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Nasal polyps
(1) Scanning electron microscopy and artifact
(2) Fine structure of mast cells

by

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(1) Summary

Nasal polyps are grossly oedematous tissue which has undergone considerable shrinkage when processed for scanning electron microscopy (SEM), converting the normally smooth surface into one with undulations. This shrinkage resulted in variable degrees of loss of the surface epithelium and thus gave rise to artifact. In its severest form, the entire surface epithelium was removed and the basement membrane exposed; lesser damage resulted in exposure of the basal cells, giving the previously described 'cobblestone' appearance. When the epithelium was intact, it was typical respiratory in type.

Introduction

Nasal polyps are a common clinical problem and, despite differing aetiologies, they have a constant histological appearance (Wilson, 1976). The surface epithelium is usually of respiratory type and consists of ciliated columnar epithelium and goblet cells; occasionally squamous metaplasia occurs. These cells rest in a basement membrane which is often thickened. Even on preparation for conventional histological examination, there is shrinkage of the specimen and the surface epithelium may be detached. It was felt that the previous scanning electron microscopic examination of polyps did not adequately take this into account and underestimated the artifact produced by preparation for SEM (Mygind *et al,* 1974). The aim of this paper is to document the findings of SEM and to evaluate the role of SEM in the study of nasal polyps.

Material and Methods

Polyps removed from nine patients both with and without allergy were studied. Polypectomy was performed under local anaesthetic, using cocaine paste 25 per cent applied to the nasal mucosa. Polyps were removed with an avulsion snare and placed into a gauze swab moistened with normal saline. The ethmoidal tag was removed and discarded and the intranasal portion was washed in normal saline to remove mucus, blood or anaesthetic paste. Polyps between 5 mm. and 1 cm. diameter were placed immediately into Karnovsky's fixative for

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four hours. The fixative was modified to contain 2 per cent paraformaldehyde, 2.5 per cent glutaraldehyde in 0.1 in sodium cacodylate buffer at pH. 7.3. They were then washed in fresh buffer (310 m. os^{-1}) and bulk stored.

Polyps were post-fixed in 1 per cent OsO_4 in 0.1 M. sodium cacodylate for up to four hours. Two samples were treated by the osmium tannic acid-uranyl acetate method to reduce tissue shrinkage (Wollweber *et al.*, 1981). Polyps were then washed in buffer and dehydrated through an acetone series before critical point drying (CO_2). After mounting, a layer of 30 nm. of gold was deposited on the specimen in a Polaron cool Splutter Coater. The polyps were viewed in a Cambridge S600 scanning electron microscope at 15 kV. using the secondary electron dilector mode. Micrographs were taken on a 35 mm. PFA Ilford film.

Results

Considerable shrinkage was produced in all specimens; this occurred mainly during critical point drying, where between 50 and 70 per cent of the bulk was lost. This resulted in collapse of the surface with resulting folding and undulating surface (Fig. 1). Specimens were best preserved using the osmium tannic acid uranylacetate method (Wollweber *et al.*, 1981). Considerable, though variable, loss of surface epithelium was produced in all polyps (Fig. 1). Where intact the epithelium was normal respiratory in type, cells had microvillae or, more commonly, cilia projecting from their surface; occasional mucus droplets were seen (Fig. 2). When the cut surface of the polyp was scanned, typical ciliated columnar epithelium was seen, with the occasional goblet cell (Fig. 3). Partial loss of epithelial

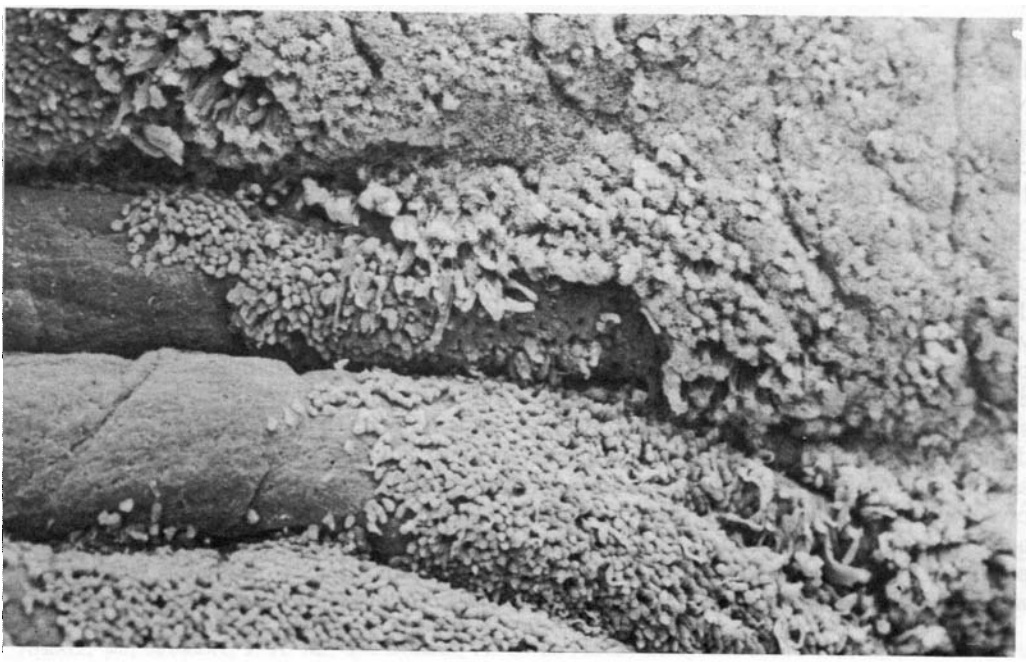


FIG. 1

SEM ×260. Shows the undulations produced by shrinkage and the loss of the epithelium. On the left is the exposed basement membrane, in the middle the basal cells giving rise to the 'cobblestone' appearance; the right-hand side shows the normal surface epithelium.

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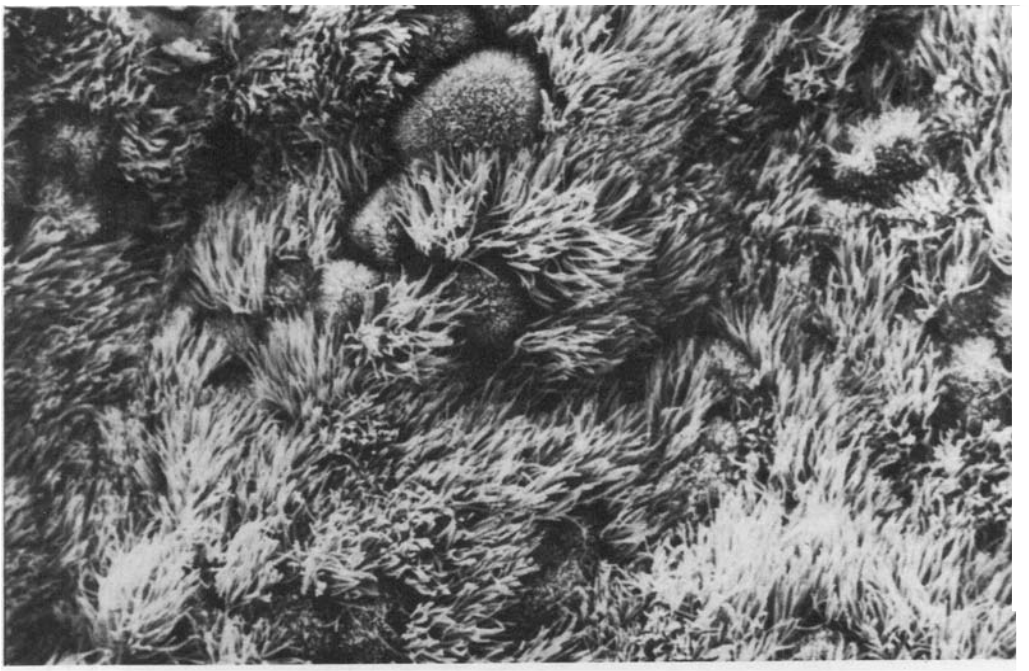


FIG. 2

SEM ×1,500. Cilia and microvillae projecting from intact epithelium.

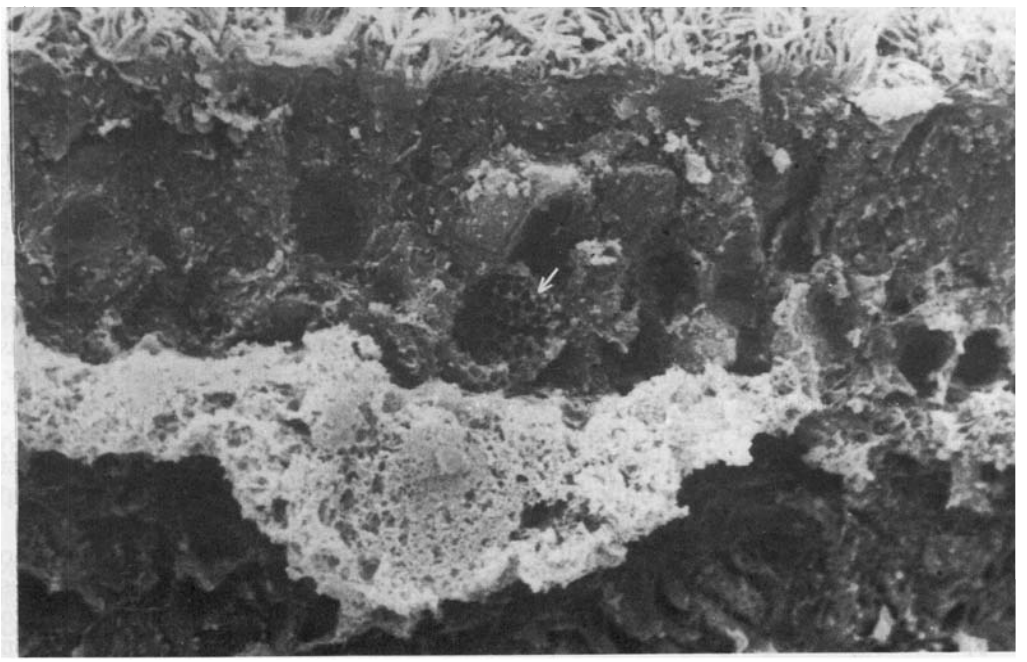


FIG. 3

SEM ×2,600. The surface epithelium is composed of columnar cells with an occasional goblet cell seen in the centre (arrow).

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cells gave rise to exposure of the basal cells. Surface cells were lost either singly or in a complete layer, giving rise to a 'cobblestone' appearance (Fig. 1). Exposure of the basement membrane resulted in a flat featureless surface.

Discussion

SEM showed that, in the polyps examined, the intact epithelium was respiratory in type. However, in all cases there was considerable artefactual shrinkage of the tissue during preparation, giving rise to potential difficulties in the correct evaluation of specimens, and even methods of preparation designed to minimize cellular shrinkage (Wollweber *et al.*, 1981) did not prevent the loss of extracellular fluid.

Previous reports (Mygind *et al.*, 1974) have considered such artefacts to be limited to widening of intercellular spaces and distortion of terminal bars. Our results, however, show that the 'cobblestone' appearance described by Mygind and his colleagues is due to loss of superficial cells and exposure of more basally situated cells. Further loss of epithelial cells leads to exposure of the basement membrane.

Tos and Morgensen (1977) suggested that nasal polyps are a result of prolapse of the lamina propria through defects in the surface epithelium and that the prolapsed tissue becomes epithelialized. Friedmann and Osborne (1982) disagreed with this view and observed that the surface epithelium of polyps is usually intact and that any defects in the epithelium were due to pressure against rigid surfaces. The present finding, of an essentially normal respiratory type of epithelium, is in accordance with the latter findings and indicates that the underlying pathological process of polyps are at a submucosal level.

Despite their easy accessibility, nasal polyps do not constitute good material for SEM. Their high fluid content makes preparation artefacts inevitable with consequential difficulty in interpretation.

(2) Introduction

Mast cells contain a large number of granules which stain metachrometically with basic dyes. These can be seen by light microscopy in polyp tissue, where they are present throughout the interstitium and particularly around the blood vessels. Degranulation produces histamines and other vasocitive chemotactic factors.

Histamine has been localized in the cytoplasm of mast cells by histofluorescent techniques (Bumsted *et al.*, 1979) but it is the free extracellular histamine which is physiologically active. Considerable amounts of free histamine have been found in fluid extracted from nasal polyps (Drake-Lee and McLaughlin, 1982).

A previous study of the fine structure of nasal polyps noted that mast cells were partly degranulated, but no comment was made on their ultrastructure (Cauna *et al.*, 1972).

The aim of this paper is to study and draw attention to the features of mast cells in nasal polyps examined by Transmission Electron Microscopy (TEM).

Materials and Methods

Polyps from six adult patients were studied; they included patients both with and without allergy, asthma, hay fever and reactive skin tests. None of the patients were taking antihistamines or any intranasal medication. The inferior turbinate was removed from a patient during an SMR following trauma; he had no nasal symptoms besides blockage, and no history of allergy. This tissue acted as control for both the method of preparation for electron microscopy and the state of normal nasal mast cells. All surgery was performed under local anaesthetic, as described previously.

The tissue was washed in normal saline to remove the local anaesthetic agent, mucus and blood and placed immediately into Karnovsky's fixative for four hours, and then bulk stored. Tissue was dehydrated through an ethanol series and gradually infiltrated with dilute Spurr resin until they were

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embedded in 100 per cent resin (Spurr, 1969). After hardening, semi-thin sections (1μ m.) were taken for light microscopy and stained with a methylene blue and azure II mixture (Richardson *et al.*, 1960). Ultra-thin sections were mounted on grid and double stained with uranyl acetate (Gibbons and Grimstone, 1963) and lead citrate (Reynolds, 1963), and then examined in a Siemens Elmiskop I electron microscope. Micrographs were recorded on Ilford 6.5 cm. by 9.0 cm. EM film.

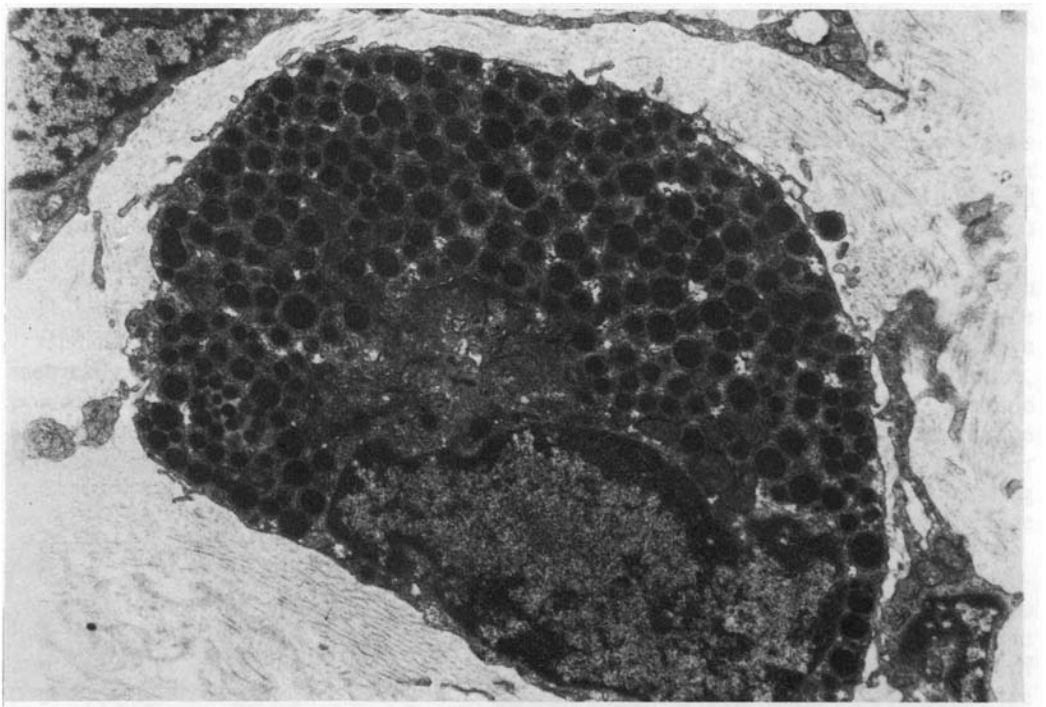


FIG. 4

TEM. Turbinate mast cell ×11,000. See text.

Results

Turbinate

Mast cells were of normal appearance, with a large eccentric nucleus containing patchy chromatin (Fig. 4). The cytoplasm contained the perinuclear Golgi apparatus and occasional mitochondria. A microtubular apparatus was present but not well developed. The cytoplasmic granules were the most prominent feature and were made up of a core of electron-dense material, most commonly crystalline in structure, but occasionally recognizable scrolls could be seen. The cell surface had characteristic cytoplasmic processes.

Nasal polyps

The basic structures of the cells were easily identified, but the striking feature was mast cell degranulation. Occasional full electron-dense granules were seen; these were smaller in size than either the partly degranulated granules or the slightly larger empty granules. The proportion of partly filled and empty granules varied from cell to cell and from patient to patient. A proportion of granules contained both electron-dense and electron-lucent areas; the dense areas had some evidence of crystalline structure (Fig. 5). The empty granules also contained structures which included preserved scrolls, although usually the material was more

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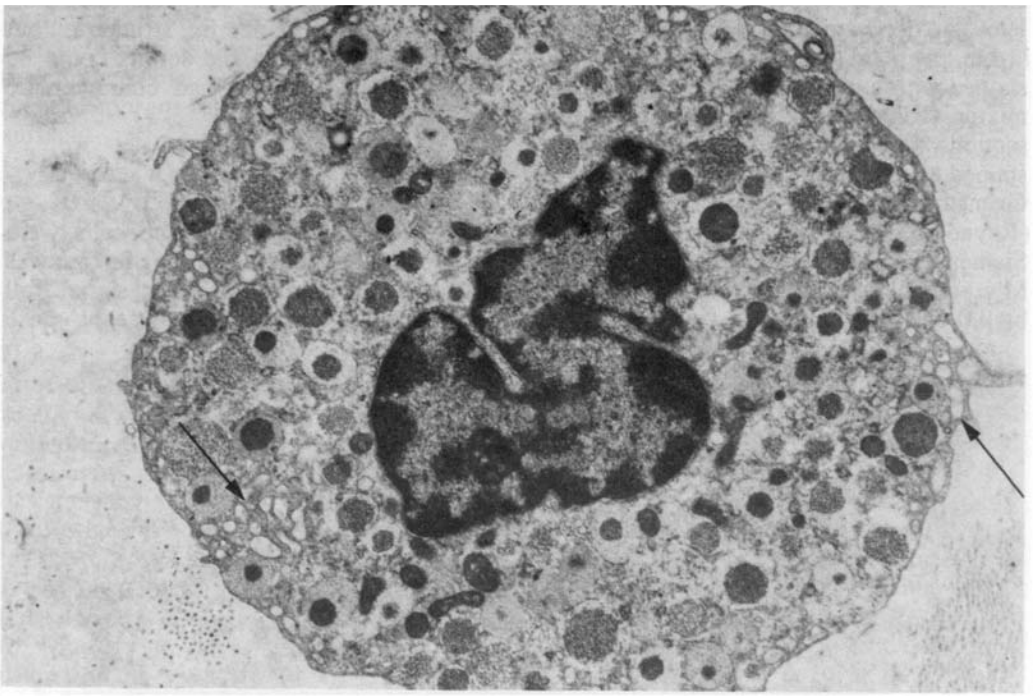


FIG. 5
TEM. Partly degranulated mast cell, ×15,000; microtubular system arrowed.

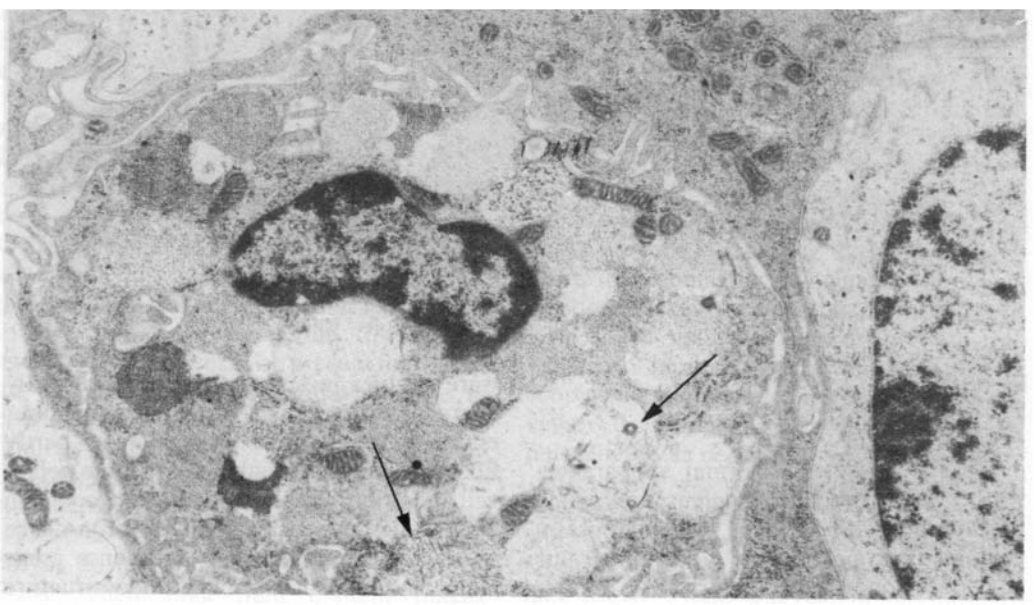


FIG. 6
TEM. Partly degranulated mast cell ×18,000. Scroll in otherwise degranulated granules (arrow).

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amorphus in nature (Fig. 6). A microtubular system was present in all cells and was particularly well developed in some. Microfilaments were noted between granules, but were not numerous. The cells also showed the characteristic surface projections.

Discussion

Mast cells are numerous in nasal polyps, and electron microscopic studies have revealed that most of these cells are partly degranulated. Mast cells release various endogenous 'primary' mediators, including histamine and eosinophil chemotactic factors, and also induce the formation of 'secondary' mediators, e.g. slow reacting substance of anaphylaxis, platelet activating and prostaglandins (Padawer, 1980). The large numbers of eosinophil leucocytes in nasal polyps is due to the secretion of eosinophil chemotactic factors. Eosinophils contain, within their granules, agents which degrade mast cell mediators, e.g. histaminase and arylsulphate-B (degrading SRS-A).

Histamine can be measured spectrofluorometrically in fluid extracted from nasal polyps. High but variable levels are present (140–7,500 ng./l., mean 1,450 ng./l.) (Drake-Lee and McLaughlin, 1982). This is far in excess of the serum level (10 pg./l.) and confirms that mast cell degranulation is occurring in polyps.

Mast cell degranulation is usually considered to be secondary to a Type I hypersensitivity reaction mediated by IgE antibodies bound to mast cells triggering degranulation when the appropriate antigen is present (Padawer, 1980). However, the majority of patients with nasal polyps do not have a history of atopy. The commonest associated atopic condition is asthma, found in 20–40 per cent of patients with nasal polyps (Moloney and Collins, 1977). Hay fever is not common and its incidence in patients with polyps may be no greater than in the general population (Drake-Lee and McLaughlin, 1982). Eczema and drug hypersensitivity are seen in a few cases, the most common being aspirin hypersensitivity which is found in approximately 8 per cent of patients with nasal polyps (Delaney, 1976).

IgE levels have been measured in extracts of nasal polyps and are higher than in serum, but it is not certain whether this represents the normal activity of the nasal mucosa, or is pathologically significant. Alternative means of mast cell degranulation have been proposed, e.g. by activated complement IgG_4, drugs and trauma (Drake-Lee and McLaughlin, 1982).

Electron microscopic studies of mast cells from human lung have shown that the granules may exhibit two different appearances (Caulfield *et al.*, 1980): the first, and most common, appearance is an organized crystalline structure, seen as a lattice network or as scrolls, depending on the plane in which the granule is examined; the second, and less frequent, appearance is that of amorphous granules. Studies on mast cells in the nose (Trotter and Orr, 1973) and gingiva (Weinstock and Albright, 1967) have produced similar results.

Mast cell degranulation caused by antigen combining with IgE on the mast cell surface is a rapid event which is complete within five minutes. Granules lose their structure, become amorphous and enlarge. Granule membranes fuse with the plasma membrane and also with the membranes of other granules producing its communicating channels through which granule contents are discharged.

The present study indicates that degranulation of nasal polyp mast cells differs in two ways from the above sequence: first, some granules contain a mixture of components of different electron density and that the electron-dense areas are often crystalline in appearance; secondly, scrolls are sometimes seen in otherwise empty vacuoles. It is suggested that these findings may indicate a different mode of mast cell activation from the classical IgE-mediated sequence, possibly occurring over a more prolonged time scale. Further studies will be required to evaluate this.

Summary

Comparison of the ultrastructure of mast cells in patients with nasal polyps with

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those found in an inferior turbinate taken from a non-allergic patient, has shown that degranulation occurred only in polyp cells. Polyp mast cells showed varying degrees of degranulation with vacuoles predominating. Electron-lucent and electron-dense material was found in some granules; crystalline structures and scrolls, normally seen in cells prior to degranulation, were found in otherwise degranulated vacuoles. These findings would support the theory that mast cell degranulation in nasal polyps is in some way different from the more rapid changes found in cells activated by the classical IgE-mediated pathway.

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