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

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(Received September 12, 1958)

> Speculating thus, a hundred years after the birth of EHRLICH, we come back to his original view of the mast cell as a "well-fed cell" of the connective tissue. But the riddle is not yet solved . . . J. F. RILEY

The mast cell has been in the focus of scientific interest for the last 80 years. This is borne out by the fact that more than a hundred valuable contributions to the subject appear every year. And yet, wide as our present knowledge concerning mast cells is, neither their morphological nor their physiological aspects can be said to have been elucidated with finality.

An accurate knowledge of the mast cells' cytophysiology has become particularly important in view of the frequently occurring injuries in connection with nuclear tests.

In connection with studies concerning mast cells it has to be borne in mind that the chemical structure of their granules and their intergranular substance respond very readily to neurohormonal changes and that they vary from species to species.

Rats and mice have been used in most of the experiments, considering that these animals have the highest number of mast cells and the cells can be easily isolated without being seriously damaged (GLICK, BONTING, DEN BOER [13]; PADAWER, GORDON [29]).

As regards their origin, the opinions differ. MAXIMOW [24] derived them from histiocytes, DANTSCHAKOVA [6] thinks they are of lymphatic origin, while according to HOLMGREN [16] they are direct mesenchymal derivatives. LEHNER [22] suggests that mast cells are cells *sui generis* which divide by both mitosis and amitosis (homeoplasty), whereas MAXIMOW, as well as MICHELS and HERZOG [25] are of the opinion that adventitial cells, lymphoid cells and other polyblasts are capable of changing into mast cells (heteroplasty).

The mast cells of connective tissue have a variable size (8 to 20 μ) and are filled with basophilic granules averaging 1 μ in diameter. Mast cells show metachromasia upon staining with thiazine dyes, a phenomenon attributed by LISON [23] to the presence of polysulphuric acid esters. These latter have been identified with heparin by JORPES, HOLMGREN and WILANDER [16] and hence the name heparinocyte, a term sometimes given to mast cells.

The present author [17] had succeeded in demonstrating heparin in the granules by histochemical and histophysiological methods, while HIRT [15] proved the same by means of luminescent-microscopic analysis; ZOLLINGER [36], under the dark-field microscope; JULÉN, SNELLMAN, SYLVEN [19], under the electron microscope.

POLLAK [31], JOHNSON and MCMINN [18] regard mast cells as migrating cells stimulated chemotactically by plasmatic impregnation. According to this theory, it is the invasion of plasma into the vessel wall, induced by colloid disaggregation, that draws mast cells to areas where lipoproteins are deposited. The latter become disintegrated by the heparin contained in the mast cells, so that the cells under consideration are significant elements in the genesis of arteriosclerosis.

Mast cells seem to carry out a multitude of functions.

Apart from producing heparin — a function confirmed by LEHNER [22] and HILL [14] — mast cells are credited by ASBOE—HANSEN [1] with the production of the ground substance of connective tissue, while, according to CARTER, HIGGINBOTHAM and DOUGERTHY [5], they are involved also in antigen-antibody reactions. A degranulation of mast cells has been observed to follow the re-injection of antigens. As RILEY [32] and KELLER [21] were able to demonstrate the presence of histamine and 5-hydroxytryptamine (serotonin) in the mast cells of rats, these cells have probably the additional function of supplying histamine to the organism. It has been claimed by BENDITT [3] that they contain chymotrypsin as well. Mast cells play further a significant role in sulphur metabolism: parenterally introduced labeled sulphur (S^{35}) was shown by ASBOE—HANSEN et al. [2] to have mostly accumulated in the mast cells. Their granules contain sulphonated polysaccharide and it seems obvious that the sulphur deposited in the cells is required for the production of the sulphuric-acid radical of the polysaccharide. SYLVEN [35], by means of centrifugation, demonstrated the presence of lipoproteins in the cells: their exact position and role are still awaiting final elucidation.

As to the role played by mast cells in pathological processes, the following should be mentioned:

DERINGER and DUNN [7, 8], further MISKOLCZY—FODOR [27] observed great numbers of mast cells in chronic inflammatory and allergic processes; SABRACES and BERGONZONI [34], in cases of tuberculosis and lymphatic congestion; MICHELS and DOUNEY [26], in toxic and allergic skin diseases; TODORO [36], in gingivitis; DOUNEY [9], DERINGER et al. [7], around neoplastic growth. Since heparin was pointed out by FISCHER [12] as an antimitotic substance, mast cells surrounding tumorous tissues may be regarded as a barrier protecting the connective tissue from cancerous invasion, a defence mechanism termed "Abwehrreaktion" by the said author. In cases of chronic polyarthritis, considerable mast cell accumulations were found in

the articular capsules by BURKL and SONNENSCHEIN [4]. Urticaria pigmentosa seems to be a disease induced by the proliferation of mast cells (NÉKÁM, [28]). The precancerous increase in the number of mast cells in the connective tissue is regarded by DUNN and MONTGOMERY [10] as a diagnostic sign. Fatal mastocytosis, suggestive of leukaemia, has been reported by EFRATI [11]. X-ray irradiation was observed by KELÉNYI [20] to induce a clumping of the mast-cell granules and to retard the coagulation of blood : he attributed these phenomena to the action of heparin set free from mast cells and concluded that mast cells contain also alkaline phosphatase and peroxydase.

According to the above, mast cells contain the following substances

1. sulphonated polysaccharide (heparin) ;
2. acetylated polysaccharide (hyaluronic acid?) ;
3. histamine ;
4. 5-hydroxytryptamine (serotonin) ;
5. chymotrypsin ;
6. lipoprotein ;
7. alkaline phosphatase ;
8. peroxydase,

and carry out the following functions

1. inhibit blood-clotting ;
2. constitute an active factor of fibrinolysis ;
3. decompose lipoprotein structures ;
4. bind and release histamine and serotonin ;
5. exert a cytotoxic action ;
6. produce the ground substance of connective tissue ;
7. exert an antitrypsin effect ;
8. promote sulphur metabolism.

It is not superfluous to bear all this in mind because the analysis of mast cells reveals an intergranular protoplasmic area the structure of which is not quite clear. Our first problem was to make this area accessible to analysis ; the second, to ascertain how the manifold functions of the cell are related to this more or less unknown structure.

In order to be able to clear up the details of any given structure and to follow its fate, it is necessary to increase its microanisotropy (ROMHÁNYI [33]). Accordingly, we had to find those stains which show affinity to mast-cell granules and at the same time make the cell substance anisotropic.

Connective tissue of rats, and sections prepared from their paraffin-embedded mesentery were used in the present experiments. Using a dissecting needle, we so stretched the connective tissue on a slide : it was then fixed by heat, chloroform-methylalcohol mixture and basic lead acetate, and then

stained. Part of these preparations was not fixed but simply dried at room temperature and so stained. No difference in respect to anisotropy seemed to exist between mast cells observed in the fixed and the unfixed preparations, nor was there a difference between the cells in the connective tissue films and those in the mesenteric preparations.

Pyronine G, toluidine blue and neutral red (Grübler) produced pronounced anisotropy, provided the last-named two stains were dissolved in an acid medium (N/10 HCl) (Fig. 1). Anisotropy was further obtained by the

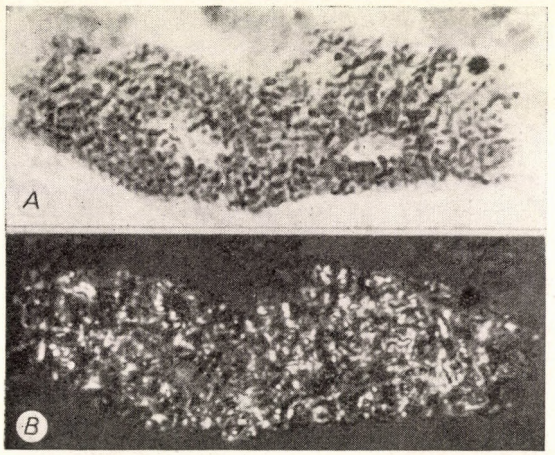


Fig. 1. Mast cell from connective tissue of rat. Unfixed. *A :* Stained with 0,1 per cent neutral red dissolved in N/10 HCl *B :* Same in polarized light ; × 1000

use of brilliant cresyl blue and thionine (likewise in acid medium). Pyronine G was the only stain to make the substance of mast cells anisotropic in a neutral medium.

At first sight it seemed as if the granules of the cells had become anisotropic ; however, a more thorough examination revealed the fact that not the granular but the intergranular substance had developed such a birefringence as could not be stopped by imbibition (Fig. 2). What this phenomenon means is that, in acid media, the intergranular substance arranges itself into paracrystals and the said semicolloidal dyes become so dissociated (change of molecular number in the semicolloid phase) as to increase the polarization effect (ROMHÁNYI [33]).

We thought that the substance, capable of adsorbing the dyes to its suitably structured surface in a way as to impart birefringence to the dye-substrate complex, might be ribonucleic acid, sulphonated polysaccharide in the stage of synthesis, or possibly a simple protein or lipid.

In order to gain insight into the structure of the intergranular area, two

methods were used. 1. Digestion (ribonuclease, elastase, hyaluronidase, trypsin, pepsin). 2. Extraction (KEILIG's method : hot chloroform—methylalcohol āā which dissolves fats of all kinds within 30 minutes (PEARS [30]).

Digestion by any of the said substances had no effect on the development of anisotropy. However, while neutral red dissolved in a neutral medium produced no anisotropy in the intergranular substance of unextracted mast cells, it gave rise to a clearly discernible birefringence in cells that had been

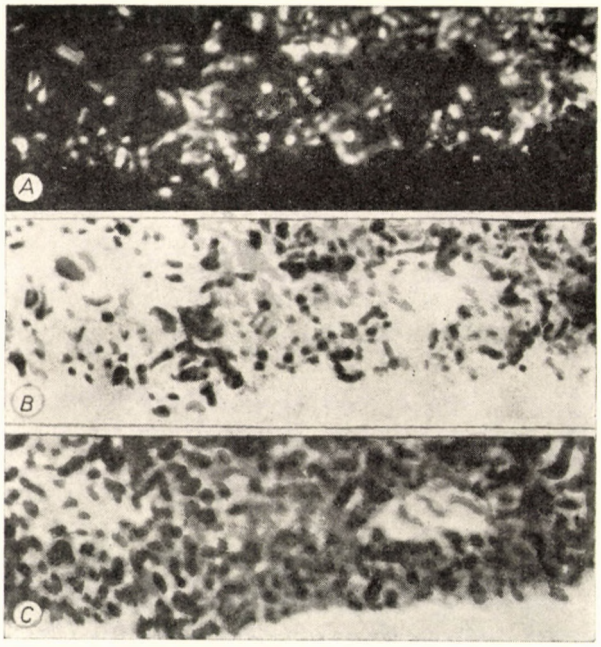


Fig. 2. Detail from cell in Fig. 1. *A :* In polarized light. *B :* Contact copy of the negative photograph of *A*. *C :* In normal light. It can be seen that *B* differs from *C*, *i.e.*, the anisotropic substance is not identical with the granules; × 3000

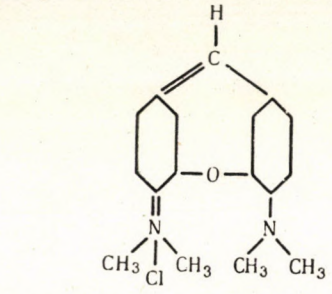
subjected to KEILIG's extraction. By adding N/10 HCl to the anisotropic intergranular substance we were able to induce its rearrangement with a consequent intensive birefringence.

This phenomenon might be explained by assuming that the intergranular substance is surrounded by a lipid layer which prevents the diffusion of enzymes. To settle this problem we subjected the connective tissue preparations to KEILIG's fat extraction, but it failed to produce any difference.

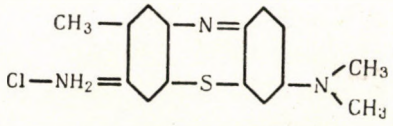
If mast cells are treated with saturated aqueous carbamide solution, or 1 per cent protamine sulphate, they will lose their faculty of binding any of the said dyes in such a manner as to produce birefringence in acid media.

The question arose whether the development of anisotropy may be associated with the semicolloidal properties of the dyes. With a view to solving this problem, we resorted to alcoholic dye solutions as stains and HCl-alcohol as acidifying agent (the said dyes give true solutions in alcohol). Anisotropy resulted.

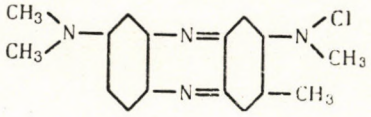
Considering that the dyes under consideration contain alkyl radicals, the idea arose that they might be adsorbed to structural lipids by the so-called van der Waals-forces (thermolabile homeopolar cohesive forces). We



Pyronin G



Toluidin-blue



Neutral red

Fig. 3. Pyronine G. Toluidine blue. Neutral red

stained and acidified the cells at a temperature of 80° C: anisotropy still resulted.

To be able to interpret the birefringence brought about in the above manner, it is indispensable to discuss the chemical structure of the dyes (Fig. 3).

Pyronine G (Grübler) is a phenylmetane derivative of the xanthone group with a molecular weight of 312.795. Mildly basic. It is divisible into 3 fractions by paper chromatography. The granules of the mast cell react with both the alcoholic (full) and the aqueous (semicolloidal) solution in neutral and acid medium alike (metachromasia). The residue after the evaporation of both the aqueous and the alcoholic solutions displays an identical birefringent crystalline structure. Crystals from the aqueous solution become

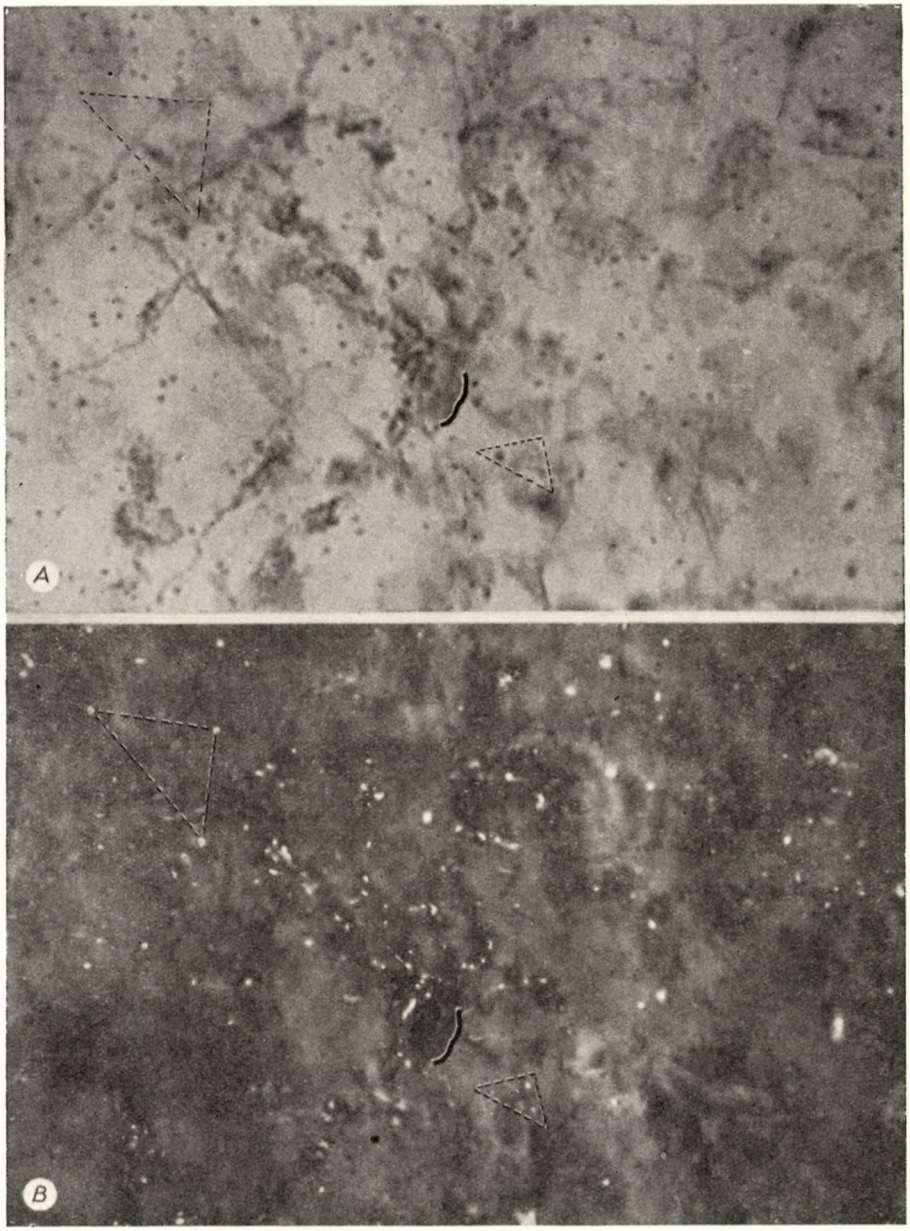


Fig. 4. Mast cell disintegrated by histamine. *A:* In normal light, *B:* The same area in polarized light. The granules at the points of the larger triangle are different from those in the smaller triangle. × 2500

isotropic when treated with heparin although no precipitation could be observed when the respective aqueous solution of the dye and heparin were brought together.

Toluidine blue (Grübler) is a basic chinone-imide dye of the thiazine group with a molecular weight of 203.819, and an absorption maximum at 620 to 622 mμ. It gives only one fraction at paper chromatography. The mast-cell granules take readily both the alcoholic and the aqueous solution in basic and acid medium alike (metachromasia). The residue after the evaporation of both the aqueous and alcoholic solutions displays an anisotropic crystalline structure which does not become isotropic on treatment with heparin. Heparin and toluidine blue give a precipitate insoluble in water.

Neutral red (Grübler) is a mildly basic chinone-imide dye of the azine group with a molecular weight of 288.775, and an absorption maximum between 540 and 542 mμ. It is divided into 2 fractions by paper chromatography. It is only in an acid medium that its alcoholic and aqueous solutions stain the mast-cell granules (orthochromasia). Crystals from both its alcoholic and aqueous solutions are anisotropic and remain so even if treated with heparin. Heparin and neutral red give a precipitate insoluble in water.

It is obvious from their structural formulas that all these dyes have two different poles (haptophore group). One pole is lipophile ($-CH_3$), the other salt-forming ($-N-Cl$). This leads to the conclusion that the intergranular substance of mast cells contains a complex lipoprotein which has partly acid and partly alkyl side chains and that both types of side chains are oriented. The salt-forming group plays the principal role in the development of anisotropy.

We followed the fate of the granules and the intergranular substance liberated from disintegrated mast cells. Disintegration was brought about by the subcutaneous administration of histamine (100 μg). The granules originating from disintegrated mast cells fall into two categories. Granules of the first category — consisting of pure intergranular substance — are doubly refractive in polarized but invisible in normal light, while those of the second category display anisotropy in polarized light but are well visible in normal light. The latter granules consist of polysaccharides to which anisotropic intergranular material is bound (Fig. 4.). Both types of granule seem to be dissolved by the ground substance of connective tissue.

Summary

After a brief survey of the chemistry and physiology of mast cells and their relations to pathologic processes, it has been demonstrated that on treatment with pyronine G, toluidine blue or neutral red, the intergranular substance of mast cells becomes anisotropic in acid media. The anisotropic substance consists of a lipoprotein which is dissolved by the ground substance of connective tissue when the cells disintegrate.

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ДАННЫЕ К СУБМИКРОСКОПИЧЕСКОЙ МОРФОЛОГИИ ТУЧНЫХ КЛЕТОК

Л. ХОРВАТ

После приведения самых существенных литературных данных, автор переходит к изложению поляризационно-микроскопического исследования субмикроскопической структуры тучных клеток.

Нейтральный красный, толуидиновая синька, пиронин Г делают в кислой среде тучные клетки анизотропными. Анизотропия относится не к зернистой, а к межзернистой субстанции. На основе экспериментов, проведенных различными пищеварительными и экстракционными методами, авторы предполагают, что внутриклеточное анизотропное вещество представляет из себя липопротеин, хорошо прослежимый при дезинтеграции клетки в основном субстрате клетчатки, в которой он растворяется.

BEITRÄGE ZUR SUBMIKROSKOPISCHEN MORPHOLOGIE DER MASTZELLEN

L. HORVÁTH

Nach einer Durchsicht der wesentlichsten literarischen Angaben wird über die Polarisationsuntersuchung der submikroskopischen Struktur von Mastzellen berichtet.

Neutralrot, Toluidinblau und Pyronin G bedingen im sauren Substrat eine Anisotropie der Mastzellen. Die Anisotropie bezieht sich nicht auf die körnige, sondern auf die Zwischenkornsubstanz. Auf Grund verschiedener mit Verdauungs- und Extraktionsmethoden durchgeführten Untersuchungen wird angenommen, daß die intrazelluläre anisotrope Substanz ein Lipoprotein sei, das bei der Desintegration der Zellen in der Grundsubstanz des Bindegewebes, in welcher es sich löst, deutlich verfolgt werden kann.

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