THE FORMATION OF MACROPHAGES, EPITHELIOID CELLS AND GIANT CELLS FROM LEUCOCYTES IN INCUBATED BLOOD *

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While tissue cultures would seem to afford an appropriate technic for the study of the part taken by the white blood-cells in various conditions, this method has seldom been employed. In 1914 Awrorow and Timofejewskij studied the white blood-cells of leukemic blood in plasma cultures, Loeb (1920) followed the amebocytes of the king crab in clotted blood, and Carrel (1921) observed the growth of the buffy coat of the centrifuged blood of the adult chicken in plasma cultures. The method by which the observations incorporated in this paper were made is simpler than that of any of the above investigators, consisting merely of the incubation of hanging drops of blood taken, by means of a paraffined pipette, either from the heart or from the peripheral circulation.

The transformation and growth of the leucocytes into macrophages, epithelioid cells, and giant cells were observed in the blood of the chick embryo, young chicken, adult hen, mouse, guinea-pig and dog, and in human blood. In every kind of blood examined there developed first a large wandering cell, several times larger than any of the normal leucocytes, which was phagocytic for red blood-cells, melanin granules, carbon particles, dead granulocytes, and tubercle bacilli. Somewhat later there appeared a cell more like a primitive mesenchyme cell, and still later the epithelioid cell was formed. This cell was sometimes binucleate and in some instances a typical multinucleated giant cell (Langhans giant cell) was formed. Since the transformation and growth of the leucocytes were much the same in the different bloods examined, the details of the phenomenon in avian and human blood only will be described.

Avian Blood. The transformed cells occurred in incubated blood taken from the adult fowl as well as from embryos of various ages, the youngest used being 6 days' incubation. Usually the blood

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studied was taken from chicks just hatched or just about to hatch, but several series were from chicks one week after hatching, and two were from young chickens two months old.

When the blood culture was studied within an hour or so after preparation, it had a number of granulocytes with large, usually spindle-shaped granules, and some non-granular cells (Fig. 1) migrating about on the coverglass. At this time these two types of leucocytes showed the usual difference in size, and all of them contained typical leucocytic nuclei. Within a few hours one or two refractive globules appeared in some of the non-granular cells (Fig. 2), which were then considerably larger than the granular cells. After twelve hours there were always several cells much larger than normal. These hypertrophied cells contained no specific granules, but usually a few refractive globules (Fig. 3). They grew in size and also divided, increasing in number so rapidly that often after two or three days the culture contained hundreds of them (1200 were counted in one drop) and only a few granulocytes. As the cells hypertrophied there was a coincident change in the nucleus, which lost its compact character and became larger, paler, and showed definite nucleoli (Fig. 4). After 48 hours, while most of them were from three to four times as large as the granulocytes, not only in length and width but also in thickness, smaller ones were still present (Fig. 5), some no larger than the original leucocyte. The cells now contained a number of refractive globules of different sizes, some quite large, most of which stained with Sudan III, while some appeared to be plasma granules. Such globules are not peculiar to the transformed leucocytes, as fat globules and plasma granules accumulate in most tissue cells grown in drawn blood or plasma. The cultures favorable for observation were those in which the cells did not form many of these globules.

After 24 to 48 hours the transformed cells were very phagocytic and took up various kinds of foreign bodies, but they seemed especially phagocytic for the red blood-cells of their own blood and most of them contained one or more erythrocytes, some of which were partly digested (Fig. 6). As many as twenty such cells were seen in a single macrophage. The ingested blood-cell soon died, as was indicated in preparations stained with neutral red by the fact that when first taken in the red blood-cell did not take the stain, but soon afterwards the nucleus became red and later the whole erythrocyte

became slightly red. Fragments of red blood-cells were also ingested and these stained red. In many instances the ingested cell crumpled up; in others it became laked, so that when stained it appeared as a fluid vacuole containing a red body — the nucleus. In later stages of digestion the nucleus of the ingested cell remained for some time after the rest of the cell had disappeared (Fig. 7). The macrophages resembled the clasmatocytes of the chick connective tissue to such an extent that it was difficult to distinguish the two cells from each other when placed side by side. The transformed leucocyte was usually somewhat larger and contained larger fat globules, otherwise each cell had the eccentrically placed nucleus, the thick cytoplasm, and the delicate, sheet-like processes continually changing shape and position. When stained with neutral red they each contained many red bodies scattered irregularly throughout the cytoplasm.

Some cells, migrating on the coverglass, were separated by a layer of serum from the red blood-cells so that they ingested few or none of the latter. These cells spread out on the glass, sometimes long and slender and joined end to end, or large and flat with a centrosphere. While these cells seldom contained ingested material they were nevertheless phagocytic; if the drop was shaken so that the erythrocytes came in touch with them, ingestion often followed and, as was observed in a few instances, the cells drew in their cytoplasm, became loosened from the glass, formed processes and became wandering cells. The clasmatocyte-like cells also occasionally became attached to the coverglass where they spread out into large flat cells in which a centrosphere was sometimes evident.

After the blood had been incubated 2 or 3 days some of the large flat cells became transformed into "epithelioid cells" (Fig. 10). By this term is meant, not all cells that resemble an epithelial cell, for that would include practically all of the large flat cells, but a specific large cell containing, around the centrosphere, a peculiar large central area which stains as a finely granular red area when neutral red is placed upon the preparation. This cell has a large nucleus with definite nucleoli usually eccentrically placed at one side of the central area. Beyond the central area, or just in its outer edges, there may be seen particles of ingested material and usually, in tissue cultures, many small fat globules in this outer region. Beyond this, the cytoplasm is extended out into an exceedingly delicate

peripheral film, the outer limits of which are almost impossible to distinguish in the living preparation, but which often become curled and drawn in, especially upon fixation, giving the characteristic curled edge so often seen in permanent preparations. Lewis and Webster (1921) described this cell in cultures of the human lymph node; Maximow (1924) observed the same type of cell in cultures inoculated with tubercle bacilli, and I have frequently obtained an abundant growth of this type of cell in cultures of isolated tubercles. The epithelioid cell developed earlier in chick blood than in the mammalian blood. The central area was neither so large nor so marked as in that of human blood, but larger than the centrosphere and stained in the characteristic manner.

Some of the transformed leucocytes became multinucleated. When this occurred, coincident with becoming epithelioid cells or later, the resulting giant cells were of the Langhans type. They contained from two to ten nuclei and were usually larger than the same type of cell containing only one nucleus, but not always so. The giant cells sometimes contained ingested material (Fig. 9) and in a few instances were observed to ingest red blood-cells, even after they contained several nuclei, but usually these cells digested what foreign bodies they contained and did not ingest any more.

The transformed leucocytes of the chick seldom lived more than seven to ten days in the incubated drops of blood, but if removed to plasma drops they lived for three or four weeks. It was in such preparations especially that the wandering cells became indistinguishable from clasmatocytes of the connective tissue subjected to the same environment. When plasma cultures of transformed leucocytes were incubated with avian tubercle bacilli the cells ingested large numbers of the latter. They digested the bacilli and behaved in every way as did the clasmatocytes when exposed to these organisms (Smith, Willis, and Lewis, 1922). In one such culture, fixed and stained after 20 days' incubation, there were observed, side by side, an elongated fibroblast-like cell (Fig. 12), a round epithelioid cell with a large central area (Fig. 11), and a clasmatocyte-like cell (Fig. 8), each containing tubercle bacilli.

When trypan blue or pyrrol blue was introduced into the culture, the granulocytes did not form blue granules; but after 48 hours the hypertrophied cells contained many, depending upon how much they came in contact with the stain.

Mammalian Blood. It was a simple matter to obtain an abundant growth of the transformed leucocytes of the chick's blood and practically every drop incubated contained many of them; but it was more difficult to get the same results with incubated drops of mammalian blood, and for this reason the following method was sometimes used as a control. Ten cubic centimeters of blood were rapidly drawn into a paraffined tube by means of an oiled cannula and centrifuged for about five minutes, most of the plasma withdrawn and the tube incubated. The buffy coat became a thick, tough layer of cells, extending up into the plasma and down among the red blood-cells. In this layer, after two to four days' incubation, the transformed leucocytes were abundant; many of them contained ingested and partly digested red blood-cells and some were multinucleated giant cells. These cells remained alive in the tube a number of days and living wandering cells were found in the bottom of the tube (two inches under the surface) even after ten days' incubation.

In the incubated drops of the blood of the mouse the cells grew well, regardless of whether the blood was taken from the heart or from the peripheral circulation. They did not multiply so extensively as those from the chick, but became many times larger than the normal cells (compare figure 14 with figures 15, 16, 17). They were phagocytic, as is shown in figures 15 and 17. The nucleus became large and contained definite nucleoli. Most of them were macrophages; a few were of the epithelioid type, as shown in figure 16.

The hanging drops of human blood were made from blood taken from the finger. These were usually injured by contact with the glass, and while the cells often lived for two or three days and displayed the beginning of the transformation, they more frequently died before the formation of an epithelioid type of cell, unless the coverglass was coated with some substance more favorable for their development. The best results were obtained by coating the covers with celloidin. In some of these cultures of human blood the white blood-cells lived for twenty days, in a few they lived nearly four weeks.

The first change in these cells also was the formation of refractive globules, which occurred after twenty-four hours, but the cells hypertrophied much more slowly than did those of the chick, so that it was often as long as four or five days before many unusually large

cells were seen. These cells were phagocytic and contained many red blood-cells; they increased in number until the eighth or ninth day. Scattered refractive globules of different sizes were present within them (Fig. 21), but these were much smaller than the globules in the hypertrophied cells of the chick blood. The nucleus became more homogeneous with more definite nucleoli. About the seventh day a few large flat cells appeared migrating on the coverslip. They were triangular, round, or spindle-shaped, and while at first they sometimes showed the remains of one or two ingested red blood-cells (Fig. 22), they soon completely digested these and took in no more. This was probably due to the fact that they did not come in contact with any, for if the blood was stirred, or fresh blood added, they again ingested red blood-cells, often in great numbers (Fig. 23). Instead of a few scattered refractive globules of different sizes, they contained many very small ones (Fig. 24). After eight or nine days these cells had increased greatly in number and had the appearance of epithelioid cells, i.e., they were large and flat with a large pale nucleus containing definite nucleoli, a large central body surrounded by a layer of fat globules and debris, and a delicate peripheral film of cytoplasm. When the preparation was stained with neutral red the central body became stained red (Fig. 26). A few of these cells became multinucleated and formed typical giant cells, but this was by no means so frequent an occurrence as in the cells of avian blood.

From the ninth day on, the majority of the hypertrophied cells formed in the human blood were of this type, although there were always present a few migrating phagocytic cells, containing red blood-cells, and some long spindle-shaped cells (Fig. 25). They did not retain the same shape from day to day; a round cell was observed to change into a long, thin, spindle-shaped cell and then into a triangular cell. These changes took place gradually and the cells migrated very slowly. They continued to increase in number for several days, after which they increased slightly in size and died when between three and four weeks old.

Although the transformed cells of the human blood lived so much longer than those of the chick, they never multiplied to the extent that the latter did. In cultures of chick blood, after a few days of incubation there were hundreds of these cells, but the greatest number ever seen in a single drop of human blood was 360 in a culture that had been incubated ten days. Occasionally a cell hypertrophied

rapidly and even ingested red blood-cells before the nucleus had lost its leucocytic character.

In regard to the red blood-cells, it is impossible to state how long those of the mammalian blood can live; they often seemed to be in good condition for as long as six or eight days. Those of the chick, however, are nucleated and form vacuoles which stain with neutral red, so that it could be determined that many of these remained alive for as long as eight days, as was shown by the fact that the nucleus did not stain with neutral red, while the vacuoles did.

Discussion. Whether more than one type of blood-cell hypertrophies and gives rise to the transformed cell is difficult to state. Awrorow and Timofejewskij conclude that the lymphocyte is the stem-cell from which arises the enlarged mononuclear cell and from it develop the other types of transformed cells found in their plasma cultures; i.e., the wandering cell, spindle-shaped cell, phagocytic cell, giant cell, and the cell which these observers call the "Auslauferzelle." They hesitate to call the cells either clasmatocytes or fibroblasts and, although they have found cells resembling the clasmatocyte and others resembling the fibroblast, these authors decided that, on the whole, the majority of the cells are not entirely like either of these types of connective-tissue cells. Carrel states that the granulocytes disappeared from his cultures after a few passages, and since he failed to find the small mononuclear after the first week, he supposed that these cells either were transformed into the large mononuclear leucocyte or died out, while the large mononuclear cells proliferated, migrated and, under certain conditions, changed into cells resembling fibroblasts and into transition forms, half fibroblast and half ameboid cells. He also observed typical macrophages in his plasma cultures. Clark and Clark (1920, 1923) were able actually to follow the living cells throughout their activity in sterile inflammation in the tadpole's tail. These investigators state the polymorphonuclear leucocytes migrated out from the blood-vessels toward the site of inflammation, where they became stationary, with spherical nuclei, sent out processes, and came to resemble fibroblasts; and that as the inflammation subsided, these cells again became ameboid and wandered away. In my cultures of chick, mammalian, and human blood, granulocytes were not observed to become transformed, and in blood containing a great

many of them there did not occur a proportionately greater number of transformed cells, nor did the hypertrophied cells contain neutrophilic or eosinophilic granules. So far as could be determined by following the cells in the incubated drops of blood, it seemed to be the mononuclear type that gave rise to the three kinds of transformed cells, i.e., the macrophage, the epithelioid cell, and the giant cell.

Sabin (1921, 1923) and her co-workers, Cunningham and Doan (1924), have been carrying on a series of most interesting observations in which they differentiate the various types of blood-cells by means of vital dyes, and on this basis attempt to establish a grouping of the blood-cells more in accordance with what they consider to be their origin. By this method these investigators, in their later publications, distinguish the monocyte from the clasmatocyte and claim that, while both the monocyte and the clasmatocyte are phagocytic, there is nevertheless a distinct difference between the two types of cells. In view of this it is rather interesting that some of the transformed leucocytes certainly resemble clasmatocytes, whether the term be employed in its usual sense of tissue macrophage or in the more restricted meaning assigned to it by these later writers. These clasmatocyte-like cells may not be true clasmatocytes, but neither can they properly be termed monocytes, owing to their increased size, changed nucleus, accumulated fat globules, irregularly scattered ingested material and neutral-red bodies. Just what term should be used to designate these cells does not decrease the importance of the fact that macrophages, epithelioid cells and giant cells do arise from the leucocytes of the blood, without, in this case, any possibility of participation in the phenomenon by the endothelium or the connective tissue.

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DESCRIPTION OF PLATE XXI

Transformed leucocytes in the incubated drops of blood, drawn while living. Ocular No. 6, 2 mm. lens. [Reduced one-half in reproduction.]

Fig. 1. The normal cells of avian blood. These were drawn one hour after the cultures were prepared. Granulocyte, mononuclear, lymphocytes, and nucleated red blood-cell.

Fig. 2. Non-granular cell in blood from a 2-months' old chicken after 6 hours' incubation.

Fig. 3. Hypertrophied non-granular cell in blood from a 2-months' old chicken, incubated 12 hours.

Fig. 4. Transformed leucocyte containing fat globules and red blood-cells in same culture as that of Figs. 2 and 3, after 24 hours' incubation. The nucleus has lost its leucocytic character.

Fig. 5. Phagocytic cell containing a red blood-cell in same culture as that of Fig. 8. This cell had not hypertrophied.

Fig. 6. A phagocytic wandering cell containing many red blood-cells. From the blood of a week-old chicken, incubated 3 days.

Fig. 7. A phagocytic wandering cell spreading out on the coverglass. It contained laked red blood-cells and the nuclei of many digested ones. From the blood of a 2-months' old chick, incubated 4 days.

Fig. 8. A phagocytic wandering cell which was transferred to a drop of plasma containing avian tubercle bacilli. This cell ingested many organisms and lived for 20 days.

Fig. 9. A giant cell containing partially digested red blood-cells. Blood from a week-old chicken — after 4 days' incubation.

Fig. 10. An epithelioid cell in a drop of blood from a 19-day old chick embryo, after 3 days' incubation.

Fig. 11. An epithelioid cell containing many tubercle bacilli. From the same preparation as that of Fig. 8 — after 4 days in plasma.

Fig. 12. A long spindle-shaped cell containing ingested avian tubercle bacilli in same culture as that of Figs. 8 and 11.

Fig. 13. Red blood-cell from the blood of the mouse.

Fig. 14. Mononuclear cell from the blood of the mouse.

Fig. 15. Phagocytic cell from a drop of mouse blood incubated 40 hours.

Fig. 16. Round flat cell of the epithelioid-cell type from a drop of blood of the mouse, taken from the abdominal circulation and incubated 48 hours.

Fig. 17. Phagocytic cell from a drop of blood from the heart of the mouse, incubated 48 hours. It contained many red blood-cells, each of which was in a different stage of digestion.

Fig. 18. Mononuclear cell from human blood, one half hour after the culture was prepared.

Fig. 19. Polymorphonuclear cell from human blood, one half hour after the culture was prepared.

Fig. 20. Human red blood-cell, one half hour after the culture was prepared.

Fig. 21. Phagocytic wandering cell of the clasmatocyte type, containing ingested erythrocytes; drawn after 7 days' incubation of human blood on celloidin.

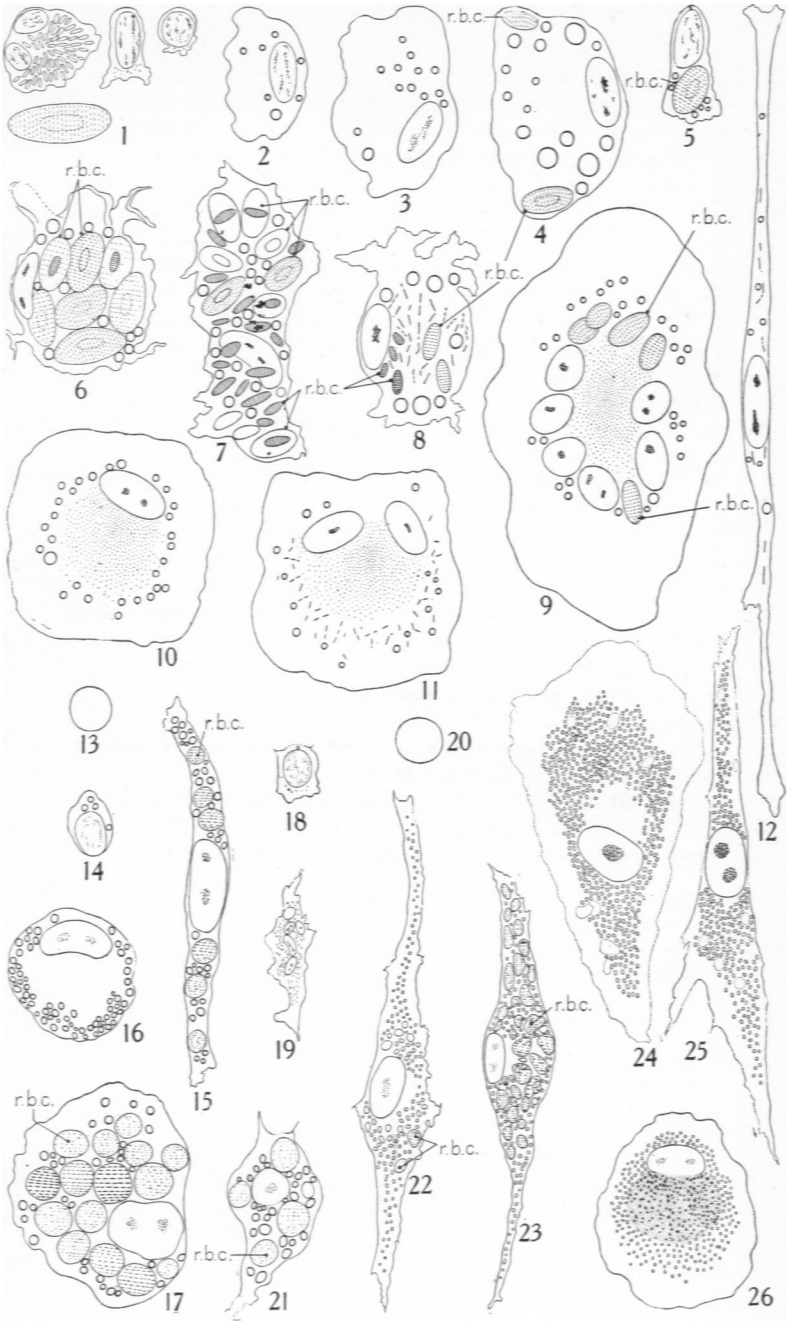
Fig. 22. A spindle-shaped cell from the human blood after 8 days' incubation.

Fig. 23. A cell of the fibroblast type, which, after it had grown eight days on celloidin, was again exposed to red blood-cells. It ingested many red blood-cells. These were partly laked and partly digested at the end of 10 days when the preparation was drawn.

Fig. 24. Epithelioid type of cell with enlarged centrosphere and large pale nucleus containing a nucleolus. Drawn after a drop of human blood had been incubated 13 days on celloidin.

Fig. 25. Spindle-shaped cell from human blood incubated 9 days.

Fig. 26. An epithelioid type of cell, showing the enlarged stained centrosphere; drawn from human blood, incubated 8 days on celloidin.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