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Electron Microscopy of Sickle Cells.

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(Introduced by F. W. Hartman.)

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Since sickle cell anemia was first described by Herrick,[1] there have been numerous descriptions of the changes in the red corpuscular structure in this disease as observed in optical studies. The corpuscles progressively transform in structure and shape from the customary discs into elongated forms curved in the mid-portion and pointed at either end, into multi-pointed forms, needlelike forms, forms resembling exaggerated crenation, oat-like forms, and other bizarre types. Many additional forms, transitional between discs and the above group collectively designated as "sickle cells," have been illustrated by Diggs and Bibb.[2]

Such changes are readily observed in sealed wet preparations of whole blood from the patient. However, "sickled" corpuscles usually revert to their rounded forms upon exposure to air. This necessitates application of a fixative to the sickled corpuscles before such exposure to air, in order to obtain permanent preparations.

Beck and Hertz[3] introduced the red corpuscles into a saline citrate solution which was then sealed with paraffin oil. After sickling had been accomplished upon standing a solution of formaldehyde was added to the blood-saline-citrate mixture and reversion to the corpuscular shape was prevented. Permanent preparations of the sickled cells were thus obtained.

For electronic studies, the Beck-Hertz technic was modified as follows. The blood of a negro patient with sickle cell anemia was employed. This patient was recovering from an acute hemolytic crisis. Blood was introduced into paraffin-lined Wassermann tubes in which had been placed a small amount of powdered heparin. The heparin-blood mixture was covered with paraffin oil and allowed to stand 21 hours. Formalin-saline was then added to the now sickled erythrocytes which were allowed to stand an additional 24 hours. The formalized sickle cells were removed from the paraffin oil-sealed tubes and washed 4 times in physiological saline solution. They were then spread over formvar-covered glass slides and dried over phosphorus pentoxide. Final direct specimen mounting was performed by the modified stripping technic previously described.[4] For a general discussion of the effects of formalin fixation upon cytoplasmic structure with electronic methods, the reader is referred to the work of Porter, Claude and Fullam.[5]

Our Fig. 1 (× 5,000) is an electron micrograph which presents one of the crescent forms

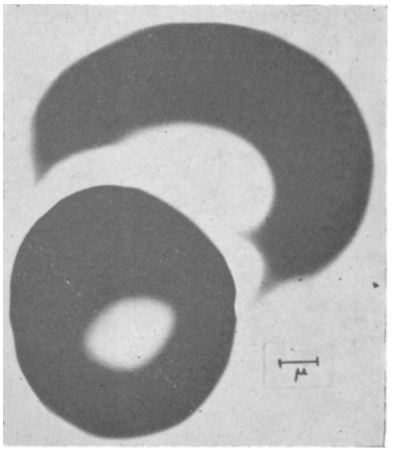

Fig. 1.

[1] Herrick, J. B., *Arch. Int. Med.*, 1910, **6**, 517.

[2] Diggs, L. W., and Bibb, J., *J.A.M.A.*, 1939, **112**, 695.

[3] Beck, J. S. P., and Hertz, C. S., *Am. J. Clin. Path.*, 1935, **5**, 325.

[4] Rebuck, J. W., and Woods, H. L., *Blood*, 1948, **3**, 175.

[5] Porter, K. R., Claude, A., and Fullam, E., *J. Exp. Med.*, 1945, **81**, 233.

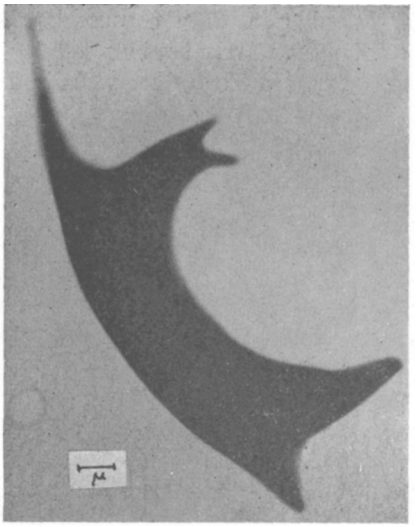

FIG. 2.

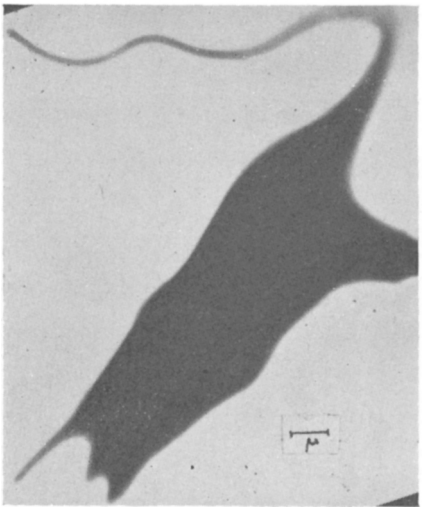

FIG. 3.

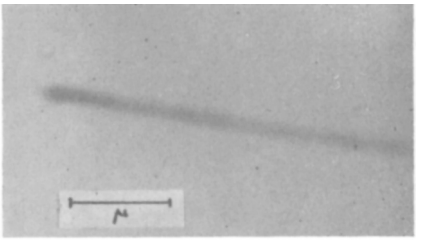

FIG. 4.

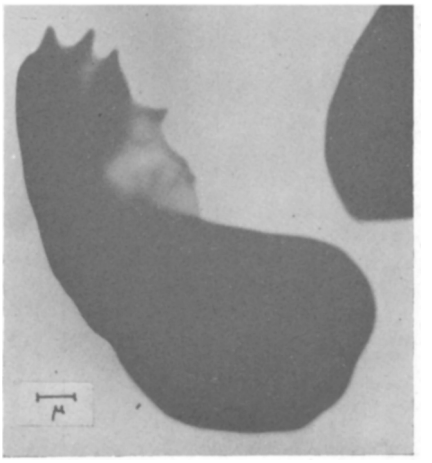

FIG. 5.

from which the disease receives its name and a rounded form with an unusually thin corpuscular center. Ordinarily normal red corpuscles present relatively dark centers with this technic. The decreasing hemoglobin content of the points of the sickled form is apparent.

Fig. 2 (× 5,000) is an electron micrograph of one of the multi-pointed forms which retains a suggestion of the characteristic crescent-like outline. One of the points has been transformed into an elongated structure.

Fig. 3 (× 5,000) depicts another multi-pointed form with an elongated filament protruding from one pole.

Fig. 4 (× 13,000) is a higher power view of a portion of another but similarly elongated polar filament. The hemoglobin does not appear to be distributed homogeneously along the filament core.

Fig. 5 (× 5,000) depicts a stage intermediate in the development of the sickled form. At one pole of the cell elongation has progressed and numerous pointed processes are in evidence. These processes vary as to hemoglobin content. In the concavity of the crescent, hemoglobin has been withdrawn so that a large area presents the opposing corpuscular membranes in near approximation.

Further electron micrographs of developmental forms intermediate between disc- and crescent-shaped and/or multi-pointed forms will be the subject of a subsequent paper.

Summary. A modification of the Beck-Hertz technic permits direct mounting of sickle cells for electron microscopy studies. Electron micrographs of sickle cells essentially confirm the findings of Diggs and Bibb[2] and substantiate pertinent optical studies as to structural detail. In addition, both corpuscular hemoglobin-membrane relationships and polar filament structure are further visualized.