ELECTRON MICROSCOPY OF THE TISSUE MAST CELL*

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PLATES 7 AND 8

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Although the tissue mast cell has been studied intensively by light microscopy during the past seventy-five years, little is known concerning the details of its structure. Such knowledge is of special interest, since the mast cell appears to contain heparin (1) and histamine (2), compounds of considerable physiological interest. The present paper is concerned with the results of electron microscopy of sectioned mast cells obtained from the peritoneal fluid of animals either untreated, irradiated, or injected with toluidine blue, protamine sulfate, or stilbamidine. Protamine and toluidine blue bind heparin (3), stilbamidine liberates histamine (2), and x-irradiation produces marked changes in the cytology of the mast cell (4).

Materials and Methods

To obtain normal mast cells, adult male Syrian hamsters (100 gm.) and Sprague-Dawley rats (200 gm.) were injected intraperitoneally with 3 and 5 cc., respectively, of buffered Tyrode's solution. After 10 minutes the animals were sacrificed by ether anaesthesia or by cervical fracture. Immediately thereafter the peritoneal fluid was withdrawn and transferred to a tube containing an equal volume of OsO_4 (2 or 4 per cent in buffered Tyrode's solution). Ten minutes later the cells were isolated by centrifugation. The supernatant was discarded and the cells were resuspended in a wash of Tyrode's solution for 10 minutes. The same procedure was used for dehydration of the cells with alcohol (50, 75, 95, and 100 per cent ethyl alcohol) and for infiltration with plastic (70 per cent butyl methacrylate-30 per cent ethyl methacrylate containing 0.5 per cent 2,4-dichlorobenzoyl peroxide as catalyst). Finally the cells were suspended in a small amount of plastic and transferred to gelatin capsules. Polymerization was accomplished under ultraviolet irradiation or in an oven at 50°C. Sections were cut on an International-Minot thin sectioning microtome and observed with the RCA EMU-2A electron microscope.

In the course of this work other technics and tissues were explored in attempts to achieve better preparations. Fixation by means of 10 per cent formalin, alcohol-formalin, chromic oxide and formalin, osmium tetroxide and potassium dichromate, 4 per cent basic lead acetate, absolute alcohol, and freeze-drying gave results that were usually inferior and never better that those obtained with osmium tetroxide

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alone. The same was true of ultrathin sections of fresh frozen subcutaneous connective tissue cut on the freezing microtome at 20 micra and placed immediately in osmium tetroxide. Variations in pH of the fixative or time of fixation, polymerization of the embedding plastic at 80°C., or embedding in partially polymerized plastic also did not improve the preparations. Our experience indicates that it is difficult to obtain well fixed preparations of the tissue mast cell. This seems especially true, since white blood cells and fibroblasts in the same preparations are well fixed and clearly show the fine structure already established for them in the literature. Electron micrographs of mast cells in thin sections of spleen, capsules of spleen and liver, mesentery, and skin prepared according to the method of Palade (5) appeared essentially the same as those of mast cells from the peritoneal fluid.

In addition to the work on normal mast cells the following experiments were carried out:

Experimental Group I.—On the 4th and 6th days after single total-body exposures to 600 r of x-radiation (250 kv., 15 ma.; 0.5 mm. Cu, and 3.0 mm. Bakelite filters; 26.7 cm. target distance; 1.5 mm. Cu half-value layer; 215 to 225 roentgens per minute) mast cells were obtained from the peritoneal cavities of rats and hamsters and subjected to the procedures outlined above.

Experimental Group II.—Three to 5 ml. of protamine sulfate or toluidine blue in concentrations of 1:2,500 to 1:5,000 and 1:1,000 to 1:200,000 respectively in Tyrode's solution were injected intraperitoneally into rats and hamsters. Upon sacrifice 10 minutes later the peritoneal fluid was removed and prepared for electron microscopy. In similar fashion the effects of stilbamidine (1:160 in Tyrode's solution) were studied.

RESULTS

Typical electron micrographs of mast cells from untreated animals are seen in Figs. 1 to 4. Sections of cells from the rat and hamster were similar in appearance and will be described as one. The nucleus, which is large and often eccentric in location, has a very irregular outline caused by indentation of its surface by the surrounding cytoplasmic granules. The interior of the nucleus is occupied by a rather dense structure consisting of dark thread-like material, vacuoles, and empty spaces. The former occurs diffusely throughout the nucleus, but is most concentrated about the periphery; while the vacuoles and empty spaces are predominant more toward the interior. Bodies resembling nucleoli were rarely found, but where present resembled the round nuclear inclusion of Fig. 1. Bounding the nucleus is a distinct membrane which in places is manifest as a double line. Cells containing two nuclei were seen occasionally. Mitotic figures were never found.

The large cytoplasmic granules, which represent the dominant feature of the mast cell under the light microscope, are represented in the electron micrographs by round, oval, or irregular bodies 0.5 to 1.0 μ across. The granules have definite

boundaries which are sometimes wavy at points of contact with other granules. and thus present the appearance of an interlocking of the surfaces of the granules Inside the granules is found a reticular or vacuolar structure consisting of thread-like material which is similar to but finer than that of the interior of the nucleus. The threads seem to be made up of or have adhering to them very fine round granules (Fig. 2). A difference in density is apparent among the large cytoplasmic granules and seems to depend upon the degree of separation of the threads of the internal reticulum.

In addition to the large granules other cytoplasmic structures are evident in the small spaces between granules and in larger granule-free areas. Common in such locations are mitochondria, which appear as elongate bodies (0.1 to 0.2 μ by 0.5 to 0.7 μ) with dark outlines and dark, linear, internal structures. Groups of mitochondria are frequently found between the nucleus and the cell boundary, as in Fig. 1, or in nuclear indentations surrounded by large cytoplasmic granules. In the same areas one also finds small vacuoles and short tube-like formations which appear identical with the endoplasmic reticulum of Porter (6).

The cells possess a distinct limiting membrane which becomes especially conspicuous when it forms folds which project away from the body of the cell (Fig. 1). In places the limiting membrane appears as a double line, the inner one of which seems at times to belong to the endoplasmic reticulum. In the same way the endoplasmic retiuclum seems to form the outer line of the nuclear envelope.

Sections through mast cells treated with toluidine blue, protamine sulfate, or stilbamidine are essentially identical in appearance. Fig. 3 is representative of the effects of these treatments. The most striking change induced by the agents is in the spacing of granules which are surrounded by clear areas and widely separated from one another. Structures seen only occasionally in untreated cells are now conspicuous. The limiting membrane of the cytoplasmic granules is obvious and appears to be made up of fine, dark granules. The intergranular cytoplasm with its vacuoles and reticulum of thread-like material is clear as are the outlines of the mitochondria. The inner structure of the large granules, however, is essentially unchanged. Measurements of granules in sections of cells from several experiments employing these treatments showed the granules to be of the same size as those in cells from untreated animals.

Many of the mast cells from irradiated animals have the characteristics shown in Fig. 4. While these cells contain some granules of normal size and appearance, large portions of the cells are occupied by bodies which are many times the size of the usual granule and which show only vestiges of an internal reticulum. The nuclei of such cells are usually elongated and markedly deformed. Only rarely were cells having these characteristcs seen in preparations from untreated animals.

DISCUSSION

Electron microscopy of sections of normal mast cells and of normal mast cell granules has been reported by Asboe-Hansen (7) and Köksal (8), respectively. Using the technic of removing the embedding plastic before microscopy, Asboe-Hansen found the mast cells to be made up of very dense, diffusely scattered cytoplasmic granules, some of which were connected by a coarse, fibrillar cytoplasm. No evidence of mitochondria, intragranular structure, or cell membranes was obtained. In our experience mast cells having such characteristics appear only after removal of the embedding plastic.

Köksal found the mast cell granule to be round or oval, to have a distinct boundary, and, occasionally, to possess canal-like structures in some locations. The latter seem identical with the structures which appear throughout the granules in our preparations. We have not found within granules the empty spaces or compact nucleus-like bodies which Köksal observed.

In electron micrographs of sections through mast cell tumors of the dog Bloom *et al.* (9) have observed mitochondria and filamentous cytoplasmic extrusions from the cell surface. The latter seem to be the same as the formations we interpret as folds in the limiting membrane. It should be pointed out that such folds occur also in the limiting membranes of white blood cells in peritoneal fluid (Fig. 1), though perhaps not as frequently as in the mast cells.

The separation of the large cytoplasmic granules and the appearance of clear spaces surrouding them after treatment with toluidine blue, protamine sulfate, and stilbamidine apparently results from an imbibition of fluid by the cell. *In vitro* and *in vivo* observations of mast cells from peritoneal fluid and in the cheek pouch or mesentery reveal that such treatments are attended by a swelling of the cell to about 1⅓ of its original size (10). The swelling does not appear to involve the granules, for as mentioned above, there are no changes in their dimensions or internal structure. The same appears true of the basic structure of the intergranular cytoplasm which is clearly revealed upon the separation of the granules.

The significance of the present findings with relation to the function of the mast cell is not readily apparent. Had the treatments with toluidine blue, protamine sulfate, and stilbamidine been followed by changes in the intragranular structure, statements concerning whether the mast cell granules contain heparin or histamine might be possible. Evidence from other experiments indicates, however, that after such treatments substantial amounts of histamine are liberated into the surrounding medium (11), and that heparin is located around the granule, as shown by orthochromatic staining of the mast cell granules and metachromatic coloration of the areas surrounding the granules (10). This suggests that the intragranular reticulum represents neither histamine nor heparin. It is possible that the intragranular structure is the mani-

festation of material having the elementary chemical composition of mitochondria found in mast cell granules isolated by differential centrifugation (12).

The effects of total-body x-irradiation on mast cells, as revealed by the electron microscope, were similar to those seen in whole mounts of skin, mesentery, and cheek pouch examined with the light microscope (4). The apparent coalescence of granules and the disappearance of intragranular structure suggest the loss of functional integrity of the granules. That such cells are dead or dying was borne out in light microscope studies, which revealed that the conglomeration of granules was followed by eventual disruption and phagocytization of the cell (4) and a decrease in the total mast cell population (13, 14).

SUMMARY

Electron microscopy was carried out on sections through tissue mast cells from the peritoneal fluid of rats and hamsters, either untreated, x-irradiated, or injected with toluidine blue, protamine sulfate, or stilbamidine. Mast cells from untreated animals have large nuclei and are filled with densely packed, cytoplasmic granules. The latter possess a distinct boundary and an internal structure which is reticular or vacuolar in nature. Between the granules are found elongate mitochondria and endoplasmic reticulum. Mitochondria often appear also in groups in granule-free areas adjacent to the nucleus. Nuclear and cell membranes of an apparent double nature are found. After treatment with toluidine blue, protamine sulfate, and stilbamidine the granules are surrounded by clear areas and are widely separated from one another; the endoplasmic reticulum is more conspicuous. The internal structure of the granule is unchanged. In the mast cells from x-irradiated animals there is an apparent coalescence of granules which is attented by a loss of intragranular structure. The findings are discussed in relation to other work on the structure and function of mast cells.

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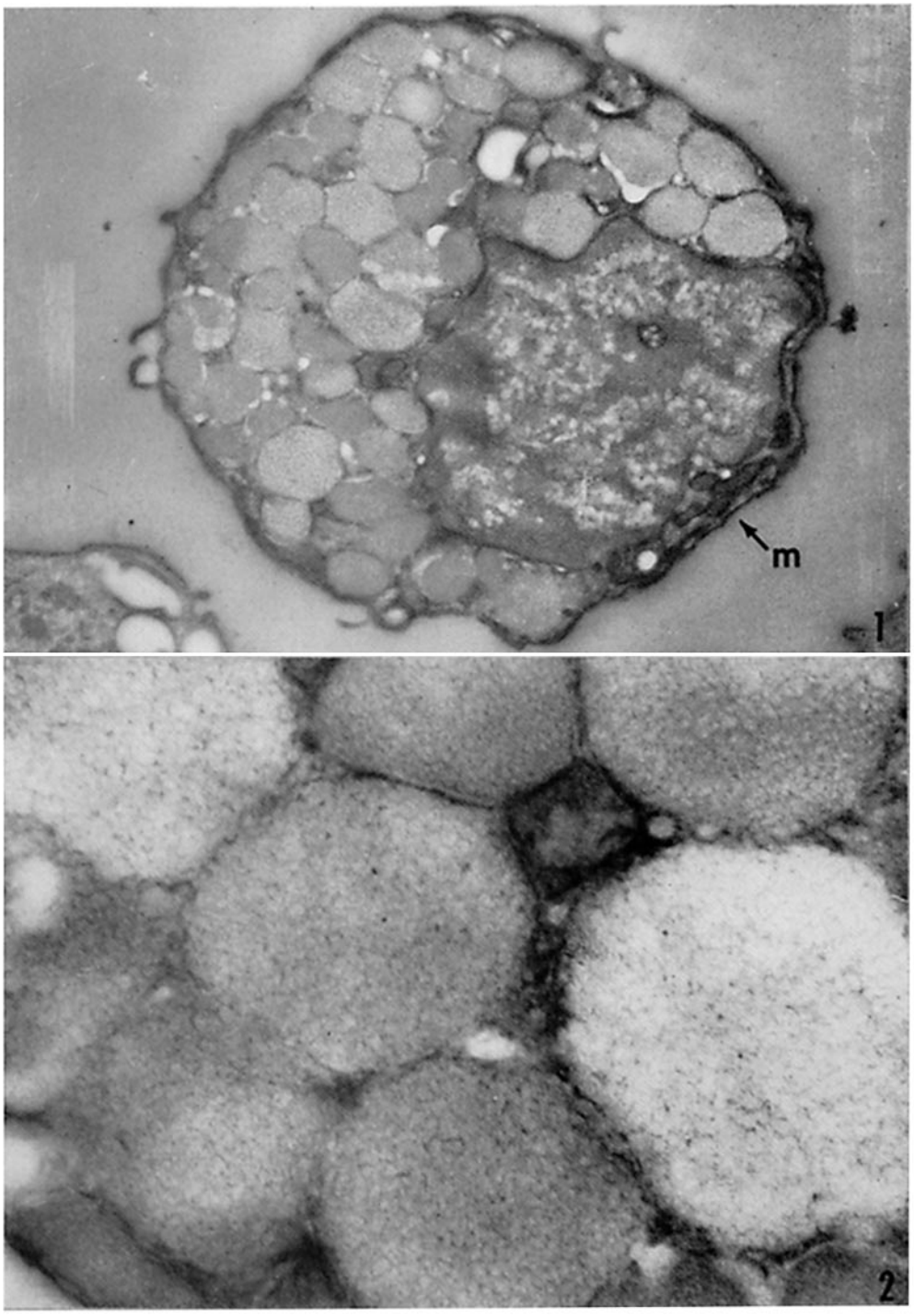
EXPLANATION OF PLATES

PLATE 7

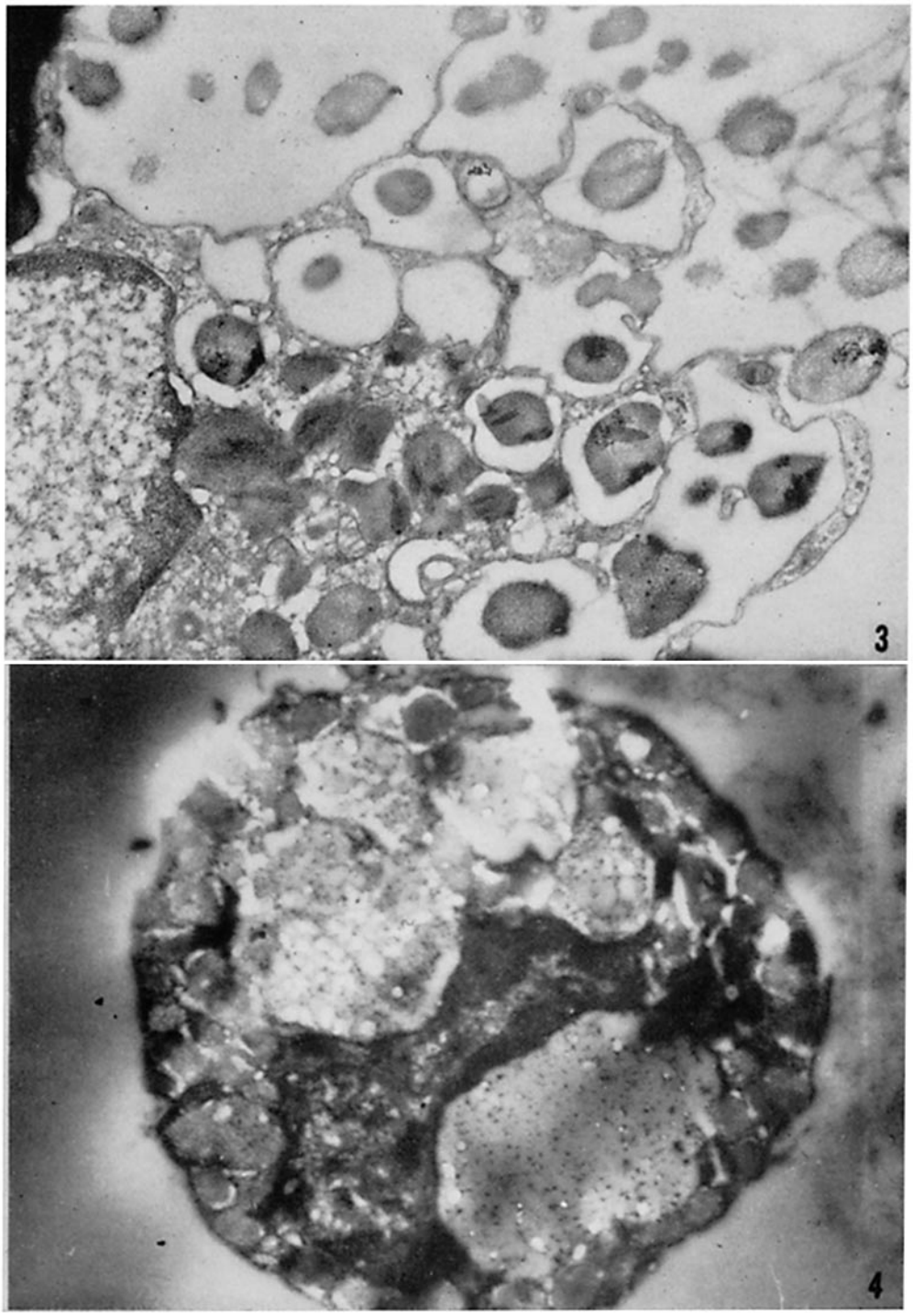
FIG. 1. Mast cell from untreated hamster. *m*, mitochondria. × 14,000.
FIG. 2. Mast cell from untreated hamster. × 45,000.



(Smith and Lewis: Electron microscopy of tissue mast cell)

PLATE 8

FIG. 3. Mast cell from protamine sulfate (1:5,000) treated hamster. × 14,000.
FIG. 4. Mast cell from x-irradiated hamster. Four days after 600 r. × 14,000.



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