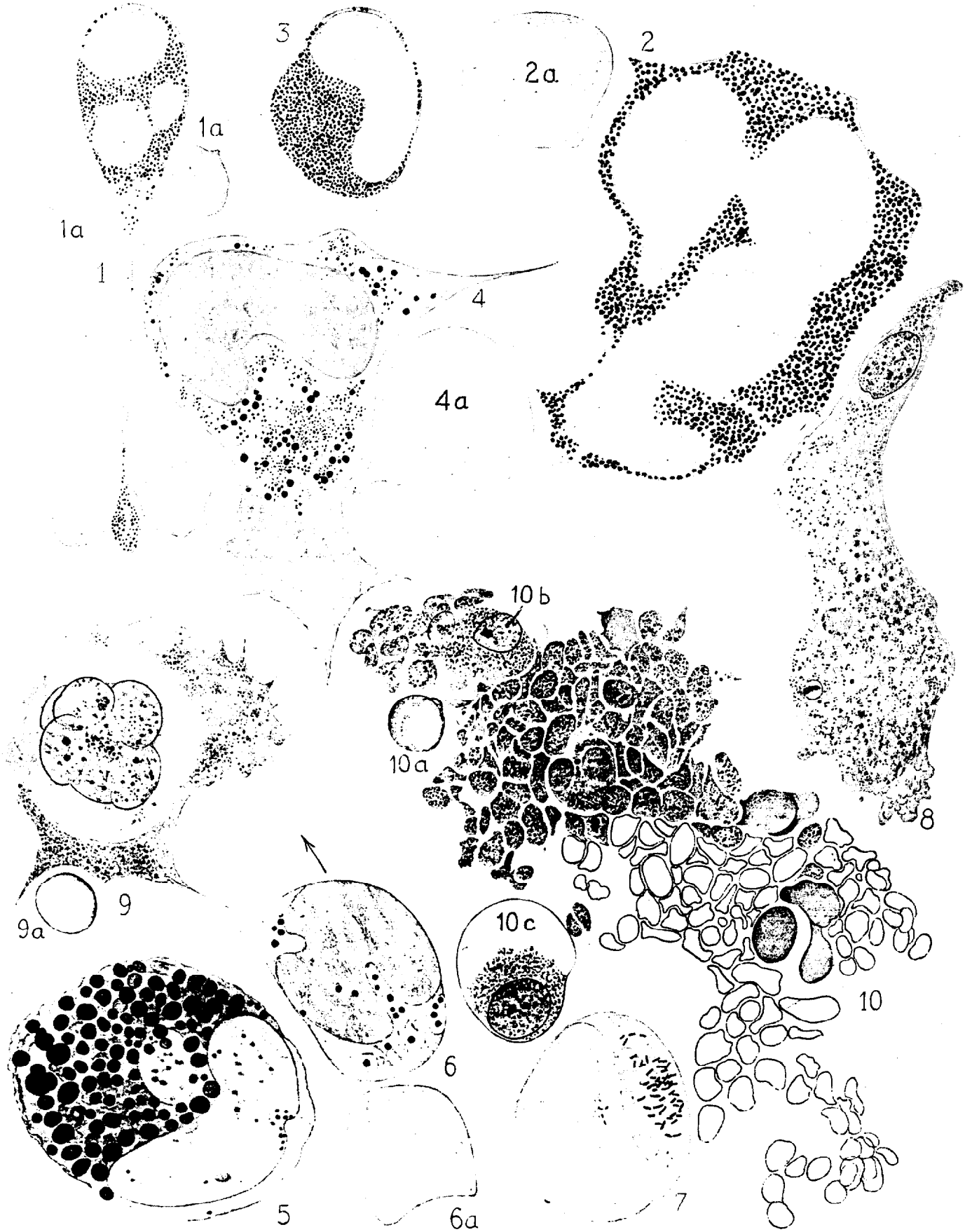
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