Nasal mast cells: a preliminary report on their ultrastructure*

by

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Summary

The ultrastructure of mast cells found in normal inferior turbinate was compared with the features found in the inferior turbinate in two groups of patients, those with allergic rhinitis due to dust mite hypersensitivity and those with nasal polyps; the latter group also had their polyps studied. Adenoid tissue was examined in children with secretory otitis media to see if there was evidence of mast cell degranulation, which would support the hypothesis that either local allergic or other mast cell-mediated reactions caused the condition.

The mast cells from five normal turbinates varied considerably in size, shape and distribution, but were found mainly in the submucosa. There was no difference in the morphology of cells of different sizes and they could not be sub-grouped into either connective tissue or mucosal mast cells. Most granules were electron dense and homogeneous, although scrolls and crystalline structures were seen occasionally. Some of the granules contained lighter material and others had become vacuoles. Mitochondria were present in all cells suggesting active metabolism.

The three patients with allergic rhinitis showed extensive but variable degranulation of the mast cells in all depths of the mucosa. Nine of the 10 cases with nasal polyps had mast cells identified in both the polyp and the turbinate. They were only normal in one turbinate and in one patient it was impossible to identify mast cells. All the mast cells were degranulated extensively in all other specimens.

The adenoids from seven children had identifiable mast cells, which were less frequently found than in the turbinates. There was some degranulation in four of the patients and in one it was fairly extensive.

Introduction

Nasal diseases form a considerable part of ENT practice, and yet the aetiologies are not well understood. The commonest diseases in adulthood are the chronic rhinitises, which are simply classified into allergic and vasomotor types. Nasal polyps may develop in patients with rhinitis.

Allergic rhinitis is diagnosed on the basis of the history, examination and skin test results. If the investigations are negative and no obvious allergen is implicated then the condition is labelled vasomotor rhinitis. The diagnosis is entirely clinical with no reference to the cellular or vascular reactions that occur within the nose.

The allergic reaction is mediated by the immunoglobulin IgE which is firmly fixed onto the surface of a mast cell or basophil.

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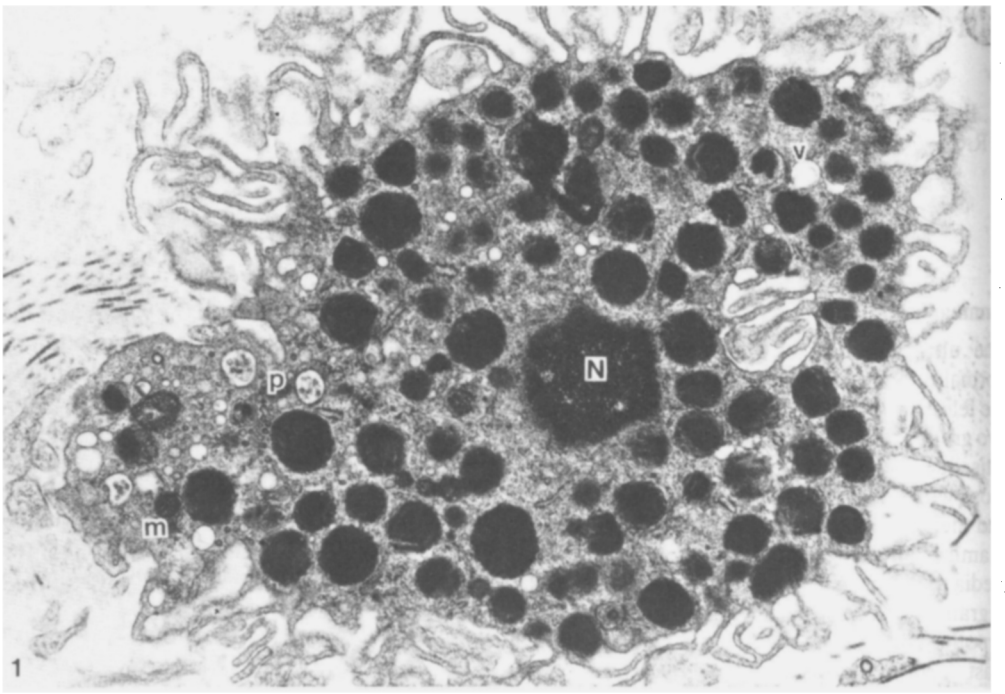

Fig. 1.

Electron micrograph ×4,090. A normal mast cell with extensive surface projections, an occasional vacuole, v, and a partly degranulated granule, g, Mitochondria, m, may be seen.

There are 3,000 receptor sites on the cells and the specific IgE for the allergen has to be present on two adjacent receptor sites for the cell to degranulate when it reacts with the allergen (Ishizaka and Ishizaka, 1967). The reaction causes the surface membrane to alter and calcium enters the cells; this initiates the formation of cyclic AMP from ATP. The initial response is the release of the preformed elements within the granules. Histamine is dissolved from the protein/heparin complex which can be seen as an electron dense matrix and is either amorphous or organized as scrolls or crystals within the granules. The granule swells and becomes less electron dense; the end-result is vacuoles (Caulfield *et al.*, 1980). The second process occurs on the cell membranes and is delayed. Arachidonic acid is mobilized and metabolized by two pathways to produce the prostaglandins and the leukotrienes. The main prostaglandin found in mast cells is D2 and the leukotrienes are LTC2, LTD2, and LTE2; these are what were previously described as slow reacting substance of anaphylaxis.

If there is no clinical evidence for an allergic reaction then it is assumed that this type of cellular reaction is absent and the symptoms are mediated by neurovascular responses which are abnormal. Autonomic imbalance results from persistent parasympathetic overactivity.

This simple classification may be challenged because there is some evidence that cellular reactions occur in non-allergic cases. The morphology of cells found in nasal smears taken from nasal secretions may show an eosinophilia. Mast cell reactions attract eosinophils. Further evidence comes from patients with nasal polyps. While the aetiology is open to debate, the majority of cases have no evidence of allergic diatheses (Drake-Lee *et al.*, 1984a). Ultrastructural examination of nasal polyps shows that the mast cells

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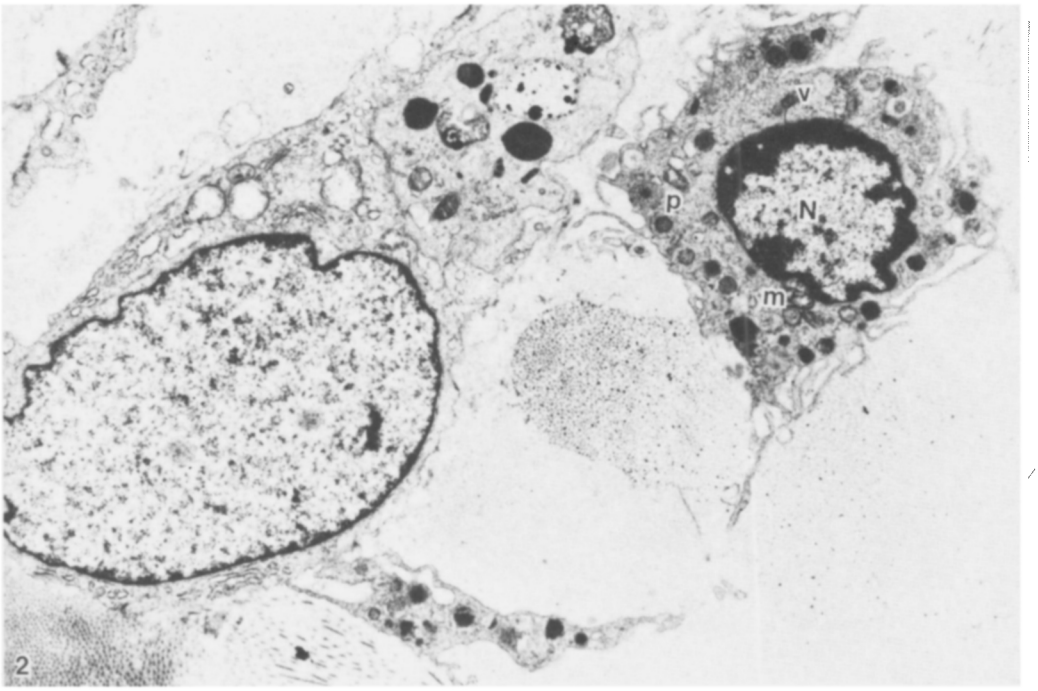

Fig. 2.
Electron micrograph ×7,777. An elongated cell with far fewer granules and surface projections.

are degranulated in all polyps examined (Cauna *et al.*, 1972; Busutill *et al.*, 1976; Drake-Lee *et al.*, 1984b). At present the mast cell degranulation has only been studied in the polyps and this study will look at the inferior turbinate to see if the reactions are more extensive.

Many children have nasal symptoms and go through a catarrhal stage. When the symptoms are at their height they frequently have middle-ear effusions in addition. The combination of ear and nose problems has given rise to the hypothesis that glue ear may be an allergic disease centred on the adenoids with the local oedema causing eustachian blockage (Dees and Lefkowitz, 1972; Collins *et al.*, 1985).

Since all allergic reactions involve mast cells it is prudent to look at the morphology of mast cells to see if there is any evidence of degranulation. Mast cells may be degranulated by a large number of stimuli besides allergic reaction, and so alterations may be due to other triggers as found in patients with nasal polyps.

The aims of this study are to examine the ultrastructure of mast cells in the normal nose and to compare the findings with cells in patients with allergic rhinitis or nasal polyps and in the adenoids of children with secretory otitis media.

Materials and methods

Normal nose

The inferior turbinate was trimmed from five patients who were admitted for septoplasty, submucous resection and septorhinoplasty; all operations were performed as a result of trauma. All patients had no history of nasal diseases, including viral rhinitis, allergic and vasomotor rhinitis. Apart from blockage due to the deflected septum, the patients had no symptoms and had negative skin tests. The surgery was performed under

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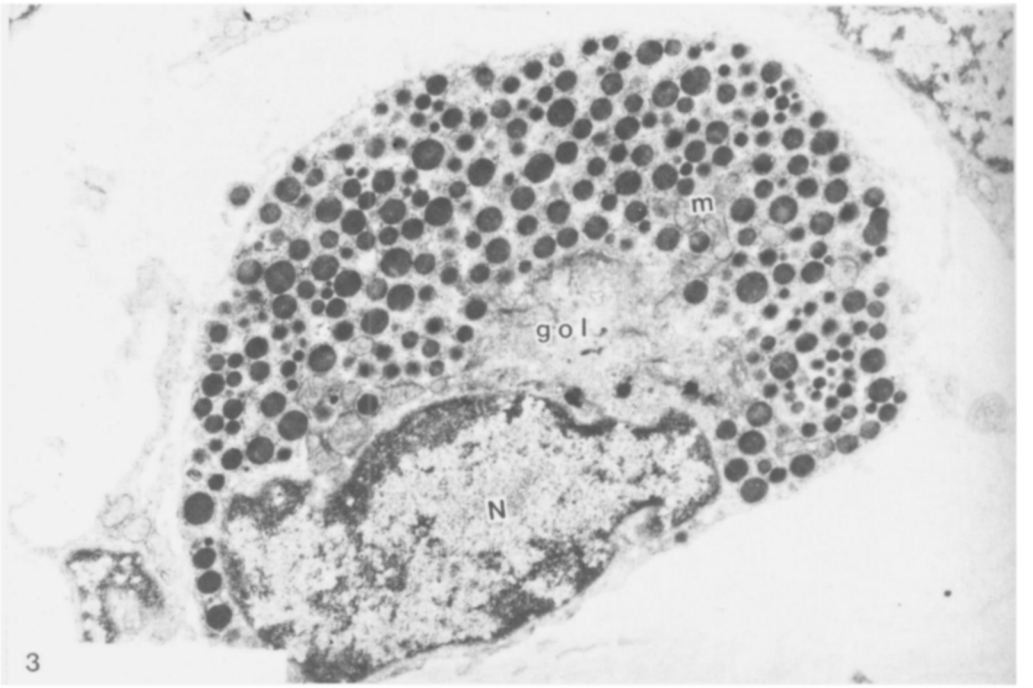

Fig. 3.

Electron micrograph ×3,500. A large cell with an excentrically placed nucleus and a more obvious golgi apparatus, gol.

general anaesthetic after the nose had been prepared with cocaine hydrochloride.

Perennial allergic rhinitis

Three patients with perennial allergic rhinitis due to house dust mite were studied. All had a positive history on exposure which was confirmed by skin tests. They were admitted for trimming of the inferior turbinates.

Nasal polyps

Ten patients with nasal polyps were examined. The polyps were removed by an avulsion snare and the part which was in the nose was used. A piece one millimeter thick and half a centimeter long was cut off the polyp. A biopsy of the inferior turbinate using Tilley-Henckel forceps was taken. All patients had bilateral polyps and both specimens examined came from the same side. The nose was prepared with cocaine in the usual manner.

Adenoids

The adenoids were removed by blunt curettage from seven children with secretory otitis media. All were removed under general anaesthesia and no local preparation was used.

Local nasal preparation

The following technique was used on all patients who required nasal preparation to reduce haemorrhage. The nose was sprayed with 10 per cent cocaine hydrochloride and neophrin nose spray at least 10 minutes before surgery. On admission to the anaesthetic room the nose was painted with cocaine crystals 25 per cent made up as a paste.

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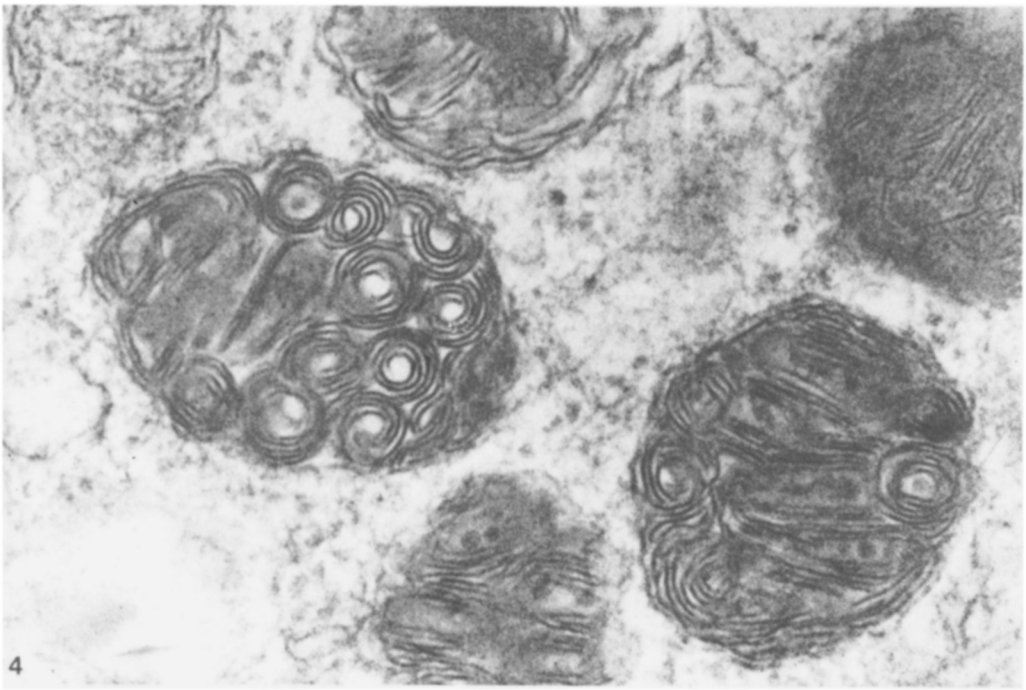

Fig. 4.
Electron micrograph ×100,000. Granule ultrastructure showing a scroll pattern.

Tissue preparation

The material was diced into cubes of approximately one millimeter and as much of the epithelium as possible was included. These were placed into 2.5 per cent glutaraldehyde in cacodylate buffer within five minutes of surgery and fixed for four hours at 4°C and allowed to come to room temperature. They were then placed into the buffer for one hour and subsequently washed in distilled and deionized water. The blocks were stained with uranyl acetate for half an hour and dehydration was carried out though an acetone series. The blocks were infiltrated with Spurr resin (Spurr, 1969). Semi-thin sections of 1 μm were taken for staining with toluidene blue, using a hot plate. Ultra-thin sections of 90 nm were mounted on a copper grid and were double-stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections were examined on a JEOL 100S transmission electron microscope and photographs were taken on Kodak 4489 EM film.

Histological examination

Routine examination for the detection of mast cells was abandoned since mast cells were frequently found on EM which were not detected on light microscopy.

Electron microscopy

Between 6 and 20 blocks were examined from each patient. Since the study was descriptive and the results were not easy to quantify because there was both considerable variation in cell type and in the degree of degranulation, representative mast cells are shown.

Results

The ultrastructural details which were representative of the findings are presented as Figs. 1 to 16. Normal cells and their inclusions are Figs. 1 to 6. The first three figures give the

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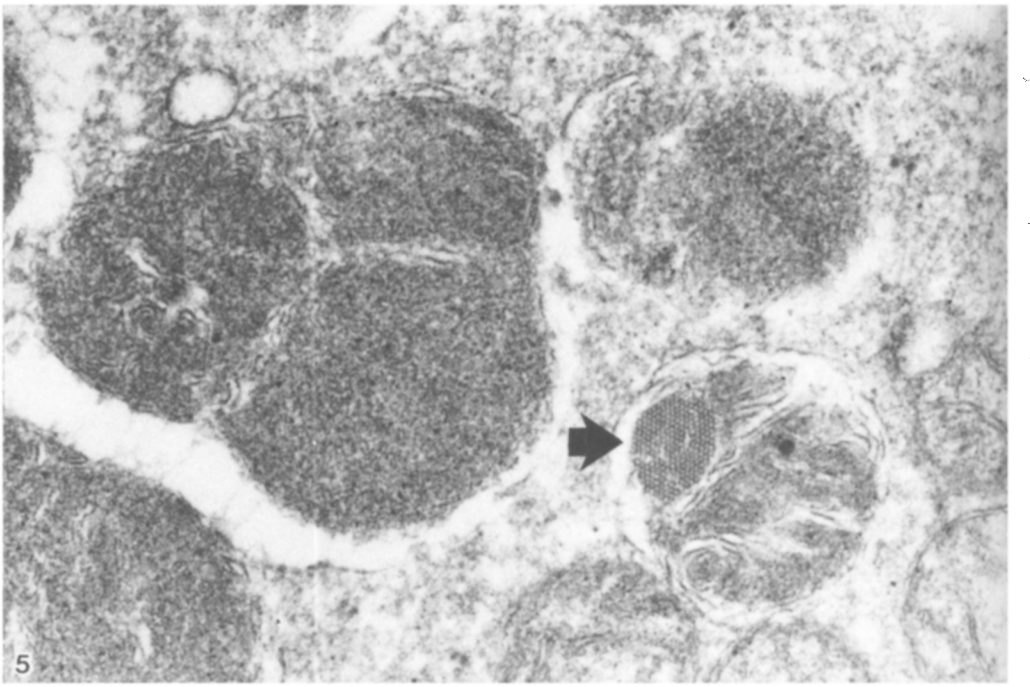

Fig. 5.

Electron micrograph ×70,900. The various types of ultrastructure within the granules, the most noticeable feature is the honeycomb crystalline structure.

overall morphology encountered in the five normal subjects and the following three figures the variations in the granules.

The three patients with allergic rhinitis were studied and they showed variation in the degree of degranulation; the changes seen in one patient are shown in Figs. 7 to 9. There was considerable degranulation encountered in all cases though it was less marked in one.

Although ten patients with polyps were studied, only nine had enough evidence for the cells studied here to be considered as mast cells. In only one patient was the morphology of the cells comparable to normal and this was found in the inferior turbinate. The degree of degranulation was greater than in cases with allergic rhinitis.

There was no obvious difference in the features in allergic rhinitis compared with those found in both the inferior turbinate and the polyp in cases with nasal polyps.

Review of the adenoid tissue showed that four of the patients had cells which showed some degree of degranulation; in only one case was it comparable to the extent of the changes found in patients with allergic rhinitis.

Normal nasal mast cells

The overall morphology encountered was very variable, although mast cells tended to be round or oval; occasionally they may be much more elongated, as Fig. 2 showed. The size varied considerably, even when the difference in magnification and the plane of section were taken into account. The relative size in the granules is useful when comparing micrographs. There was no difference in the distribution of either smaller cells or larger cells within the connective tissue, and mast cells were rarely encountered within the surface epithelium.

The nucleus (N) varied in position from the

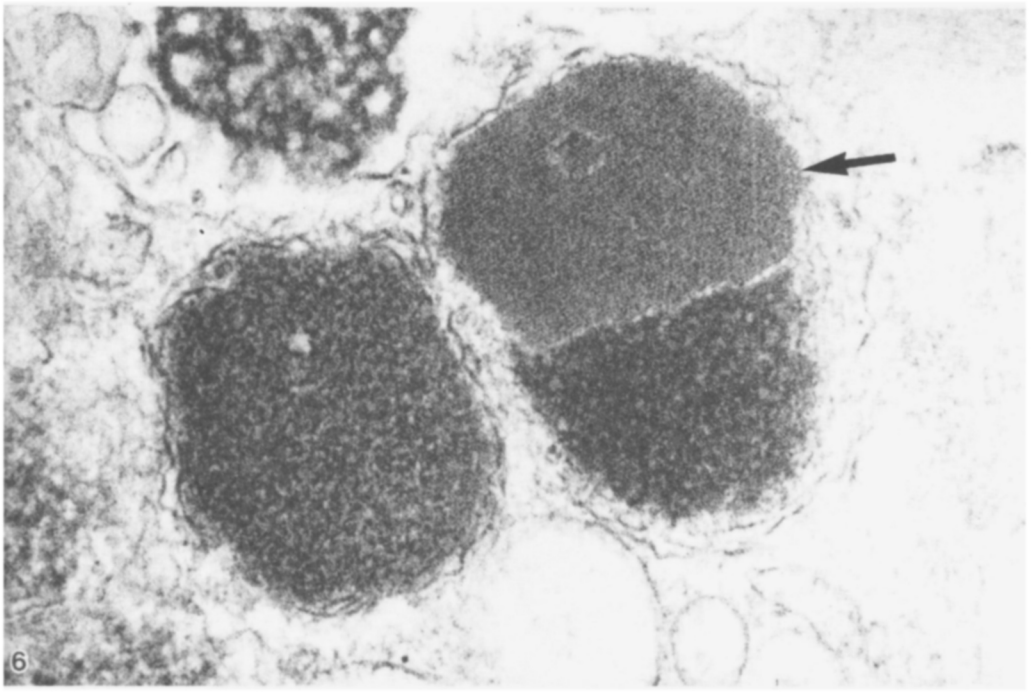

Fig. 6.
Electron micrograph ×93,180. This shows the long axis of the honeycomb as a series of parallel lines.

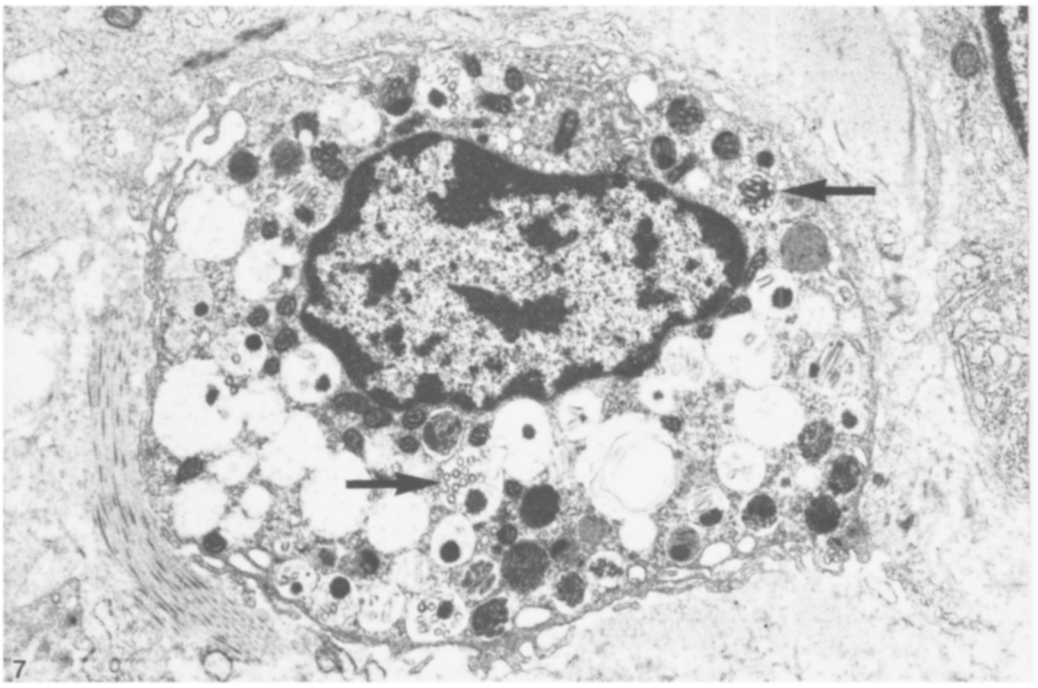

Fig. 7.
Electron micrograph ×10,450. Partial degranulation in an allergic patient, the scrolls may just be seen.

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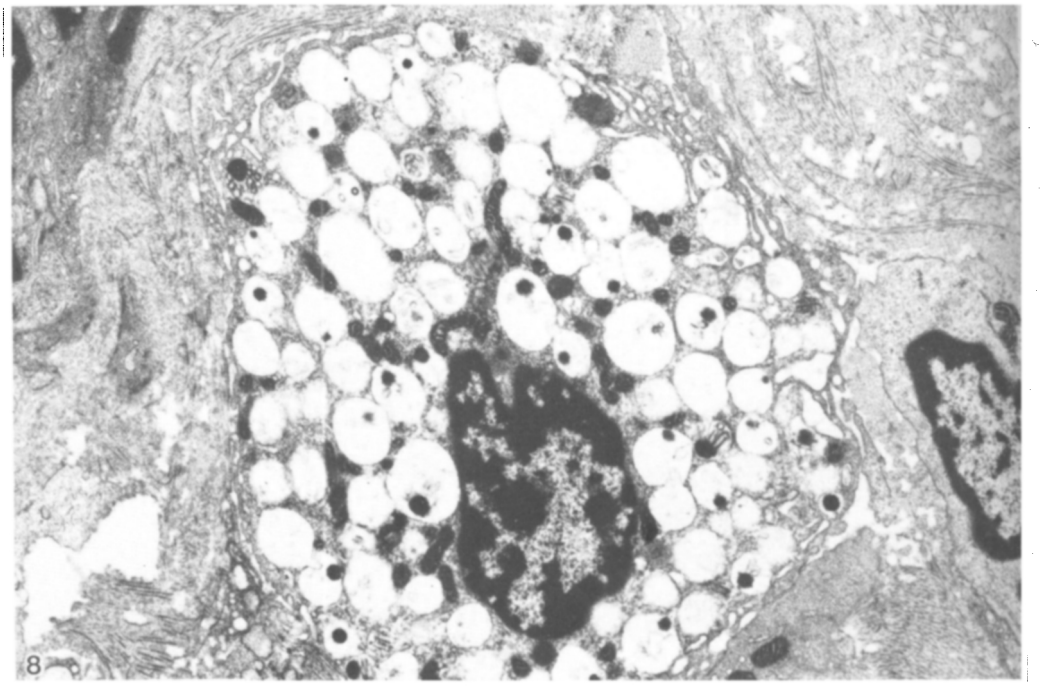

Fig. 8.
Electron micrograph ×9,772. The changes are more extensive, but the cell is still recognisable as a mast cell.

centre of the cell to the side (Fig. 3). Electron dense chromatin was patchy and was more common on the edge of the nucleus.

There was marked difference in the surface of the cells. Projections were usual but could be as extensive as in Fig. 1 or virtually absent, as was the case in Fig. 3. The cytoplasm was fibrillar in all cells.

Although not well seen at these magnifications, mitochondria (m) were present in all cells. Their position in the cell was not constant nor was their relationship with any intracellular features. They could be towards the edge of the cell, clumped together or scattered around the nucleus.

The golgi apparatus (gol) was not encountered frequently and it was only well developed occasionally, as in Fig. 3.

There was little interreaction with other cells, but Fig. 14 from the adenoid shows a granule (g) in the cytoplasm of a fibrocyte. The most striking feature and that which identified the cells was the electron dense granule. The majority were amorphous and electron dense with some organization seen. A few were of a lighter matrix (p) and vacuoles were found (v).

The number of granules was very variable with some cells having few (Fig. 2) and other cells having very large numbers (Fig. 3).

Granule morphology

Most of the granules were electron dense and amorphous. When further details were visible then scrolls were most frequently encountered (Fig. 4). They are called scrolls because they resemble parchment scrolls, but they were often cut along their long axis when they appeared as tubes. Both planes of section were seen in Fig. 4. The ghost of a scroll may be the only feature to identify a degranulated mast cell. Granules can contain more than one structure in the granule; Figs. 5 and 6 show both electron dense material and a crystalline matrix.

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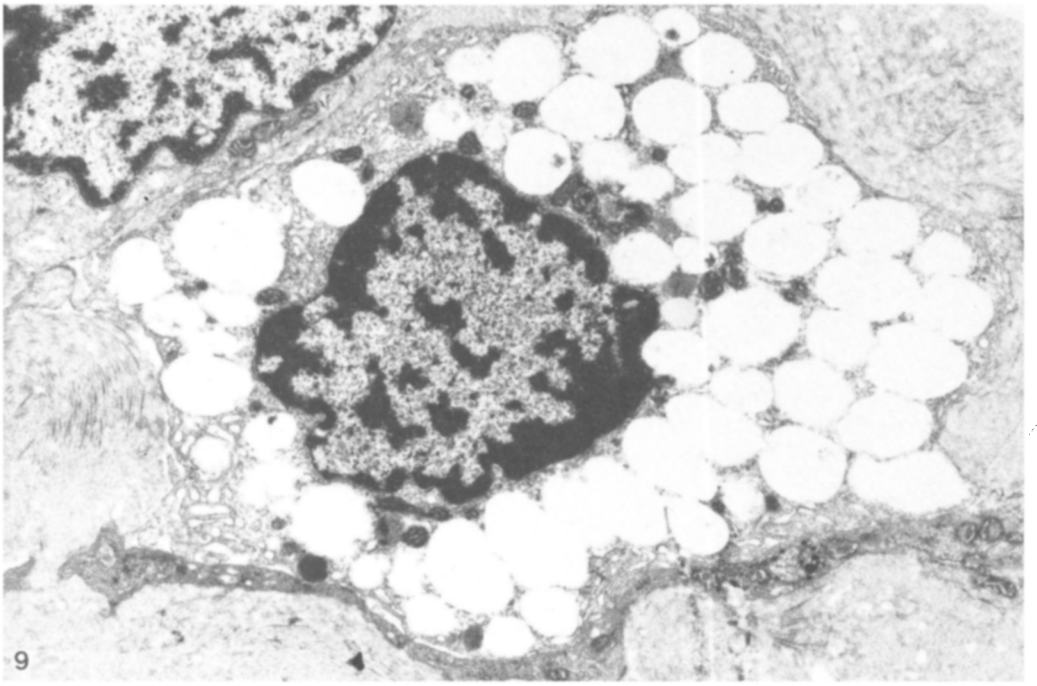

Fig. 9.

Electron micrograph ×11,810. A fully degranulated cell, comparison with the previous two figures would suggest the cell to be a mast cell. The vacuoles almost fill the cytoplasm.

A crystalline matrix is the other distinctive feature which was seen rarely in nasal tissue. The most marked hexagonal crystalline pattern is seen in Fig. 5. When this was cut in the other axis then it appeared as a row of parallel lines (Fig. 6, arrowed).

Very large 'multi-granules' were encountered rarely. They can occur in any mast cell, but are more frequently seen in the skin. They appeared to be three or more granules within the same structure (Fig. 5).

Allergic rhinitis

The three figures were taken from the same patient and showed the variable degree of degranulation which was seen (Figs. 7 to 9). It is just possible to see scrolls at this magnification (Fig. 7, arrowed). Vacuoles were larger than granules and took up more of the cytoplasm. There was no gradation of the changes seen in the cells between the submucosa and the deeper layers.

Similar changes were encountered in the other two patients, but cells were more granulated in one.

Nasal polyps and inferior turbinates

The representative morphology of the mast cells within the polyps and the inferior turbinates is shown in Figs. 10 to 13. Almost all cells showed extensive degranulation. Figs. 10 and 12 came from polyps and the other two from the inferior turbinate: both polyp and inferior turbinate cells were from the same patients.

Some of the cells were very difficult to identify, but the surface projections, the occasional electron dense granule and the vacuolated remnants are suggestive. Other cells were much easier, as the figures here show. Some of the granules have lighter outer

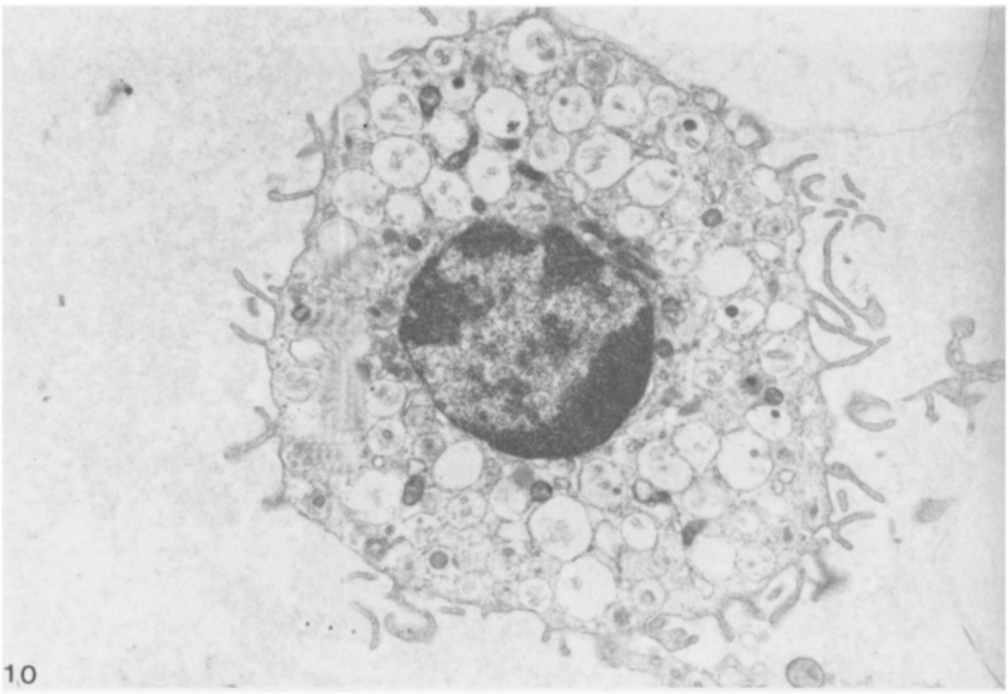

Fig. 10.
Electron micrograph ×9,772. Fairly extensive degranulation in a polyp mast cell.

parts and electron dense cores; this was encountered in normal cells (Fig. 2) and was helpful in identifying almost degranulated cells. These changes should not be confused with lipid droplets, which are best shown in Fig. 11. Lipid droplets were uncommon and occurred in a number of cells from the same patients. There was no aggregation of mitochondria by the lipid droplets.

Only one specimen had almost normal mast cells and that was an inferior turbinate (Fig. 13). In general the polyps had a greater degree of degranulation than the cases with allergic rhinitis. Destruction of a mast cell with loss of the surface membrane occurred occasionally and this was seen very rarely in the normal nasal mucosa.

Adenoid tissue

The morphology of mast cells within the adenoid was similar to that found in the normal adult inferior turbinate, although they were less numerous. All cells were easy to identify as mast cells. There was a spectrum of changes from patient to patient. Cells from three patients were normal (Figs. 14 and 16). There was considerable variation in the degree of degranulation in one patient. In the other three patients, there was evidence which suggested degranulation and in one it was quite marked (Fig. 15).

Discussion

A cell type may be defined by three different factors; its morphology, its biochemical reactions and its function within the organism. Studies in each area are open to limitations. This discussion will concentrate on morphology.

Light microscopy of mast cells shows cells with large numbers of metachromatically staining basophilic granules, which are found in the connective tissues of the body. There are both larger and smaller mast cells and this

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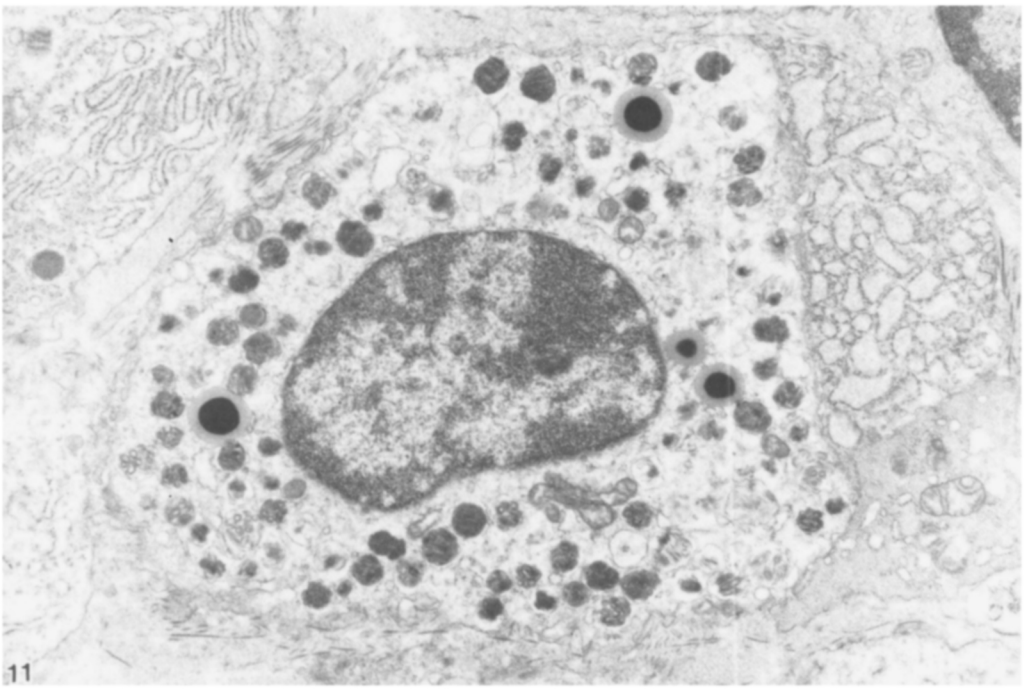

Fig. 11.

Electron micrograph ×10,222. The corresponding cell from the inferior turbinate shows less degranulation. The double density bodies are lipid bodies.

has lead to mast cells being classified in the gut, particularly in animals, into mucosal and connective tissue types. The classification is based on the staining properties and the *in vitro* cellular responses of rodent mast cells. Mucosal mast cells are smaller and found near the surface epithelium whereas connective tissue mast cells are larger, have more granules and are found deeper, often near blood vessels. Both types are closely related morphologically to the blood basophils, the latter having multilobed nucleoli and spindles. Granules tend to be fewer in blood basophils as well as in the smaller mast cells.

Studies at the light microscopic level use a number of different tissue fixatives and stains. Most routine histology is performed on tissues fixed in a formol solution and the mast cells are stained deep purple by an aqueous solution of toluidine blue. It has been suggested that fixation techniques dissolve the water soluble granules of the mucosal mast cells (Enerback, 1981) and that Carnoys fixative should be used when studying mast cell populations in the human gut (Strobel *et al.*, 1981). Mast cell granules may be stained metachromatically with a number of different stains including toluidine blue, Azure A, Bismarck brown, thionin, Csaba's alcian blue safranin and by the choline esterase technique.

There is evidence to suggest that mast cells develop from two distinct groups of cells. Connective tissue mast cells develop from purified human blood monocytes (Czarnetzki *et al.*, 1984) and that, in rodents, mucosal mast cells are derived from the local lymph nodes and the development is dependent on the thymus (Miller, 1980). The older established view was that mast cells arose from connective tissue elements *in situ*. They are migrating cells and this would account for the surface projections which are seen on some cells, and very well developed on a few. Since

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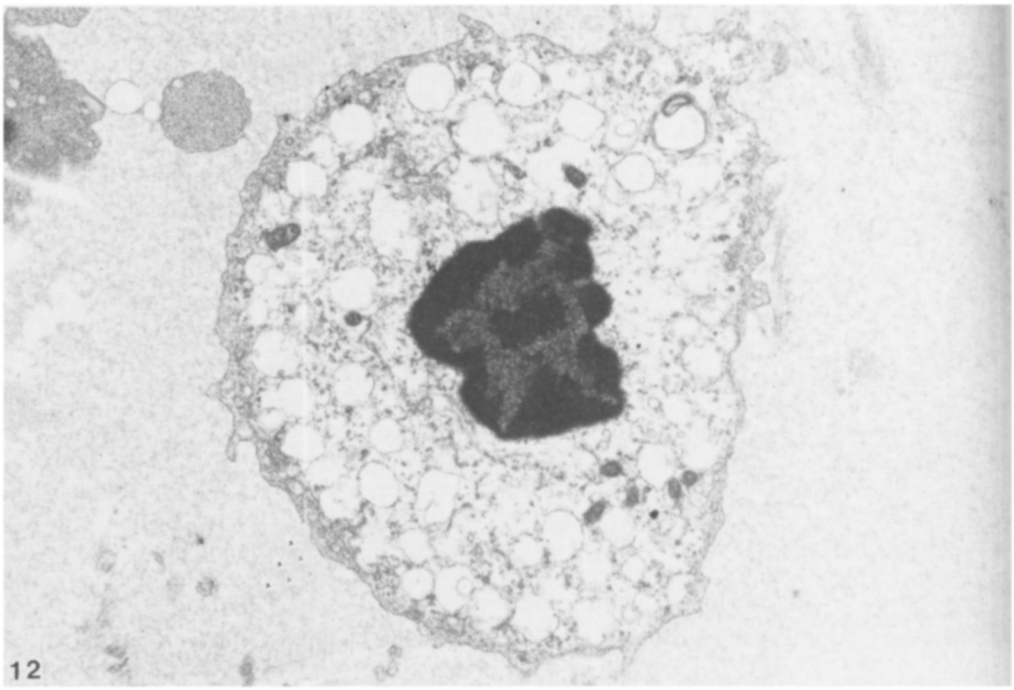

Fig. 12.
Electron micrograph ×10,222. A completely degranulated cell from a polyp which is completely different from those found in the inferior turbinate of this patient.

mast cells appear to be mainly submucosal, it is the authors' view that cells within the epithelium and on the surface do not account for the major site of reactions. Surface cells may represent degenerating cells, and studies on surface cells obtained by brushing may be open to limitations.

Mucosal and connective tissue mast cells have some differences in experimental reactions to degranulating agents *in vitro* and also to drug reactions. Mucosal mast cells do not have their cell membrane stabilized by sodium chromoglycate (Bienenstock *et al.*, 1982).

The studies at light level should be confirmed at the ultrastructural level. Tissues are fixed by different methods for electron microscopy. Such tissues will not stain with some of the light stains. Toluidine blue stains well for light and electron microscopy and so is frequently used for tissue orientation. Mast cell granules continue to stain metachromatically, but this study confirms the view of others that light staining is of little value in detecting mast cells before EM (Galli *et al.*, 1984). Many of the smaller cells are missed, but this is not related to the ultrastructure of the granules which are similar in both larger and smaller mast cells.

The characteristic of a normal mast cell at the EM level is the electron dense granule which can be amorphous, organized as a scroll or as a crystalline matrix. The cell has a single nucleus. All the other features are variable. Unfortunately a cell may lose many of its features when it is either taking part in reactions or diseased.

It is a mistake to assume that the normal cells in the nose are resting, not involved in reactions. Although there is no evidence of extensive changes which would suggest degranulation, normal cells in this study possessed vacuoles and granules that are less dense. These alterations could be due to two processes, the maturation of developing gran-

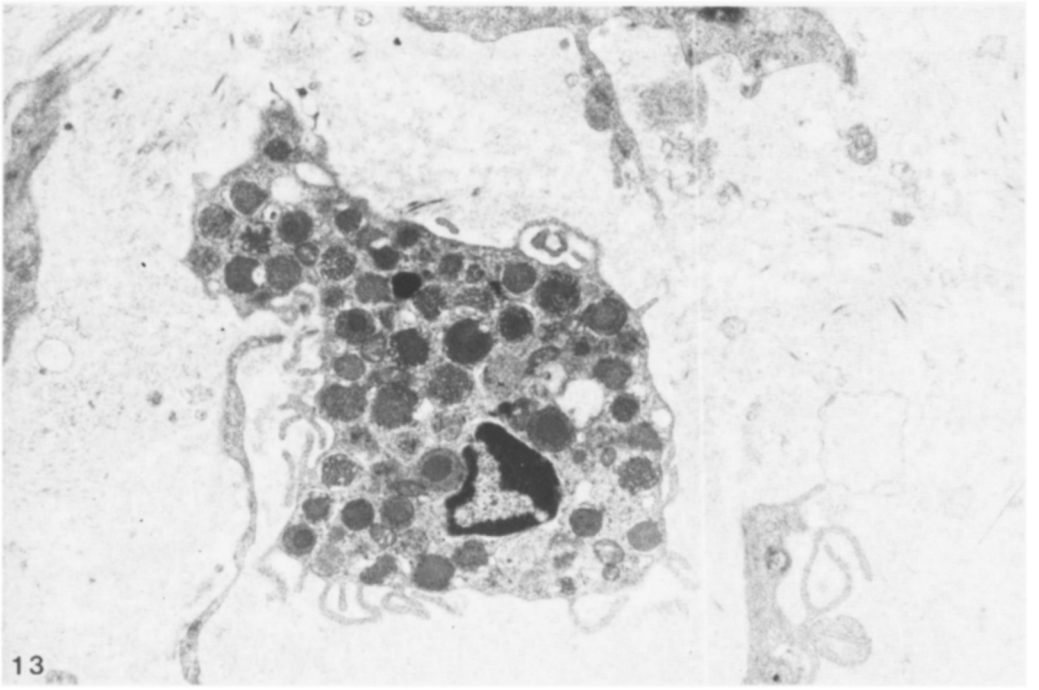

Fig. 13.
Electron micrograph ×12,270. A normal mast cell from the inferior turbinate showing a few vacuoles.

ules or the events that occur in degranulation. Maturation is largely ignored and emphasis on *in vitro* studies is on degranulation. A biopsy is a freeze frame of tissue events, cells developing, reacting and dying.

This study showed that normal mast cells within the nasal epithelium varied considerably in size, shape, numbers of surface projections, distribution of mitochondria and the presence of Golgi apparatus. Mast cells were rarely encountered in the surface epithelium, which suggests that their main line of defence is submucosal. Except in Fig. 3, all cells showed some variation in granule morphology, including the occasional vacuole. Most of the granules were homogeneous and very electron dense. It is not possible to determine whether the difference in size was because it was the same cell in different stages or cells of different types. This study does not support the view that there are two distinct groups of mast cells in man, but it does suggest that there is a spectrum of cells sizes. Mast cells are involved in the defence of the airway and, since degranulation may be caused by a large number of different factors, some degranulation would be encountered normally, and was seen here.

Mast cell degranulation

Rodents release their granules direct into the extra-cellular fluid (Kessler and Kuhn, 1975; Dvorak *et al.*, 1983). Occasionally the granules may dissolve prior to release as is the norm in humans. Dissociated human pulmonary mast cells have scrolls as the commonest intra-cellular feature. Degranulation with anti-IgE is a rapid process and goes through the following sequence: the granules enlarge and become amorphous; then, either by the formation of a microtubular system or by direct contact between the granules, the contents are discharged into the extracellular fluid which result in vacuoles. The process is complete in about twenty minutes (Caulfield

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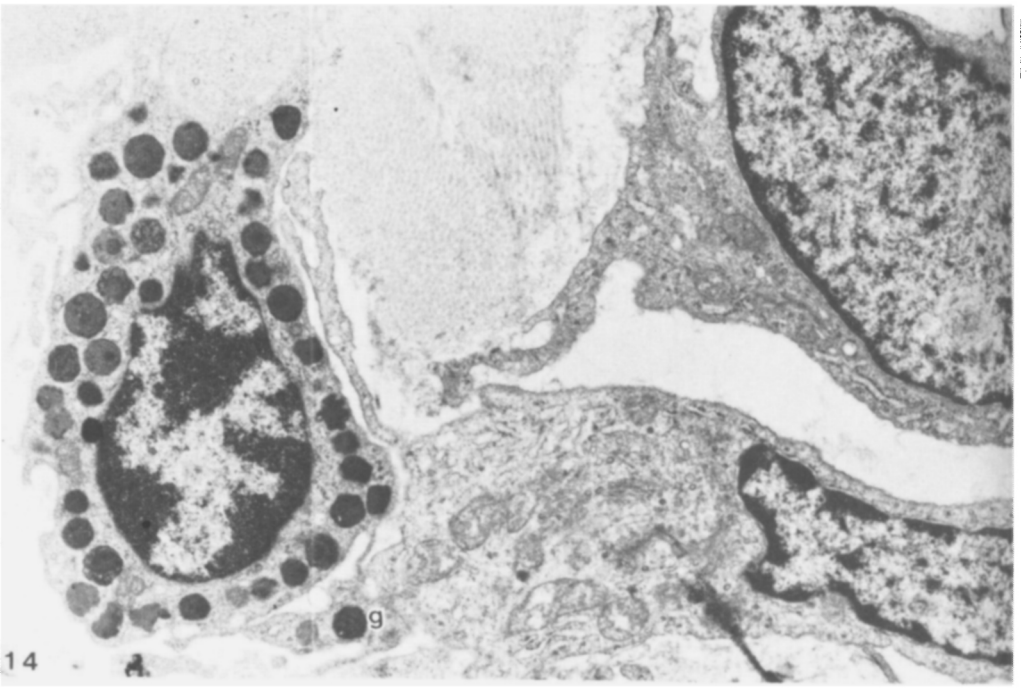

Fig. 14.
Electron micrograph ×12,000. This is a normal adenoid mast cell, which is fairly small. The fibroblast next to this cell has two mast cell granules in its cytoplasm.

et al., 1980). There has been one study using biopsies of nasal tissue (Trotter and Orr, 1973). Unfortunately the paper did not give details of the number of patients studied nor any clinical details. It included biopsies from the bronchioles as well as the nose. It is, however, possible to compare this study with the material presented here, and in general there is considerable agreement. Degranultion does not produce intact granules into the extracellular fluid as the norm, but the matrix dissolves initially and then is discharged from granules which are swollen. Microfilaments were not observed in either normal or degranulating mast cells.

In vivo challenge has been studied in humans by Kawabori and his colleagues (Kawabori *et al.*, 1983) and they suggested that the features differ. Five patients who had perennial allergic rhinitis but were receiving immunotherapy were challenged intra-nasally with allergen. The changes were slower and were more evident nearer epithelial surface.

The changes of the cells in nasal secretions have been studied and Okuda and his colleagues have suggested that most of the allergic reactions occur on or above the epithelium and are mediated by basophils (Okuda *et al.*, 1983). Nasal secretions are difficult to examine cytologically and quantitative examination is not possible (Wihl, 1979). Autolysis and degeneration are often seen.

This study, which evaluated patients with perennial allergic rhinitis to house dust mite, indicated that degranulation occurred throughout the epithelium and that the end-result was complete degranulation of the cell. No obvious basophils were demonstrated. If perennial allergic rhinitis was due to basophils reacting on the epithelial surface then they should be encounted migrating from the capillaries through the mucosa onto the surface of the epithelium. We believe that this mechanism is unlikely and further studies will be needed to confirm this impression.

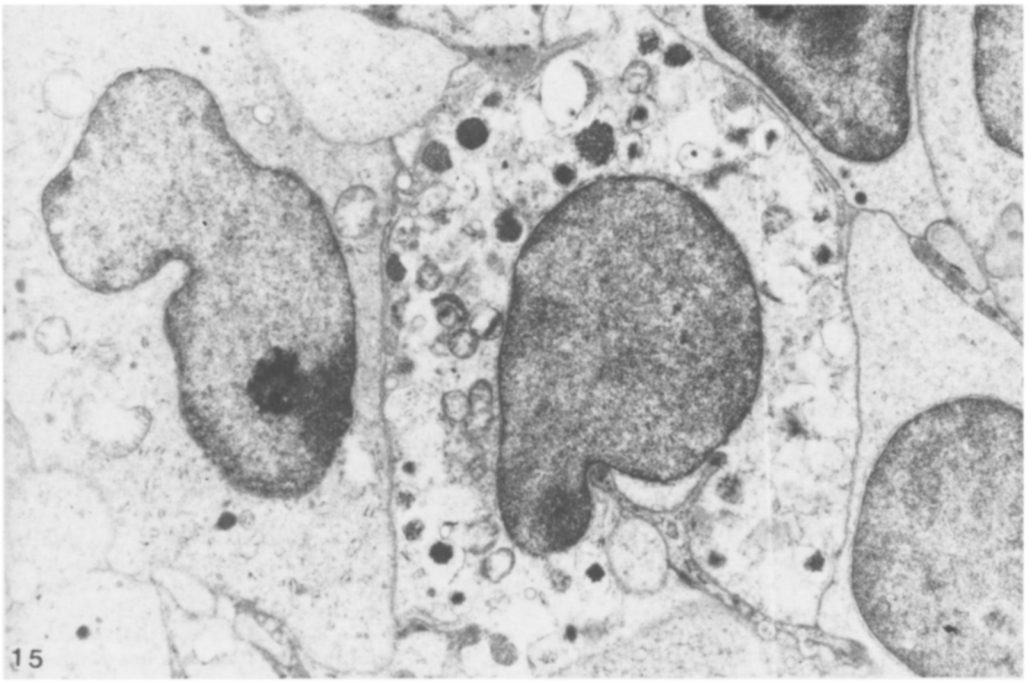

Fig. 15.
Electron micrograph ×10,220. An extensively degranulated adenoid mast cell.

Mast cells in polyps have been shown to be degranulated, and this study showed that, in the majority of cases, the process may extend into the rest of the nose. This could indicate that mast cell degranulation is a generalized feature in the disease, and not secondary to the reactions occurring within the polyp. The degranulation is usually extensive and sometimes it is difficult to identify the cell as a mast cell. There were no obvious features which differentiated mast cells in the polyps from those found in either the inferior turbinate of the same patient or from patients with allergic rhinitis.

Adenoids

The role of the adenoids in the pathogenesis of secretory otitis is unclear, but adenoidectomy aids the resolution of the condition (Maw, 1983). Neither size alone nor their ability to act as a reservoir of infection causes the middle-ear effusion (Hibbert and Stell, 1982). Allergy may be a factor in the development of effusions (Dees and Lefkowitz, 1972; Collins *et al.*, 1985). The allergic response may occur throughout the nose or be confined to the post-nasal space. The local inflammation causes mucous gland hyperplasia and oedema at the nasal end of the eustachian tube. It is equally possible that other reactions beside allergy could cause mast cell degranulation. However, adenoid tissue has been used to study the reactions of normal mast cells (Behrent *et al.*, 1978). The majority of children have nasal symptoms prior to adenoidectomy so that the mast cells may be abnormal.

There was a range of both cell sizes and ultrastructural changes in the seven children studied here. Three of the patients had relatively normal mast cells, in four there was evidence of degranulutation and in one of these it was marked. Although three were less granulated than the normal cells the changes were less marked than in patients with allergic rhinitis and nasal polyps. Further study will be

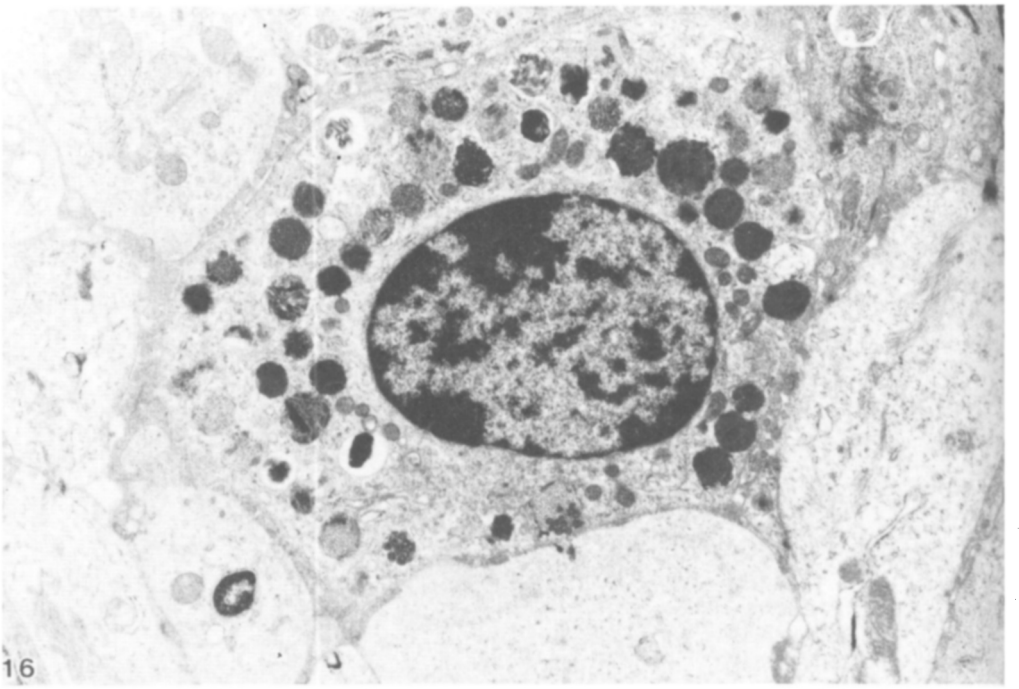

Fig. 16.

Electron micrograph ×10,000. Several of the granules contain material of light density, and others contain scrolls. One of the granules contains both structures. The cell shows early features of degranulation.

required to evaluate mast cell reactions in secretory otitis media.

Conclusions

Nasal tissue is a complex structure and its reactions are controlled by both cellular and vasomotor responses which complement each other. Normal mast cells present a spectrum of both cell size and granule organization. Cellular responses have been seen in patients with allergic and non-allergic rhinitis and in those with nasal polyps. Adenoids have been found to have some evidence of mast cell degranulation in half the cases studied so far. Further work is necessary to evaluate these findings and to relate them to the morphology of patients with allergic rhinitis.

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