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SOME DATA CONCERNING THE SUBMICROSCOPIC MORPHOLOGY OF MAST CELLS. II.

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An earlier report [1] gave account of our observation that, upon treatment with acid neutral red, toluidine blue and pyronine G (Grübler), the mast cells of rats become anisotropic. We concluded that mast cells had an oriented structure which — according to ROMHÁNYI [2] — becomes more pronounced on the addition of dye molecules. We isolated mast cells with a view to studying their structure and ascertaining the nature of their birefringence.

Method

Mast cells were isolated by the somewhat modified technique of GLICK, BONTING, DEN BOER [3], PADAWER, GORDON [4] and KELLER [5].

The procedure was as follows. Lower median laparotomy by means of electrocautery (which does not cause haemorrhage so that the cell suspension remains free of erythrocytes) is performed on rats under aether anaesthesia. A mixture composed of four parts of Tyrode and one part of physiological sodium citrate solution is then carefully instilled into the open abdominal cavity (it is advisable to bring the solution to a temperature of 37° C before administration). This done, the lips of the wound are held together by a long-limbed Péan's clamp. The pH of the Tyrode solution should not exceed 7.2; that of the physiological sodium-citrate solution, 7.35.

After massaging the animal's abdomen for 1½ to 2 minutes the clamp is removed and the wound cranially lengthened by 2 cm (the cautery should avoid the umbilical depression). The animal is then brought into a semirecumbant position and approximately half of the introduced solution is withdrawn by means of a syringe. The solution thus extracted is turbid, non-coagulable, and fatty to an extent which depends on the nature and time of the animal's last meal. Although PADAWER and GORDON recommend fractional centrifugation in order to separate mast cells from other cells (granulocytes, lymphocytes, mesothelial cells), we omitted this unphysiological procedure. The cell suspension obtained from the abdomen is centrifuged (300 r. p. m. for 3 minutes) and the supernatant discarded; from the cells a smear is prepared which — while still wet — is fixed in OsO_4 vapour. Dried, the preparation is washed with distilled water in order to remove crystallized salts.

The use of the following dye combination is recommended:

0.1 g of neutral red,
01. g of toluidine blue,
100.0 g of *N*/10 HCl.

Staining, 5 minutes; rinsing with distilled water, drying. Covering with dammar resin or immersion oil.

Results

The preparations obtained by the technique described were examined under a phase contrast microscope provided with polarization filtres. A picture was revealed as illustrated in Figs. 1 and 2.

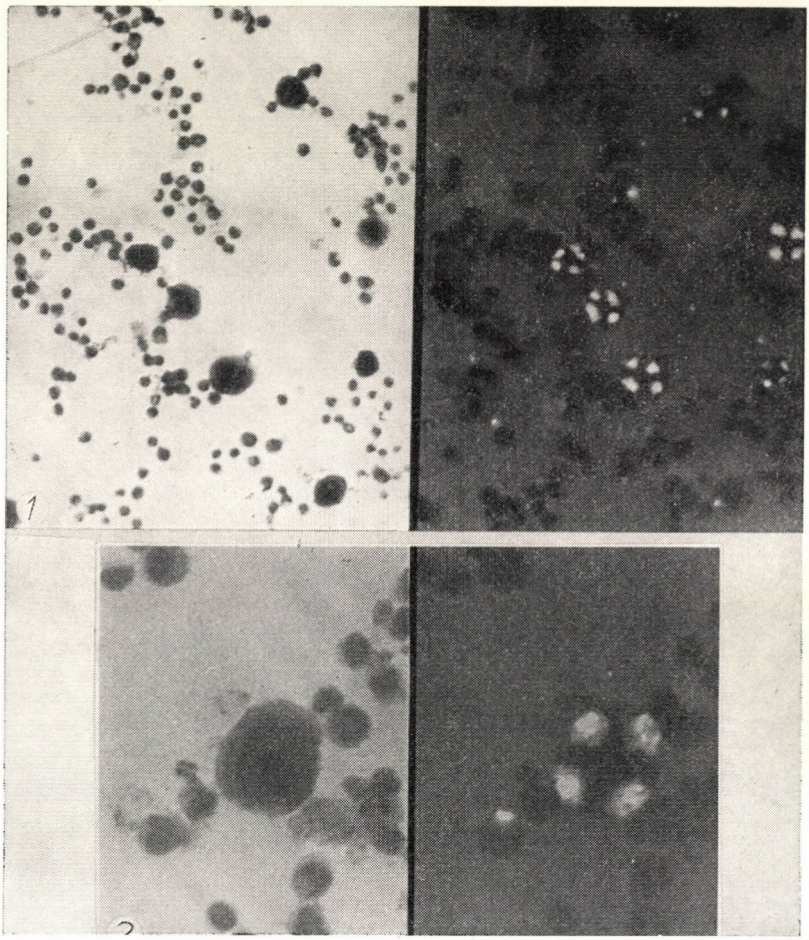


Fig. 1. Cells isolated from the abdomen of rats. Stain : acid neutral red mixed with toluidine blue. Normal and polarization-microphotograph. × 100
Fig. 2. Same as Fig. 1, enlarged × 300. Polarization cross clearly visible

The polarization cross was found to have a positive value. From this it follows that anisotropy was due to the circularly arranged intracellular substance. This substance was not intragranular but intergranular ; this means that the granules of the mast cells were embedded in a circularly oriented protoplasmic substance (Fig. 3).

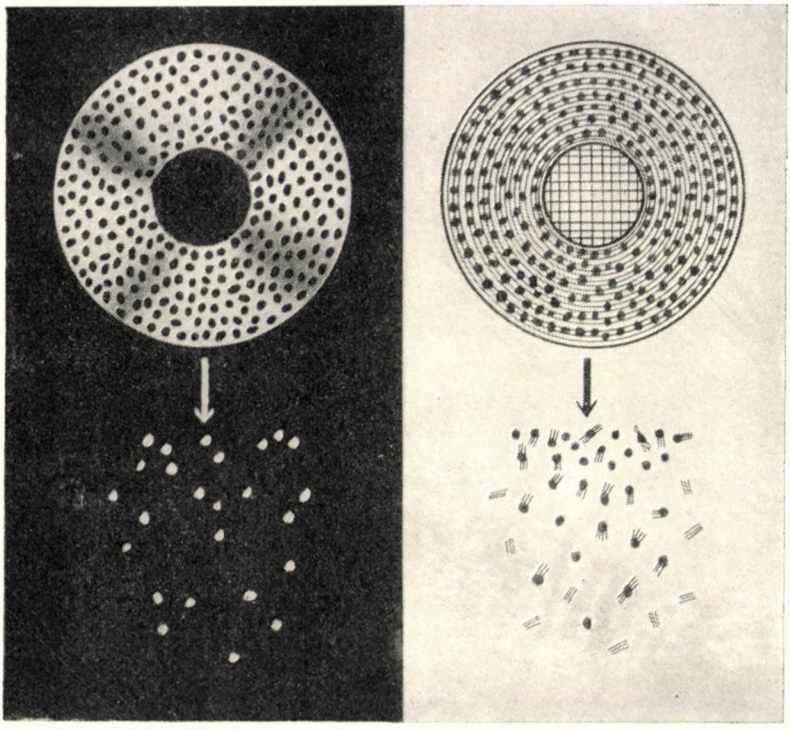
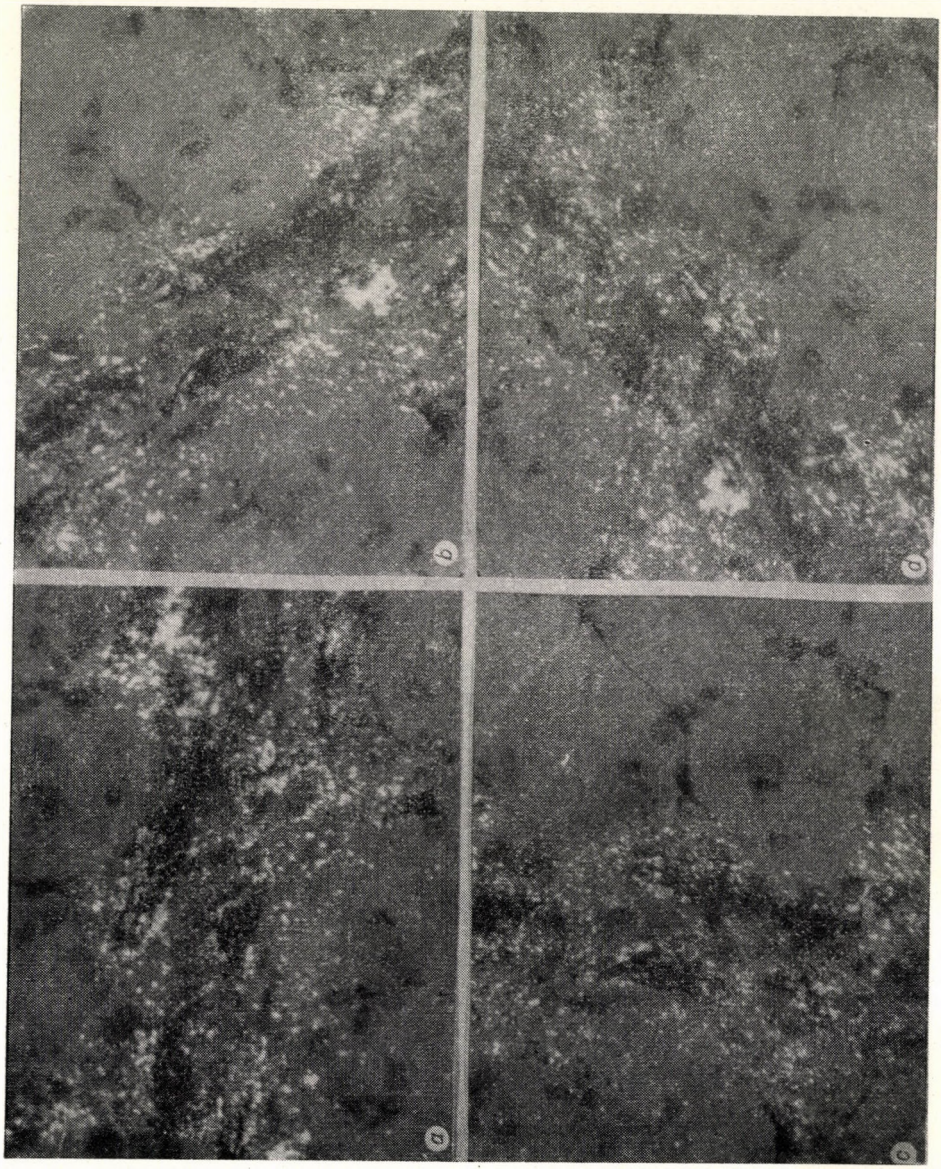


Fig. 3. Polarographic outline of mast cell, and explanatory drawing made on the basis of the positive polarization cross. Arrows point to anisotropic granules released by disintegrated cells ; the figure presents the orientation responsible for anisotropy

Discussion

The above described observation elucidates the phenomenon presented by way of illustrations in our previous publication [1] : around the disintegrated mast cell there are anisotropic granules, invisible in normal light, so that no visible quantity of dye is adsorbed to them. These granules contain, therefore, purely intergranular oriented substance. Worthy of note is further the phenomenon that anisotrophy is displayed also by the granules which bind the dye liberated by the disintegrating cell. We suggest the explanation that, simultaneously with disintegration, the intergranular substance is adsorbed to the granule, or that the latter imbibes the former : optically, the anisotropic substance is sometimes distinguishable from the dye-adsorbing granules in which case the two are observable side by side. It was likewise under the phase contrast microscope combined with polarization filtres that we were able to observe this phenomenon.



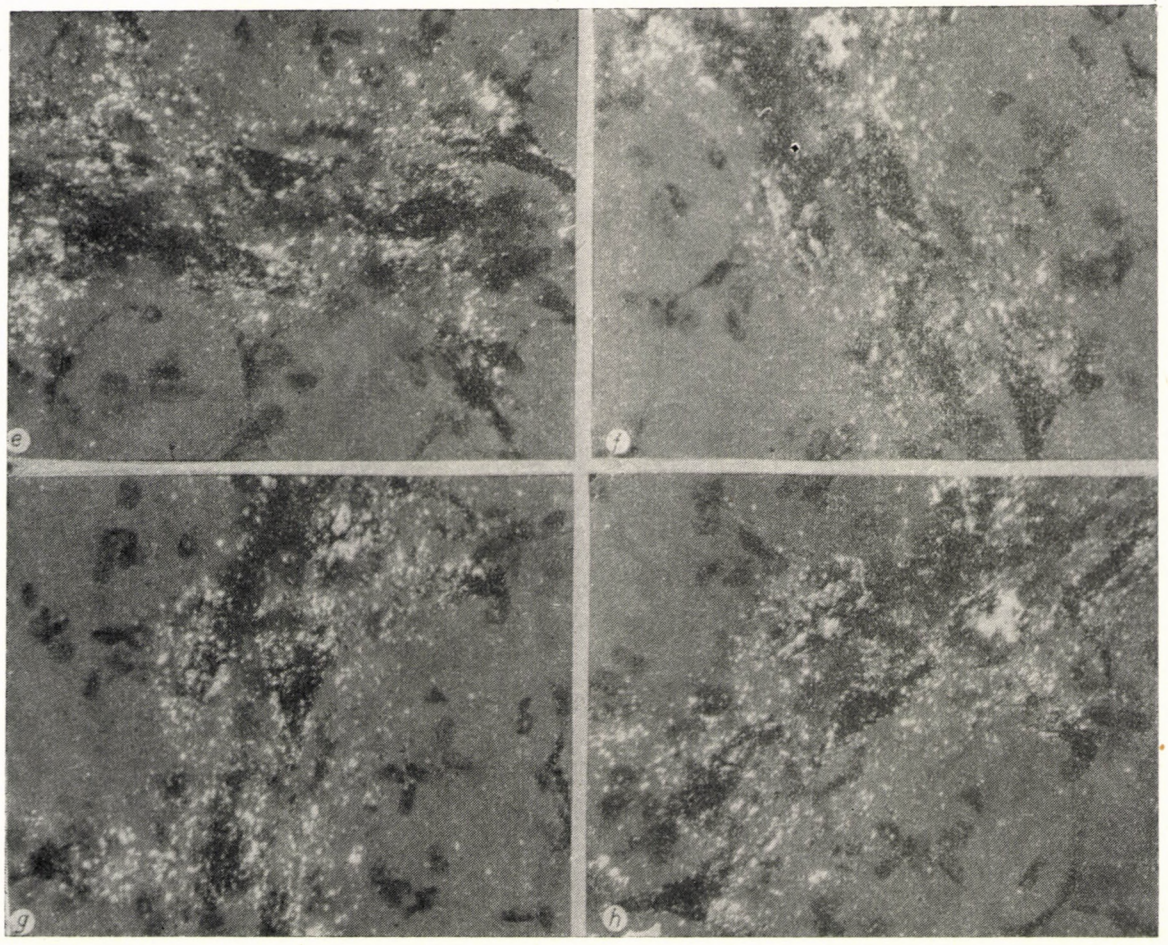
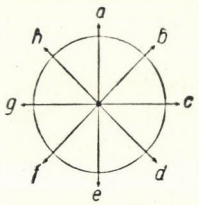


Fig. 4. Preparation of granules from disintegrated mast cell in polarized light, turned over angles of 45°

To facilitate a detailed study of their optical orientation, serial photographs were made of the granules released by disintegrated mast cells. Placing the latter into the optical axis of the microscope, it was turned over an angle of 360°, and after each turn of 1° a picture was taken. We have enlarged



every 45th film and, having no space to present all of the 360 photographs, we present these enlarged films so that there is an angular difference of 45° between each of the eight photographs here reproduced.

Summary

The submicroscopic structure of mast cells, isolated from the abdominal cavity of rats, has been examined under the polarization microscope. Stained with a mixture of acid neutral red and toluidine blue, the mast cells display a positive polarization cross which points to the existence of a circularly arranged protoplasmic structure. Anisotropy is due to the intergranular substance.

Polarized photomicrographs were taken in order to facilitate a closer study of the orientation of the granules liberated by disintegrated mast cells.

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ДАННЫЕ К СУБМИКРОСКОПИЧЕСКОЙ МОРФОЛОГИИ ТУЧНЫХ КЛЕТОК. II

Л. ХОРВАТ

Автор исследовал с помощью фазово-контрастного поляризационного микроскопа на основании предлагаемого Гликком и Падавером метода субмикроскопическую структуру изолированных тучных клеток. Он изолировал тучные клетки из брюшой полости белых крыс и фиксированные парами OsO_4 смазки окрасил смесью красителей нейтральный красный и толуидин, растворенной в N/10 HCl. Препарированные и окрашенные этим методом тучные клетки показали положительный поляризационный крест. Данное явление основывается на двойном преломлении межзернистого вещества протоплазмы. Из этого можно заключить, что вещество между зернышками распределено циркулярно.

Зарнышки тучных клеток, в нормальных неповрежденных клетках оптически изотропны. С другой стороны зернышки распавших тучных клеток показывают отчасти анизотропию. Это явление объясняется тем, что при распаде анизотропное межзернистое вещество пропитывает зернышки тучных клеток. В целях более подробного изучения описанного явления автор изготовил от исследованных в поляризационном микроскопе тучных клеток скоростную ротационную киноленту.

BEITRÄGE ZUR SUBMIKROSKOPISCHEN MORPHOLOGIE DER MASTZELLEN, II.

L. HORVÁTH

Es wurde die submikroskopische Struktur von isolierten Mastzellen mittels der Glick und Padawerschen Methode unter dem Phasenkontrast-Polarisationsmikroskop untersucht. Die aus der Bauchhöhle von Albinoratten isolierten Mastzellen wurden in mit OsO_4-Dampf fixierten Ausstrichen mit einem in *N*/10 HCl gelöstem Gemisch von Neutralrot und Toluidinblau gefärbt. Die auf diese Weise präparierten und gefärbten Mastzellen zeigten ein positives Polarisationskreuz. Dieses Phänomen ist durch die Doppelbrechung der intergranulären Protoplasmasubstanz bedingt. Hieraus wurde gefolgert, dass die Intergranularsubstanz zirkulär orientiert ist. Die Körnchen der Mastzellen sind innerhalb der intakten normalen Zellen optisch isotrop. Die Körnchen der zerfallenen Mastzellen hingegen zeigen zum Teil Anisotropie. Diese Erscheinung wird dahingegend erklärt, dass beim Zerfall die anisotrope Intergranularsubstanz die Körnchen der Mastzelle durchtränkt. Zwecks genauerer Untersuchung der beschriebenen Erscheinung wurde ein Raffer-Rotationsfilm von den im Polarisationsapparat untersuchten Mastzellen hergestellt.

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