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ULTRASTRUCTURE OF MAST-CELL GRANULES IN SQUAMOUS CELL CARCINOMA

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An accumulation of mast cells is regularly found in human basal and squamous cell carcinoma, and an association with a defensive process against cancer growth has been suggested (1, 4).

In normal and urticaria pigmentosa skin, mast-cell granules show two subgranular components, i.e. lamellar structures and a fine granular material (2). In basal cell carcinomas the mast-cell granules have revealed various pathological ultrastructural changes (3). The present report is concerned with electron microscopic observations of mast-cell granules in a squamous cell carcinoma.

Material and Method

Numerous specimens were removed for electron microscopy from the tissue of a squamous cell carcinoma of the vulva. Clinically, the lesions consisted of an atrophic scaly part and a slightly elevated papillomatous part. The specimens were fixed with a 4 % glutaraldehyde solution in veronal acetate buffer pH 7.4 with 4.5 % sucrose for one hour, washed in the same buffer overnight, fixed with a 1 % osmic acid solution in the buffer for 30 minutes and washed in the buffer. The fixation and washing were carried out in a refrigerator controlled at a temperature of 4°C. The samples were dehydrated in a series of graded alcohols and embedded in Epon 812. To select areas for ultramicrotomy, 1 μ thick sections were stained with toluidine blue and examined under the light microscope. Mast cells were found in a dermal infiltrate as well as scattered in the epidermis. The dermal cell infiltrate under the scaly or verrucous areas consisted of oval or spindle-shaped, large cells with ample cytoplasm and mitotic figures. Ultrathin sections from various areas were cut by an LKB ultramicrotome and stained with uranyl acetate and lead citrate. A Siemens electron microscope "Elmiskop I A" was operated at 80 kV with double condensors.

Observations

The mast-cell forms were irregular, oval or spindle-shaped with several cytoplasmic protrusions of various forms and lengths. The mast cells contained a nucleus and cytoplasmic organelles, *i.e.* mitochondria, non-granular endoplasmic reticulum and ribosomes. A Golgi apparatus covered a wide area and showed dilated vacuoles and saccules (Fig. 5).

The mast cells contained numerous granules in their cytoplasm, while no granules were found in the extracellular spaces. The granules revealed various figures, and according to the differences in the subgranular components, they were classified into three types. The *first* type showed the two subgranular components previously described in normal mast cells (2). These cells were mostly seen between the dermal fibers outside the dermal infiltrate (Fig. 1, Table 1), but also in the cell infiltrate (Fig. 2, 3, 6, Table 1) and in the epidermis

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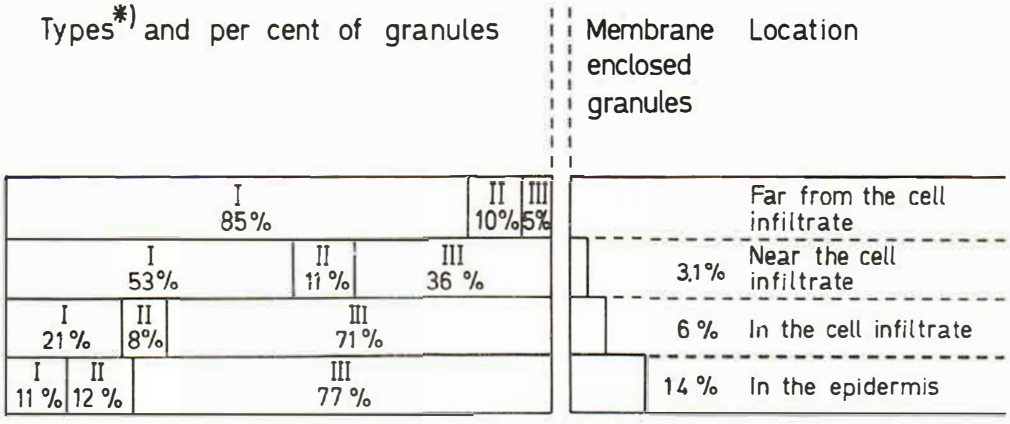
16 – 337-1384. Acta Derm. 49: 3

Table 1. *Distribution of mast-cell granules in squamous cell carcinoma*



(Fig. 10, 12, Table 1). The forms of the granules were round, oval or polygonal. They covered an area of approximately 0.165–0.306 μ^2. The diameter of the largest granules was 0.66 μ, while the average diameter of most granules was within the range of 0.4–0.5 μ. The fine granular material was dense and revealed crystalline structures in most of the granules (Fig. 1, 2, 10, 12). The parallel lines of these crystalline structures occurred with intervals of either 60 Å or 120 Å. The 120 Å lines crossed each other at an angle of 60°, while the 120 Å lines crossed the 60 Å lines at an angle of 30° (Fig. 2). The lamellae were parallel and formed scrolls. Each lamella showed a periodicity of 60 Å (Fig. 1, 3). Some of the granules of the mast cells in the cell infiltrate and the epidermis revealed both subgranular components, many granules being filled with lamellar scrolls (Fig. 3) or with dense fine granular material, which, in some granules, was covered by crystalline patterns (Fig. 2, 12). Rarely, the granules were completely enclosed by membranes.

The *second* granule type showed two subgranular components, but the patterns were irregular. This type was also found between the dermal fibers (Fig. 1), in the cell infiltrate (Fig. 4), and in the epidermis (Fig. 10). The forms of these granules were round or oval, and they covered an area of approximately 0.16 μ^2, the diameter being 0.4–0.5 μ. The granular material was relatively coarse with a round dense mass in the center, which, although rarely, showed faint crystalline structures. The lamellae were multilayered and mostly located in the peripheral zone of the granules. They were arranged concentrically and formed scrolls, but the periodicity was faint. No granule was enclosed by a membrane.

The *third* granule type showed unusual subgranular components. Such mast cells were found mainly in the cell infiltrate or in the epidermis. They were round, oval, crescent or spindle-shaped, and they occupied an area of approximately 0.098 μ^2 corresponding to a diameter of 0.3–0.4 μ. The granules contained both subgranular components or only one. The main material was fine or coarse granular with a central denser core (Fig. 5). Inside the central core of some granules lucent spots were observed (Fig. 5). Some granules revealed dense bands and strands (Fig. 4, 12), or grouped dense particles (Fig. 4, 6) were observed instead of the central cores. A few granules were entirely filled with grouped dense particles (Fig. 6) or dense strands with anastomosing branches (Fig. 4). Blurred scrolls or straight lamellae often

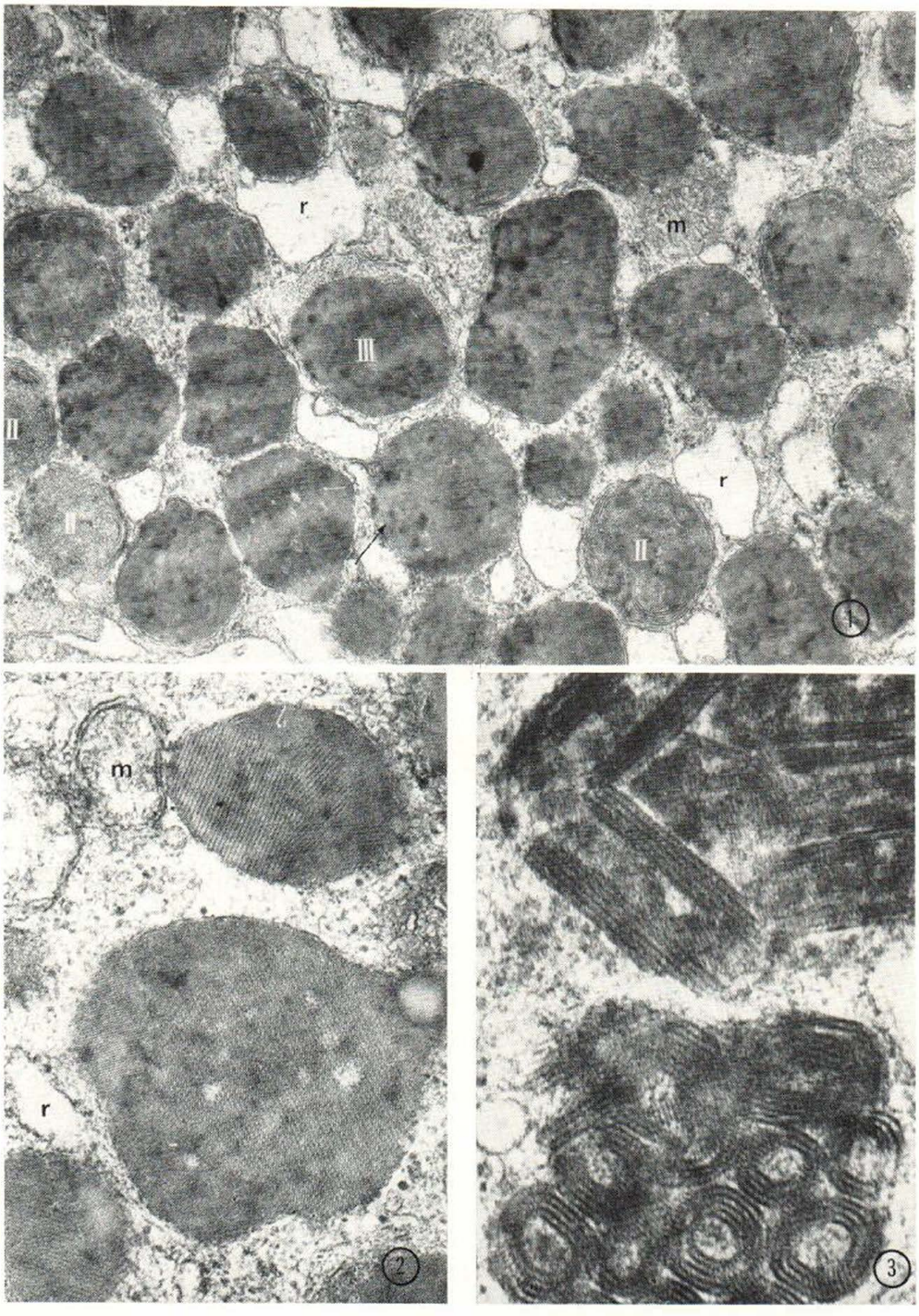


Fig. 1. Mast cell between dermal fibers far from dermal cell infiltrate. Most of the granules belong to the first, three granules to the second (II), and one to the third (III) type. The type I granules showed two kinds of crystalline structures and their crossings (arrow). The lamellae show a periodicity in the first and the second type of granules. (m) indicates mitochondrion, (r) endoplasmic reticulum. ×60,000.

Fig. 2. Granules of a mast cell in the cell infiltrate. Three granules of type I show a crystalline structure and faint lamellar figures. The lines of the crystalline pattern show two different widths, *i.e.* 60 Å and 120 Å, and their crossings at angles of 30° and 60°. (m) indicates mitochondrion, (r) endoplasmic reticulum. ×120,000.

Fig. 3. Granules of a mast cell in the cell infiltrate. Three granules show straight and scrolled lamellar structures both with a periodicity of 60 Å. ×240,000.

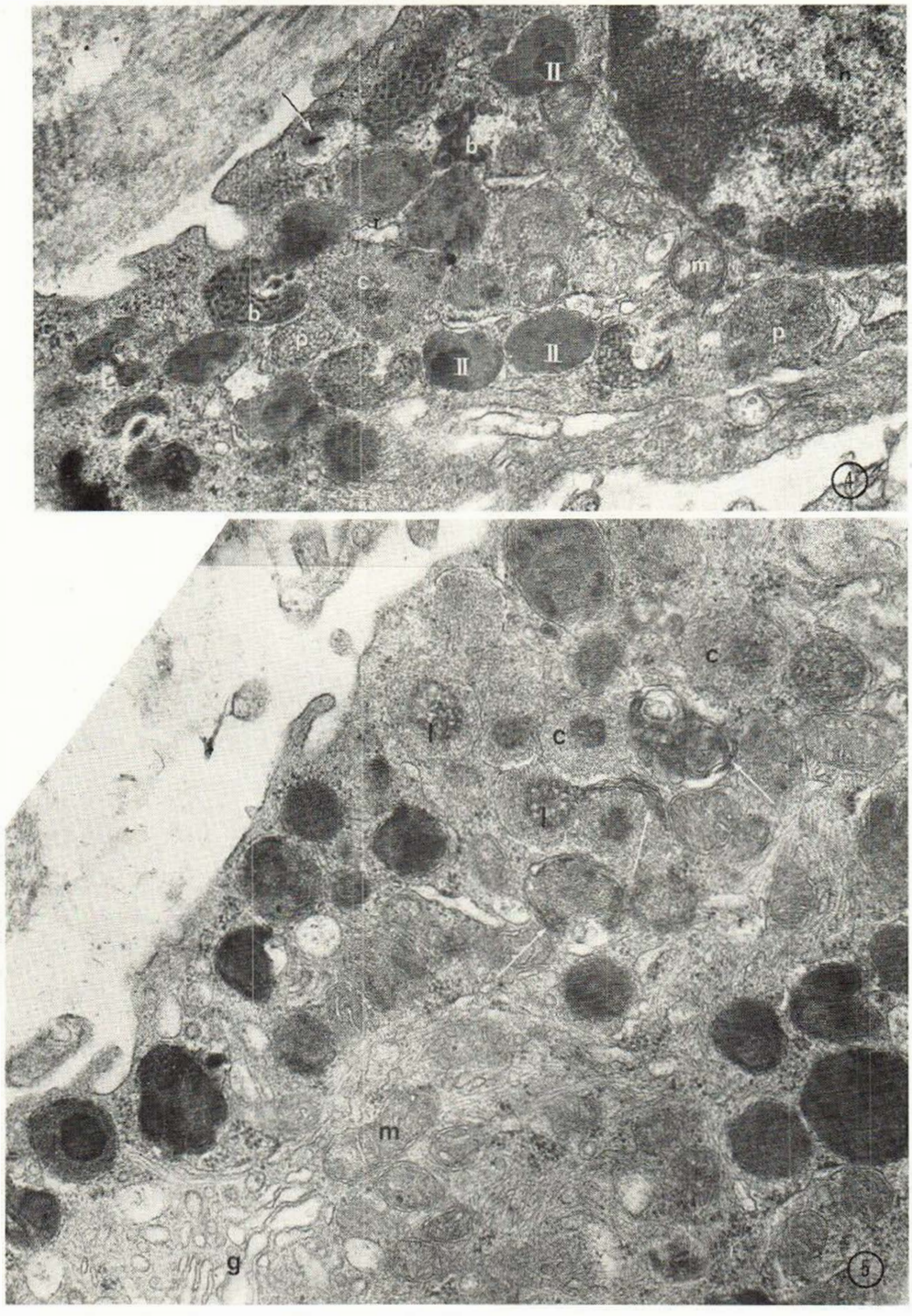


Fig. 4. Granules of a mast cell in the cell infiltrate. Second type granules are marked by II. All the rest of the granules belong to the third type showing different forms and sizes and various pathological subgranular components, *i.e.* bands (b), dense particles (p), and a scattered appearance of coarse granular material (c). (m) indicates mitochondrion, (r) endoplasmic reticulum, and (n) nucleus. An arrow indicates a membrane-enclosed granule. ×24,000.

Fig. 5. A mast cell of the cell infiltrate. The granules in the upper part of the cytoplasm show scattering of the coarse granular material (c). (l) indicates a central area showing lucent spots in the central cores. Arrows indicate lamellar structures showing concentrically arranged wavy appearances like myelin. (g) indicates dilated saccules and vesicles of the Golgi apparatus. (m) indicates a mitochondrion. ×24,000.

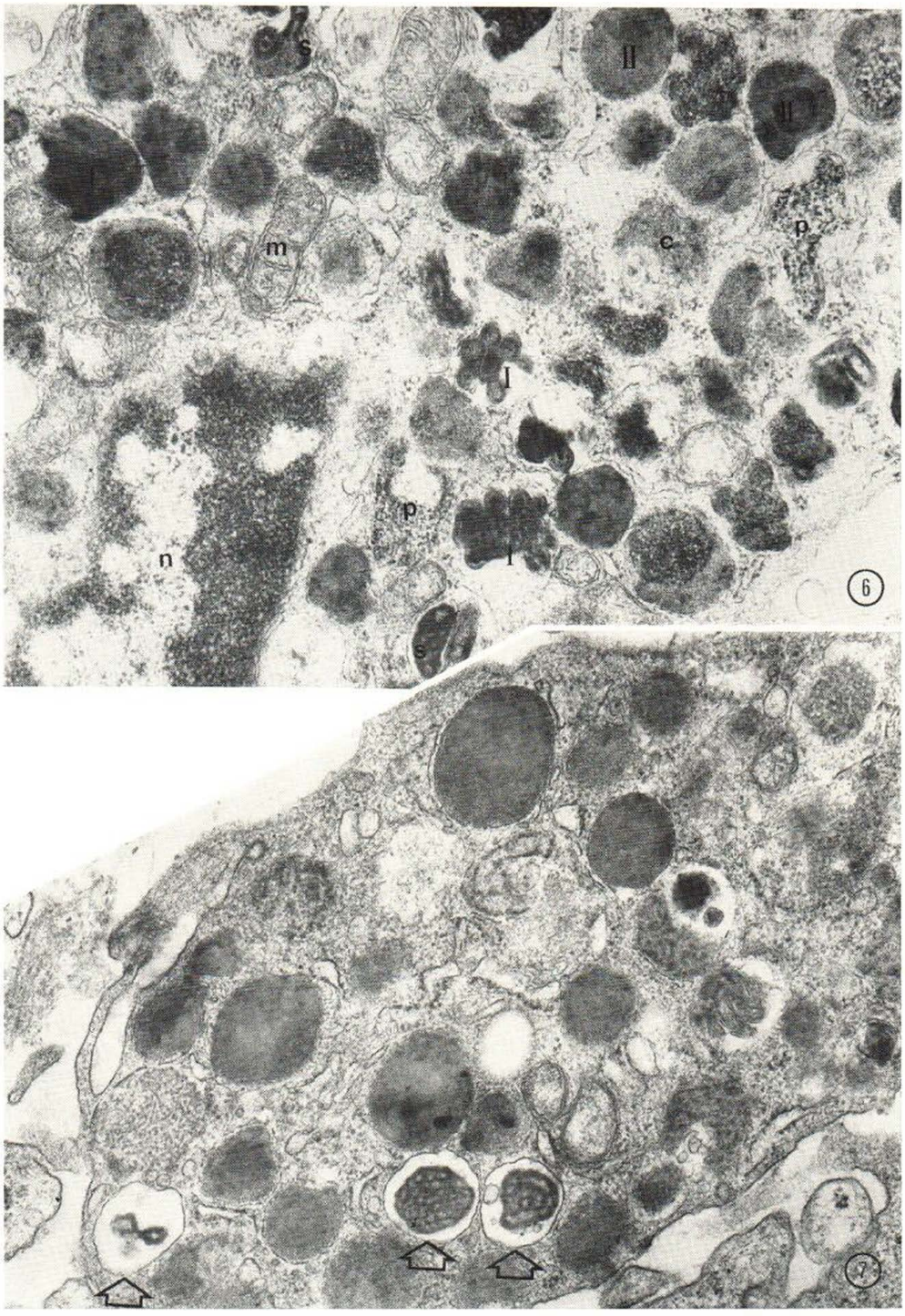


Fig. 6. Part of a mast cell in the cell infiltrate. The granules show various figures. Numbers I and II indicate the categories to which the granules belong. Number I granules show scrolls or straight lamellae. The third type of granules are not numbered. (s) indicates strands, (p) dense particles, and (c) scattered coarse granular material. Note the coexistence of these different figures in one cell. (n) indicates nucleus, and (m) mitochondria. ×60,000.

Fig. 7. A mast cell of the cell infiltrate. The granules show figures of the first, second, and third types. In the lower part of the photograph, there are three membrane-enclosed granules containing dense strands of fine granular material (arrows). ×60,000.

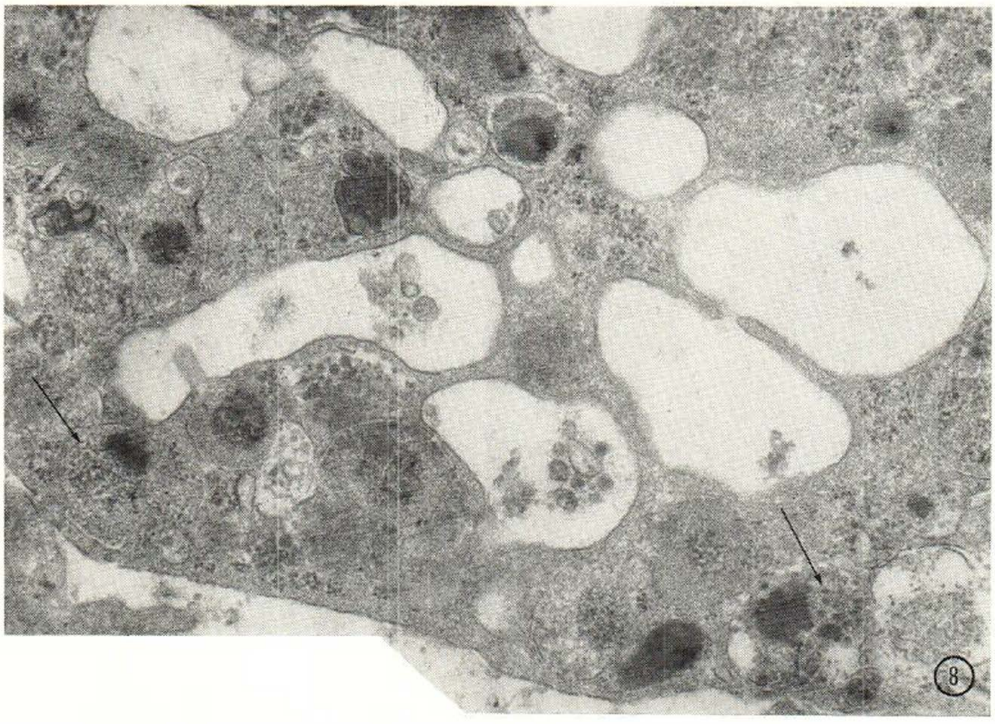


Fig. 8. A mast cell of the epidermis. Cytoplasm with a honey-comb like area and third type granules. Arrows indicate dense particles enclosed by a membrane. ×60,000.

coexisted with the above described granular material (Fig. 4, 6). Some granules contained concentrically arranged, wavy lamellae like myelin-figures without periodicity (Fig. 5, 10).

The distribution of the different granules in the cytoplasm differed with the locations of the mast cells in the tissue. Mean values of each granule type in individual cells, which were found in one area, was compared with those of other areas (Table 1).

The granules surrounded by a membrane were found at rates of 3.1 % between dermal fibers, 6 % in the cell infiltrate and 14 % in the epidermis. The contents of the membrane-enclosed granules were blurred lamellar structures (Fig. 4), dense particles (Fig. 8), coarse granular material (Fig. 9), and dense bands and strands (Fig. 7).

Some of the mast cells in the cell infiltrate (Fig. 7) and in the epidermis (Fig. 8) revealed honey-comb like structures.

Discussion

The metachromatically stained granules characterizing the mast cells revealed various figures under the electron microscope. One type of granules showed subgranular components like those in normal mature granules, and their sizes were almost the same as these (2). The second type of granules was supposed to be either abnormal immature or under abnormal disintegration as judged by the subgranular figures. The distribution of this type of granule in different areas (Table 1) suggests that they are presumably a transitional form either to the first or the third granule types. The third type of granules was smaller than the other types and showed special subgranular figures. The most extraordinary structures were the dense particles and the concentrically arranged wavy lamellae simulating myelin. Both pathological subgranular components were never found in one and the

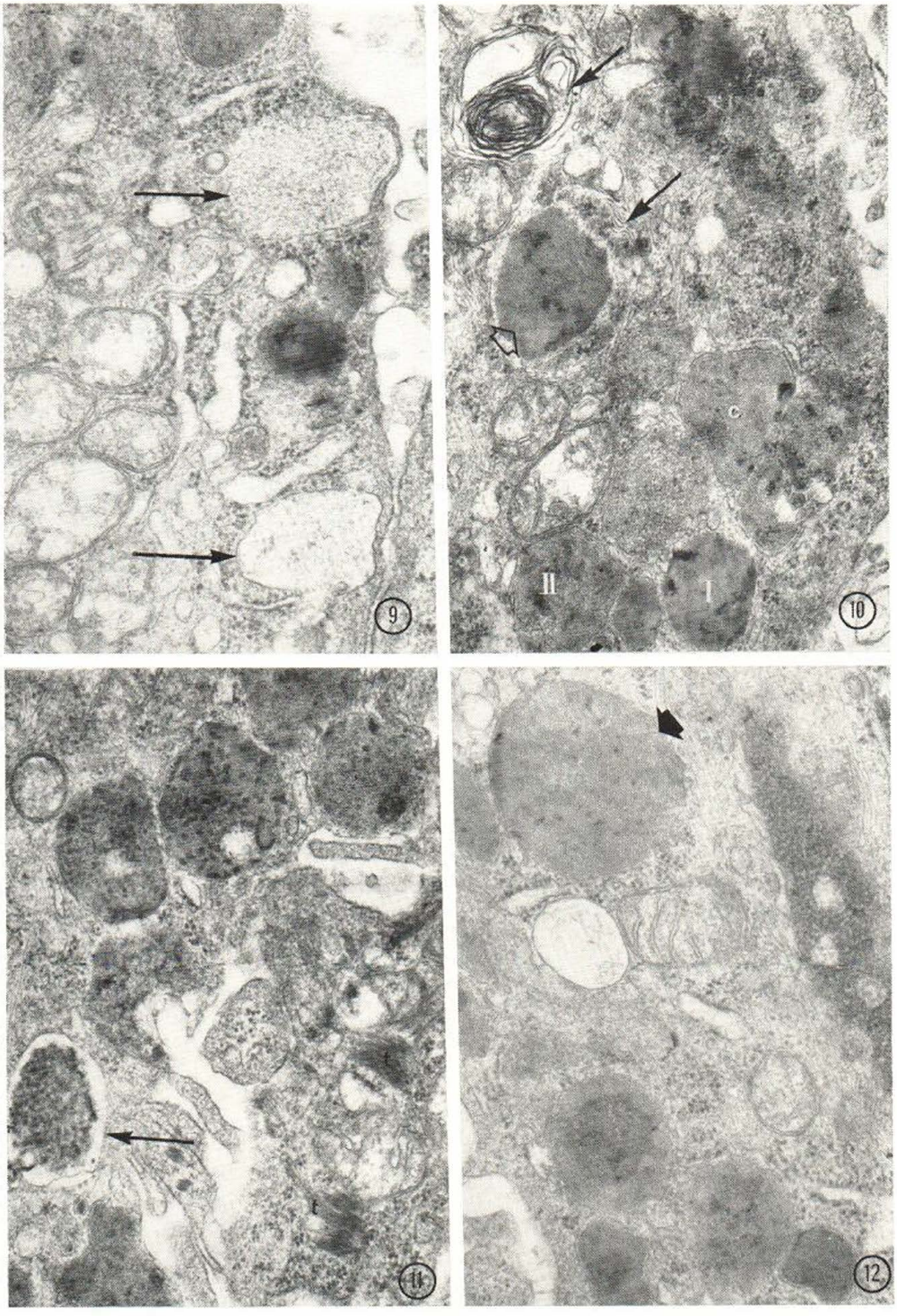


Fig. 9, 10, 11, and 12. Different figures of mast-cell granules from the epidermis. In Fig. 9 two membrane-enclosed granules are seen (arrows). Between them is a granule of the first type. In Fig. 10 wavy myelin-like lamellae (arrows), crystalline structures (framed arrow), scattering of coarse granular material (c) and type I and II granules (I, II). In Fig. 11 coarse granular material, dense particles, and lamellar structures are seen in every granule. One is enclosed by a membrane (arrow). (t) indicates tonofilaments in an epidermal cell. In Fig. 12 a large type I granule with a crystalline pattern and lamellae is seen (arrow). Others show figures of type III. ×60,000.

same granule. These observations suggest that in the third granule type the formation of the granule substance is severely disturbed. Not only the subgranular components but also the volumes of the granules were changed. The location of cells containing such granules suggests that, in some way, these mast cells are influenced by the carcinoma. The granules enclosed by a distinct membrane and the existence of honey-comb like structures in the mast cytoplasm suggest a discharge of granules. The occurrence of such granules suggests that a discharge and disintegration of granules was accelerated in the epidermis (Table 1). In view of the understanding that mast-cell granules are formed in the Golgi apparatus (2), the demonstration of a widened Golgi apparatus showing dilated saccules and vesicles might reflect the formation of abnormal mast-cell granules.

When comparing the mast-cell granules of this study with those previously seen in basal cell carcinoma (3), several differences are evident. While in the basal cell carcinoma, there were granules consistently showing both subgranular components, swelling of numerous granules, no honey-comb like structures, and no dense membrane, squamous cell carcinoma mast cells showed granules revealing extremely well-developed lamellae and crystalline structures, granules showing pathological subgranular components, and membrane-enclosed granules and honey-comb like structures indicating degranulation. These observations indicate that the alteration of the mast-cell granules in squamous cell carcinoma involved mainly the subgranular components, while changes of the granule volumes characterized the mast cells of the basal cell carcinoma.

SUMMARY

The granules of dermal mast cells in a squamous cell carcinoma were studied by the electron microscope. The granules showed 1. subgranular components like those of normal mature granules of human mast cells; 2. relatively coarse granular material with a central dense mass, and concentrically arranged or scrolled lamellae; 3. various pathological figures, *i.e.* concentrically arranged, myelin-like lamellar structures, and grouped dense particles, dense bands, and central dense cores. Membrane-enclosed granules were found at the rates of 3.1–6 % near or in the cell infiltrate, and 14 % in the epidermis.

REFERENCES

1. Cawley, E. P., and Hoch-Ligeti, C.: Association of tissue mast cells and skin tumors. *AMA Arch. Derm. 83:* 145–50, 1961.
2. Kobayasi, T., Midtgård, K., and Asboe-Hansen, G.: Ultrastructure of human mast-cell granules. *J. Ultrastructure Res. 23:* 153–65, 1968.
3. Kobayasi, T., and Asboe-Hansen, G.: Ultrastructural studies of mast-cell granules in basal cell carcinoma. *Acta derm.-venereol.* In press.
4. Lascano, E. F.: Mast cells in human tumors. *Cancer 11:* 1110–4, 1958.