

## A Method for the Electronmicroscopy of Tissue Mast-Cells

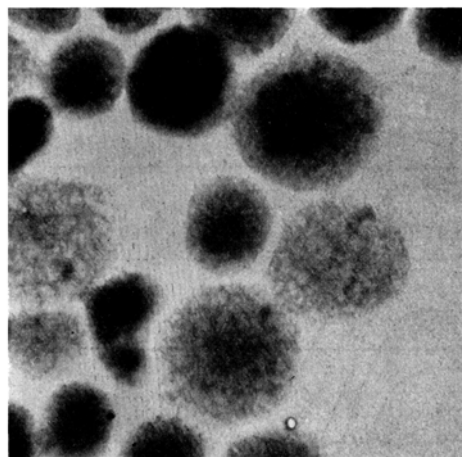
It is generally concluded nowadays that the heparin in the animal organism is stored and probably also formed in the mast-cells. Considerable work has been done to make clear the cytochemistry of these cells. Besides heparin and heparin-monosulphuric acid and/or other heparinprecursors, the presence of quite a number of substances in the mast-cells has been shown, as well as an absence of other substances normally occurring in cells. A review of these investigations has recently been given<sup>1</sup>.

In examining fresh peritoneal smears from rat or mouse with a phase-contrast microscope, numerous mast cells appear with densely packed, luminous granules. With toluidin blue the granules are metachromatically stained. In respect to osmosis, the granules react in a manner similar to that of the mitochondria<sup>2</sup>. If the cells are suspended in distilled water, the granules suddenly swell and lose their brilliancy. In such cells the intergranular cytoplasm is also metachromatically stained, and obviously the heparin has been dissolved from the granules. The granules of the mastcells can thus be considered as a specialized heparin-carrying type of mitochondria.

Nothing is known of the submicroscopic morphology of the mastcells. It thus seemed to us of interest to develop a method for electronmicroscopic examination of these cells. As experimental animals, rats were chosen, as it is very easy to obtain mast-cells from the peritoneum of these animals, and furthermore rat mast-cells are rather resistant to manipulations. The mast-cells were isolated in the following way: The animals were killed by a blow on their skull, their abdomen was opened and a few drops of physiological saline were dropped on the peritoneum. The peritoneal surface was cautiously scraped with a micropipette and the fluid, rich in peritoneal cells, was then withdrawn.

For the continued preparation we used a modification of the methods for obtaining blood cell preparations for electron microscopical examinations<sup>3</sup>. A glass slide is dipped in a solution of polyvinylformaldehyde, so-called formvar, to obtain a thin coating on the glass. This formvar film can easily be loosened and transferred first to a water surface and then from there to another slide on which is placed a specimen screen for electron microscopy. After drying, the screen lies between the glass and the formvar film. The cell suspension is then expressed onto the formvar film covering the specimen screen and in a few minutes the mast-cells have settled and adhered to the film. Erythrocytes, which are present in the sus-

pension in great numbers, are easily washed away with physiological saline. After fixation in different fixatives the preparations are dried in air and then shadowed with Pt + Pd, and examined in a SIEGBAHN-SCHÖNANDER electron microscope. This method offers the advantage that the preparations can be examined as well in the light—as in the electron microscope.



Granules from a mast-cell of a rat mesenterium. Some of the granules are too thick to be penetrated, but others have swollen and become thinner, thus bringing out an internal structure. Electronmicrograph. The preparation has been treated with distilled water. Shadowed with Platinum/palladium. Magnification 10000  $\times$ .

The majority of the mast-cells are very dense and transmit the electron rays in their periphery only. Occasional cells however, stretch considerably on the film and become thin enough to permit a closer study even of their central parts. Thus pictures have been taken of the nucleus and nucleolus of such cells. The granules (Fig.) are as a rule completely electron-impermeable, their size varies from 0.5–1.0  $\mu$ . Granules that have taken up water, however, and become swollen to twice this diameter, permit the passage of the electron ray and intragranular structures are observed. These may represent the granule-skeleton of lipid-protein which has been postulated by ZOLLINGER<sup>1</sup>.

A detailed report of the electronmicroscopical findings in the present investigation will be published elsewhere.

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### Zusammenfassung

Es wird eine Methode zur elektronenoptischen Prüfung der peritonealen Mastzellen der Ratte beschrieben. Bestimmte elektronenmikroskopische Beobachtungen an diesen Zellstrukturen werden aufgezeigt.

<sup>1</sup> H. V. ZOLLINGER, *Exper.* 6, 384 (1950).

<sup>1</sup> U. FRIBERG, W. GRAF, and B. ÅBERG, *Acta Path. Microbiol. Scand.* 29, 197 (1951).

<sup>2</sup> H. V. ZOLLINGER, *Exper.* 6, 384 (1950).

<sup>3</sup> M. BESSIS, *Blood* 5, 1083 (1950). – M. BERNHARD, H. BRAUNSTEINER, H. L. FEBRE, and J. HAREL, *Presse méd.* 58, 472 (1950). – J. REBUCK, *Amer. J. Clin. Path.* 19, 217 (1949).