Phase Contrast and Electron Microscope Studies of
the Appearance and Behaviour of the White Cells of
Normal Human Blood

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With three plates (figs. 3-5)

SUMMARY

1. The phase-contrast microscope has been used to study form and movement of human leucocytes and blood platelets in three types of preparation; (a) warm sealed blood drops, (b) warm sealed films of leucocytes adhering to coverslips after incubation of blood drops at 37° C. for 30 minutes or more, and (c) warm sealed films of blood platelets obtained by similarly incubating drops of saline suspensions of these cells. Similar preparations to (b) and (c) were obtained on formvar films and studied in the electron microscope. Phagocytosis was studied in all the above preparations after adding suspensions of collodion particles or of Staphylococcus albus.

2. The neutrophils were the most versatile cells seen in the above preparations. The amoeboid forms were the most mobile and phagocytic. Another form showed rapidly waving processes which gradually spread into a flat membrane on the glass. Flattened immobile neutrophils were still capable of phagocytosis. Bacteria which adhered to the cell surface moved over it towards the thicker central region of the cell where ingestion occurred.

3. Eosinophils showed a mobile amoeboid form and flattened forms which were often very bizarre in shape. They did not phagocytose readily.

4. Monocytes showed slowly moving amoeboid forms or forms with ruffle-like membranes around the cell. They were phagocytic.

5. Lymphocytes were capable of amoeboid movement but did not flatten on glass and were not phagocytic.

6. Blood platelets showed dendritic and flattened forms. Bacteria or collodion particles adhered to the surfaces of the latter and travelled over them, clustering at their thicker centres.

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INTRODUCTION

DURING the past decade several authors have published studies of leucocytes and blood platelets, using the phase-contrast and electron microscopes. The flattening of leucocytes was studied with the phase-contrast microscope by Bessis and Bricka (1949 a and b), and their structure described by Bessis (1949a) and by Feissly and Lüdin (1949). These authors mentioned briefly a few observations on the movement of living leucocytes and Bessis described various degenerate changes in living leucocytes kept at room temperature. Feissly and Lüdin also described the structure of blood platelets.

Using the electron microscope Bessis (1949b) and Rebuck and Woods (1948) studied the ultra-structure of leucocytes. The fine structure so revealed included that of the chromatin of lymphocyte nuclei (Rebuck, 1949), the pseudopodia of leucocytes (Bernhard and his co-workers, 1950 a and b), and the granules (Bessis and Bricka, 1950 a and b). The flattening of leucocytes was described by Bessis and Bricka (1949c). Rebuck and Woods (1948) published the only electron microscope picture of phagocytosis by a leucocyte. Pictures showing the ultra-structure of blood platelet cytoplasm have been published by Bessis and Bricka (1948), Bessis and Burstein (1948), and by Bessis (1949b). Their structure and its changes during flattening have been described by Wolpers and Ruska (1939) and by Bessis (1949b).

In the work presented here the phase-contrast and electron microscopes have been used to study both the structure and the behaviour of normal human leucocytes and blood platelets. Special attention has been given to movement and phagocytosis, and a very simple method of obtaining phagocytosing leucocytes for electron microscopy was evolved.

TECHNIQUES

Methods of concentrating leucocytes and platelets for study

The method used for concentrating leucocytes other than lymphocytes was that described by Wright and Colebrook (1921). A wax ring 1 cm. in diameter was made on a glass slide or coverslip. The cell thus obtained was filled with fresh finger-prick blood and placed in a Petri dish lined with moist filter paper. After incubation at 38° C. for 30 minutes or more, the blood clot was removed and the preparation washed free from red cells with normal saline. A film of leucocytes adhered to the glass in approximately the following proportions; neutrophils 80 per cent., eosinophils 10 per cent., monocytes 10 per cent. Lymphocytes were rarely present. When citrated blood was used, no adherence of leucocytes was obtained.
To obtain blood platelets, a few drops of blood were mixed in a wide tube with about 5 c.c. of citrate saline containing 0·6 per cent. dextran. The dextran accelerated the sedimentation of red cells (Minor and Burnett, 1948). When this was complete the supernatant fluid was pipetted off and the suspended platelets were centrifuged and washed with normal saline. A drop of the concentrated platelet suspension was then incubated in the same manner as the fresh blood (described above) for periods of up to 30 minutes. Examination of these preparations showed platelets in the process of flattening themselves against the glass surface.

Blood of group A<sub>2</sub>, R<sub>T</sub> from a single normal human being was used in these studies.

**Preparation of materials for phagocytosis**

Collodion particles have previously been used in phagocytosis experiments (see Mudd and others (1934)). In my experiments suspensions of collodion particles were prepared in the following manner: a 1 per cent. solution of collodion in alcohol-ether was diluted 15 times with acetone and carefully poured on top of an equal volume of distilled water in a separating cylinder. The acetone mixed slowly with the water, so precipitating collodion particles which settled and were run off at the bottom. The suspension of particles was then dialysed in a collodion membrane against distilled water, or centrifuged and washed several times. The final suspension was made in normal saline.

Suspensions of *Staphylococcus albus* for phagocytosis were prepared by gently rubbing the surface of agar slope cultures with a glass rod and washing with a few c.c. of normal saline.

**Ordinary light microscopy**

Preparations fixed and stained by various techniques were observed in the ordinary light microscope to facilitate their interpretation when observed by phase-contrast microscopy.

**Phase-contrast microscopy**

A Cooke, Troughton, and Simms binocular phase-contrast microscope was used in observing living and fixed cells and platelets. A thin sheet of copper, bearing a heating element controlled by a rheostat potentiometer, could be fitted to the mechanical stage. Living preparations were placed over a hole in the plate.

Suitable preparations were obtained by placing a very small drop of blood or a film of leucocytes or platelets, together with bacterial or collodion particle suspensions when required, on a thin coverslip and carefully lowering on to it a slightly smaller coverslip. The slips were then sealed with paraffin wax and the preparation clipped over the hole in the warm stage of the microscope with the larger slip below. It was then possible to use the ×95 oil-immersion objective.

Phase-contrast photographs were taken with a ciné outfit designed by
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Walker and Davies of the Biophysics Research Unit, King’s College, London. They were taken on Ilford extra-fine-grain panchromatic film with an exposure time of 2 seconds.

Electron microscopy

A solution of 0.5 per cent. formvar in dioxane was used to prepare formvar films on glass slides. When the film was dry a square about 2 cm. × 2 cm. was scored round with a sharp knife and floated off on to distilled water. A wax ring was made on this by dipping a piece of 1 cm.-bore glass tubing into hot paraffin wax and carefully touching it down on the formvar film. It was then refloated on to a slide and drained of excess water.

A dense population of leucocytes or platelets was obtained on the film by Wright and Colebrook’s (1921) technique. In studies on phagocytosis the suspension of collodion particles, or of bacteria and fresh human serum, was then added and the preparation reincubated. In some cases the particles for phagocytosis were mixed with whole blood at the beginning of incubation.

After washing in warm saline the preparation was fixed. Many simple and mixed fixatives were tried, but the most life-like appearance was given by osmium tetroxide. Fixation for 5 to 10 minutes in a fresh 2 per cent. solution, or in the vapour, was used in this study. Occasionally absolute alcohol was used. The preparation was then washed in distilled water and the formvar film carefully stripped from the slide. The wax ring caused the film to float so that it was easily mounted on the specimen screen used in the microscope. Preparations were stored in a desiccator and shadowed with palladium gold at an angle of about 45°.

The electron microscope used was a Metropolitan-Vickers E.M.3. Photographs were taken at 75 kv.

Results

Comparison of the information yielded by the microscopical methods used

The study of living leucocytes by ordinary light microscopy was very difficult on account of their extreme transparency, which makes their contents almost or quite indistinguishable from one another. In order to obtain information about the cell constituents by this method it was necessary to resort to leucocytes which had been fixed and stained. The disadvantage of this was that one could no longer study living processes such as movement or phagocytosis as they occurred in any particular cell. Phase-contrast microscopy has overcome these difficulties. The particles in living leucocytes which were invisible by ordinary light were made darker or lighter than their background and their movements could be followed readily. Phase contrast is best used on very thin or transparent objects which have little phase difference. It is difficult to film rapidly with phase-contrast illumination since long exposures are required.

Both the above forms of microscopy suffer from the fact that resolution is limited by the wave-length of visible light. The electron microscope enables
a far higher resolution to be obtained by using a beam of electrons instead of light-rays. Its chief drawbacks in a study such as this were that the cells had to be extremely flattened and obtained on delicate films to allow penetration by the electron beam. The cells had to be fixed and vacuum-dried and these processes produce artifacts. However, one simple fixative of the many which were tried gave a fairly life-like picture of the leucocytes. This was osmium tetroxide. It preserved the homogeneity of the cytoplasm, avoiding the sponge-work appearance produced by protein precipitants. Little shrinkage appeared to be caused by it in the cell as a whole, but collodion particles and sometimes eosinophil granules had a shrunken appearance.

Morphology and movement of leucocytes and platelets

In the two types of preparation studied (sealed blood drops and sealed films of leucocytes obtained by Wright and Colebrook’s technique) the same type of leucocyte assumed a number of quite distinct forms. The greatest variety of form was seen amongst the neutrophils. In sealed blood drops kept at 38° C. and viewed by phase-contrast microscopy the neutrophil immediately began to flatten from the spherical form of 10 to 12 μ in diameter to a roughly pear-shaped form and began to move, broad end first. The most typical size and shape of these fresh, active neutrophils was that seen in fig. 3, A–D, perhaps 20 to 25 μ long, 10 to 15 μ in width at the broad end, narrowing slightly for 15 to 20 μ and then more suddenly to leave a short, narrow tail. Sometimes there was no tail (fig. 3, D), but at other times, particularly as the preparation aged, it was 20 μ or more long and occasionally divided into a number of fine cytoplasmic processes (filopodia).

The fresh active neutrophil was capable of moving roughly 30 μ per minute at 38° C. At the broad front end of the cell was a hyaloplasmic border up to 4 μ in depth which advanced in a continuous flow. Behind this was a region of cytoplasm containing many neutrophil granules, those in the central zone showing a fairly continuous Brownian movement, combined with a forward and outward streaming as the cell flowed forward (fig. 1). The nucleus, which comprised 1–5 unequal lobes, occupied most of the main body of the cell and was surrounded by more granules. The tail was almost or quite free from granules and was a relatively permanent structure. As the front end of the neutrophil flowed forward, the tail moved more slowly, with the result that the whole cell became somewhat elongated, the nucleus often being considerably deformed in the process. The granules streamed slowly up the centre of the cell, vibrated at the front end and then moved slowly out towards each side, where they remained more or less stationary. At intervals of about 20 seconds to 2 or 3 minutes, according to the rate of movement of the cell, the region just in front of the tail appeared to become more fluid. Nucleus and granules streamed rapidly to the front region, causing the main cell body to become more rounded, the tail being drawn up later. Tail filopodia were quite stiff structures, as could be seen when a granulocyte changed direction and they were swung through the medium.
When a neutrophil came up against an obstacle such as a platelet clump in its path, it either pushed its way round one side of it or it would pass round both sides of it and become almost divided into two. The halves would continue to advance until they were perhaps 100 μ apart, remaining attached to one another by a slender thread of cytoplasm (fig. 3, E). The nuclear lobes would be distributed between the two halves, remaining connected by a very fine strand of nuclear substance. Eventually one half would continue its advance, whilst the hyaloplasmic tip of the other became invaded by granules showing very vigorous Brownian movement. This half would then stream rapidly back into the other half and the whole cell might subsequently move in the normal manner.

In ageing blood drop preparations neutrophils often put out numerous pseudopodia which were shortly withdrawn again into the main cell body after showing Brownian movement of the granules up to the cytoplasmic edge. After 40 minutes' or so incubation by Wright's technique, motile, pear-shaped forms were sometimes obtained which were rarely smooth in outline like those seen in sealed blood drops, but had their cytoplasmic surfaces irregularly thrown up into folds.
Phase contrast observations also revealed another form of neutrophil in Wright’s preparations incubated for an hour or more and a few were sometimes seen in sealed blood drops. The cell body had taken on a flattened oval or circular shape about 22 to 25μ in diameter, often surrounded by numerous processes (fig. 3, L–O). The flattest forms of all were obtained on the lower surface of a coverslip when this had been placed on top of a blood filled wax cell to form a roof in Wright and Colebrook’s technique. These flattened neutrophils were capable of changing their outlines slightly and of phagocytosing foreign particles which came into contact with them (fig. 3, L–O). Granules within the cell showed some slight rearrangements and sometimes vigorous Brownian movement in small localized areas of the cytoplasm.

The flattened neutrophils were the most suitable forms for electron microscopy and the details revealed by it will be described below. There was great variation in the cell outline. Sometimes it was fairly smooth or gently undulating, sometimes surrounded by numerous processes which might be long or short, fine or stout, branched or unbranched. Processes of one neutrophil were apparently capable of fusing with those of another (fig. 4, A). Often something of a pear-shaped outline was retained by these flattened neutrophils. In such cases there would be a blunt end with undulating outline, sometimes having the appearance of two overlapping folds of cytoplasm, and a tail end bearing filopodia. The hyaloplasmic border of the cell varied from 1 to 5μ in width. Surrounding the central nuclear lobes was an area of granular cytoplasm 2 to 6μ in width. The granules were round, about 300 to 400 in number, and varied in diameter from 0.6μ down to 0.1μ, most being about 0.3μ. Among them it was sometimes possible to distinguish some 20 to 30 rod-shaped mitochondria 0.1μ to 0.3μ in width and 0.6μ to 1.0μ in length.

With the phase contrast microscope it was occasionally possible to find a dendritic form of neutrophil in a freshly sealed Wright’s preparation. In these the nucleus and granular cytoplasm were concentrated into a roughly spherical shape, whilst the surrounding hyaloplasm was drawn out into narrow, unbranched dendrites up to about 10μ in length and 0.6μ in diameter, which waved in the surrounding medium (fig. 3, H). These dendrites grew rapidly to a width of 1.0 to 1.5μ and ceased to wave. Then from their bases they began to broaden and to merge with one another, with the result that a hyaloplasmic membrane gradually spread outwards around the cell producing a flattened neutrophil (fig. 3, I–K).

Flattened neutrophils obtained after Wright’s technique were sometimes seen to change into ‘prickle cells’ by a sudden contraction and rounding up of the main cell body, whilst the hyaloplasmic processes remained fixed to the substratum, becoming drawn out into long straight filopodia (fig. 3, P).

Yet another form of neutrophil was seen in some ageing Wright’s preparations. It had a dense, central perinuclear region bearing short hyaloplasmic processes or a membrane; but the hyaloplasm in contact with the substratum was spread out farther. Thus there were overlying hyaloplasmic membranes or processes. Some of the cells studied by electron microscopy were
believed to be neutrophils of this form, and they were capable of phagocytosis
(fig. 4, r).

The eosinophil was in many ways similar in behaviour to the neutrophil. It was about the same size and in sealed blood drops viewed by phase contrast illumination at 38° C. it assumed a similar pear-shaped, motile form, though generally it showed a smoother outline. The nucleus consisted almost invariably of two equal lobes, oval in shape (occasionally of one or of three unequal ones) which occupied a position side by side towards the narrow end of the cell. Almost all of the large eosinophil granules were concentrated towards the broad front end of the cell in front of the nucleus, where there was a narrow hyaloplasmic border 1 to 2μ in depth. The hyaloplasmic tail region was often furnished with filopodia of varying lengths and complexity.

Movement of the pear-shaped eosinophil was similar to that of the neutrophil, but in most cases a little slower. Some slight Brownian movement of granules in the forward central zone was distinguished, but streaming movements were often rapid and clearly seen. There was the typical fountain-shaped current of granules moving up the centre of the cell to the front and out towards the sides where they came to rest as described in the neutrophils (fig. 2, a). There were at other times currents which were the reverse of the fountain. Granules from the sides near the front end travelled swiftly forwards and then moved in towards the centre where they came to rest until carried backwards and outwards to the sides as other granules piled up in front (fig. 2, b). Both of these types of currents resulted in movement straight ahead. Asymmetrical currents as seen in fig. 2, c and d accompanied a turning of the cell to one side.

In Wright's preparations seen by phase contrast illumination a few flattened eosinophils were found with central nucleus surrounded by granules and an outer border of hyaloplasm (fig. 3, r). They were often somewhat elongated with the nuclear lobes placed end to end. Most of the granules were clustered around the nucleus. The hyaloplasmic border of the cell was of variable width and extended into processes of many shapes and sizes, some of which contained isolated granules.

The flattened eosinophils were the cells most easily recognizable in the electron microscope by reason of their large, opaque granules. These num-

Fig. 3. Phase-contrast photographs of living leucocytes. A-G in sealed blood drops, H-T in sealed Wright's preparations. A-D, a lymphocyte and a moving neutrophil taken at intervals of 10 seconds. Note the sudden acceleration of the neutrophil from C to D. Three blood platelets 'p' were also present. E, an elongated neutrophil phagocytosing a bacterium. 'Digestive' vacuoles containing several bacteria were already present. F and G, phagocytosis of two bacteria by a neutrophil and formation of a 'digestive' vacuole in 20 seconds. H-K, a dendritic neutrophil in various stages of flattening. This took 7 minutes. L-O, a flattened neutrophil phagocytosing a bacterium 'b'. Six minutes elapsed between L and O. P, the same neutrophil as above taken 30 minutes later when it had become transformed into a 'prickle' cell. Q, a nearly flattened dendritic neutrophil. R, a bizarre flattened eosinophil. S and T, a monocyte showing waving of the ruffle-like membrane, 40 seconds between exposures.
bered about 100 to 150 and were usually round, oval, or shaped like short rods. They varied in size from 0.3 to 1.7 μ, but the majority were about 0.6 μ in diameter (fig. 5, A). Some eosinophils also showed a few much smaller, less opaque particles down to about 0.01 μ in diameter. In some electron microscope pictures there appeared to be fine strands of black material connecting some of the granules to one another. The same effect was observed when collodion particles were fixed in osmium and looked at in the electron microscope. Fig. 4, D is an electron microscope photograph of an eosinophil which has retained its pear shape.

**Basiphils** were not usually seen, being very few in number, but it was considered that fig. 4, C might be an electron microscope picture of one. It showed a large, bilobed nucleus and a small number of granules which were not distinct and appeared to be far fewer and more irregular in size than those of typical eosinophils.

In sealed blood drops and in Wright's preparations monocytes were usually extremely flattened and indistinguishable from their background by phase contrast illumination. The nucleus was round, oval, indented or kidney-shaped, measuring 7 to 11 μ in diameter. It was more or less centrally placed in the cytoplasm which measured 17 to 21 μ in diameter. The cytoplasm was peppered with fine granules except for an outer border 2 μ in width. The cell shape was roughly circular or oval, the outline smooth and usually gently undulating. These flattened monocytes showed a slow change in shape of nucleus and cytoplasm with little change in position of the cell.

In the electron microscope the granules in flattened monocytes were seen to number up to about 120 (fig. 4, B). Up to 20 of these were usually distinct and measured 0.3 to 0.6 μ in diameter, the rest being smaller, less opaque and difficult to distinguish. Up to 30 were very pale rod-shaped mitochondria measuring 0.4 to 1.5 μ in length and 0.1 to 0.2 μ in diameter.

In fresh Wright's preparations the monocytic cytoplasm seen by phase contrast illumination was not always spread out flat in one layer on the substratum. Sometimes the cell appeared fairly spherical with one folded membrane, or possibly several independent membranes thrown out in different planes around it. Such membranes were described by Goodrich (1919) in leucocytes of invertebrates. Waves passed fairly rapidly and regularly around these membranes (fig. 3, S and T). At other times the cell was more flattened and the overlying membranes irregularly shaped. Sometimes the membranes were folded or ridged with the ridges elongated into fine processes. The membranes

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**Fig. 4.** Electron microscope photographs of osmium-fixed leucocytes after Wright's technique. A, a flattened neutrophil capturing 4 bacteria 'b'. Note apparent fusion of two processes with those of a neighbouring cell. B, a flattened monocyte capturing bacteria 'b'. C, a basophil? or eosinophil. D, an eosinophil. E, a neutrophil phagocytosing collodion particles. The cell contained many particles but was continuing to phagocytose. Various stages in the process may be seen. F, a double membranated neutrophil showing a 'digestive' vacuole containing 4 bacteria. Note the rounded cell body surrounded by a hyaloplasmic membrane which is overlain by cytoplasmic processes at a higher level.
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were capable of extending and withdrawing slowly so that there was some change in shape of the cell. Such movement was probably similar to that of the undulating membrane of monocytes in tissue culture described by Carrel in 1926.

In Wright's preparations lymphocytes almost always failed to adhere to the substratum and they were subsequently washed away with the red cells. Thus they were not obtained for electron microscopy. In sealed blood drops they were often rounded and immobile, but when the glass surfaces were closely pressed together they often showed great activity.

The large lymphocyte was about 12 to 15 \( \mu \) in diameter and tended to show an elongated or pear-shaped form. At the broader end was a border of cytoplasm, up to 3 \( \mu \) in depth, and this was followed by the nucleus which measured 8 or 9 \( \mu \) in diameter. The bulk of the cytoplasm lay behind this and it tapered slightly towards the end.

The process of lymphocyte movement was similar to that of the granulocytes and the cell was capable of moving at over 20 \( \mu \) per minute. The broad end of the cell flowed forward more rapidly than the narrow end, so that the cell and its nucleus became elongated. Then at intervals the lymphocyte would draw up its tail and the nucleus would round up again. The deformability of the nucleus was very striking.

Many of the small lymphocytes appeared as rounded non-motile cells with a smooth outline, measuring 8 or 9 \( \mu \) in diameter, thus occupying most of the cell volume. The cytoplasm was scanty and clear, but 20 to 30 grey, rod-shaped mitochondria were usually distinguishable by phase contrast microscopy, mostly clustered at one side of the nucleus. In about half of these cells 1 or 2 large, dark granules were also distinguishable, with occasionally a few small ones as well.

Some of the small lymphocytes were capable of directional movement at a rate of about 10 to 15 \( \mu \) per minute in a similar manner to the large lymphocyte, though the nucleus was not so deformable. It was capable of becoming indented, heart or dumb-bell shaped. The mitochondria and granules usually followed after the nucleus during movement. Other small lymphocytes showed a very small degree of movement (fig. 3, A–D).

Blood platelets in freshly sealed blood drops appeared either singly or in clumps as dark grey bodies about 2 \( \mu \) in diameter with a lighter, irregular periphery by phase contrast illumination (fig. 3, A, 'p'). Occasionally some were seen shaped like tadpoles. On incubation the single platelets and the outer ones of a clump appeared to swell and become a lighter grey. Eventually they became refractile circles about 3 to 7 \( \mu \) in diameter, or even larger. Some then became free from their neighbours in a clump and floated away in the serum. Often a number of minute granules in rapid Brownian motion were distinguishable within them.

Platelets separated from citrated blood were studied by electron microscopy. A series of preparations incubated for gradually increasing time intervals revealed the method of flattening against the substratum. At first the platelets
appeared with a dense, irregularly shaped centre of 1 to 2μ in diameter bearing numerous finger-like projections 4μ or more in length (fig. 5, c, ‘d’). These were the dendritic forms described by Bessis (19496). Later forms showed dendrites with widened bases which joined to form a web or membrane (fig. 5, b). Finally, a flattened form was reached which measured 4 to 16μ in diameter and was often circular (fig. 5, c). Some giants of up to 30μ in diameter were believed to be the result of fusion of several platelets (fig. 5, c). Similar preparations of unfixed platelets and osmium fixed platelets showed identical forms when studied by phase contrast microscopy. The cytoplasm of several platelets showed holes (fig. 5, D and G). These were probably formed by the fusion of the tips of dendrites as they expanded. The lower half of the platelet in fig. 5, E shows how this might occur. The central region, occupying about one third of the platelet diameter, was the densest part, but it sometimes contained small vacuoles (fig. 5, G). The main bulk of the cytoplasm was less opaque, often showing denser ribs running radially to the tips of any remaining dendrites. Some platelets revealed cytoplasmic fibres running radially and merging with other fibres running parallel to the border (fig. 5, D). Many appeared to be non-granular, but a few had up to 100 round granules 0.1 to 0.3μ in diameter distributed throughout the peripheral cytoplasm (fig. 5, c and G). In others only the dense central region of the platelet had a granular appearance.

Phagocytosis by leucocytes

Phagocytosis was studied in both sealed blood drops and in Wright’s preparations by phase contrast and electron microscopy. The presence of fresh serum, in practice human serum, was required for phagocytosis of the bacteria used, but not for collodion particles. The latter tended to clump together, but the former moved about singly or in small groups.

By far the most actively phagocytic cell seen in the sealed blood drops by phase contrast microscopy was the neutrophil. The advancing hyaloplasmic edge of the fresh active forms would come into contact with bacteria or particles either singly or in groups and after a few seconds the hyaloplasm would flow round and over them to engulf them completely. The ingested material then traversed the hyaloplasm and entered the granular perinuclear zone of the cytoplasm where, if it was a bacterium, a digestive vacuole was formed round it in a few moments (fig. 3, F and G). When other digestive vacuoles were already present the newly entered bacterium sometimes travelled up to one of these and burst into it, joining the other bacteria there in Brownian motion. Several vacuoles formed around individual bacteria sometimes coalesced to form larger ones, so that a neutrophil might come to possess a few enormous vacuoles, each containing many bacteria. No digestive vacuoles were formed around collodion particles.

The whole process of phagocytosis up to the beginning of vacuole formation took sometimes as little as 1 minute, but often 3 or 4 minutes or longer. A fast moving neutrophil was capable of performing a separate act of phago-
cytosis every minute, or more frequently. These neutrophils could ingest a large clump of 20 to 30 collodion particles all at once by slowly flowing around it. This might take as much as an hour to complete, the cell being then so full as to be incapable of further movement.

Fig. 3, E shows phagocytosis by an elongated neutrophil. Sometimes, as in this case, a little tongue of hyaloplasm was thrown out which flowed round the bacterium.

Flattened neutrophils in Wright’s preparations were also capable of phagocytosis in spite of their immobility. When a bacterium contacted a part of the cell surface which had a smooth outline there was a slight local outflowing of hyaloplasm to engulf the bacterium. If it contacted the tip of a short stout process the bacterium might be engulfed there and then pass along the process towards the perinuclear zone. If the process was a slender filopodium, the bacterium was sometimes engulfed by the tip and then it travelled along the process, causing local swelling on the journey, or the filopodium was retracted slowly into the cell. Frequently a bacterium did not appear to be engulfed when it contacted a filopodium, but it passed centrally along the filopodial surface at about 3 to 10 μ per minute and then entered the hyaloplasm (fig. 3, L–O), sometimes causing neighbouring filopodia to be pushed aside through an angle of perhaps 20°. If a small group of bacteria were involved usually only one of them would be in contact with the filopodium, the others being towed along until the main cell body was reached. Ingestion was effected either by the bacterium merely sinking into the cytoplasm, or by the outflowing of a small tongue of hyaloplasm which wrapped round it.

Electron microscope pictures show cells fixed in various stages of the act of phagocytosis. Ingested collodion particles were seen within the cells as grey, circular bodies measuring 0.2 to 2.0 μ or more in diameter with a darker border. When not ingested they usually underwent shrinkage, appearing as dense, black bodies with an irregular surface and with bridges between them. The shadows thrown by them were often wider than the particles themselves. Fig. 4, E is a neutrophil showing various stages in phagocytosis of collodion particles. The bacteria used (Staphylococcus albus) appeared as circular or rod-shaped black bodies 0.4 to 0.8 μ in diameter.

It was difficult to study phagocytosis by eosinophils in the phase contrast and electron microscopes as it was often impossible to distinguish ingested material from eosinophil granules. In fixed and stained specimens

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**Fig. 5.** Electron microscope pictures of a leucocyte and of blood platelets, all osmium-fixed except B, which was fixed in absolute alcohol. A, an eosinophil. B, 2 platelets, one nearly flattened, the other transitional from the dendritic phase. C, 3 platelets. A dendritic one lay on top of a completely flattened one which was granular. D, 3 platelets incubated 15 minutes after adding collodion particles. The large platelet showed cytoplasmic fibres, two large holes and clusters of collodion particles at the centre of the cell. E, a platelet incubated for 1 minute after adding collodion particles. They are seen near the edge of the cell, but are not clustered at the centre. F, 2 platelets from the same preparation as D. One had an unusual frilled appearance. G, 3 more platelets from the same preparation as D and F. One was a giant with granular cytoplasm and a thicker centre which had a vacuolar appearance.
seen by ordinary light microscopy it was seen that they were only feebly phagocytic.

The monocytes, on the other hand, were more phagocytic than the eosinophils, though less so than the neutrophils. They accomplished this by enclosing bacteria in the folds of their undulating membranes, or by merely sliding over adherent bacteria (fig. 4, B).

Bacteria often appeared to adhere to the surfaces of lymphocytes and in the case of cells believed to be large lymphocytes some of them seemed to have been ingested, though this was difficult to determine.

When the bacteria or collodion particles were washed from a preparation of leucocytes after a period of phagocytosis, it was found that within 5 minutes all ingested ones had reached the perinuclear granular zones of the cells.

Studies of platelets with particles

When suspensions of blood platelets were mixed with collodion particles, or bacteria and serum, the platelets did not flatten out on the substratum. However, if the platelets were allowed to flatten first and the bacterial or particle suspension was added later, some very interesting results were obtained. After 10 to 15 minutes incubation with collodion particles the electron microscope showed the platelets to have collected about 20 or more particles at their thickened centres, with a small number towards the periphery (fig. 5, D and E). Bacteria were not captured so readily. Collodion particles at the centres of the platelets had the shrunken, black appearance with connecting bridges and not the smooth, circular, grey appearance characteristic of particles well inside leucocytes. The adhesive forces between the particles or bacteria and the platelet were strong enough to withstand vigorous washing with a pipette.

When a collodion suspension was added to flattened platelets for only one minute before fixation all the particles were found towards the periphery of the platelet and none in the centre. The nearest particle to the centre was 2 or 3 μ from the periphery (fig. 5, E). Particles at the periphery were adherent to any part of the cell outline, often to the tips of the remaining dendrites.

DISCUSSION

The electron microscope techniques described in this work had the advantage of being simple to carry out and gave preparations comparable with those obtained by other workers who used much larger quantities of blood containing anticoagulants.

The extreme diversity of form seen amongst living leucocytes in the preparations studied in this work is, I believe, dependent chiefly on the fluidity of the medium and on the presence or absence of a solid substratum. When being swept along in the blood stream under pressure, leucocytes probably remain spherical in shape. When suspended in static fluid such as serum or anticoagulated shed blood the granulocytes may take on the dendritic form seen in fig. 3, H with lashing dendrites all round. Dendritic forms were described by Bessis and Bricka (1949a) in preparations made from anticoagulated
blood and Hope Simpson (1942) has described pus cells (granulocytes) covered with a coat of vigorously waving tentacles each 2μ in length. The dendritic neutrophil photographed in fig. 3, H was found in a Wright's preparation which had just been set up. Such forms were not found in older preparations, so presumably they were adapted to their environment immediately before the preparation was made. This environment would probably be serum exuded on the surface of the slide from the shrinking clot of blood above.

In this static fluid environment the form of monocyte equivalent to the dendritic form of neutrophil seemed to be that seen in fig. 3, s and T, surrounded by a rapidly waving membrane. Goodrich (1919) found that when invertebrate leucocytes were floating freely in a hanging drop their undulating membranes were motile in a similar way. One may assume that the lashing of dendrites or rapid waving of an undulating membrane might result in active movement of the leucocyte through the fluid medium.

I have found no evidence from my own preparations or in the literature of previous authors to suggest that lymphocytes have an analogous form which might be capable of movement through a static fluid medium.

When the dendritic neutrophils in Wright's preparations came into contact with a flat surface such as a glass slide they spread themselves out as shown in fig. 3, H–K. Bessis and Bricka (1949a) have also described various stages in the flattening of these dendritic forms. Conversion of flattened neutrophils into 'prickle' cells as described by Sabin (1923) was also noted (fig. 3, o and p). Similarly, monocytes tend to spread themselves out as an elongated film on a glass surface and the rapid mobility of their membranes slows considerably. Goodrich (1919) and Fauré-Fremiet (1929) observed similar flattening of invertebrate leucocytes on glass. They attributed the phenomenon to surface tension forces. In the process of flattening the membrane sometimes appears to become folded and by ordinary light only these folds are visible and they have the appearance of flagellate pseudopodia. The existence of the membrane between them was first recognized by Carrel in 1926.

In my preparations I have rarely seen anything resembling the degenerative changes seen in leucocytes by Bessis (1949a). I believe that the formation of a vacuole completely surrounding the nucleus, which he described, and the phenomenon of surface blistering or potocytosis, also described by Zollinger (1948), occurred only in cells which had been damaged mechanically.

When a spherical granulocyte or lymphocyte from a fresh drop of blood is placed between a warm slide and coverslip, rather than spreading itself out like the dendritic cells in Wright's preparations it may assume a polarity and amoeboid movement ensue. The movement is similar to that of the monopodal Amoeba proteus described by Mast in 1926. The theories of amoeboid movement are reviewed by de Bruyn (1946). At present the elastic tension of the superficial plasmagel layer is thought to provide the locomotor force. The superficial cytoplasm is thought to be in the gelled state, the gelled layer being thicker at the tail end and thinnest towards the anterior end of the cell, while
the rest of the cytoplasm is in the sol state. The gelated cytoplasm forces the fluid cytoplasm forward by its contraction. The plasmagel layer extends forwards by gelation of plasmasol at the sides of the anterior end of the cell, and continuous solation occurs near the posterior end of the cell.

Lewis (1939) described constriction rings during the movement of lymphocytes and granulocytes in tissue culture, and ascribed them to a thickening of the plasmagel in an equatorial band which remained fixed in relation to the cell environment until all the cytoplasm except part of the tail had passed through it. De Bruyn (1946) on the other hand considered they were caused by external factors in the medium such as fibrin strands or irregularities in the glass surface. In my preparations it appeared that the tail end of the cell often did not advance so fast as the anterior end so that the leucocyte gradually became elongated as shown in fig. 1, A–C. Suddenly there was a release of the tail which was rapidly drawn up and the cell assumed a more rounded form. It seemed as though the elastic tension of the plasmagel had suddenly overcome some resistance such as the adherence of the tail to the substrate. The regularity of contraction in any particular cell would seem to indicate that it was part of the regular mechanism of the movement rather than a contingent effect. If it were due to irregularities in the medium one would expect to see cells with more than one constriction ring at times, but I have not observed this.

I cannot offer support to Mast’s theory that the plasmalemma executes a rolling movement during cell locomotion. Particles contacted by amoeboid neutrophils were either phagocytosed or ignored and they did not adhere to or travel forwards over the cell surface as in the Amoeba described by Schaeffer (1920). However, bacteria sometimes adhered to lymphocytes and in these cells they tended to collect at the posterior end of the cell and were not seen to travel forwards over the upper cell surface.

Blood platelets showed several features in common with leucocytes. When suspended in saline they assumed a dendritic form, but the dendrites did not appear to be capable of movement like those of neutrophils. On contacting a flat surface the platelets would spread out by a gradual widening of the dendrites from their bases towards their tips, just as in the case of neutrophils. The flattened forms were immobile. No amoeboid forms of platelets were seen. Other features common to platelets and leucocytes will be discussed later.

Since the work of Mudd and his collaborators in the early 1930’s, little work has been reported on the mechanism of phagocytosis. The process falls naturally into three parts. Firstly, there is the approximation of particle and phagocyte, implying mobility on the part of one or other or both and ending in their contact. Secondly, there must be adhesion of cell and particle and, thirdly, the actual ingestion of the particle which is affected by the viscosity and elasticity of the cytoplasm.

When cells are able to move about their chances of contact with particles are increased. Leucocytes in their amoeboid form were capable of rapid move-
ment and therefore able to make frequent contacts with particles. In the case of flattened immobile leucocytes in the preparations studied, contacts with particles were made by their sinking through the suspending medium or by the Brownian movement of the particles themselves.

The adhesive stage of phagocytosis was not clearly seen in the amoeboid neutrophils which ingested them almost immediately on contact. However, it was seen in many other leucocytes. Amoeboid lymphocytes were seen with bacteria adhering to their tails. Particles adhered to the surfaces of flattened immobile neutrophils and platelets. They were then moved slowly over the plasmalemma towards the central region of the cell without any visible accompanying movement on the part of the cell as a whole. In the case of the neutrophils ingestion usually occurred when the particles reached the thicker granular region of the cell, but in the thinner platelets particles remained clustered at the centre of the flattened surface. The forces which cause adhesion of cell and particle are probably akin to those concerned in agglutination phenomena. Movement of particles towards the centre of a flattened cell surface probably results in a state of equilibrium between the forces participating in the adhesion.

The final ingestive phase of phagocytosis can only be accomplished when the cell or part of it is in a relatively fluid state so that the plasmalemma is able to make use of its elastic properties. An amoeboid neutrophil is in such a state and behaves rather like a fluid droplet, spreading as a result of surface tension forces.

In conclusion we may say that leucocytes and blood platelets are extremely versatile cells capable of adapting themselves to different environments in order to perform their cellular functions of movement and phagocytosis. The different leucocyte types participate in these processes to different extents, the neutrophil being the most active under the conditions of my experiments.

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FIG. 3
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Fig. 4
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