Progress in HEMATOLOGY

VOLUME II

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with 19 contributors

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Introduction

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VOLUME 1 of *Progress in Hematology* had not been long off the press before it became obvious that there were many subjects so advanced that another survey of hematologic frontiers was desirable.

Advances in hematology continue to be largely the result of the application of physicochemical methods to biologic phenomena. Knowledge thus acquired is more or less promptly relayed to, or seized by, serious workers struggling with the mechanisms and management of disease. This is nowhere better illustrated than in the hemoglobinopathies, particularly that represented by the bizarre deformity, sickling, a constant source of wonder to inquisitive investigators. Clinical skill and perspicacity coupled with intellectual detachment accounts for some of the advances in other areas, such as, notably, in the prevention of kernicterus.

The inventory of fact and thought represented by these reviews is impressive. The extent of the invasion of new fields may surprise those who have not followed closely the paths of the probers. The pace of biologic investigation today is so rapid that, in some directions, ground is being gained exponentially. Though man well realizes that his very limitations will always deny him a "complete" knowledge of the world, the fruits of search and research will ever continue to answer partially man's yearning to explain, foresee and control his environment.

The Editor is indebted to the contributors for the cheerfulness and willingness with which they attended to their tasks. The Publishers have, as ever, been patient and helpful. To his colleagues and associates of the Charlotte Drake Cardeza Foundation, the Editor is grateful for continuing enthusiastic support and cooperation.

PHILADELPHIA, DECEMBER 1958

Contents

Recent, Significant Contributions of Dynamic Cytology to Hematology

MARCEL BESSIS

In the last ten years, important discoveries have been made in microscopy. Those which have brought the most important, immediate results are phase microscopy and electron microscopy. One permits us to see living cells, the other their ultra-structure. These two types of microscopy are, in fact, complementary. In order to interpret properly the complex images presented by the electron microscope, it is useful to be familiar with the behavior of cellular organites in the living state, and vice versa.

With phase contrast it becomes possible to study the cell, its behavior, function, pathology, its response to noxious stimuli and to various drugs, *during its life*. Heretofore, cytologic observations were based on the examination of the corpses of cells which, though brilliantly stained, nevertheless were dead objects. It is now possible to observe directly the behavior of these elements and to bring cytology closer to physiology and clinical medicine.

The value of examination by phase contrast is further increased when combined with cinematography. Almost always, movements of cells are extraordinarily slow when compared to movements to which we are accustomed at the human level. Many are so slow that even a trained eye cannot notice them. Cinematography makes it possible to accelerate phenomena from 30 to 60,000 times, depending on the interval between 1 second and 30 minutes. By this means, between the years 1910 and 1930, pioneers such as Comandon,[23-25] Fauré Frémiet,[28] Jolly,[36] Lewis and Lewis[40] discovered important phenomena which had hitherto passed unnoticed with simple ocular observation.

However, these experiments, although of fundamental significance, remained limited by the absence of clear images of intracellular organellae. This limitation has been overcome today. Thanks to phase contrast microscopy combined with cinematography, a precise analysis of all the cellular and intracellular movements can be made, and the effects of various media or stimuli on the morphology and behavior of the smallest organites may be observed. The action of poisons or drugs on cells can be also analyzed.

When one looks at a moving picture of a drop of blood between slide and coverslip, accelerated some 50 times, the blood cells which appear almost inert when otherwise examined become intensely and strangely alive. Emission of pseudopods, appearance and disappearance of dendrites, movements of the whole cell which crosses the field in a few seconds, oscillation of nuclei, rhythmic movements of the centrosome, quivering of granules, dance of the mitochondria, undulation of the chondriocontes follow one another. Inversely, slowing of the

From the Centre Nationale de Transfusion Sanguine, Paris, France.

process allows study of the various phases of certain very rapid, explosive biologic reactions, such as certain types of hemolysis.

For the study of living cells, we have at our disposal today, besides the phase contrast microscope, "interference" microscopes. There are various types. In general the image they produce is not as good as that with phase contrast, but on the other hand they make possible the measurement of the optical densities of certain parts of the cell. We shall not discuss them here, except to mention a particular system (called Nomarski's system[14]) which has proved to be superior to phase contrast for examining thick preparations, particularly rouleaux and crystals of erythrocytes (figs. 2 and 3).

In this article we shall briefly review the new facts about blood cells, acquired in the last ten years by examining them in the living state combined with the aid of microcinematography. Our remarks concerning general cytology will be condensed so as to devote special attention to those facts which should be of interest to the hematologist and the pathologist. For details of equipment and technics, the reader is referred to the pertinent papers in the bibliography.

The descriptions which follow, as well as the diagrams and photographs in the articles cited as references, give only a vague idea of what is seen on a film. The films cannot accurately be described; they must be seen. It is for this reason that we indicate in the Appendix the main films available and those who prepared them.

1. Red Cells

The mammalian erythrocyte has no power of motion. It is, however, not completely inert, for one can observe with phase contrast a scintillation of its palest central portion (3 or 4 variations of luminosity per second). The cause of this phenomenon is not known. It is the result either of slight changes in the thickness or of changes in the refractive index.[48-52, 65, 66]

Agglutination of erythrocytes. The plasticity of erythrocytes is very great. This plasticity is increased in the presence of antibodies.[6, 8, 9] When several agglutinated erythrocytes are stretched, they take the form of spindles connected with threads. These threads are very elastic, can attain a length of 20 microns and retract to only a few microns. When they break, their free extremity swells, they become agitated with brownian movement and form the so-called myelinic figures.[10]

Expulsion of the nucleus of the erythroblast. The expulsion of the nucleus of the erythroblast has been filmed. Under the experimental conditions (blood between slide and coverslip, heparin, room temperature) this expulsion occurs in acidophilic erythroblasts. The cells present lively movements, and suddenly some rounded excrescences appear at different spots along the cell border. One of these excrescences contains the nucleus, which after several convulsive movements is expelled.[2, 8] The actual phenomenon of expulsion takes about 10 minutes, and its various phases can be observed on Giemsa-stained smears of sternal marrow (fig. 1).

Movements of reticulocytes. The reticulocytes present very characteristic movements which are clearly discernible with cinematography. These cells, when

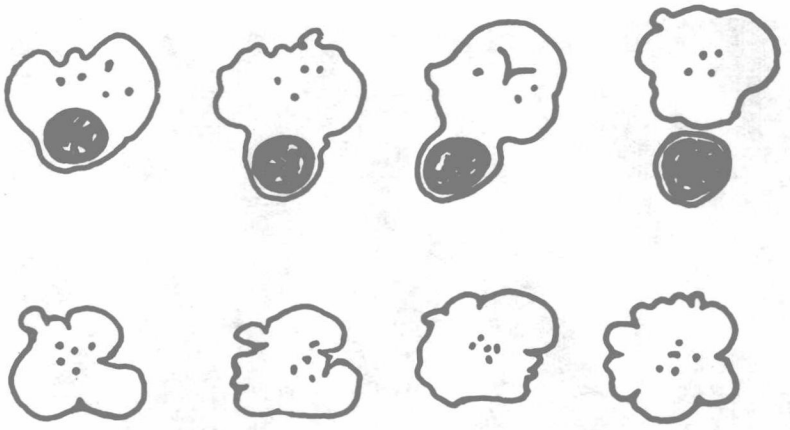

FIG. 1.—Enucleation of an erythroblast. *Bottom*: movements of a reticulocyte immediately after the expulsion of the nucleus (from a filmed sequence).

examined in vital preparations (*a l'état frais*), have a three- or four-leafed appearance (called hilar form). This is not due to permanent alteration of the cell as is generally thought, but to very slow movements of protoplasm, contracting and shrinking in certain areas, sending out large rounded expansions (fig. 1). These are the reticulocytes that Ralph[51, 52] studied under the name of "mobile erythrocytes."

2. White Cells

Granulocytes. Like the other types of leukocytes, granulocytes, which are spherical when in circulation, become deformed as soon as they come up against a solid surface along which they crawl. The shapes they can acquire are extraordinarily varied, their nucleus and cytoplasm being highly plastic (see figs. 4–11). White cells move like amoebae, as many authors, and recently De Bruyn,[16-19] have established.

As the granulocyte moves, its nucleus appears completely passive; its consistency seems fluid. The nucleus is extremely plastic and molds itself over the contour of any rough surface over which the granulocyte passes. As a leukocyte advances, its posterior portion remains attached firmly to the supporting surface and, progressively, pulls itself away from it, sometimes breaking in the process: this demonstrates its great rigidity. This posterior part, this "tail" of the moving leukocyte, has been studied particularly by Senda.[59] The speed of motion of granulocytes varies from 19, 39 and 36 microns per minute[33] at 37°C. Jolly and Comandon[23, 36] showed that this speed varies with the temperature. Frédéric and Robineaux[30] showed that granulocytes could move by emitting not only their well-known pseudopodia but also thin sheetlike expansions.

Spontaneous spreading of granulocytes. Like certain histiocytes, granulocytes possess the property of spreading out on certain surfaces.[7] Certain extracellular conditions play an important role in this phenomenon. The *milieu* (normal saline is more favorable than plasma) and type of *surface* (nonwettable surfaces,

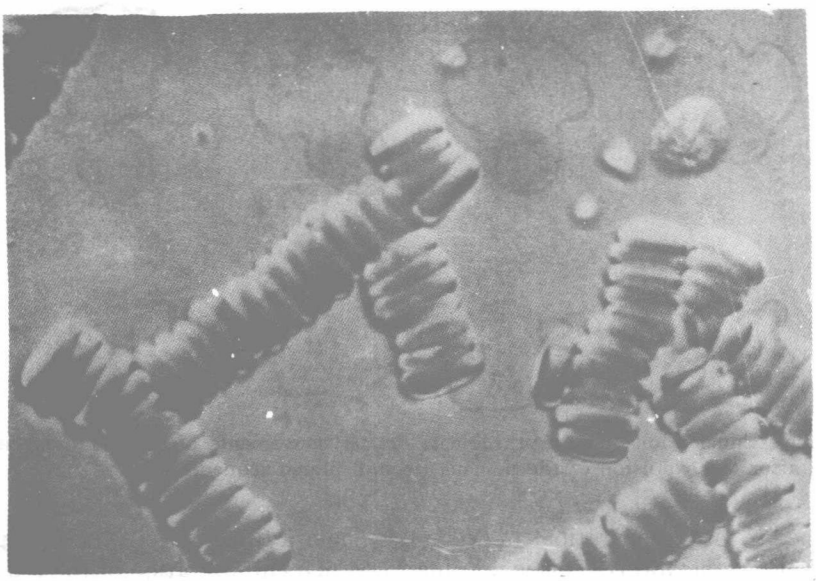

FIG. 2.—Appearance of red cell rouleaux, examined by interference contrast (Nomarski system). Note the absence of a halo around the cells in this thick preparation.

plastics) influence spreading. The cell thus spread out is particularly suitable for observation: the granules are generally spread into a single layer, the nucleus is well visualized, the cell is immobile, and the halos caused by phase contrast are minimized because of the thinness of the preparation (fig. 5).

Intracytoplasmic movements. Of all the intracytoplasmic movements, the most remarkable is certainly that of the cell center.[11, 44, 45] The cell center appears as a region paler than the surrounding cytoplasm, agranular and measures from 0.5 to 1 micron. The neighboring granules are radially oriented. In the concavity of the nucleus this center moves in a to-and-from pattern, with a periodicity of about 30 seconds and a variable amplitude (from 5 to 10 microns). The nucleus passively submits to the action of the cell center which pushes it away and even deforms it (fig. 6).

Vacuoles. Four types of vacuoles have been described in granulocytes (the lipid bodies being considered among the granules): (1) the neutral red vacuoles which appear and develop particularly in vitro; (2) the vacuoles of degeneration, particularly the inter-nucleocytoplasmic vacuoles (Dustin,[27] Bessis[6]); (3) vacuole of pinocytosis (Lewis[39]); and (4) contractile vacuoles. The secretory vacuoles demonstrated by Richter[54] should also be mentioned. We will briefly discuss these last two.

Bessis and Locquin[11] pointed out the contractile character of certain cytoplasmic vacuoles in granulocytes. Cinematography confirmed these observations and showed, as we shall see later, the presence of such vacuoles in platelets as well. One or two of these vacuoles are found, rarely more. Their expansion takes a variable length of time, on the average from 10 to 20 minutes. Once having

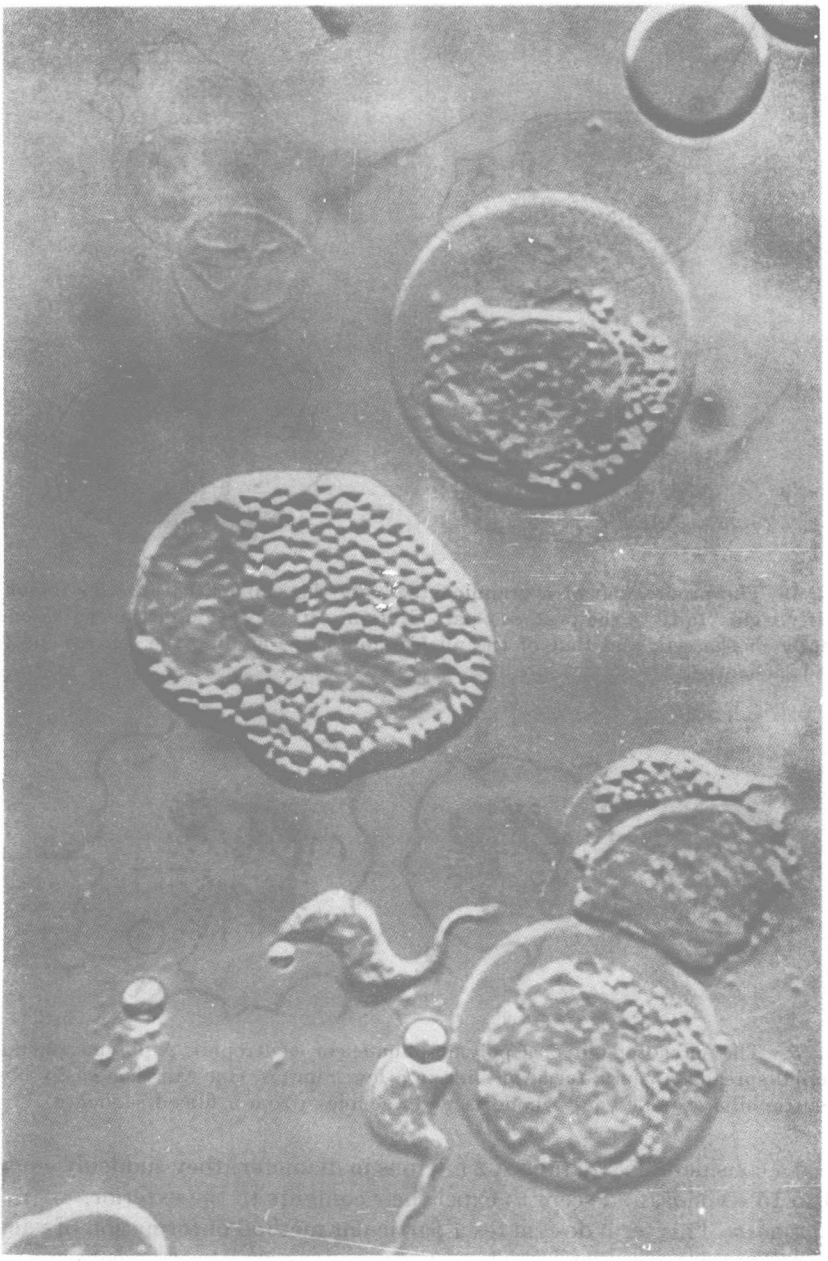

FIG. 3.—Appearance of varying cells by interference contrast (Nomarski system). *Center*. an eosinophilic granulocyte surrounded by three lymphocytes undergoing lysis (note the juxta-nuclear vacuole). *Bottom:* two trypanosomes (blood of guinea pig infected with trypanosomes).

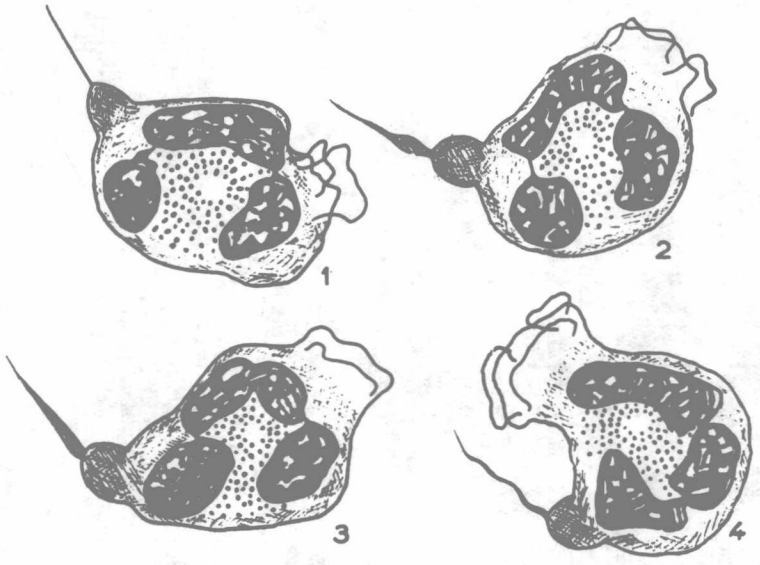

FIG. 4.—The progression of a granulocyte. Note the veil in the anterior region and the fairly rigid "tail" in the posterior part. Note also the location of the centrosome, in the center of the cell, and that of the nuclear lobes surrounding the centrosome (from a filmed sequence).

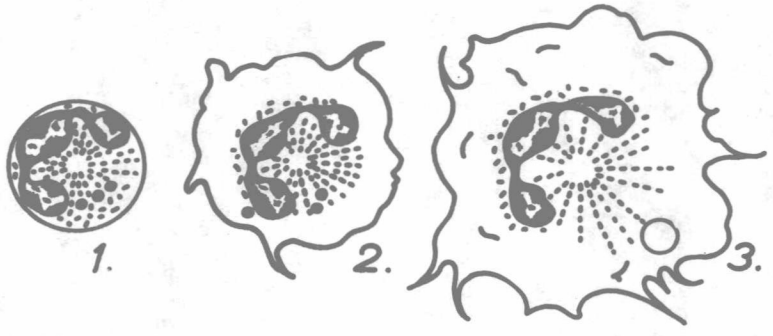

FIG. 5.—The spreading-out of a polymorphonuclear neutrophil. A polymorphonuclear neutrophil spreads out on a formwar slide in a few minutes. One can then easily observe the neutrophilic granules, mitochondria and vacuoles (from a filmed sequence).

reached a considerable size (about 2 microns in diameter) they suddenly contract, in 10 to 15 seconds, and seem to expel their contents to the exterior.

Pinocytosis. This term designates a particular method of formation of vacuoles (fig. 7). The cell periphery sends out projections which then converge, thus forming a small vacuole which gradually penetrates into the cytoplasm. It is a particular way of absorbing liquids and large molecules. Pinocytosis must play a vital physiologic role. It can be observed in many types of cells,[32] and recently it has been demonstrated[21] to be enhanced in the presence of albumin, lactoglobulin or gamma globulin.

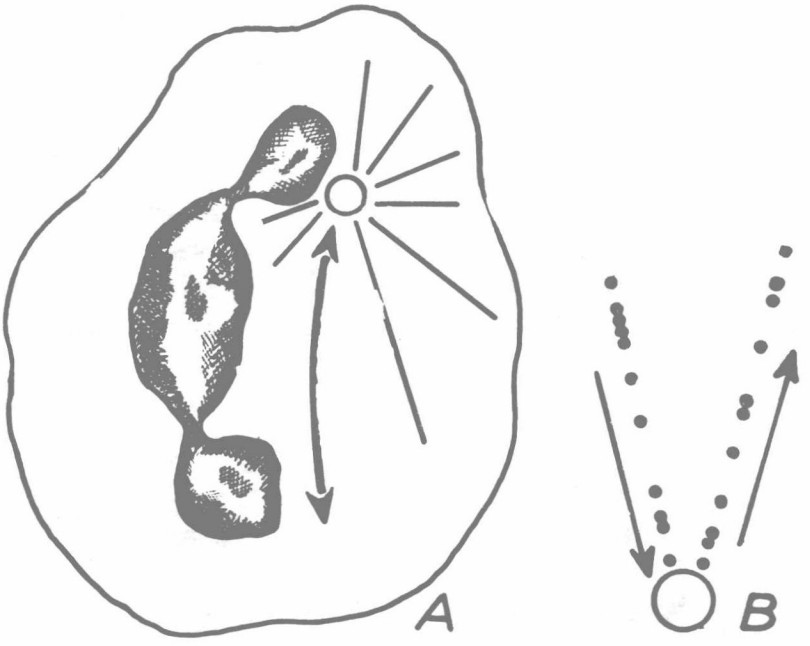

FIG. 6.—A. The movement of the centrosome with relation to the nucleus. B. The movement of the granule in the aster. The points denote equal intervals in time.

Lymphocytes. Contrary to what has been maintained by certain authors, lymphocytes also present active movements; they move as rapidly as do granulocytes (about 35 microns per minute), but without becoming as deformed during their movement. The lymphocyte in motion frequently looks like a hand-mirror[16–19, 53] (fig. 8). The same may be said for plasmocytes, which also frequently present this appearance.[12] Lymphocytes do not possess the property of spreading, whatever the "milieu" or surface, a fact undoubtedly related to their lack of phagocytic power.

Intracytoplasmic movements of lymphocytes are very active. The mitochondria and vacuoles of lymphocytes do not, however, have any characteristics which distinguish them from those of other cells. The centrosome is in contact with the nucleus, which molds itself over it, being slightly deformed by it. The little "notch" in the nucleus of the lymphocyte is, in fact, due to a depression in the surface of the nucleus caused by the centrosome. Microcinematography at accelerated speed demonstrates these phenomena in an unquestionable manner; on such films, the nucleus always appears to be easily molded, being constantly deformed. In contrast, the cell center is seen to be the firmer element, much more resistant, mechanically.

The monocytes. In tissue cultures, monocytes present the same type of movement as histiocytes. This is not surprising, since these two cells are very closely related, if not identical. The movements characteristic of histiocytes and macrophages are well known and have been studied for many years by Policard,[43] Carrel,[20]

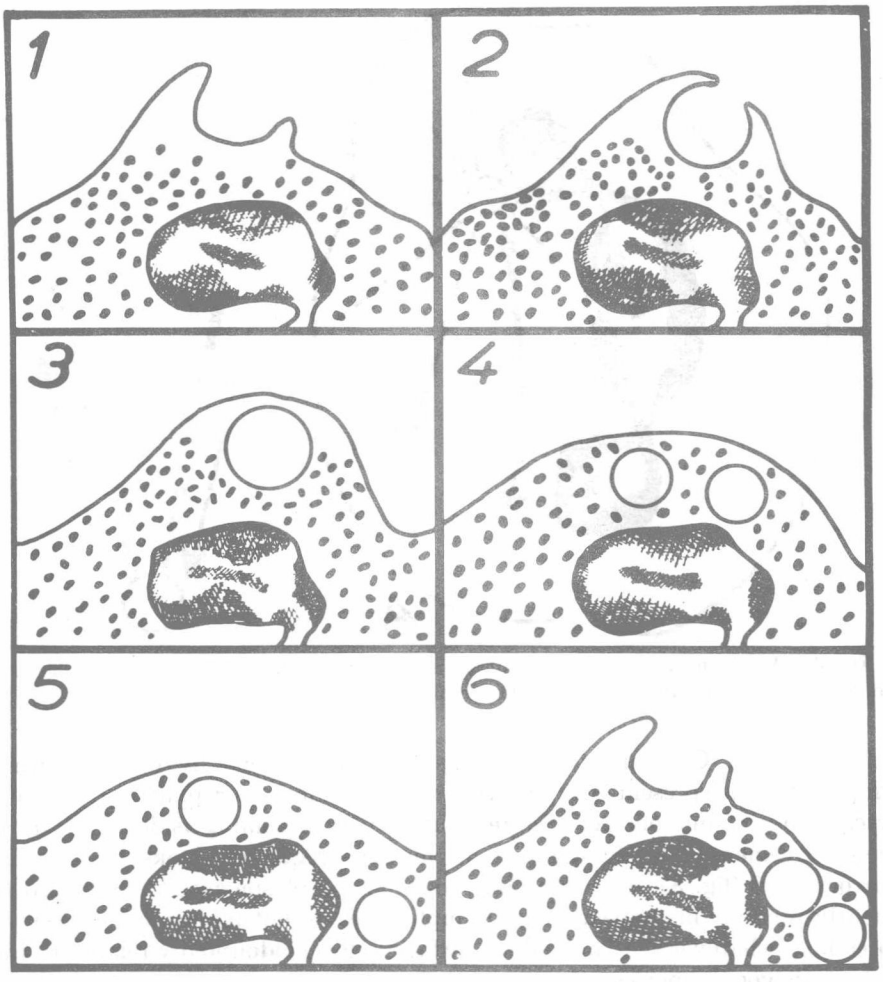

FIG. 7.—Pinocytosis in a polymorphonuclear neutrophil (from a filmed sequence).

Lison,[41] Fauré Frémiet,[28] André Thomas,[64] Tompkins,[65, 66] Chévremont[22] and others.

The histiocyte and the monocyte, as they move, generally display a triangularly shaped form. One of the angles is in the rear, the hyaloplasmic veils appearing near the angles. Monocytes can be differentiated from granulocytes by the presence of these veils, which appear even when the cell is not attached to a surface. Monocytes have a great tendency to spread out on glass, in contrast to granulocytes which, as we have seen, spread much more rapidly on plastic or nonwettable surfaces.

Plasmocytes. The phase contrast microscope has made possible the observation of the ergastoplasm (endoplasmic reticulum) of living plasmocytes.[60–62] This appears as a network of little lakes and canaliculi arranged in a parallel pattern around the nucleus and the cell center (fig. 9). When these cells are compressed,

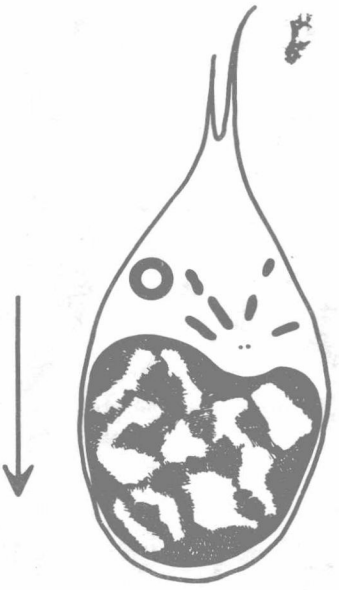

FIG. 8.—A lymphocyte as seen by phase contrast. Note the position of the centrioles, the centrosome, the Gall body and the caudal filaments.

or during their autolysis, these lakes swell and become rounded vacuoles. The formation of Russell bodies and crystals and the expulsion of vacuoles from the nucleus[62] can also be observed.

Phagocytosis. Phagocytosis of bacteria has been recently studied with the help of new technics, particularly by Frédéric and Robineaux,[30] Robineaux,[55, 57] Pulvertaft[48-50] and Pollack.[47] Wilson[67] described the ejection of bacteria after their ingestion. Robineaux and Nelson[58] described the curious phenomenon of phagocytosis of bacteria fixed by immuno-adherence onto erythrocytes. The granulocytes seize the bacteria and ingest them immediately, leaving the erythrocytes intact. *Phagocytosis of mineral particles* has recently been studied by Policard and Collet.[46] The observations were highly interesting, in view of the role of this phenomenon in the production of silicosis. Coal, kaolin free of silica, feldspars, and red iron oxide from England, may be phagocytosed in great quantities with essentially no injury to the cell and no alteration in its movements. Quartz, micas, calcite, fluorspar and especially amorphous silica in submicroscopic particles are on the other hand very noxious.

Phagocytosis of aged cells or cells which have been sensitized by an antibody is a well-known phenomenon which plays an important role in immunohematology. Phase contrast has made possible its detailed study which we shall briefly discuss. Phagocytosis of an erythrocyte or of cell stroma appears to necessitate its prior adherence to a localized point on the surface of the leukocyte. Thus, we discover here a familiar principle which applies to all types of phagocytosis, namely, that the body to be phagocytosed must first adhere to the phagocyte. Without this adherence, the hyaloplasmic veils push back the erythrocyte but

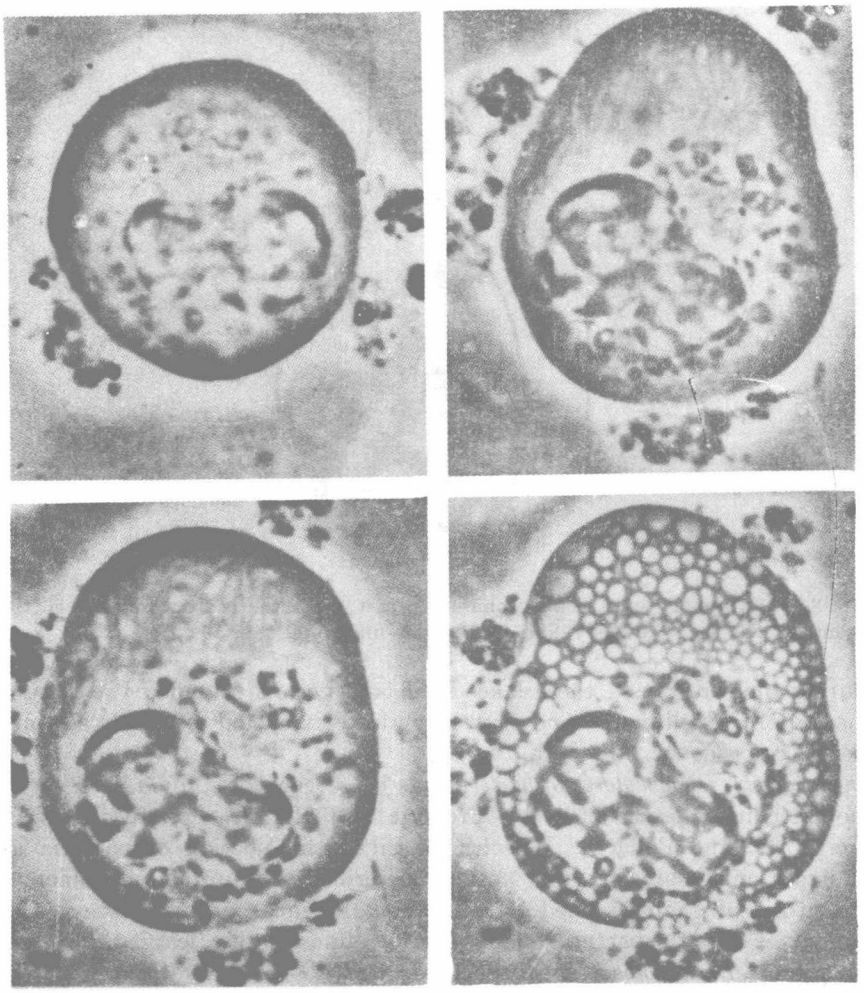

FIG. 9.—A plasmocyte undergoing progressive autolysis (phase contrast). Progressive appearance of the ergastoplasmic lakes which enlarge and finally produce the vacuoles characteristic of this cell (from Thiery).

do not succeed in enveloping it. At times, the veil can section the erythrocyte into two parts. The fact that this sectioning is not accompanied by hemolysis confirms results previously known from micromanipulation (fig. 10).

These and other observations seem to indicate that the phenomenon must be considered as a generalized one. It may explain the well-known fact that in cells holding phagocytosed erythrocytes, the latter are often of variable size, and almost always their diameter is smaller than that of a normal erythrocyte. Furthermore, one wonders whether the schizocytes—or at least some of them—seen in certain forms of anemia may not originate in a similar manner.