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Mast cells of the human stomach

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INTRODUCTION

Mast cells are widely distributed in the body, and despite earlier doubts as to their occurrence in certain regions of the gastrointestinal tract, they can now be easily demonstrated after appropriate fixation (Enerback, 1966). They have even been observed in the intestinal epithelium (Dobbins, Tomasini & Rollins, 1969).

Previous investigations on the morphological aspects of mast cell function have been performed on animal material under experimental conditions *in vivo* or *in vitro*. The human subject has not been studied. Though degranulation and vacuolation of mast cells subjected to histamine-liberating substances have already been described (Bloom, Larsson & Smith, 1957; Singleton & Clark, 1965), evidence for the occurrence of these processes *in vivo* in the human subject has not been reported.

MATERIAL AND METHODS

Five normal people and 30 patients with gastric ulceration were submitted to gastroscopy, and biopsy material was obtained from four different sites in each case, viz. prepyloric, incisura angularis, high on the lesser curve and high on the greater curve. The material was fixed in 5% cacodylate buffered glutaraldehyde (pH 7·3) for 4–24 hours at 4 °C. It was subsequently washed in cacodylate buffered 10% sucrose for 24 hours at 4 °C and then post-fixed in veronal acetate buffered 1% osmium tetroxide (pH 7·3) for 2 hours at 4 °C, rinsed in chilled tap water and then in chilled 70% ethyl alcohol at 4 °C, dehydrated in a graded series of ethyl alcohol solutions and cleared in propylene oxide. The cleared tissues were embedded in Araldite.

Sections 1 μm thick were cut with a glass knife on an ultramicrotome and stained by the method of Richardson, Jarret & Finke (1960) with methylene blue–Azur II. These sections were examined with a light microscope and, as a result, certain limited areas of the block were selected from which sections approximately 25 nm thick were cut with a glass knife. These were stained with 1% uranyl acetate and Reynold's lead citrate and examined with a Philips 300 electron microscope.

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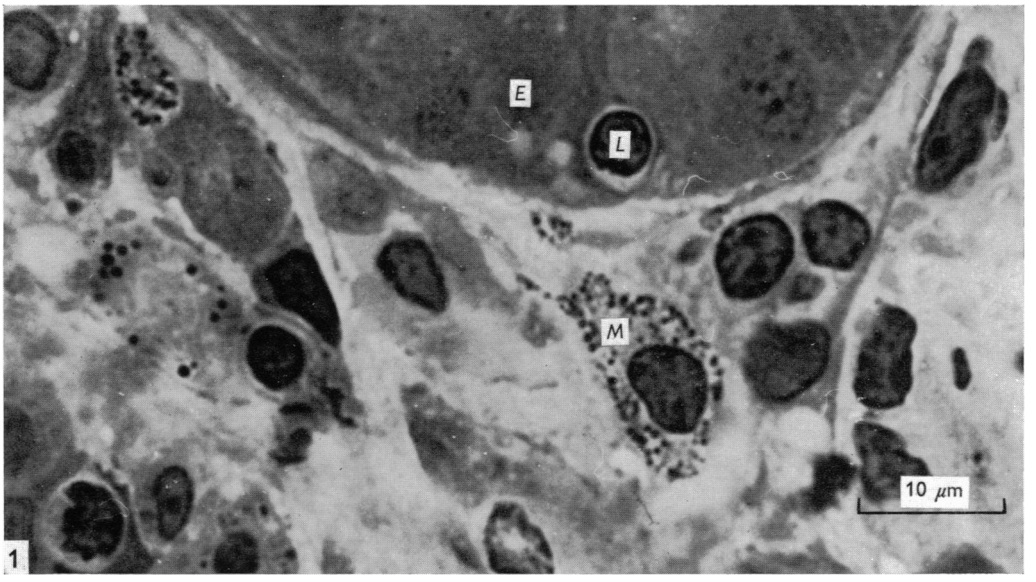

Fig. 1. A mast cell (*M*) in the connective tissue of the gastric mucosa. The mast cell contains numerous cytoplasmic granules. A lymphocyte (*L*) is seen in the epithelium (*E*).

Table 1. *Subepithelial mast cells in the normal human stomach*

Region	Mean percentage of orthochromatic granules compared with the total number of granules	Standard error of the mean	Regions compared	*P* value
Prepyloric	62·6	4·2	Prepyloric/any other region	N.S.
Incisura angularis	53·9	4·0	—	—
High lesser curve	69·6	5·2	Incisura angularis/ high lesser curve	0·05
High greater curve	69·8	4·7	Incisura angularis/ high greater curve	0·02

RESULTS

Light microscopy

Mast cells were present in all the regions of the stomach which were studied. The mast cells in the subepithelial layer were of irregular outline (Fig. 1) and were easily identified by the metachromatic granules in their cytoplasm. The granules varied in size and were stained either dark blue or red. The granules of some mast cells were almost all dark blue, whereas in other cells there was a varying proportion of red granules. The distribution of the two types of granule varied in the different regions of the stomach. In the normal stomachs there was a statistically significant difference

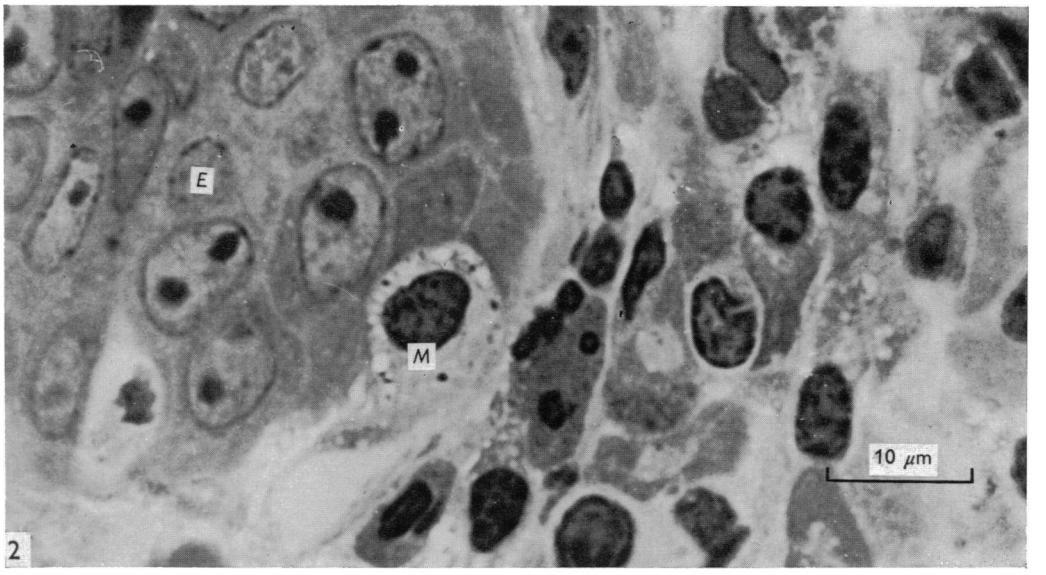

Fig. 2. A mast cell (*M*) is situated in the basal region of the gastric epithelium (*E*). The cytoplasm of the mast cell contains numerous vacuoles as well as some granules.

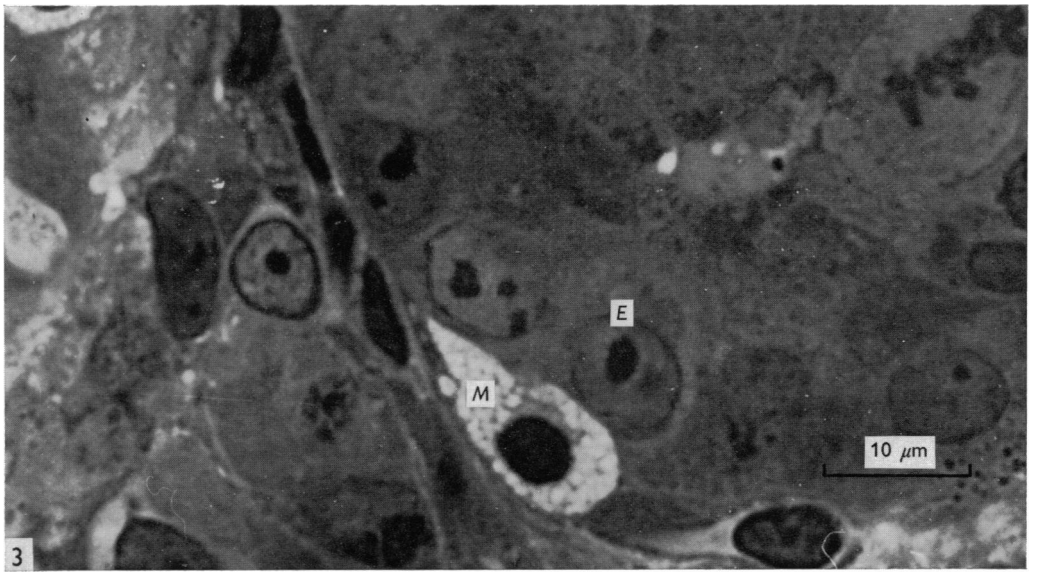

Fig. 3. The mast cell (*M*) in the basal region of the gastric epithelium (*E*) is devoid of mast cells granules and the cytoplasm appears to be full of vacuoles.

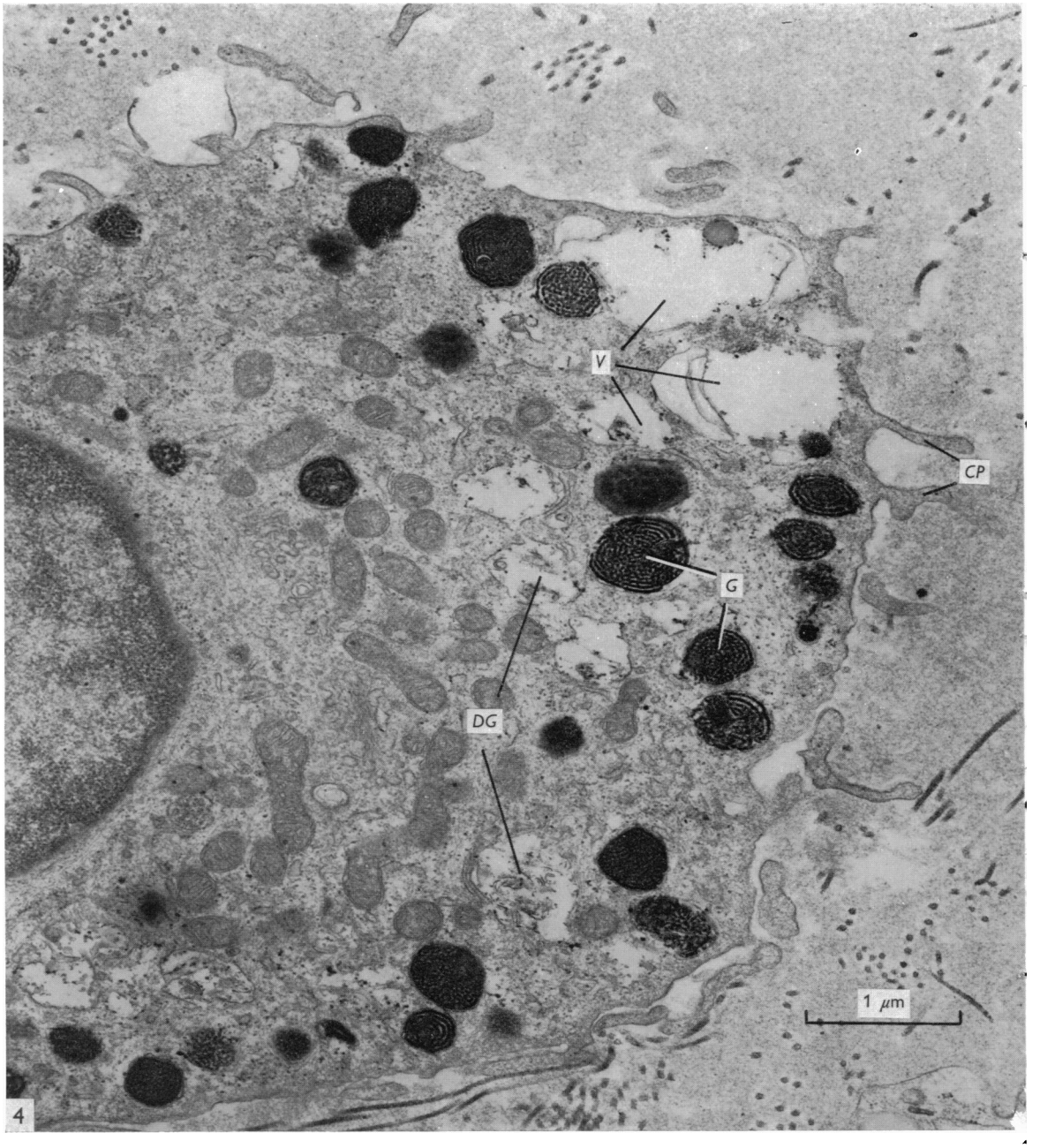

Fig. 4. A connective tissue mast cell with numerous mast cell granules (*G*). The mast cell has numerous cytoplasmic processes (*CP*) projecting into the surrounding connective tissue. Occasional mast cell granules communicate with cytoplasmic vacuoles (*V*) and such vacuoles contain disintegrating granular components (*DG*) or a particulate material.

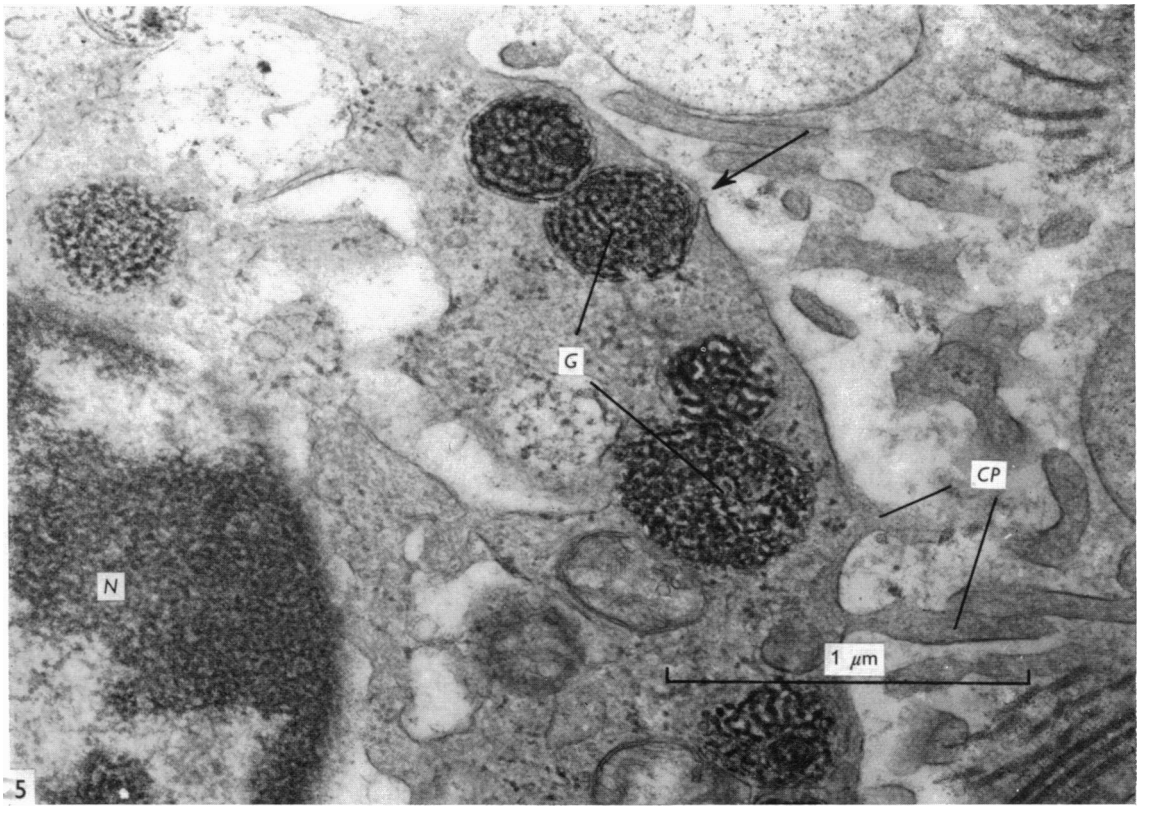

Fig. 5. Connective tissue mast cell with part of the nucleus (*N*) shown. Cytoplasmic processes (*CP*) and mast cell granules (*G*) are apparent. At one place (marked by the arrow), the limiting membrane of a mast cell granule is fused with the cell membrane.

(Student's *t* test) between the subepithelial mast cells at the incisura angularis and those high on the lesser curve and high on the greater curve (Table 1). Cytoplasmic vacuoles were seen occasionally in the mast cells of the subepithelial layer.

Cells which had certain features characteristic of mast cells were found in the epithelial layer. These cells contained granules, some of which stained metachromatically; they showed a higher content of cytoplasmic vacuoles than the subepithelial mast cells (Fig. 2), and all these vacuoles contained a metachromatically staining material. The greater the number of vacuoles in these cells, the fewer the number of granules (Fig. 3). The nuclei of the sparsely granulated cells were densely stained (Fig. 3). Occasional cells containing only vacuoles were seen in the lumen of the gastric glands.

Electron microscopy

Mast cells in the subepithelial connective tissue were of irregular outline, with numerous cytoplasmic processes up to 1 μm in length extending from their surface (Figs. 4, 5). They were not associated with a basement lamina. The cytoplasm

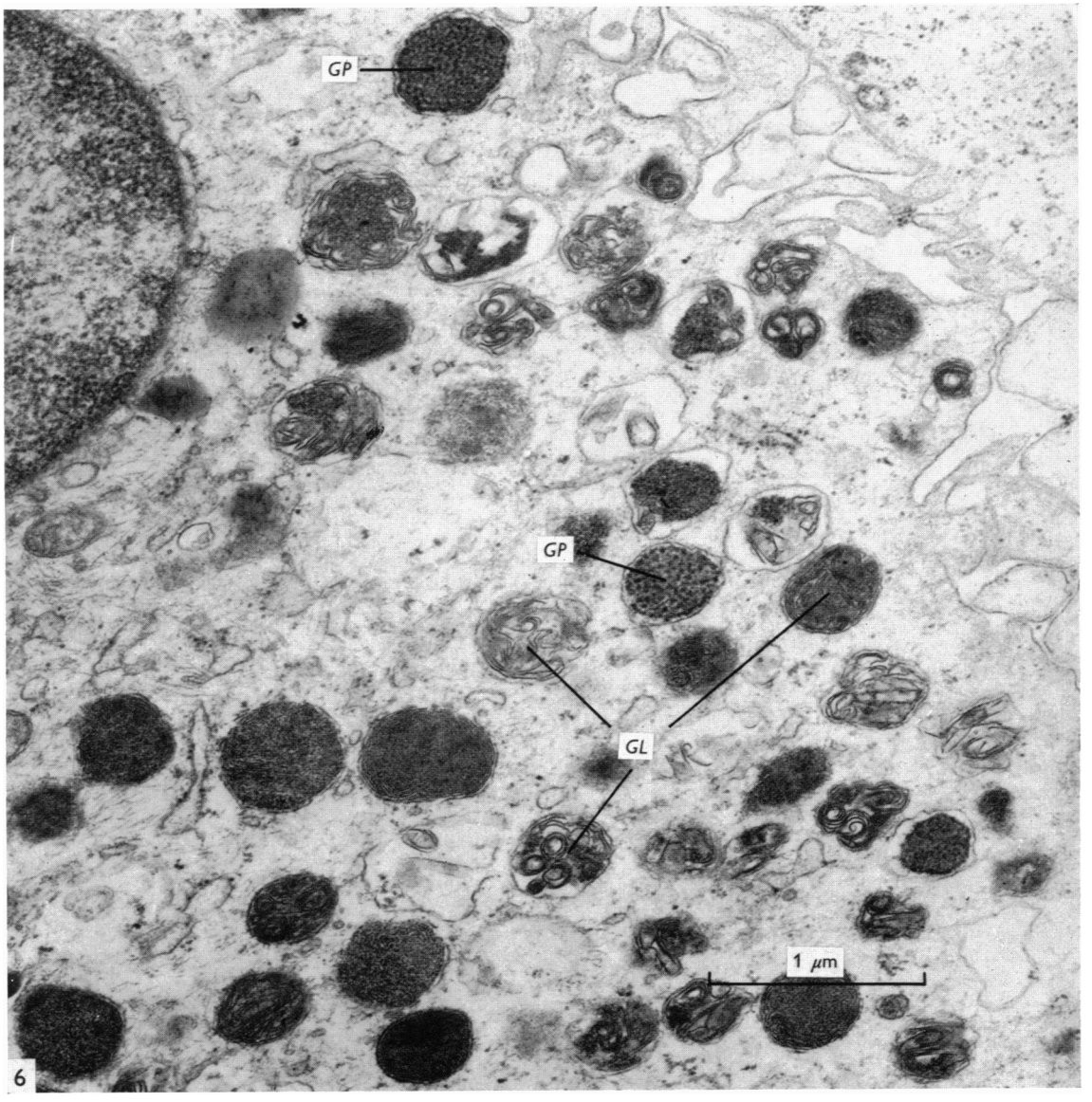

Fig. 6. A connective tissue mast cell with numerous cytoplasmic granules. Some have a particulate structure (*GP*) whereas others have a lamellar arrangement (*GL*).

exhibited rough and smooth surfaced endoplasmic reticula, mitochondria, lysosomes, characteristic granules (Fig. 6) and vacuoles. The granules, approximately 0·2 to 0·8 μm in diameter, were membrane-bound (Figs. 5–7) and showed particulate and lamellar components. The constituent particles were about 15 nm in diameter. Some mast cells contained granules with a predominance of one or other type of component. The cell membrane was sometimes fused with the limiting membrane of a granule

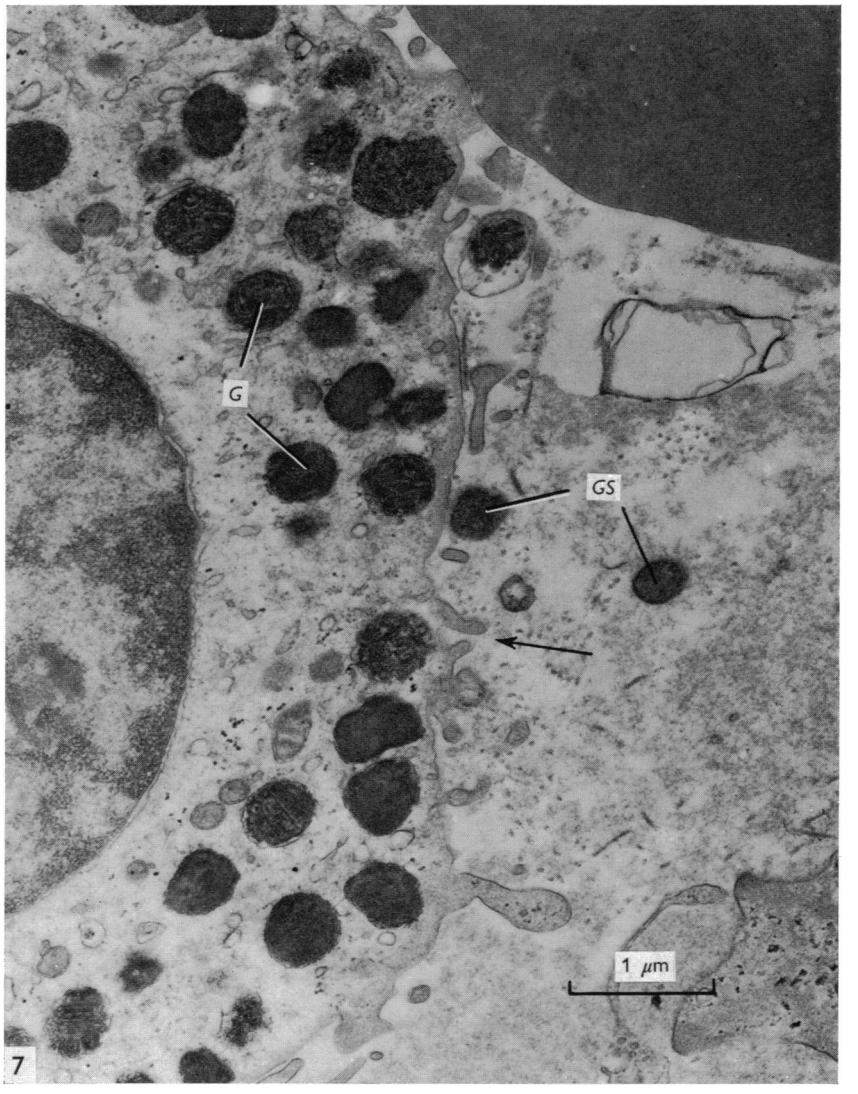

Fig. 7. A subepithelial mast cell with numerous cytoplasmic granules (*G*). One lamellar type granule appears to be in the process of being shed into the connective tissue at the break in the surface membrane indicated by the arrow. Shed granules (*GS*) can be seen in the connective tissue.

so that the granular content was in communication with the surrounding connective tissue (Fig. 5). Some granules which had apparently been shed were lying within the connective tissue (Fig. 7). Vacuoles, bounded by a membrane approximately 7 nm thick, were occasionally seen in the subepithelial mast cells (Fig. 4). These contained the remnants of disintegrating granules or were connected to disintegrating granules.

A gradation was seen between the subepithelial mast cells and cells found in the

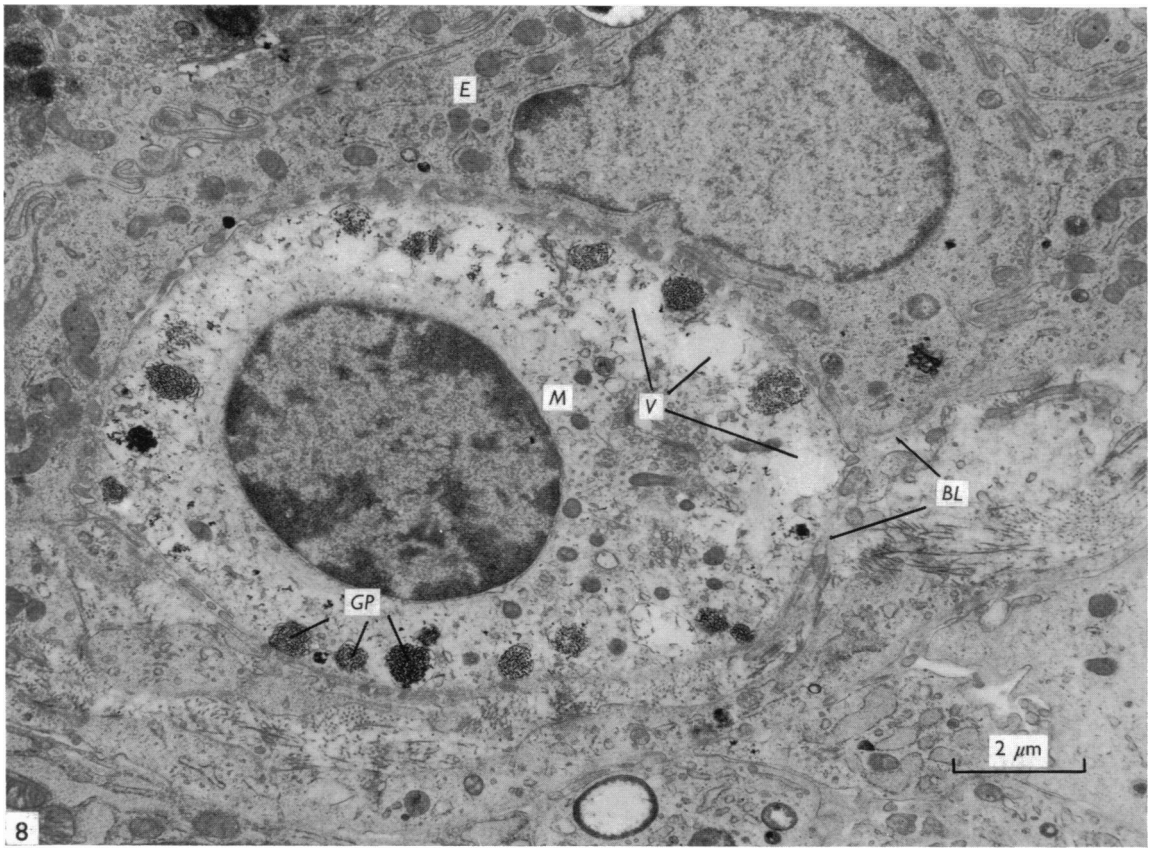

Fig. 8. A mast cell (*M*) which is situated between the gastric mucosal epithelium (*E*) and the underlying basement lamina (*BL*). The mast cell contains granules (*GP*) with a particulate substructure, and also vacuoles (*V*). The vacuoles contain the remnants of disintegrated mast cell granules.

basal region of the gastric epithelium (Figs 8, 9). Cells having ultrastructural characteristics of mast cells and identical to those already described in the subepithelial connective tissue were seen breaching the basement lamina separating the epithelium from the subepithelial connective tissue. These cells in the basal region of the gastric epithelium possessed numerous cytoplasmic processes some of which penetrated the underlying basement lamina (Fig. 10). One notable difference between these intraepithelial cells and the mast cells of the subepithelial connective tissue was their increased content of vacuoles and the presence of the remnants of disintegrated granules. The granules which were present had a particulate substructure, with subunits of about 15 nm diameter. The limiting membrane was frequently continuous with that of a cytoplasmic vacuole and such vacuoles were occasionally in continuity with the cell membrane. The process of granule disintegration with the formation of additional cytoplasmic vacuoles apparently continued until the cell became

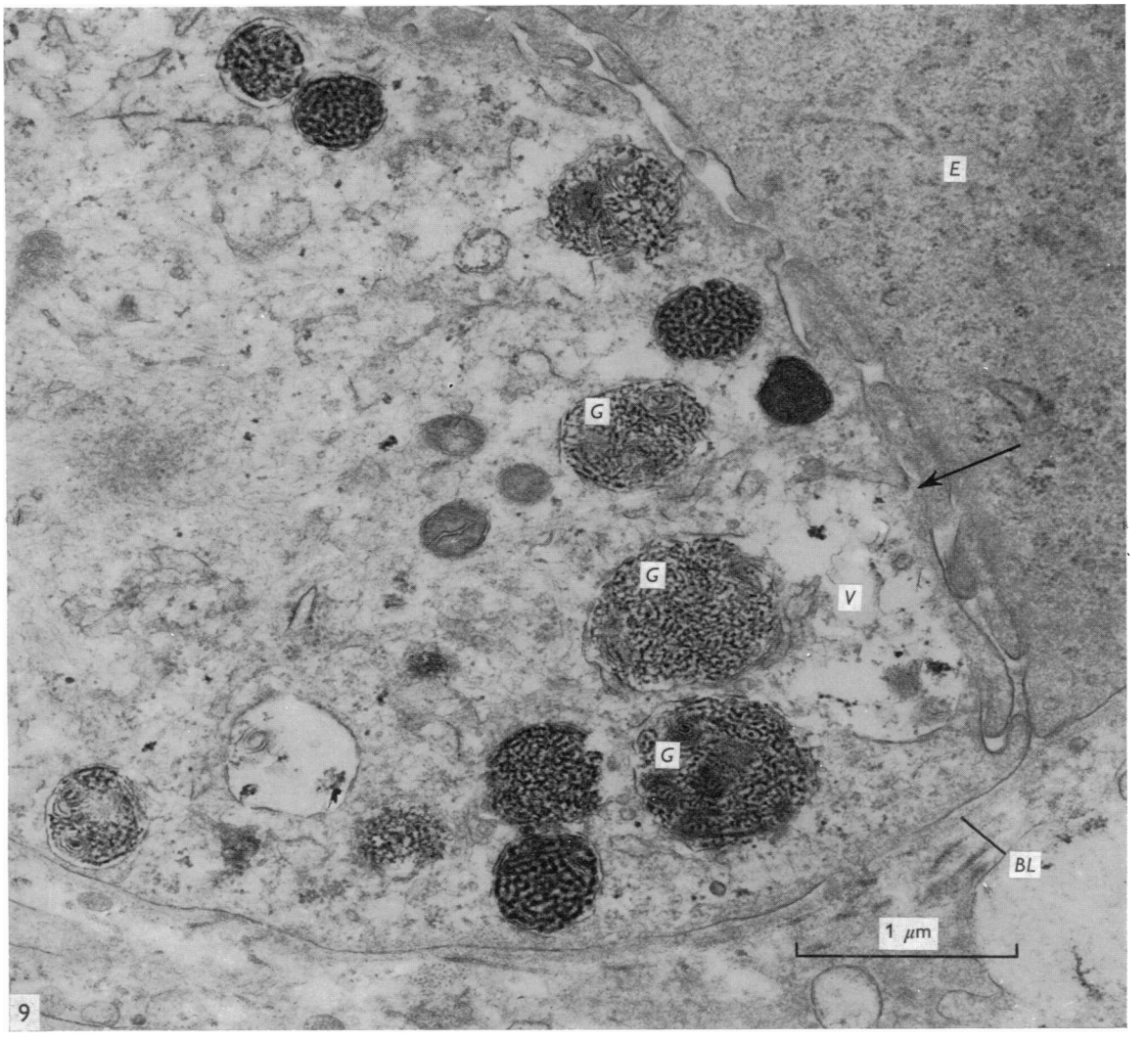

Fig. 9. A mast cell which lies between the gastric mucosal epithelium (*E*) and the basement lamina (*BL*). Mast cell granules (*G*) are seen with a particulate and lamellar substructure. The limiting membrane of a granule is continuous with that of an adjacent vacuole (*V*). This vacuole contains material from a disentegrating mast cell granule and is continuous with the cell membrane at the place indicated by the arrow.

devoid of granules (Fig. 11) and the cytoplasm was filled with membrane-bound vacuoles. There was no evidence of the shedding of intact granules from these intraepithelial cells.

DISCUSSION

The cells described in the present study were identified as mast cells on the basis of several criteria. They stained metachromatically, showed granules with characteristic ultrastructural features, did not possess a basement lamina and were normally

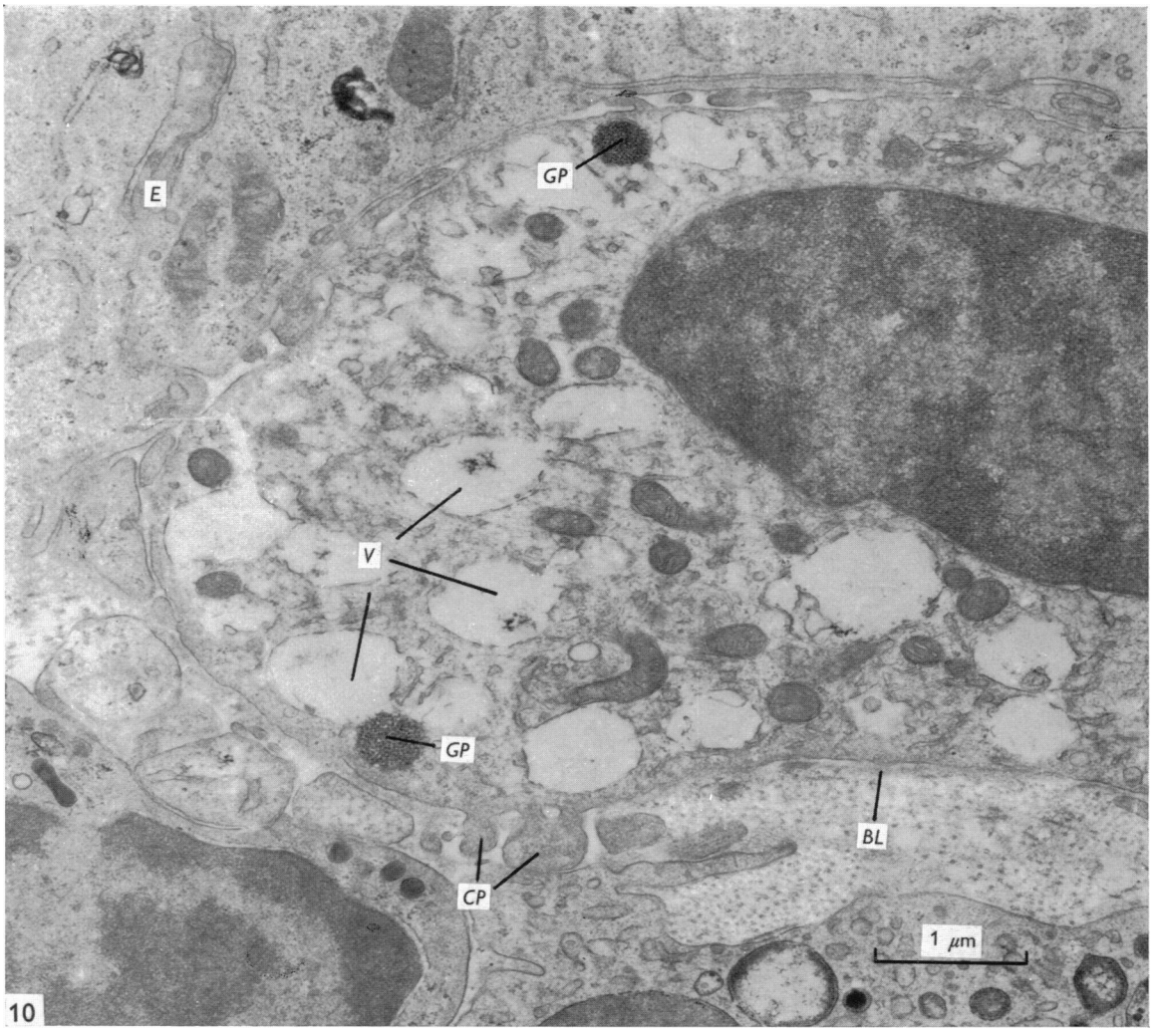

Fig. 10. A mast cell which lies between the gastric mucosal epithelium (*E*) and the basement lamina (*BL*). Cytoplasmic processes (*CP*) from the mast cell project through this basement lamina. The mast cell contains two mast cell granules (*GP*) with a particulate substructure and numerous vacuoles (*V*).

found in connective tissue. Both with the light microscope and with the electron microscope a gradation was found between the typical mast cells seen in the sub-epithelial connective tissue and certain cells found in the gastric epithelium. For this reason, and also because they contained some of the ultrastructurally characteristic granules, these intraepithelial cells were regarded as mast cells. Mast cells have already been observed in the intestinal epithelium (Dobbins, Tomasinif & Rollins, 1969).

Previous investigators have produced evidence for variation in mast cell granules (Bloom & Haegermark, 1965; Enerback, 1966; Hibbs, Phillips & Burch, 1960) and

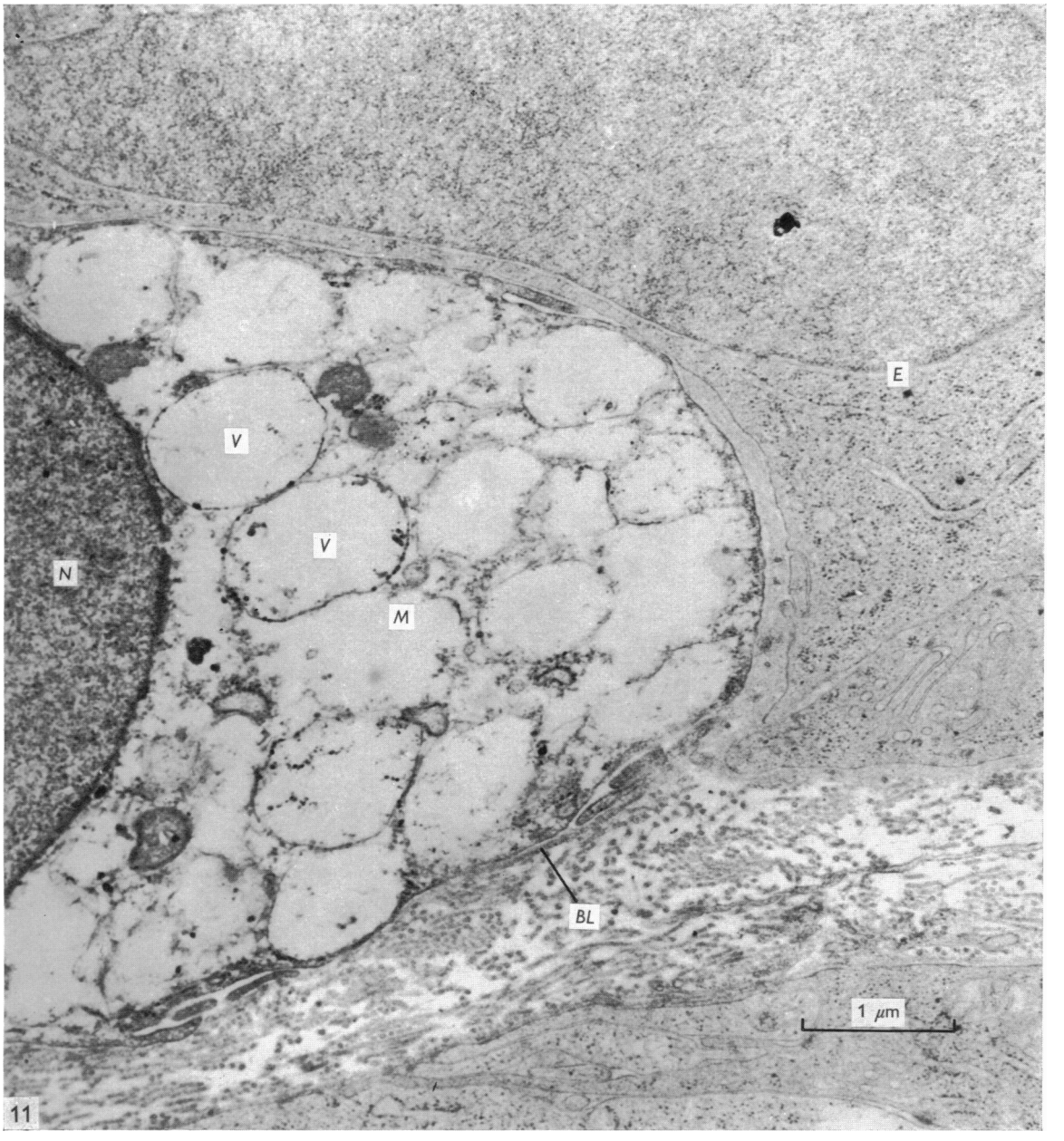

Fig. 11. A mast cell (*M*) which lies between the gastric mucosal epithelium (*E*) and the basement lamina (*BL*). The nucleus (*N*) of the mast cell is markedly electron-dense. The mast cell cytoplasm shows numerous vacuoles (*V*) which contain remnants of disintegrated mast cell granules.

now in human gastric mucosa two types of mast cell granule have been demonstrated by methylene blue-Azur II staining and with the electron microscope. The present study has revealed differences between the subepithelial mast cells and the intraepithelial mast cells and a gradation was noticed between them. Evidence of degranulation – that is the shedding of intact mast cell granules – was found in the subepithelial mast cells, but not in the epithelial mast cells. Vacuolation, with intracytoplasmic granule disintegration, apparently occurs mainly in the epithelial mast cells but is present, to a limited extent, in the subepithelial mast cells. The subepithelial mast cells were found to contain both orthochromatically and metachromatically stained granules. The granules of the intraepithelial mast cells usually stained orthochromatically, and had either a particulate or a mixed type of substructure. No granules consisting entirely of a lamellar substructure were observed in epithelial mast cells. The absence of the lamellar type of mast cell granules in the epithelial mast cells, the limitation of degranulation to the subepithelial mast cells, and the observation that the shed granules were always of the lamellar type, indicates that only the lamellar type of granule undergoes degranulation. Using methylene blue – Azur II staining, metachromatic granules were seen mainly in the subepithelial mast cells whereas the metachromasia of the epithelial mast cells was demonstrated principally in the vacuoles. Metachromatic vacuoles were also seen in some subepithelial mast cells. The disappearance of metachromatic granules from the mast cells which have migrated into the gastric epithelium apparently coincided with the disappearance of those granules which are entirely lamellar in structure. It is therefore considered that the lamellar granules represent the metachromatic granules seen with the light microscope.

Yamasaki, Fujita, Ohara & Komoto (1970) observed *in vitro* that the degranulation of rat mast cells was not an essential condition for the release of histamine, nor was it a reliable criterion of histamine release. However, vacuolation seems to be a more concrete structural manifestation of histamine release, and *in vitro* experiments with rat mast cells have demonstrated that histamine liberators invariably produce mast cell vacuolation (Bloom & Haegermark, 1965; Horsfield, 1965; Singleton & Clarke, 1965; Yamasaki *et al.* 1970). Thus histamine or other amines (see Uvnäs, 1964) could be released in the human stomach by the disintegration of the particulate orthochromatic granules with the resultant production of vacuoles and the passage of the products of granule disintegration through the minute pores connecting the vacuoles to the cell membrane. Evidence for the preferential location of mast cells with orthochromatic granules in the parietal cell area has been provided by the statistically significant increase in the percentage of orthochromatic granules in individual mast cells in the high lesser curve and high greater curve regions when compared with the region of the incisura angularis. This observation would concur with the established theory implicating histamine in normal acid secretion.

When granule disintegration with vacuolation becomes complete the nucleus appears to become uniformly electron-dense, and the mast cell is shed into the gastric lumen.

SUMMARY

Mast cells were examined from various sites in the normal human stomach and in the stomachs of patients with gastric ulceration.

The distribution of the different types of mast cell granules was determined in the subepithelial region of the normal human stomach. There was a significant difference in this respect between the mast cells at the incisura angularis as compared with those high on the lesser curve or high on the greater curve.

Mast cell degranulation (i.e. the shedding of intact granules) and vacuolation were examined with the electron microscope.

Degranulation and vacuolation were observed in subepithelial mast cells, whereas only vacuolation was seen in intraepithelial mast cells.

The significance of degranulation and vacuolation is discussed.

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