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# DEGRANULATION AND REGRANULATION OF HUMAN MAST CELLS

## An electron microscopic study of the whealing reaction in urticaria pigmentosa

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In pigmented lesions of urticaria pigmentosa whealing can be produced about one minute after stroking with a blunt instrument. This phenomenon called urticarial dermographism is considered to be brought about by release of histamine from tissue mast cells. The structural equivalent to the release of histamine is a release of granules from tissue mast cells to the extracellular compartment.

Previous electron microscopic studies of degranulation were performed on skin of urticaria pigmentosa at 3 minutes (3) and 15 minutes (4, 5) after stroking, while the development of the degranulation process with the lapse of seconds or minutes has not been satisfactorily investigated. In the present study, the processes of degranulation and regranulation were observed within very short periods.

### Material and Method

Skin samples of eight patients with dermal mastocytosis (urticaria pigmentosa) were studied by means of the electron microscope. Ten pigmented macules or papules of eight patients were stroked with a blunt steel instrument and biopsied with a 4 mm punch without anesthesia. As controls, eight non-traumatized macules or papules of the symmetrical regions were also biopsied, one from each patient. The time interval between stroking and the removal of the skin sample were 30 seconds, 1 minute, 2 minutes, 5 minutes, and 10 minutes. The tissue specimens were divided by a sharp knife into 9 pieces of about 1 × 1 mm.

The specimens were immediately fixed (overnight) in a 4 % glutaraldehyde solution in veronal-acetate buffer pH 7.2 with 4.5 % sucrose at 4°C. The specimens were then washed in the same buffer at 4°C overnight, fixed again in a 1 % solution of osmic acid in the same buffer at 4°C for one hour and subsequently washed in the buffer. After dehydration, the specimens were embedded in Epon 812 and sectioned with an ultramicrotome.[1] Ultrathin sections were stained with uranyl acetate and lead citrate and studied by means of the electron microscope[2] at 80 kV and with a double condensor system.

The mast-cell granules were classified by their structural figures, and the various categories as well as the villi were listed in Fig. 1. The areas where the components in question were located were measured on a transparent graphic sheet covering electron micrographs magnified 60,000 times, and each component was calculated per unit area ($\mu^2$). Fig. 1 indicates the number of the components per $\mu^2$ of unstroked specimens. The number per $\mu^2$ of every

[1] Reichert Om U2.

[2] Siemens "Elmiskop I A".

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stroked specimen was divided by that of the corresponding control to show the quantitative variations with the lapse of time (Fig. 2).

## Observations

The components of the mast cells were mature, immature, abnormal immature, disintegrating and extracellular granules. Also empty spaces and honey-comb like areas as well as villi were demonstrated (see 6). Fig. 1 shows that the distribution of the components per $\mu^2$ in unstroked lesions of seven cases of urticaria pigmentosa varied considerably. The variations during the response to stroking appear from Fig. 2. A degranulation and a regranulation were demonstrated in the middle layer of the corium, while, in the uppermost and deeper corium, the mast cells showed no distinct changes after stroking (Fig. 4, 5, 6, 7). The degranulation and the regranulation were evident at thirty seconds (Fig. 3), and reached a peak at one minute after stroking (Fig. 2). From two to five minutes (Fig. 8, 9 A) after the trauma, the mast cells were in a resting phase in which, evidently, the extracellular granules were dissolved in the ground substance. Mast cells in the resting phase (Fig. 9 A) resembled mast cells of normal skin (Fig. 9 B), both containing mature granules. Besides, longer villi and extraordinarily numerous mitochondria were demonstrated, and the crystalline structures of the mature granules were less evident. After the resting phase, the mast cells started regranulation, which occurred simultaneously with a degranulation.

Two minutes after stroking, macrophages were found in the areas where dissolved granule material was demonstrated (Fig. 8). These cells contained occasional melanin granules; two mast-cell granules were seen in the cell body of one macrophage. After ten minutes the above-mentioned components were increased in number. In the extracellular space no dissolved granule material was found (Fig. 10).

The ultrastructural changes produced by stroking were as follows. The mature granules showed few and indistinct crystalline patterns and less fine granular material, while there were no pronounced changes in the lamellae. The abnormal immature granules revealed profiles resembling to some extent those of normal immature granules. Unlike these, they were enclosed by distinct membranes, and besides they contained dense fine granular material as well as distinct or blurred lamellae. The lamellae showed unparallel as well as definitely parallel arrangements (Fig. 14). The abnormal immature and the disintegrating granules showed no pronounced changes after stroking.

The mast cells degranulate either by extrusion of whole granules (Fig. 13) or by intracellular dissolution of the granule material leaving honey-comb like areas behind in the cytoplasm (Fig. 3). After stroking, the honey-comb like patterns showed figures similar to those before stroking, but the extracellular granules were different. Their outlines could not be discerned, and they were immediately surrounded by amorphous material. This was in contrast to the distinct outlines of the extracellular granules before stroking (Fig. 11, 12).

After stroking, the villi showed marked variations in length. At one minute, they were elongated (Fig. 6, 12). At five minutes, they were the shortest of all experimental and control samples, but still longer than those in normal skin (Fig. 9 A, B). At thirty seconds, two and ten minutes the lengths of the villi were almost the same as in the controls. At one, two and five minutes occasional bifurcations and irregular widths were noticed (Fig. 6, 8, 9 A).

The mitochondria were few and small at thirty seconds (Fig. 3), while numerous round or oblong forms were found at one, two to five and at ten minutes after stroking (Fig. 6, 8, 9 B, 10, 12). The latter revealed regular cristae and a double membrane. Widened Golgi zones were found at one, two and ten minutes after stroking (Fig. 6, 8, 10), at one minute mainly consisting of vesicles in a relatively disorderly array (Fig. 12). Ribosomes and granular endoplasmic reticulum were distinct at one, two and ten minutes after stroking.

## Discussion

The ratio of each component was calculated, because the distribution of the components in unstroked samples varied considerably between different indviduals (Fig. 1). The response at *thirty seconds* seemed to be represented by a degranulation, mainly whole-granule extrusion, and an increasing new-formation of granules. Low values of mature granules, and high values of extracellular and non-mature granules, as well as low values of disintegrating granules and honey-comb like patterns, support this interpretation. At *one minute*, the decreased ratios of non-mature granules and more or less increased ratios of mature granules indicate a tendency towards maturation, while the increased ratios of disintegrating granules and honeycomb like patterns suggest an accelerated dissolution of the granules. The increased value of extracellular granules is evidence of accelerated extrusion of whole granules. Indistinct outlines of the extracellular granules at one minute after stroking suggest an accelerated dissolution. Thus, both whole-granule extrusion and dissolution of granules appear to be stimulated by the trauma.

Previous electron microscopic studies revealed halos around the granules 15 minutes after stroking (4, 5) similar to the perigranular halos seen after injection of the chemical histamine liberator 48/80 (7, 8) and after local infiltration anesthesia of normal human skin (5). In this study, no perigranular halos were seen after stroking. Only after local anesthesia could halos be demonstrated (Fig. 9 B).

That no degranulation was found in the uppermost corium remains unexplained. The cause of the variation in the shapes of the villi is obscure, and the question whether they have any relation to cell activity or to degranulation is unanswered.

The mast-cell granules are possibly formed in the Golgi zone (1, 2, 6). A widening of this area, as well as the presence of numerous mitochondria and granular endoplasmic reticulum may well represent a regranulation process. Other authors (2, 7, 9) have described phagocytosis of rat mast-cell granules by macrophages, eosinophiles and neutrophiles. In the present study mast-cell granules were recognized in the cell body of only one macrophage. A reason for this may be that within two minutes most of the extruded granules are dissolved in the extracellular ground substance, appearing no longer as particles.

## SUMMARY

The response of human mast cells to stroking was studied by means of the electron microscope in skin samples of 8 patients with urticaria pigmentosa. Thirty seconds after stroking degranulation and regranulation were noticed and at one minute maximum degranulation was demonstrated. The mast cells degranulated by extrusion of whole granules and, subsequently, by intracellular dissolution of granule material. The dissolution of the granules in the extracellular ground substance was accelerated. This phase was followed by a resting phase two to five minutes after stroking. After five to ten minutes the mast cells showed regranulation and degranulation. Two minutes after stroking macrophages were found in the area where mast-cell granules were dissolved in the extracellular material. A widened Golgi area, numerous mitochondria and granular endoplasmic reticula were also found at one, two and ten minutes. These phenomena were considered to represent the regranulation process.

## Acknowledgements

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## REFERENCES

1. Combs, J. W.: Maturation of rat mast cells. An electron microscope study. *J. Cell. Biol. 31:* 563–75, 1966.
2. Fawcett, Don W.: An experimental study

of mast cell degranulation and regranulation. *Anat. Rec. 121:* 29–51, 1955.
3. Freeman, R. G.: Diffuse urticaria pigmentosa. An ultrastructural study before and after whealing. *Am. J. Clin. Path. 48:* 187–99, 1967.
4. Hashimoto, K., Gross, B. G. and Lever, W. P.: An electron microscopic study of degranulation of mast-cell granules in urticaria pigmentosa. *J. Invest. Derm. 46:* 139–49, 1966.
5. Horiki, M.: Fine structure of mast cells in urticaria pigmentosa. *Rynshyo Hifuka 21:* 403–11, 1967. (Japanese.)
6. Kobayasi, T., Midtgaard, K. and Asboe-Hansen, G.: Ultrastructure of human mast-cell granules. *J. Ultrastruct. Res. 23:* 153–65, 1968.
7. Michel-Singleton, E. and Clark, S. L.: The response of mast cells to compound 48/80. Studied with the electron microscope. *Lab. Invest. 14:* 1744–63, 1965.
8. Orfanos, C. and Stüttgen, G.: Elektronenmikroskopische Beobachtungen zur Mastzellendegranulation bei der diffusen Mastocytose des Menschen. *Arch. klin. exp. Derm. 214:* 521–48, 1962.
9. Welsh, R. A. and Geer, J. C.: Phagocytosis of mast cell granules by the eosinophilic leucocyte in the rat. *Am. J. Path. 35:* 103–11, 1959.

# FIGURES

*Figs. 1 and 2. Mature granules:* Intracellular granules which are not enclosed by a distinct membrane and show lamellae and fine dense granular material (6). *Non-mature granules:* Normal immature and abnormal immature granules. *Disintegrating granules:* Intracellular granules which are enclosed by a distinct single membrane and contain destroyed lamellae and coarse granular material (6). *Honey-comb like patterns:* A pattern of intracellular spaces surrounded and crossed by distinct single membranes. Some spaces contain remnants of granules. *Extracellular granules:* Granules found outside the double cell membrane, *i.e.* in the extracellular compartment. *Villi:* Cytoplasmic protrusions bordered by a double cell membrane. *The cell area* within which the counted subcellular components are located includes the entire cytoplasmic area plus a pericellular zone surrounding the cell body. This zone includes villi and dissolving granules, but no dermal fibers. The counted area was around 800 $\mu^2$ for each specimen. The ratio represents

$$\frac{\text{Calculated value of each experimental sample}}{\text{Calculated value of the corresponding control sample}}$$

*Fig. 3.* Urticaria pigmentosa mast-cell response in the middle dermis thirty seconds after stroking. Six mast cells are shown. Granules appear both intra- and extracellularly. Honey-comb like pattern (H). Villi (V). × 6000.

*Figs. 4, 5, 6, and 7.* Urticaria pigmentosa mast cells of one and the same tissue sample, but at different depths one minute after stroking.

*Fig. 4.* From upper part of the corium. EP indicates epidermal cells. The mast cells in the uppermost part of the corium show no degranulation, and the villi are long and narrow. In the lower left corner, the mast cells show disintegrating granules, and some extracellular granules are found (arrows). × 6000.

*Fig. 5.* Higher power of framed area of Fig. 4. Most of the granules are mature. No extracellular granules and one distintegrating granule are seen (arrow). EP indicates epidermal cells; G Golgi zone; M mitochondria. × 24,000.

*Fig. 6.* Mast cells of the middle layer of the corium. Note numerous and distinct villi and extracellular granules (arrows). G indicates Golgi zone; M mitochondria. × 6000.

*Fig. 7.* Mast cells of the deep corium. No extracellular and no disintegrating granules are seen. × 6000.

*Fig. 8.* Two mast cells of the middle dermal layer two minutes after stroking. Both contain mature granules, mitochondria and villi. Four macrophages (P) and fine granular homogeneous extracellular granule-material (H) are seen in the corium. × 6000.

*Fig. 9 A.* A mast cell of the middle layer of the corium five minutes after stroking. No halos were found around the granules, and no disintegrating nor extracellular granules. The villi are longer than those in Fig. 9 B. Several mitochondria are seen. The specimen was removed without anesthesia. × 6000.

*Fig. 9 B.* A mast cell of normal skin. The specimen was biopsied five minutes after infiltration with lidocaine®. Around all granules note light halos probably produced by anesthesia. The granules are mature, and the villi are few and short. × 6000.

*Fig. 10.* Urticaria pigmentosa mast cells of the middle corium ten minutes after stroking. Numerous cytoplasmic granules and villi are seen. Parts of macrophages (P) are seen, but no homogeneous material like that in Fig. 8. G indicates Golgi zone; M mitochondria. × 6000.

*Fig. 11.* Urticaria pigmentosa mast cells of the middle dermal layer. These cells are controls to Fig. 12. Most granules show disintegration figures (see legends to Figs. 1 and 2). The extracellular granules (E) show no advanced dissolution. × 24,000.

*Fig. 12.* Mast cells of the middle dermal layer one minute after stroking. Most granules are located extracellularly and in the process of dissolution (E). The dissolution of both intra- and extracellular granules is more pronounced than in Fig. 11. The villi are narrow and longer than those of Fig. 11. Some show bifurcation (arrows). G: Golgi zone showing dilated vesicles. M: mitochondria. × 24,000.

*Fig. 13.* Extrusion of a mast-cell granule (MG). Arrows indicate cell membrane continuing in "granule membrane". V: Villus. ×60,000.

*Fig. 14.* Abnormal immature granules (AG) showing dense granular material and distinct or blurred, parallel or unparallel lamellae. The granules are enclosed by distinct membranes. H indicates partially emptied spaces; D: disintegrating granules; G: Widened Golgi area; M: mitochondria. ×60,000.

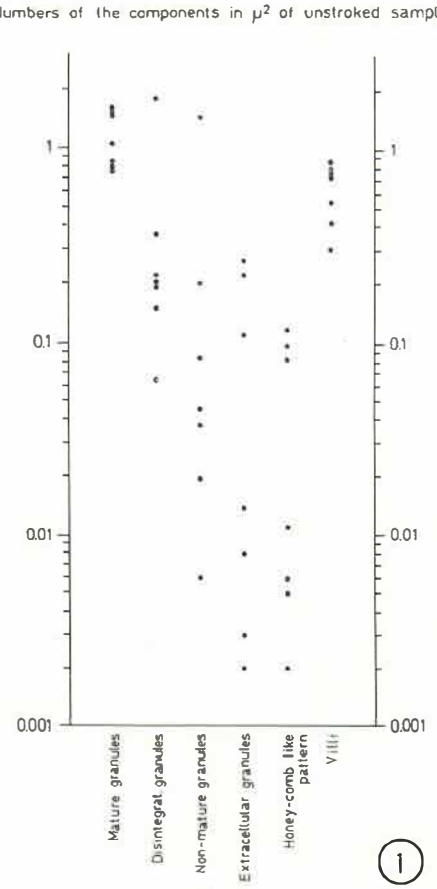

Numbers of the components in μ² of unstroked samples
1
0.1
0.01
0.001
Mature granules
Disintegrat granules
Non-mature granules
Extracellular granules
Honey-comb like pattern
Villi
1

Fig. 1.

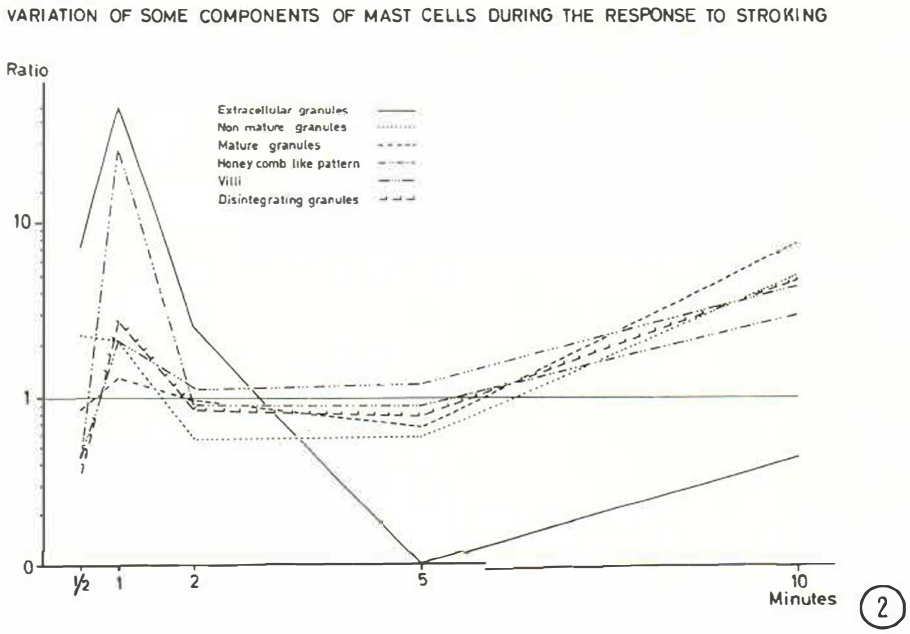
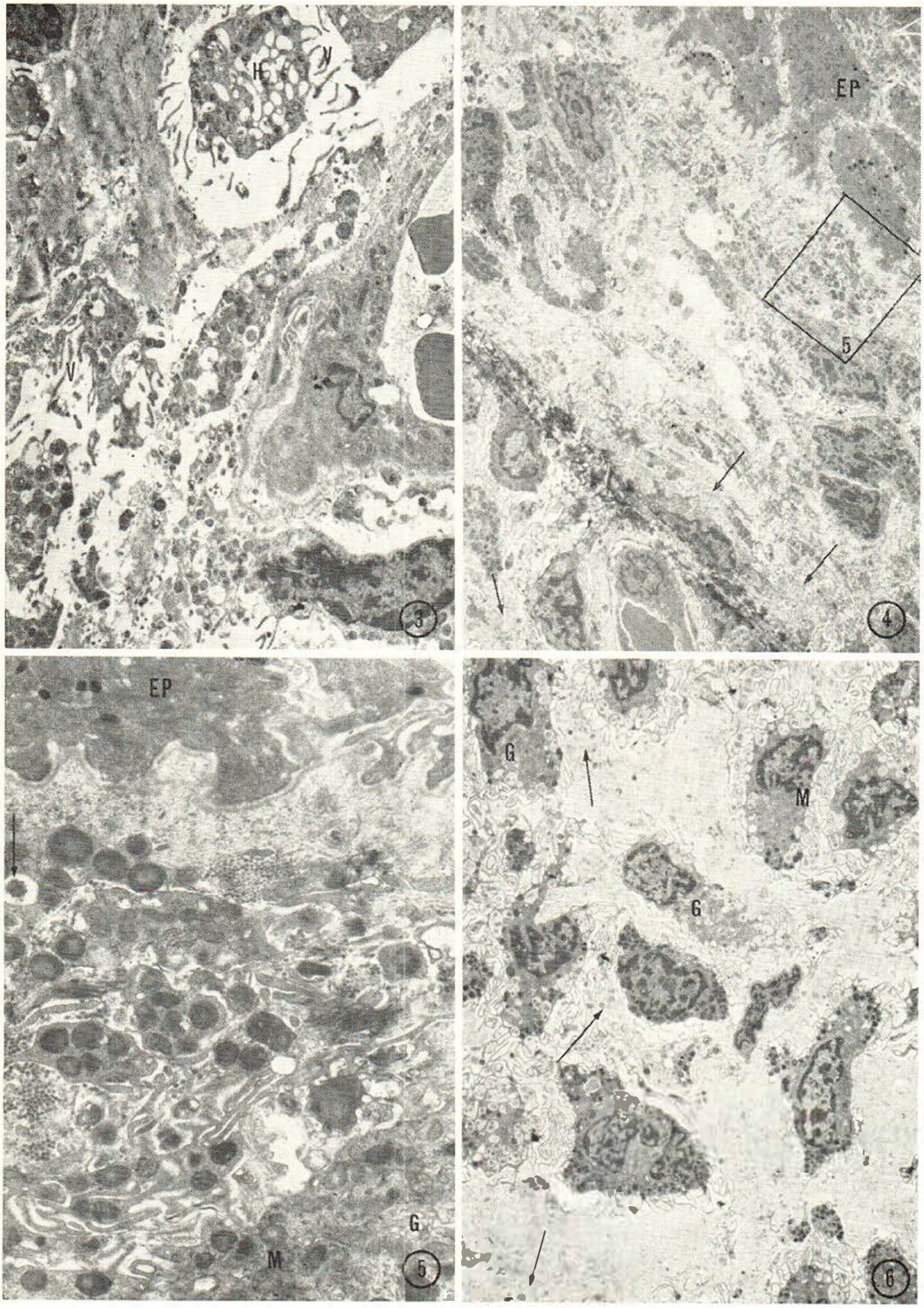
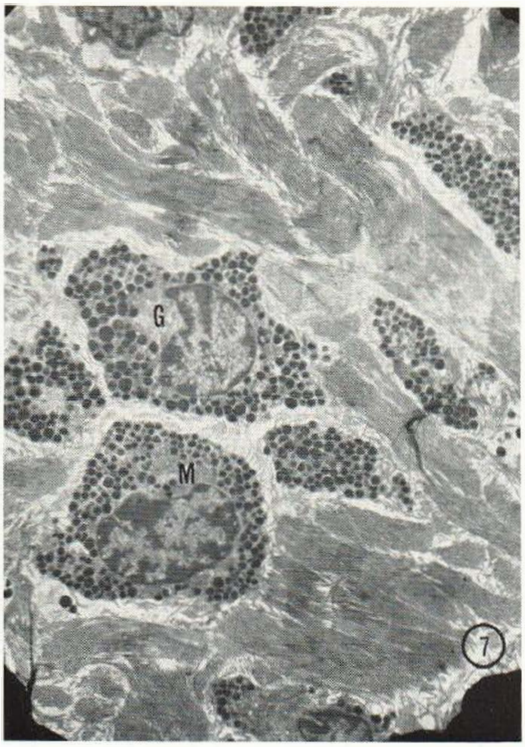
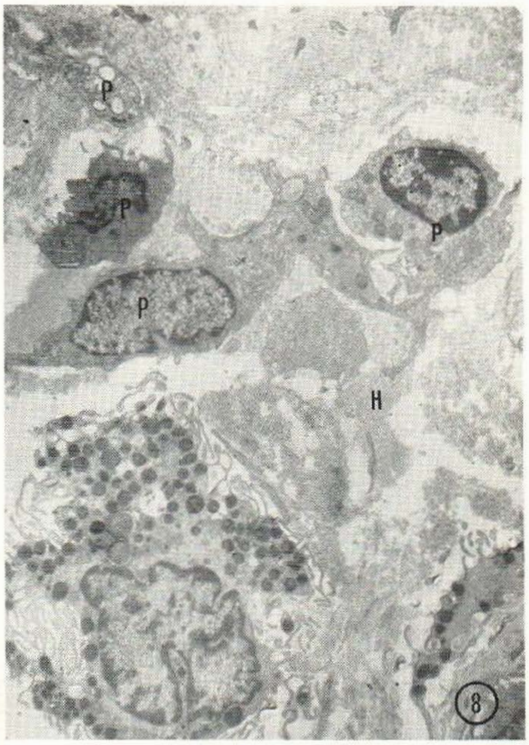

VARIATION OF SOME COMPONENTS OF MAST CELLS DURING THE RESPONSE TO STROKING
Ratio
Extracellular granules
Non mature granules
Mature granules
Honey comb like pattern
Villi
Disintegrating granules
10
1
0
½
1
2
5
10
Minutes
2

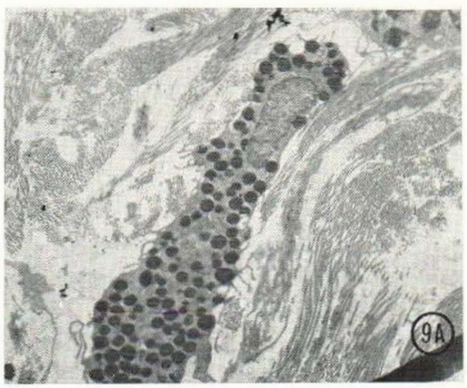
Fig. 2.

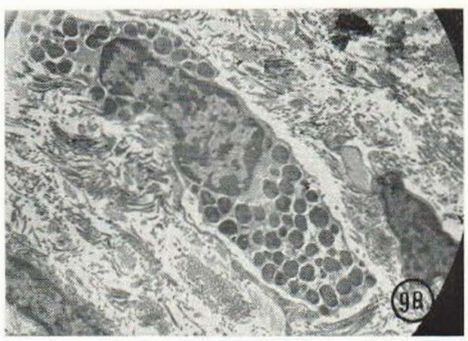


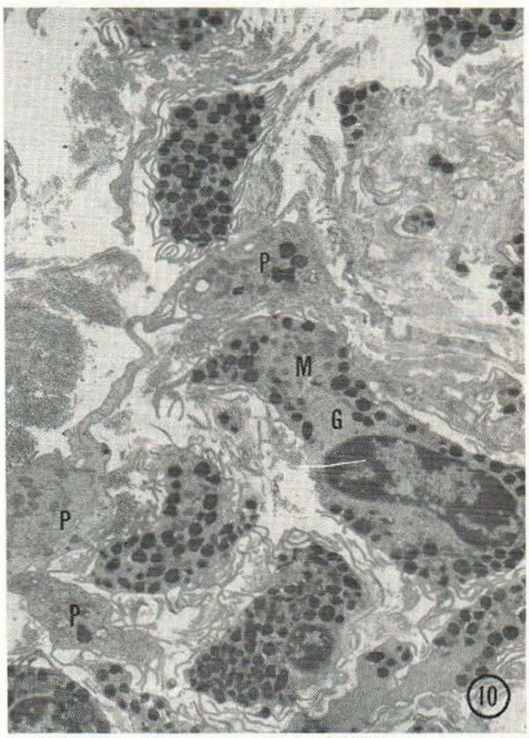

Figs. 3–6.



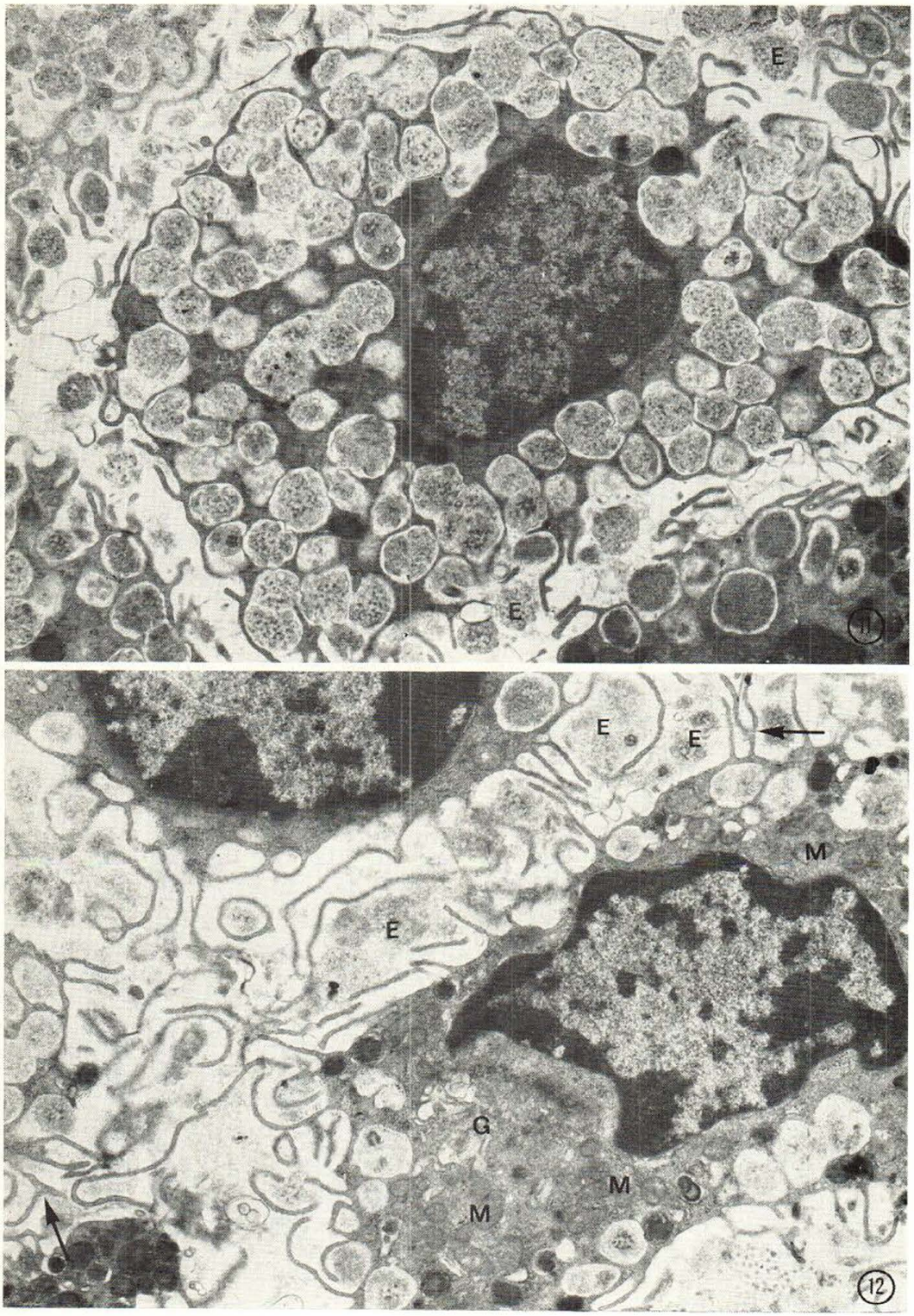



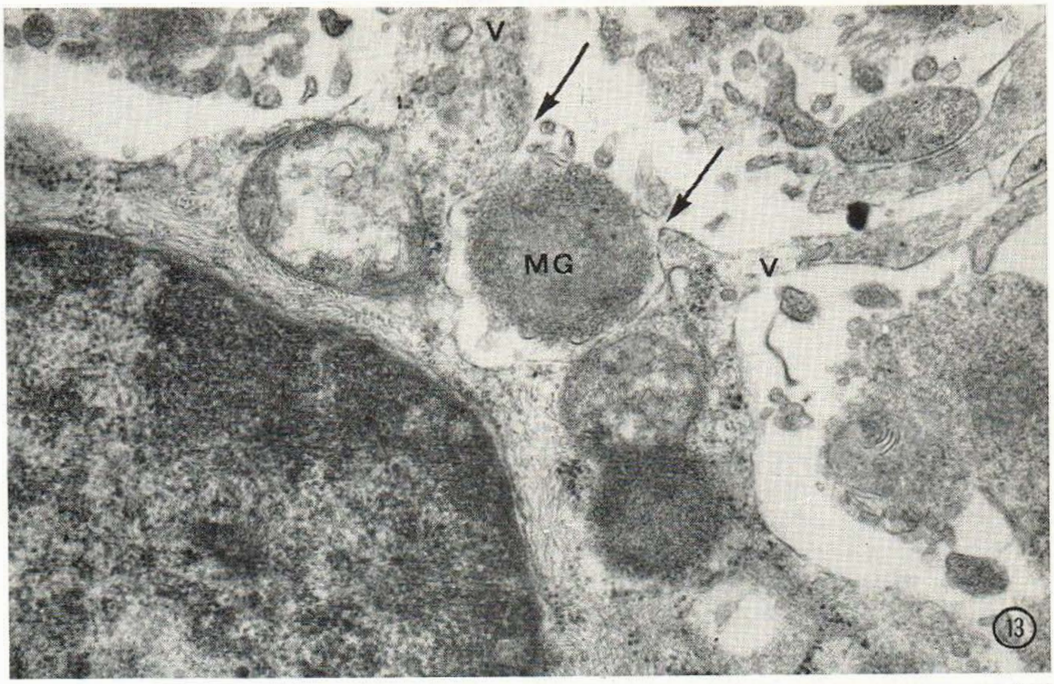



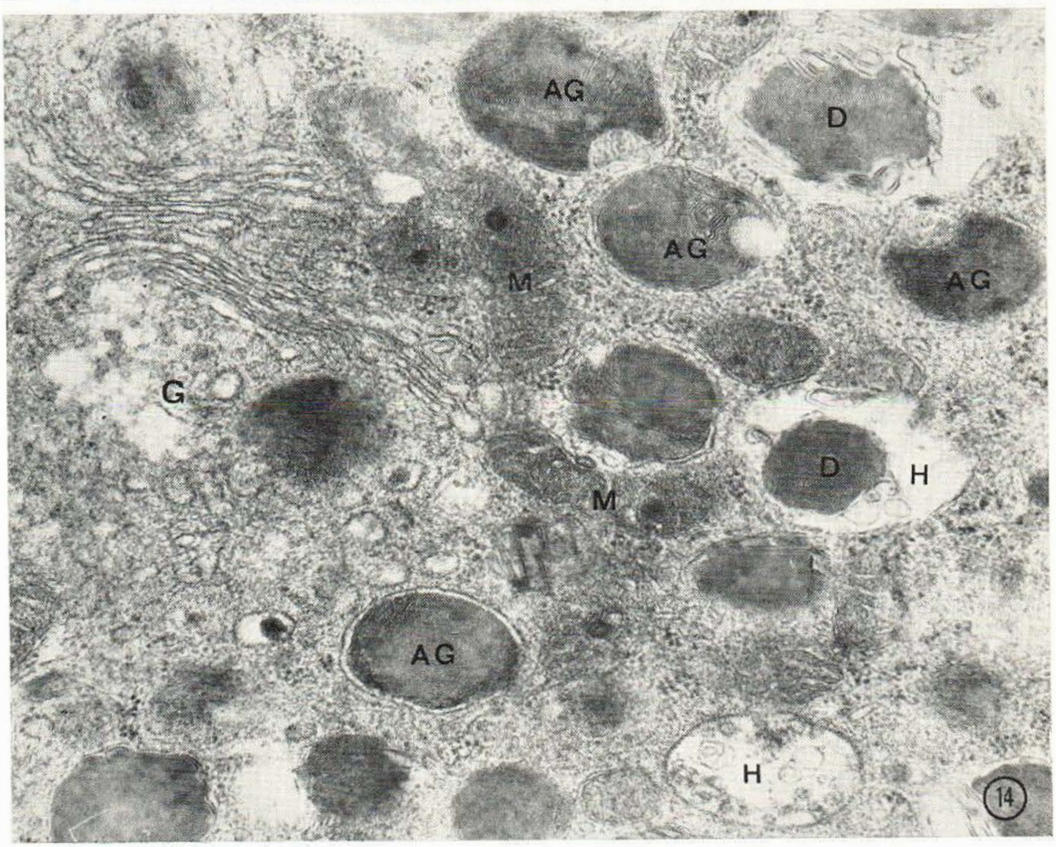




Figs. 7–10.




Figs. 11–12.




Figs. 13–14.