STUDIES IN ELECTRON MICROSCOPY OF BLOOD CELLS

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THE PURPOSE of this report is to give a summary, illustrated with electron micrographs, of our work on the electron microscopy of blood cells.

As soon as an electron microscope suitable for biologic purposes had become available, and especially after the publication of the first results obtained in the study of viruses, many workers endeavored to study normal and pathologic cells with this apparatus. However, despite the great progress that is being made in the study of bacteria, viruses, and certain specialized tissue fibers, the advances which have taken place in the field of cytology have been few.

The failure of cytologists to make more rapid progress in this work is due chiefly to the technical difficulties involved in this relatively new investigative approach. Today the electron microscopist is in a position not unlike that of the pioneer microscopists before the technics of sectioning and differential histologic staining were available. The lack of technical aids in electron microscopy is also complicated by the fact that, to date, we have been able to examine only a few special cells which must be at the same time small and thinly spread. Still, as is the case with every new technic, new results are brought forward every day, and the laboratories using this microscope are continually becoming more numerous.

In this presentation we intend to discuss the principal technics we have been using, review the findings in our studies of the cellular elements of normal blood, and in conclusion present a brief discussion of the electron microscopic findings in leukemic cells.

MATERIAL AND METHODS

The work with electron microscopy was begun in 1947. The material used for these studies was either human blood or that of laboratory animals. In all cases, blood was collected in anticoagulant solution (heparin or sodium citrate), following which it was centrifuged for a short time at low speed. Specimens were then withdrawn from different layers in order to obtain platelets, leukocytes and erythrocytes separately. As we have said, the main obstacle confronting the study of cells is their thickness, which either makes them totally impenetrable to electron rays or results in the production of an ill-defined image. To overcome this difficulty we have resorted to the following technical maneuvers.

Methods of destruction.*Cells can be destroyed by various processes, mechanical, chemical or physical. The mechanical methods which have given the best results consist in crushing the cells of a wet smear by direct pressure on the glass slide with a cover slip. The cellular elements, as a rule, remain clustered around the nucleus, the latter being considerably altered. The methods of enzymatic digestion and of treatment by chemicals, such as hydrochloric acid or potassium hydroxide may also yield interesting
results but must be used with the utmost care. Lastly, Policard's micro-incineration technic, with or without previous impregnation, can also give useful information.

**Methods of spreading.** The technic which has proved to be the most interesting to date is the smearing of cells on a glass slide. It has long been known that thrombocytes, which have a round shape in the circulating blood, assume a dendritic shape when placed in a glass tube. If we examine such shapes between a slide and cover slip the dendrites will soon adhere to the glass, then gradually spread into a wide halo around the thrombocyte center. This represents the normal spread pattern, and such spreading of cells can be found in tissue cultures of most cells of mesenchymal origin. In their normal state, human blood granulocytes do not possess this property, but with Brickaootnote{1} we have shown that it is possible to obtain the spreading of polynuclears by changing the slide material.

If either glass or Plexiglas slides covered with Parlodion, Formvar, Cellophane or Silicone were used the spreading of the polynuclear cells were readily seen. In certain diseases, such as septicemia perfringens and pneumonia, polynuclear cells will spread directly on a glass slide.

**Metal impregnations.** We also have used metal impregnation by means of osmic acid or uranium salt. These impregnations in electron microscopy are intended to replace the dyes used in light microscopy. The images thus obtained are of greater contrast in relation to the amount of reagent bound by the examined object, and in progressive proportion to the atomic weight of the reagent used. This technic has already been used successfully by Schmitt et al.ootnote{29} for muscular fibers, Schmitt and Grossootnote{30} for collagen, Hallootnote{23} for fibrin, and Mudd and Andersonootnote{23} for bacteria studies. We have used this method for our studies of the cytoplasm of spread cells.

The problem of the fixation of cellular material is of greatest importance and special consideration must be given to this question in cytologic investigations with the electron microscope. To date it has not been ascertained whether the destroyed fixed ultrastructure actually corresponds to the morphologic state existing in the living cell. The two fixatives which we have used most regularly are osmic acid and absolute alcohol.

We have been using methods which belong particularly to electron microscopy: Claude's method of cell mouldingootnote{7} and, of course, Williams and Wyckoff's method of shadow casting.ootnote{32} The microscope employed in this work was a C.S.F. apparatus of the electrostatic type.
Fig. 2.—Different forms of thrombocytes (gold shadowing technic) (6800×). Above, right and left: Dendritic form. Center: Transitional form. Left below: Spread form. Right below: Azurophil granulations visible after destruction of the cell.
In order to obtain "circulating forms" it has been necessary to fix the blood directly after puncturing the vein. In such preparations the platelets appear as rounded or slightly oval discs and in most instances are not electron-transparent even with maximum tensions. This makes it impossible to study the internal structures of these forms. One sometimes sees, however, a central mass, irregularly outlined, composed of small granules, extremely black, averaging 300 millimicrons in diameter.

Some of these granules located at the periphery are distinctly visible and sharply defined. The dendritic shapes of thrombocytes suggest the form of spiders or of stars with pseudopodes which are originally threadlike and exhibit occasional enlargements. These dendrites adhere to the slide at a spot where a terminal enlargement develops and widens slowly. All of these pseudopodes continue to enlarge until they unite and give the spread shapes of thrombocytes. This form appears as a round or oval surface, 5 to 10 microns in diameter and is composed of an extremely dense area at the center where the chromomere grains collect. Surrounding this area is the hyalomere which is quite thin, thus permitting a detailed study of its ultrastructure by electron microscopy.

In some successful preparations of spread platelets it is noted that the hyaloplasm consists of fibrils with the fibrils themselves being composed of granules. The fiber
pattern varies from one cell to another, probably being dependent upon the condition under which the cell spreads, and to the moment when the fixation arrests its spreading. The orientation of the hyaloplasmic fibers varies considerably in some specimens: one can see many closely packed radiating fibers connecting the opaque center to the slightly darker rim (figs. 1 and 2). Many circular or arclike...
fibers are frequently seen, and often a mixture of irregularly oriented fibers will be observed.

The circular fibers are worthy of special mention as they seldom consist of a single chain of granules, but form a thick cord of some ten fibrils. These circular fiber rings probably correspond to the circular waves noted by Faure-Fremiet in

Fig. 5.—Spreading polymorphonuclear leukocytes with optic microscope using gold shadowing technic (1000x). Above: Dendritic form. Below, left: Transitional form. Below, right: Spread form. Here one can recognize the three nuclear lobes and the perinuclear zone which contains the neutrophilic granulations.
choanoleukocytes examined by autocollimation and described by us in thrombocytes and polynuclears. The different positions which these rings occupy between the nucleus and the peripheral cell border no doubt depend on the moment when the fixation takes place. In some cells it is impossible to distinguish the general direction of fibrils which seem either to gather network-like or to be just scattered at random.

One may wonder whether the granules described above in the hyaloplasm of thrombocytes and which, as we shall see later, are found in the cytoplasm of all blood cells, exist in the living cells. The possibility that they are artefacts or the result of precipitation of protoplasmic gel at the time of the desiccation of fixation must be considered. It is not easy to tell at the present moment, but the following arguments can be put forward in favor of the idea that they actually represent pre-existing granular "unities" present in the living cells.

(a) First, these granular structures are found to be morphologically identical in the various fixation processes; dried smear fixed afterwards, moist smear, fixation in alcohol at −20°C. or +20°C. or fixation with osmic acid. All these preparations show granules of the same size. However, in preparations with osmic acid the granules are not so precisely defined as in those fixed with alcohol. In some cases the granules appear to be coated with a thin membrane which might be a lipoid film. In spite of the fact that these fixation processes tend to yield the same morphologic picture, we must keep in mind that the pre-existence, in the living state, of...
a structure totally different from the granular one is a real possibility if it is admitted that its unknown unstable state in vivo changes into a stable granular state at the slight denaturation.

(b) The presence of a living structure seems to be indicated by the tyndallian opalescence of the hyaloplasmic veils of certain cells (triton choanoleukocytes of Faure-Fremiet), but neither the form—granular, fibrillar, or reticulated—nor the location of these structures can be ascertained; it could be entirely due to a superficial folding pattern.

(c) For some time past, much data has been gathered proving the existence in vitro of at least one kind of submicroscopic granules, namely Claude's and Brachet's microsomes. Recently, Chantrenne and Jeener's work seems to point to the existence of a series of different sized granules ranging from 50 millimicrons to 1 micron (mitochondria), with the chemical constitution varying according to size. In some of our articles we have termed the granules we described in blood cells 'microsomes.' This word is thus used in its broader meaning, for in many cases the granules do not contain ribonucleic acid.

(d) Other authors (Claude et al., Porter et al., Faure-Fremiet, Bessis and Theaureau) have described granular structures in such widely varying material as chick cells, choanoleukocytes of snails and liver cells of mammals.

(e) We shall finally point out that, when thrombocytes are destroyed mechanically or by allowing a certain amount of autolysis to take place during the preparation, it is possible to find these granulations in their free state.

At the present time it can be said that nearly all workers concerned with the
Fig. 8.—Structure of the erythrocyte as seen with gold shadow technic (7500X). Left above: A casting of an erythrocyte. Right above: Erythrocyte stroma void of hemoglobin. Center: Erythrocyte in which the superior part of the membrane has been partly torn. Below: Erythrocytes in which the upper part of the membrane has been completely removed.
ultrastructure of cytoplasm, employing widely different means of investigation, have discarded the theory of a basic alveolar structure and favor the concept of a granular, or fibrillar, or granular-fibrillar structural arrangement. This theory is much more in keeping with the cytoplasmic physical properties of elasticity, contractility of a constantly changing state.

Such a picture of the cytoplasm would be in keeping with the results of the electron microscope observations, but it should be stressed that the granular chains we described are larger than molecules. It could thus be concluded that such structures condition the physical properties of the cytoplasm without necessarily draw-

![Figure 9](https://www.bloodjournal.org/content/bloodjournal/35/3/1092/F1)

**Fig. 9.**—Reticulocyte from a case of hemolytic disease of the newborn after hemolysis in distilled water. Notice the folds in the membrane and the rounded plaques which stain with the dyes (10,000×).

...ing definite conclusions concerning the molecular arrangement. These observations give us information chiefly related to an intermediate state between the actual chain of molecules, which cannot yet be well observed with the light microscope.

It is worth noting that, from our observations, the pure fibril theory does not seem worthy of support, as we failed to obtain isolated fibers and from the moment when destruction begins, whether mechanical or autolytic, the only structure to remain apparent is granular. One is thus entitled to infer that if the chains may assume the fibril-like forms under the influence of linkages, their shape in the free state is spherical, and they revert to this shape when any destruction of the "linkages" occurs. These naturally could destroy themselves and form again, "the labile character of some linkages ensuring the flexibility and deformability of the whole" (Faure-Fremiet). One finds here without doubt, an explanation of the peculiar
Fig. 10. (top).—A gold shadow casting of the filament which unites two erythrocytes. This is seen when one breaks up an agglutinated mass of erythrocytes (10,000X).

Fig. 11. (bottom).—A gold shadow cast of an altered erythrocyte showing "hematexodies" (10,000X).

orientation of the structure we have mentioned, which varies according to the nature of the cells and the moment of observation.

Studies of Leukocytes

The leukocytes have been studied after spreading and by the technics of mounting and destruction.
(a) **Description of the granulocytic leukocytes spreading.** The cell first exhibits long and thin dendrites occasionally terminating in a spherule (fig. 5, top). After a few minutes the dendrites disappear by a progressive widening of their base (fig. 5, bottom left), petal-like membranes gather round the cell border and soon fuse into an undulating membrane surrounding the cell. In a few minutes the spreading is completed, the surface of the membrane grows still larger and fixes on the slide (fig. 5, bottom right). Three distinct areas can then be seen in the cell, a central area which contains the nucleus, 2 to 3 microns in width, a median area that contains the granules and lastly a wide peripheral hyaloplasmic area of pure cytoplasm devoid of any kind of inclusion.

We see by this description and by the photographs that this spreading is absolutely identical to that of the histiocytes of mammals, the latter being up to now considered as the only cells that could spread.*

The hyaloplasmic structure is identical to that of thrombocytes as we have described above, in most instances the granules apparently constitute several layers, making it difficult to separate the chains. In some preparations, spherules of about the same magnitude as the cytoplasmic granules can be seen scattered around the intact cells.

(b) **Specific granules of neutrophilic and eosinophilic leukocytes.** The study of leukocytic granulation has been made possible by the technic of casting and destruction. Neutrophilic granulations as a rule appear as rods or ellipoids, as Rebuck and Woods have already observed, and probably constitute a transitory stage in the autoduplication of the granules, a process that makes the granules undergo division very much like the "plasts" of plant cells. This division of the granules may explain the gradual undifferentiation of leukemic cells, according to Darlington's opinion of undifferentiation of cancer cells, the granules being unable to keep pace with the rapid multiplication of leukemic cells.

(c) **Granulations in lymphocytes and monocytes.** The study of these granules could also be approached by means of destruction on slide. The mitochondria in lymphocytes appear as spherules measuring about 0.2 to 0.7 microns, very much like the granules in the center of thrombocytes.

**Study of Erythrocytes**

We have carried out electron microscope studies on structure and shape of the erythrocytes, osmotic and mechanical hemolysis and agglutination.  

(a) **Structure.** A preparation device has enabled us to study the structure. This technic consists of rehydration of formerly vacuum dried but unfixed smears. The

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* Further particulars on this point are to be found in the articles of Bessis and Bricka, and Thomas.
rehydration results in a "swelling" of the blood cells; their lower part is strongly
attached to the glass plate and thus cannot be deformed. As a result the membrane
is ruptured. The manner in which the membrane ruptures can vary considerably,
ranging from a mere hole to the complete breakage of the upper part of the mem-
brane. The action of an antiserum will agglutinate the membrane fragments thus
set free. These membrane fragments show the same ultrastructure as the other cells,
i.e., they are formed of spherules measuring about 100 millimicrons in diameter
(alcohol fixation).

The hemolyzed reticulocytes appear as stromata containing numerous rounded
areas.1

(b) Agglutination of red blood cells. When a clump of agglutinated red blood cells
is carefully dissociated, one will note that the cells are spindle-shaped, two ag-
glutinated red cells being united by a thread. This phenomenon can be noted both
in nonspecific agglutination (as by glucose*) and in specific agglutination by
blood group antiseraums. Various considerations suggest that this thread is com-
posed of lipo-glucoprotein, and that it originates from the outer layer of the red
cells. When the threads break they give birth to "hematexodies" (see fig. 11).

(c) Change in the shape of red blood cells. Electron microscope studies on red blood
cells rendered spherical by washing in saline then restored to their normal shape
by the addition of albumin (Ponder) have shown that (1) this phenomenon can
be noted both on hemoglobin-free stroma and on whole red blood cells; (2) the
spherical blood cell, when dried, exhibits either spicules or irregular folds on an
even stroma; (3) after antisphering substances have been added one clearly sees a
distinct thickening of the red cell border and an absence of folds in the stroma.11

Studies of Leukemic Cells*

Studies on leukemic cells are now being actively carried on but unless an etiologic
virus causing this disease exists, the future success of such studies demands a good
knowledge of normal cells. At the moment we are unable to tell whether or not
these cells possess specific characters. Recently Oberling et al.26 described long chains
of spherules, 140 millimicrons in diameter, which they observed in acute leukemic
cells spread on Formvar. These authors later, however, found similar formations in
normal granulocytes. We ourselves have not yet observed such formations, but
have frequently seen, in about one spreading cell out of ten, very small granules
(80 millimicrons) strongly osmiophilic and frequently grouped in pairs. It is worth
noting that we have not as yet examined nonleukemic cells at the same stage of
differentiation, and it seems probable that what we have observed were very small
mitochondria or secretory granules. Auer bodies and Charcot bodies, in acute
leukemia and in chronic myeloid leukemias, respectively, are particularly well
seen. Besides these two paracrystals, the rods associated with lymphoid leukemias
can also be noted. These appear only after destruction of the cells, numbering from
one to ten in each cell, and measuring from 0.5 to 2 microns in length (fig. 7).
As was said above, neither of these findings is constant or specific.

* A detailed report on these studies will be published in the Proceedings of the International Cancer
SUMMARY

The study of blood cells with the electron microscope necessitates special methods which as yet are not completely precise. The methods of smearing on Formvar, of destruction by pressing on a wet smear, and of shadow casting are those which have been used most frequently.

The author reviews the electron microscopy of thrombocytes, granulocytes and erythrocytes and discusses the ultrastructure of the hyaloplasm. Azurophilic, neutrophilic and eosinophilic granules can be very well observed with the electron microscope. Chronic and acute leukemic cells exhibit certain granules and para-crystals, the significance and specificity of which are not yet clearly established.

REFERENCES

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