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Mast cell ultrastructure in the adenoids of children with and without secretory otitis media

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Abstract

This study was designed to compare the differences in morphology of the mast cells from the adenoid in children with otitis media with effusion (OME) and those from children with recurrent tonsillitis. Tissue for electron microscopy was prepared in the standard manner and between three and 10 blocks were examined for each child. All the mast cells with nuclei were photographed and the condition of the granules noted. The number of electron dense granules in each cell was assessed on a scale between zero and 10. Sixteen unselected children with OME were compared with 19 children with recurrent tonsillitis. There were no obvious differences in the degree of degranulation between the two groups although there was more vacuolation than previously described in the normal nose but less than in those patients with perennial allergic rhinitis. Allergy and mast cell reactions do not seem to predispose to OME. It was concluded that the adenoids are not the ideal tissue in which to study normal mast cells.

Key words: Otitis media with effusion; Mast cells, ultrastructure; Adenoid; Tonsil

Introduction

The aetiology of otitis media with effusion (OME) is debated and a large number of theories of pathogenesis have been put forward to try and explain a condition that is probably multifactorial in its aetiology. The condition may be linked with respiratory disease in general, including rhinitis. Nasal allergy, as a factor, has received support from some authors (Phillips *et al.*, 1974; Bain, 1981) although this view is not widely upheld. OME varies in severity and atopy is probably associated with recurrence (Kjellman *et al.*, 1976). Certainly, children with OME have a greater incidence of nasal symptoms (Van Cauwenberge and Derycke, 1983) which may be due to adenoid hypertrophy but it is worth assessing patients with nasal symptoms for allergy (Kjellman *et al.*, 1976).

The role of the adenoids in giving rise to nasal symptoms and their removal in the management of the condition is also debated but there is some evidence that removal results in quicker resolution of the condition in the middle ears that are not treated by ventilation tubes (Maw, 1983).

Mast cells have been investigated extensively and their central role in allergy is accepted although there is much controversy in the cellular mechanisms involved in the allergic response. Degranulation of mast cells by allergen acting with surface IgE is the initiating event. The preformed mediators, including histamine, are found in the granules and are released immediately. In rodents, the process is by granule exocytosis: in man, the granule contents dissolve within the cell leaving a vacuole. Intact mast cell granules are electron dense on electron microscopy and become vacuolated during degranulation. However allergy is not the only cause of mast cell degranulation and many other triggers will induce degranulation to some degree.

Collins *et al.* (1985) believe that the raised adenoid-free histamine in patients with OME is important in its pathogenesis. Since mast cells are the source of tissue histamine, adenoidal mast cells should be more degranulated irrespective of the cause in children with OME.

We have demonstrated that the majority of mast cells from the inferior turbinate of the normal nose were electron dense but some cells had a large number of granules that were less electron dense and degranulation was probable here. These changes were very different from those found in patients with perennial allergic rhinitis where the mast cells were usually degranulated but there were also a small number of normal cells (Drake-Lee and Price, 1991). These changes would fit well with the accepted role of mast cells in allergic rhinitis.

The aims of this study were twofold: firstly, to evaluate the morphology of mast cells in the adenoid in general and secondly, to look at any differences in degranulation between two groups of patients supporting local allergy or mast cell degranulation in the adenoids in patients with OME. The ultrastructural morphology of mast cells in adenoidal tissue of children with OME will be compared with those found in children with recurrent tonsillitis and other conditions but with no history of ear disease.

Materials and methods

Patient selection

Adenoidectomy was undertaken in children with OME

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who had either nasal symptoms, recurrent OME which had been treated previously or recurrent sore throat in addition to OME. Sixteen children, who were admitted at random and with at least a six-month history of persistent middle ear effusions present on admission and confirmed at the time of surgery, were studied. Children with active rhinosinusitis were excluded.

OME was diagnosed by the history, otoscopy findings, conductive hearing loss on pure tone audiometry and a flat impedance tympanogram. The effusion was confirmed at surgery prior to the insertion of ventilation tubes.

An allergic and nasal history which included symptoms of rhinitis, asthma, eczema and drug allergies was documented both in the patients and the first degree relatives. Patients with a positive history had skin tests performed, by the prick method, to 10 common allergens (Bencard, R.). If the flare was greater than 3 mm the result was positive.

Nineteen children without OME, who were admitted with either a history of either recurrent tonsillitis or nasal obstruction due to adenoid hypertrophy, were studied. To be selected they had to have had no history of middle ear infections during the preceding 12 months and never to have had documented OME. A normal pure tone audiogram and an impedance tympanogram within normal limits were also required. A full allergic history both of the patients and first degree relatives was taken. Children who had nasal symptoms had skin tests undertaken, by the prick method, to 10 common allergens.

None of the children in either group were on any antihistamine nor any anti-allergy therapy.

Tissue preparation

Adenoids were removed by curetting in the standard manner and measured by eye following curettage into small, medium and large pads. Following removal, diced cubes of under 1 mm square were placed into fixative at the time of the operation. Glutaraldehyde at 4°C was allowed to return to room temperature and the samples were processed and prepared in a standard manner as previously described (Drake-Lee and Price 1991).

Between three and 10 blocks were examined for each patient and every mast cell that had a nucleus was photographed for study. We have previously described a method of assessing the degree of degranulation which involves counting all the granules and vacuoles in the cells under a transparent plastic grid of squares with sides of 2.5 cm (Drake-Lee and Price, 1991). All completely electron dense granules of any morphology were counted and a degranulation index was evaluated. The number of electron dense granules were divided by the total number of granules and scaled between in whole numbers between zero and 10, zero being a completely degranulated cell. A value of 10 was used since the number of mast cell granules was very variable and ranged between 11 and 204.

Results

The ages of the children with OME ranged from three to

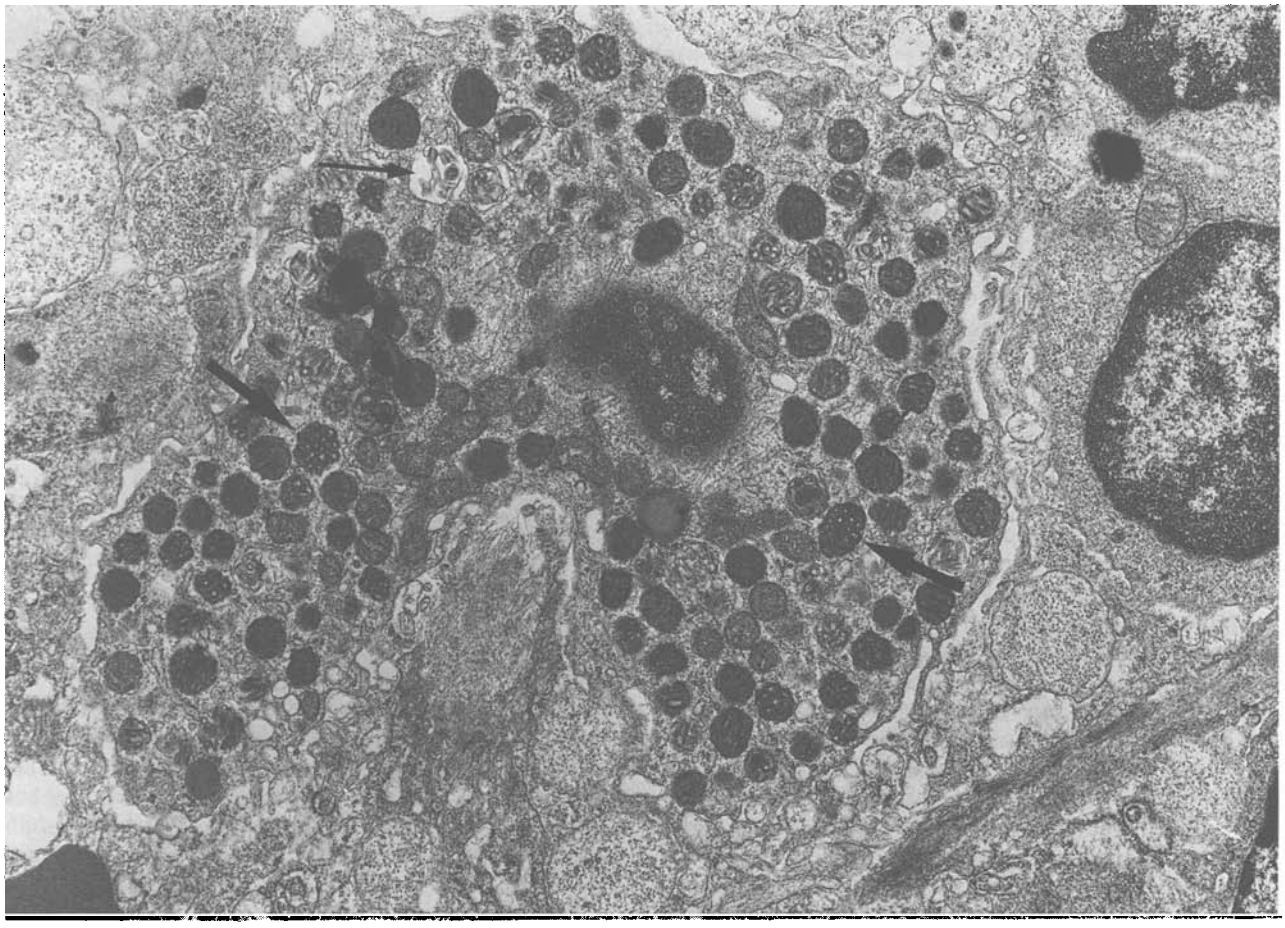

FIG. 1

A large mast cell from the adenoid showing normal features. The granules in this cell are organized into scrolls (large arrows) and amorphous granules. There are also some granules that contain only a few remnants of scrolls in otherwise empty granules (small arrow) (× 3000).

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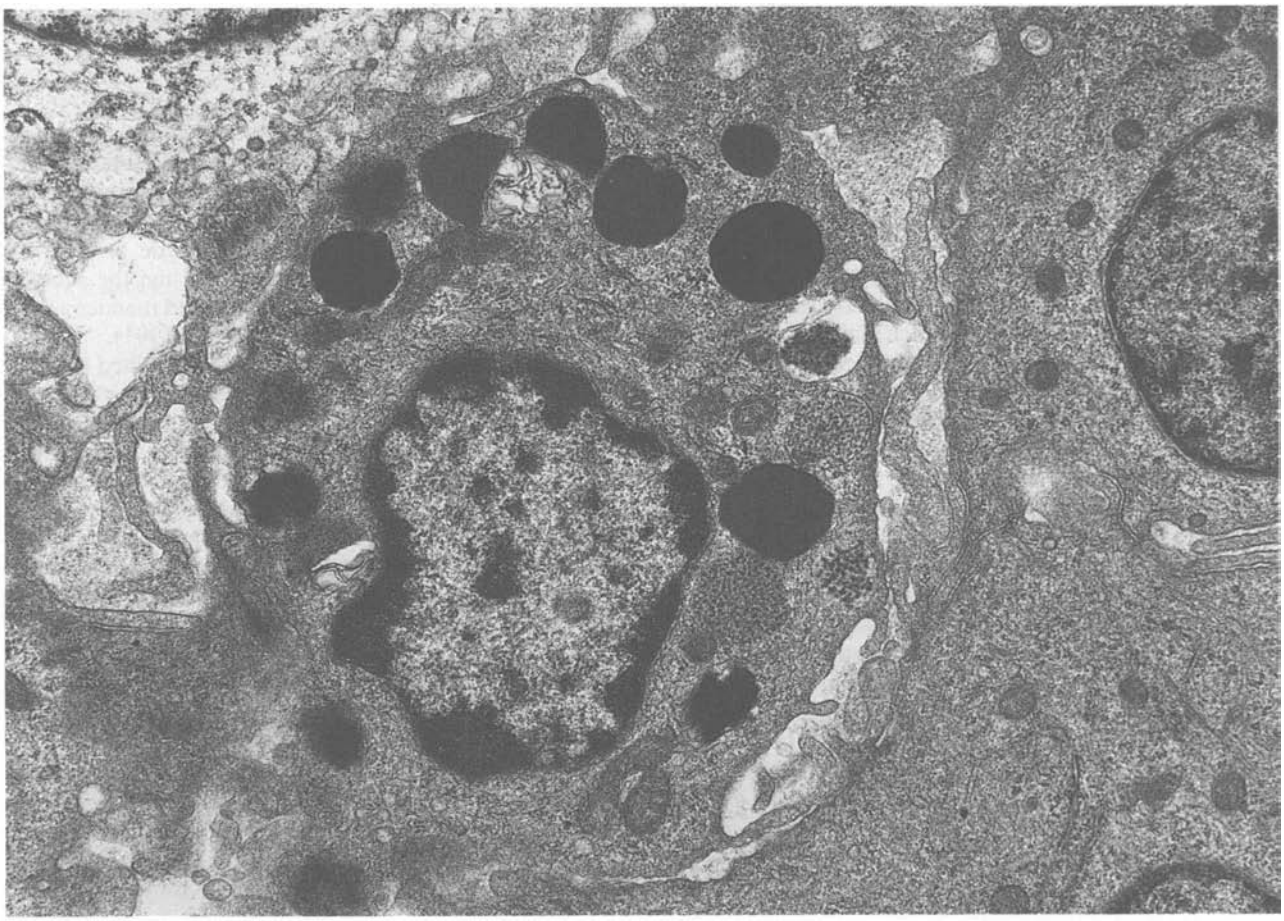

FIG. 2

A smaller mast cell showing amorphous electron dense granules which are both intensely electron dense and of variable degrees of density (× 4000).

eight years (mean 5.5; median six years) and there were five girls. The 'control' group had an age range of 1.5–12 years (mean and median seven years) and 13 were girls.

None of the children with OME had a history of asthma but nine had a history of nasal obstruction: all had negative skin tests and three had large adenoids. One child had a history of eczema and one was allergic to penicillin. Only two first degree relatives had a history of allergic problems: one had asthma and one had seasonal allergic rhinitis. The effusion was serous in four cases but in none was it purulent.

In contrast in the control group two children had asthma and one had perennial allergic rhinitis with a positive skin test to house dust mite extract. Three children had hypersensitivity to penicillin. Two first degree relatives had a history of allergy: one had asthma and one had season allergic rhinitis. The youngest child had obstructive sleep apnoea syndrome due to adenoid hypertrophy and in four more the adenoids were classified as large.

Mast cells

The morphology of human mast cells has been well documented and the cells studied here conformed to previous descriptions (Figures 1–4). Mast cells were less numerous than in the nasal mucosa and the number of granules ranged between 11 and 204 (mean 69) for patients with OME and 30 and 187 (mean 72) in the control group. Although scrolls are found in most granules (see Fig 1) the majority of electron dense granules are amorphous (see Figures 2–4).

While Figure 1 shows a large cell with the majority of its granules electron dense, Figure 2 shows an apparently much smaller cell with electron dense granules. Figure 3 demonstrates a smaller cell with both electron dense granules and vacuoles and in one of these scrolls can easily be seen. Figure 4 illustrates a large cell with granules in varying degrees of electron density.

Figure 5 summarizes the degree of degranulation of mast cells. There appeared to be a slightly greater degranulation in patients with OME but the differences were not statistically significant.

Discussion

The morphology of human mast cells has been studied extensively and the ultrastructure has been well documented (Galli *et al.*, 1984). The cell has electron dense granules but all other features are variable (Drake-Lee and Price 1991). It has not been possible to subdivide the cells on ultrastructural criteria into mucosal and connective tissue types: the number of granules within cells appear to be normally distributed (Drake-Lee and Price, 1991).

The electron dense granules can be arranged into recognizable patterns in humans. The most obvious is parchment-like scrolls and hence the name. Sometimes the

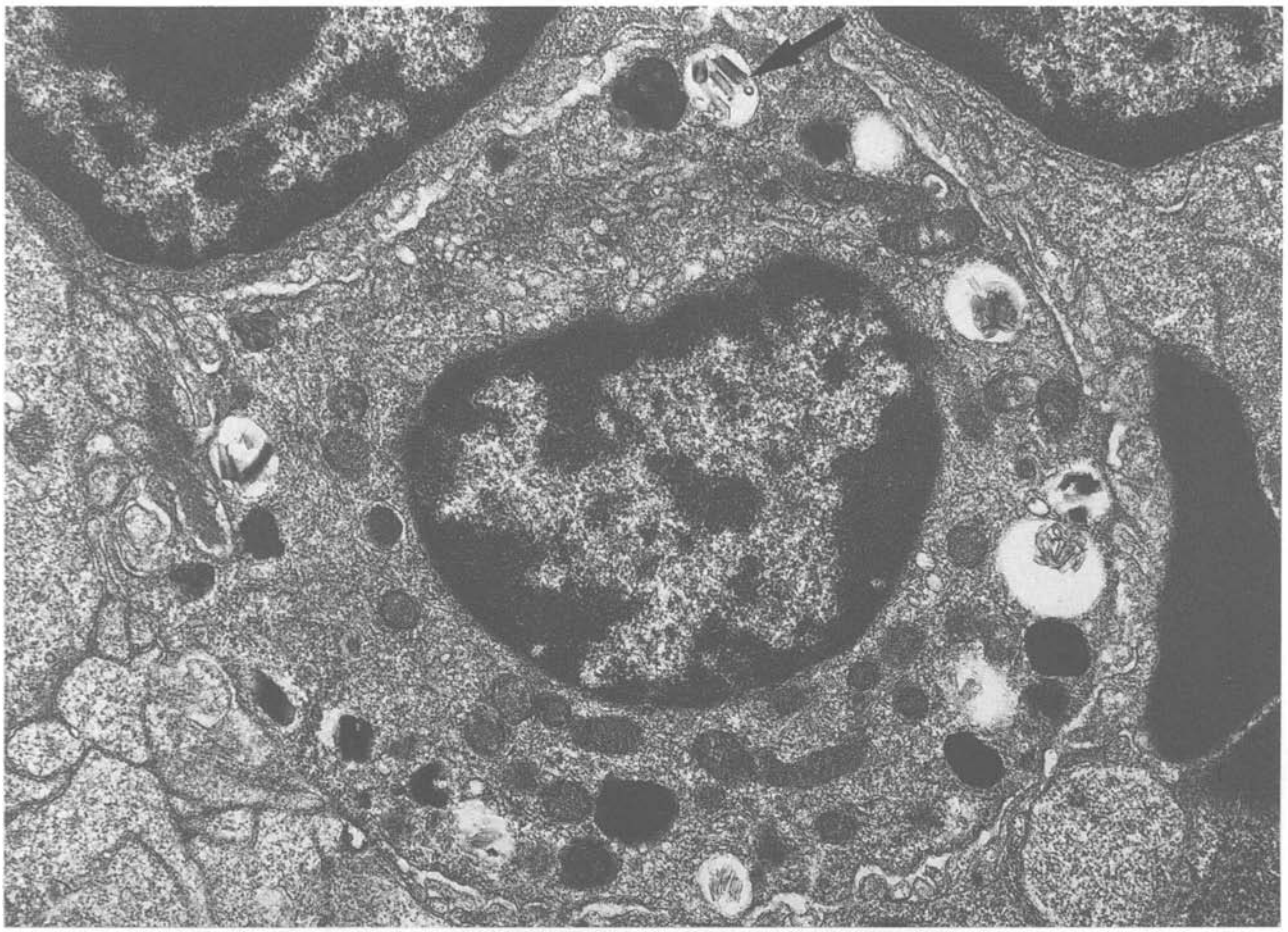

FIG. 3

A smaller mast cell where most of the granules are partly degranulated. The remnants of scrolls can be seen but here they have been cut along their long axes (large arrow) (× 4000).

organization appears as a honeycomb lattice, but this is found more frequently in the skin. An amorphous granule is seen most often in the human nose. It is believed that the more organized the granule, the more stable it is. Some evidence of granule loss has been found in the normal nasal mucosa and probably indicates normal reactions occurring because of the protective nature of the mucosal surface (Drake-Lee and Price, 1991).

The changes in mast cell morphology that occur in patients with allergic rhinitis were first documented by Trotter and Orr (1973) but this study included patients with asthma and there were no patient details included. *In vitro* allergic degranulation of isolated lung mast cells produced major changes within five minutes which included gross degranulation and vacuolation (Caulfield *et al.*, 1980).

Degranulation undergoes the following changes when studied *in vitro;* the organized structure of the granule is lost and the granule becomes larger and amorphous, then the granule enlarges further and through a series of microtubules, the material of the granule is discharged from the cell until a vacuole remains. These changes are different in different species, rodents degranulate through a process of granule exocytosis so care has to be exercised when animal studies are applied to man.

Unfortunately degranulation removes the most striking feature of mast cells, the electron dense granule, so that vacuolated cells may be difficult to identify in tissue sections. The changes described *in vitro* appear to differ from those seen *in vivo* and involve differences both in degree and in speed: the *in vivo* changes being slower and less drastic. The microtubular system is less obvious (Drake-Lee and Price, 1991). Scrolls are not always lost and can be seen in an almost empty granule, as seen in Figure 3, and this together with electron dense material helps to identify mast cells.

We looked at patients with perennial allergic rhinitis and found that there was a subpopulation of normal mast cells but the majority had lost over half the material from their granules when compared with normal nasal mucosa where there were only a few degranulated cells.

The possible reasons for degranulation of mast cells in the adenoid and middle ear are diverse and include not only allergy but also infection and complement reactions. Children have an increased incidence of upper respiratory tract infections. Some of the infections may be subclinical. While allergic children do not appear to have an increased incidence of upper respiratory tract infections, these patients may have respiratory hyperreactivity. Although histamine does not appear to produce the symptoms of rhinovirus colds Naclerio *et al.*, 1988), viral infections may give an increased sensitivity to histamine (Clementsen *et al.*, 1988) probably mediated by indirect mechanisms. The mode of action is debated; viruses may degranulate mast cells nonspecifically as a consequence of infection or, as in dogs, they may act with allergens to produce an experimental atopic disease (Gold, 1986).

Palva and his colleagues looked at mast cell numbers in

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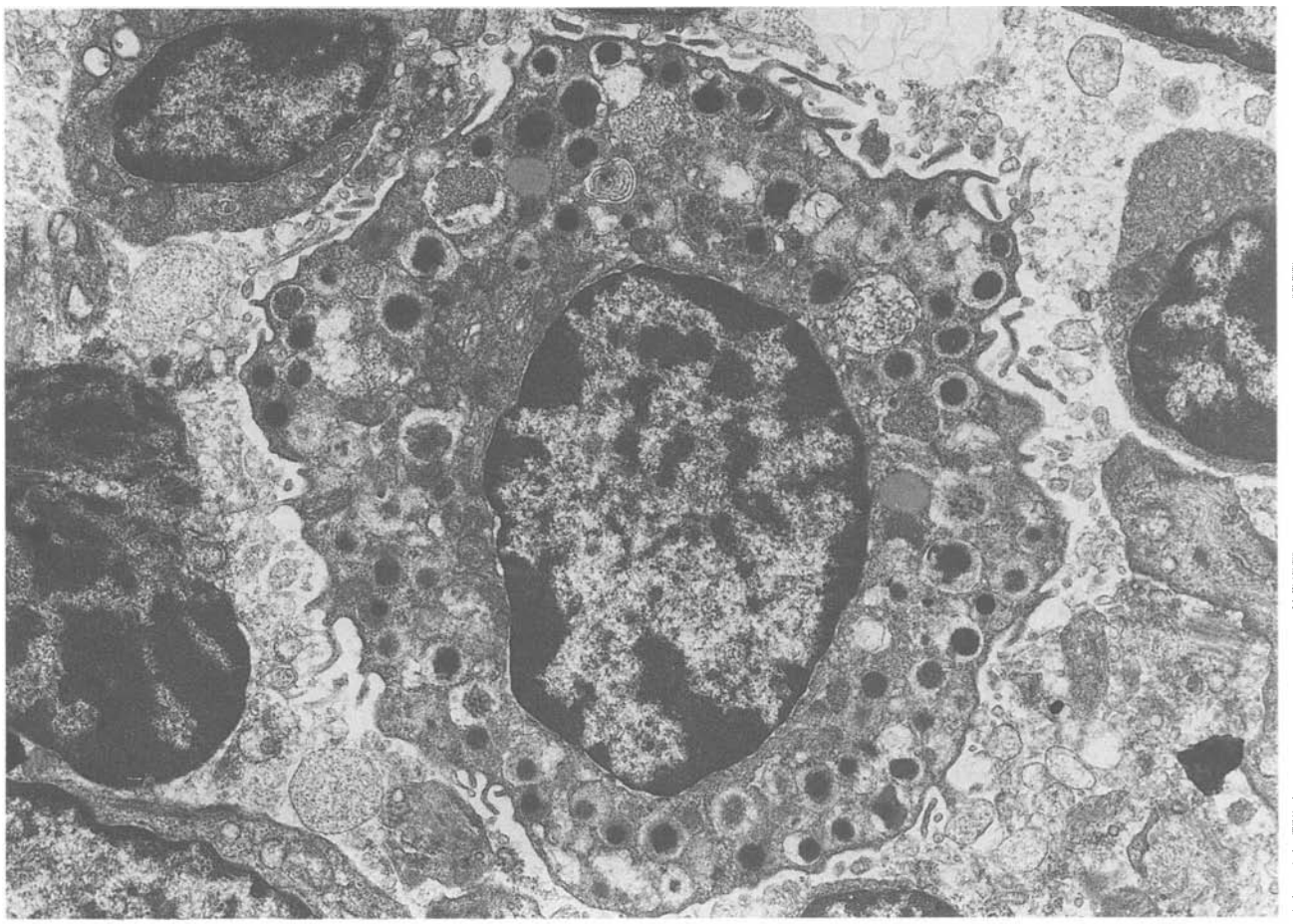

FIG. 4

A large mast cell showing a few completely electron dense granules. The size and amount of the cell occupied by the less dense granules can be seen to have increased (× 3000).

the adenoid and middle ear mucosa as well as the levels of free histamine in nasopharyngeal secretions and the middle ear effusions. They also looked at the adenoidal tissue histamine. The levels of free histamine in nasopharyngeal secretions were lower than within the adenoid, suggesting that histamine was present by diffusion: mast cells were less numerous in the ear than in the adenoids and levels of histamine in the effusions were higher than serum but significantly lower than in nasopharyngeal secretions (Palva *et al.*, 1991). These findings and those of Collins *et al.* (1985) of increased tissue histamine levels may reflect the increased degranulation that occurs in adenoidal tissue since the mast cells take part in both non-specific and specific immunological reactions.

Children who have an increased incidence of upper respiratory tract infections should have more evidence of mast cell degranulation, from whatever cause, compared to those without them. It is virtually impossible to find children who do not have a history of upper respiratory tract infections and it is not ethical to remove normal adenoid tissue from normal children. Similarly surgery was not undertaken on children with an active upper respiratory tract infection. The 'control' group were chosen since they had no evidence of middle ear disease.

This study showed that mast cells were more degranulated than we had previously found in the normal nose. This was consistent in both groups with an increased incidence of upper respiratory tract infections but less in those for patients with perennial allergic rhinitis (Drake-Lee and Price, 1991). There was little difference between those patients who had OME and those who had a recurrent sore throat. It would appear that in an unselected population allergic reactions are unlikely. It would also

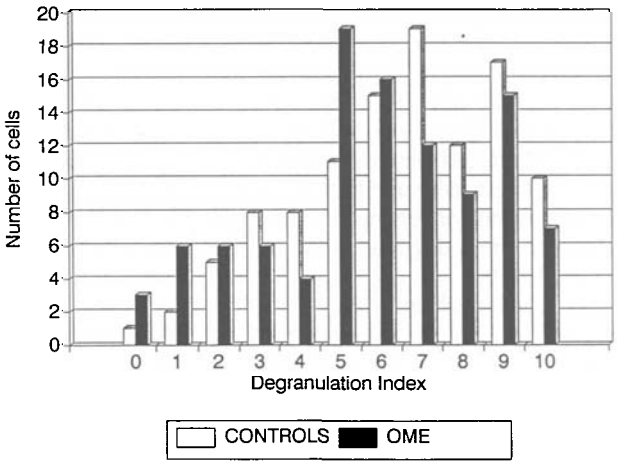

FIG. 5

The degree of degranulation of mast cells in the adenoid assessed by the number of electron dense granules expressed as a degranulation index. On a scale zero to 10 (zero being a completely degranulated cell). The degranulation index is compared with the number of cells in all the blocks from each group of patients to show gross evidence from the two populations.

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appear that mast cell reactions and the release of histamine into the post-nasal space were unlikely to predispose the child to the development of OME. This study supports the conclusions drawn from clinical studies that allergy in the upper respiratory tract is not a predisposing factor in the development of OME.

Adenoidal tissue has been used to study mast cell reactions (Behrendt *et al.*, 1978). The findings in this study of an increased mast cell degranulation compared with the normal nose would indicate that adenoidal tissue is not the ideal tissue with which to study mast cell reactions.

Conclusions

Mast cells were more degranulated than those studied previously in the normal nose and less degranulated than those previously described in patients with perennial allergic rhinitis. Adenoids are not a good source for studying the normal reactions of mast cells *in vitro*. There was no evidence that mast cells of the adenoids of children with OME showed increased degranulation when their morphology was compared with those from children without the condition and would not support the hypothesis that local allergy predisposes the child for the development of OME.

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