Rhinology, 30, 229-239, 1992

A review of the morphology of human nasal mast cells as studied by light and electron microscopy

A.B. Drake-Lee[1] and J. Price[2]

[1] Queen Elizabeth Hospital, Birmingham, U.K.
[2] E.M. Unit, Royal United Hospital, Bath, U.K.

SUMMARY

This review discusses the distribution and classification of human nasal mast cells after the use of different fixatives, and some of their staining characteristics, both at the light- and electron-microscopical level. The problems encountered with alcoholic and formaldehyde fixation are discussed as well as the limitations of different stains (including the basic aniline dyes), esterase cytochemistry and immunological techniques. Also, the respective limitations of light and electron microscopy are compared. Cells studied by means of electron microscopy are much more difficult to quantify objectively. It is concluded that classification of mast cells – by means of their morphology, fixation and staining characteristics – into two categories (mucosal vs. connective tissue; T- vs. T/C cells) is simplistic, especially since human nasal mast cells are both heterogeneous and pleomorphic.

INTRODUCTION

The nose is the preferred respiratory channel at quiet respiration and it protects the lower airway by selectively filtering out all particles down to 30 μm as well as some smaller ones which includes many of the pollen (Mygind, 1978). Since mast cells are present in the submucosa of the normal nose, some reactions should be encountered as part of the normal homoeostatic mechanisms as well as those occurring in disease such as allergic rhinitis.

In vivo challenge and biopsy studies in seasonal allergic rhinitis have suggested that the allergic reaction is due to mast cell degranulation with basophilic cells increasing during allergen exposure (Enerback et al., 1986; Viegas et al., 1987; Naclerio et al., 1985). The first phase is the release of preformed mediators from mast cells following antigen interaction with surface-bound IgE. Arachidonic acid mediators are then generated from mast cell membranes. A later phase is due to the influx of other cells, including basophils, and their release of com-

pounds which give rise to the second peak of symptoms. The reactions in patients with perennial allergic rhinitis are less easy to evaluate, and in those who suffer from dust mite sensitivity all three phases should be present because of the repeated antigen exposure.

A biopsy is a freeze-frame of tissue events, i.e. cells developing, resting, reacting, and dying. It is only by the systematic study of cells in that particular tissue that events can be understood. The morphology of mast cells may be dependent on the function of the tissue in which they are found, and alterations in mast cell granules could be due to two processes, that is their development or the events that occur in degranulation. Maturation is largely ignored and emphasis on in vitro studies is on degranulation. Skin and gingiva are covered by an impervious squamous epithelium and there is little activity in the subepithelial tissues; whereas the respiratory tract and gastrointestinal tract are designed to permit rapid transit through the epithelium, and hence more mast cell reactions. It would follow that in more reactive tissues there would be a greater variety of mast cells, many of which are more immature.

A cell type may be defined by three different factors: (1) its morphology; (2) its biochemical reactions; and (3) its function within the organism. Studies in each area are complementary, but may yield different (and sometimes contradictory) results. Great care has to be undertaken when the results from the same cell type are compared between different species; the results from one species are not necessarily applicable to another.

Limitations of study may also be due to the methods used in examining the cells. Electron microscopy is invariably descriptive when applied to human mast cells and no attempt is made at quantifying results, which is partly due to the length of time needed to study mast cells and partly due to the small size of the pieces of tissue examined.

The aim of this study is to review the methods and the problems of demonstrating mast cells by both light and electron microscopy.

MAST CELL HETEROGENEITY

Light-microscopically, basophilic metachromatic cells can be divided into two distinct populations, i.e. mast cells and basophilic granulocytes. There are considerable species-dependent differences in these cell types. Some animals (e.g. rat) have few basophils, and "two subpopulations" of mast cells; whereas other animals (e.g. guinea pig) have large numbers of basophils and less readily-identifiable mast cell subpopulations. Man is somewhere in the middle with both circulating basophils and tissue mast cells.

Mast cells shows large numbers of metachromatically-staining basophilic granules, and are found in the connective tissues of the body. They were first described by Erhlich (1879) – when a medical student – who noticed that some

cells that had been previously considered as plasma cells stained metachromatically with aniline dyes. He used the term "masten" (meaning: "stuffed" or "fatten") to describe these cells, which he thought were phagocytic. The first suggestion of two mast cell populations, based on fixation, was made in 1895 by Hardy and Wesbrook. They studied a number of different species (excluding man) and stated that: "The differences between the splanchnic and coelomic basophilic cells of the rat are exceedingly striking both as regards for the size and stability of the granules and the size of the cells." More recently, this work has been re-explored by Enerback et al. (1981).

There are larger and smaller mast cells, and this has led to a morphological classification of intestinal mast cells, based on their fixation and staining characteristics. Rodent mast cells are divided into mucosal and connective-tissue types. 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. They are closely related to the blood basophils, the latter showing multilobated nuclei and spindles. Granules tend to be fewer in blood basophils and the smaller mast cells. Connective-tissue mast cells are fixed by alcoholic and aqueous fixatives, whereas mucosal mast cells are fixed better by alcoholic fixatives. Other features will be considered later.

Several investigators have tried to prove that there are two distinct populations in humans. Greenwood and colleagues reviewed the literature and concluded that there were two types of mast cells applicable to humans (Greenwood et al., 1986). At the same time, a review by Pearce (1986) stated that: "The widespread use of the term mucosal mast cell [...] is not only distressing to the purist, but is potentially dangerous, ambiguous and confusing." It would appear that the matter is not as clear-cut as is sometimes expressed. Pearce considers that mast cells may not only be species specific, but also organ specific.

LIGHT MICROSCOPY

When mast cells are studied by light microscopy, a number of different fixatives and stains are used. Most histology is performed on tissues fixed in a formol solution, and the mast cells are stained deeply 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 Carnoy's fixative should be used when studying mast cells populations in the human gut (Stobel et al., 1981). However, there have also been reservations on the use of Carnoy's fixative when studying mast cells, because it may be very toxic to tissues (Michels, 1938).

Fixation is a complex process and may be reversible, particularly if dilute aqueous solutions of formaldehyde are used for over twenty-four hours (Hopwood, 1982). Ideally, a fixative should penetrate tissues quickly and preserve as

much antigenicity as possible. Aqueous formaldehyde solutions are the best compromise, because they preserve antigenicity, penetrate tissue well and will store, whereas paraformaldehyde solutions have to be made fresh. Carnoy's fixative destroys many of the proteins, and so is of little use if immunochemistry is required, but it does preserve nucleic acids. Formaldehyde has been combined with acetic acid to fix mast cells and it has been suggested that, because of the different sensitivity of tissues to staining following postfixation treatment with formol, human mast cells may indeed be subgrouped (Pipkorn et al., 1988).

Tissue is sometimes modified before it is fixed, so that cells are studied in greater numbers. Enzymatic degradation of tissues and separation of mast cells on a gradient has been undertaken, but there is little work in humans to show whether the process itself alters mast cells.

Mast cell granules may be stained metachromatically with different stains including toluidine blue, azure A, Bismarck brown, thionin, Csaba's alcian blue-safranin, and by enzyme (such as the cholinesterase technique) and immunoenzyme techniques. Trotter and co-workers found that alcian blue and safranin gave problems with overstaining when tissue was fixed in neutrally buffered formaldehyde – but gave good results after fixation with paraformaldehyde – and that staining was variable with toluidine blue (Trotter et al., 1990). We have found that azure A gives the most consistent results; the dye fades least on storage, and may be used with neutrally buffered formaldehyde.

It has been suggested that Carnoy's fixation followed by the chloroacetate esterase technique is satisfactory for demonstrating mast cells in seasonal allergic rhinitis (Viegas et al., 1987). Since the naphthol AS-D chloroacetate esterase method is not specific and stains polymorphic leukocytes as well, these results must be viewed with caution. Immature polymorphs may not have multiple indentations of their nucleus, some mast cells are small and the late phase of the allergic response may give rise to a leukocytosis.

Interest in enzyme-cytochemical staining of granules present in human mast cell started in 1953 when Gomori used them as a histochemical substrate. The only specific substrate for mast cells is naphthol AS aminocaproate, which demonstrates a trypsin-like activity (Li et al., 1973), but we have been unable to obtain this commercially. Garrett et al. (1987) found that D-Val-Leu-Arg-methoxy-2-naphthylamine is the best substrate. They were concerned that many of the esters used lacked specificity and therefore unacceptable for routine use. They also emphasized that positive staining is dependent on two factors, i.e. the amount of the enzyme present, and its bio-availability. Other workers have felt that human mast cells may be classified by the presence of tryptase and chymase within the granules (Schwartz et al., 1987). Tryptase-containing cells (T cells) are the equivalent of mucosal mast cells. Cells containing both chymase and tryptase (T/C cells) are the same as connective-tissue mast cells, but Craig et al. (1988) raised the possibility that these reactions were due to differences in maturation.

Trotter et al. (1990) confirm the view that Carnoy's is destructive to the tissues and should not be used to demonstrate mast cells in the nose. Others have suggested that the increased numbers of mast cells is due to the poor fixation of the background (Drake-Lee et al., 1988). Comparison of two different fixation techniques in perennial rhinitis failed to demonstrate two cell types (unpublished data), as it did in patients with nasal polyps (Drake-Lee et al., 1988).

Mucosal and connective-tissue mast cells may have different staining reactions with alcian blue-safranin, but the work of Combs and co-workers on embryonic rat tissue suggests that these reactions are due to variation in the maturation of mast cells, with the granules changing from red to blue as the cells mature (Combs et al., 1988). Similar considerations hold true for the immunoenzyme techniques; cells may possess different enzymes at different stages of development rather than being two different cell types. It is equally possible for the tissues themselves to alter the way a cell develops and, therefore, the way by which it reacts.

Rat mast cells may be subgrouped by berberine fluorescence and by their staining reactions with toluidine blue following pre-treatment with nitrous acid; berberine fluorescence is normally present in connective-tissue mast cells, and nitrous acid treatment prevents binding of toluidine blue to connective-tissue mast cells. In contrast, human mast cells show only weak fluorescence with berberine and fail to stain with toluidine blue following treatment with nitrous oxide (Pipkorn et al., 1988). Although these authors tried to justify the subgrouping of mast cells on their susceptability to formaldehyde, they had to admit that the differences were less pronounced in man.

One of the features of the mast cell is its ability to bind IgE, and this may be demonstrated by immunoperoxidase staining. Plasma cells are of a similar size, so they should be identified as well. It is possible to combine both the techniques of immunochemistry and metachromasia. Toluidine blue may be used in a dilute solution and will fade, so it is possible to see both the brown staining of the immunoperoxidase and the metachromasia as well (Drake-Lee and Barker, 1984). Caution must be taken when immunostaining of mast cell granules is undertaken, since they have a natural affinity for foreign proteins and can stain non-specifically.

It is equally important that the size of the piece of tissue is representative. We agree with the view that small punch biopsies and scrappings may well not be representative (Trotter et al., 1990). Larger pieces are needed for light-microscopical evaluation of distribution, and this limits one to surgical specimens or to larger biopsies, particularly when different methods are being compared. Our work confirms the findings of Trotter et al. (1990), who state that there are very few mast cells in the epithelium, but our studies have not included patients with seasonal allergic rhinitis. There is debate on the submucosal

distribution and Trotter and colleagues feel that most mast cells are found in the superficial glandular area.

Unfortunately, electron microscopy requires small pieces of tissue for adequate penetration of the fixative. Fixation is hampered with by local anaesthesia with cocaine which produces marked vascular shrinkage. Moreover, since the biopsy goes right down to the periosteum, bone is included and this has to be removed. The specimen may then be divided into smaller pieces for fixation and a suitable number of blocks can be studied.

ELECTRON MICROSCOPY

Tissues are fixed by different methods for electron microscopy (EM). Such tissues will not be stained with most stains used for light microscopy. Toluidine blue stains tissues well for both light and electron microscopy and is, therefore, frequently used for tissue orientation. Mast cell granules continue to stain metachromatically, but our work confirms the view of others that light microscopy is of little value in detecting mast cells prior to sectioning for EM (Galli et al., 1984). Many of the smaller cells are not demonstrated, but this is not related to the ultrastructure of the granules which is similar in both larger and smaller mast cells. There is only one study on the in vivo ultrastructural development of human mast cells (Fujita et al., 1969), and this has identified two cell types by their shape, i.e. spindle-shaped or spherical. Granule morphology was very similar to that found in the normal adult nose. The authors felt that organized structures, such as scrolls, were the final stage of granule maturation.

The most characteristic feature of a normal mast cell at the EM level are the electron-dense granules which contents can be either amorphous, organized as a scroll, or crystalloid (Figure 1). The cell has a single nucleus; all other features are variable. Unfortunately, a cell may loose many of its features when it is either taking part in reactions or is diseased. The electron-dense granules disappear and the resultant vacuolated cells may be difficult to classify.

Most of the studies on normal human mast cells have been performed in tissues other than the nose. The granules in alveolar mast cells have scrolls, reticular material, or a mixture of these, and may even be empty (Caulfield et al., 1980; Fox et al., 1981). The mean number of granules was 39, ranging from 9 to 100. Some degree of degranulation may be encountered in normal lung tissue (Fox et al., 1981). Alveolar mast cells differ from tissue mast cells in that the granules are homogeneous in the former and crystalloid in the latter, and this has led some authors to believe that there are two different mast cell types in human lung (Ts'ao et al., 1977). This is at variation with the work of Craig et al. (1988), who state that the scrolls are a feature of mucosal (or T-type) mast cells. Immunochemistry using gold markers suggested that over 90% of mast cells in the lung are T-type cells. Craig et al. (1988) also stated that numbers of T-type cells were very

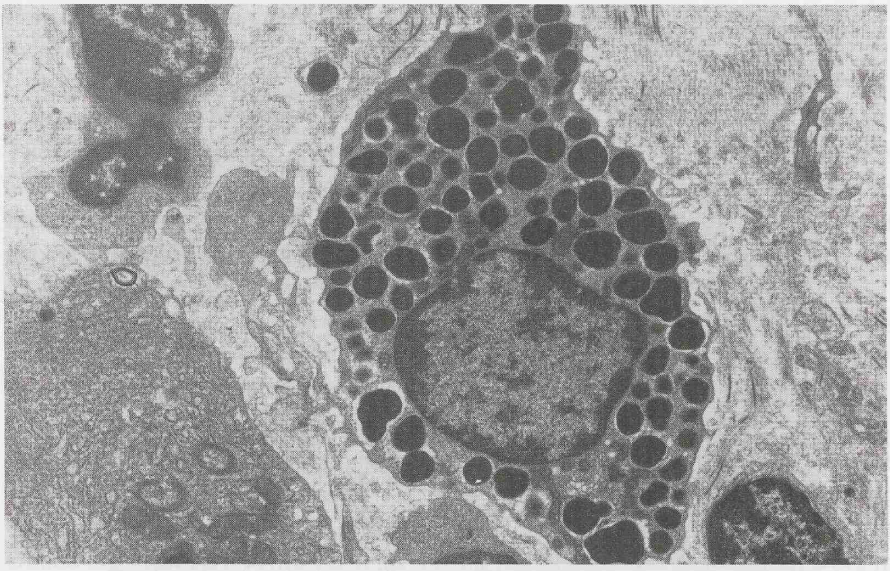

Figure 1. A normal mast cell as viewed in the electron microscope. The electron-dense granules are evident and most are homogeneous in this cell.

different in foreskin, where almost all the cells were of the T/C- or connective-tissue type. Although we could confirm that there are more crystalloid granules, we also found large numbers of scrolls in skin mast cells. Our work on the nose suggests that granule ultrastructure tends to be similar in the same subject rather than to be site-variable.

Degranulation of mast cells can be studied in five areas: (1) *in vitro* degranulation following allergen challenge; (2) *in situ* degranulation following allergen challenge; (3) degranulation in diseases (excluding allergy); (4) degranulation found *in vivo* during natural allergen challenge; and (5) degranulation occurring during natural exposure. The *in vitro* changes with anti-human IgE occur within 3 min (Caulfield et al., 1980). Degranulation involves three stages: First, granule architecture is lost, and then the amorphous granules become larger. The second stage is the formation of microtubular structures and the fusion of granules with the cell membrane. The final stage is the loss of granule contents with vacuoles filling the cell's cytoplasm. The only other change noted is the movement of filaments from around the nucleus to surround the granules (Caulfield et al., 1980; Galli et al., 1984).

The first ultrastructural study on in situ degranulation in the human nose was undertaken by Trotter and Orr (1973) who believed that degranulation underwent the following sequence:

- loss of granule architecture;

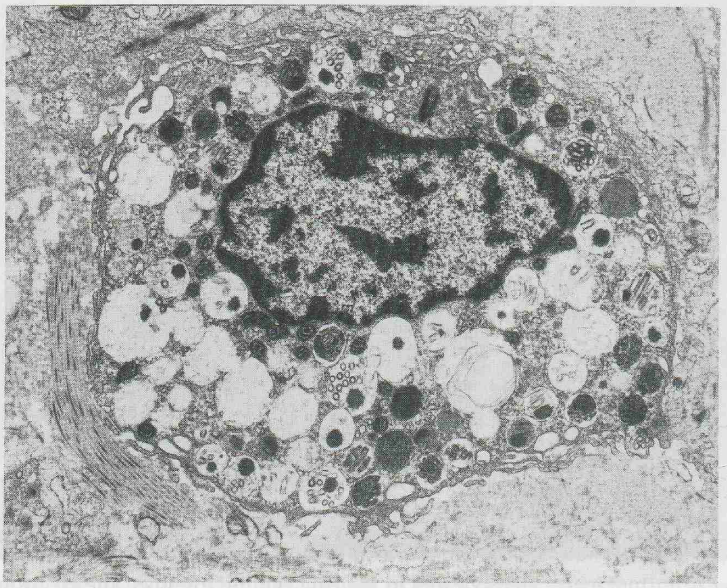

Figure 2. The variable degrees of degranulation in patients with perennial allergic rhinitis may be seen in this figure and the following one.

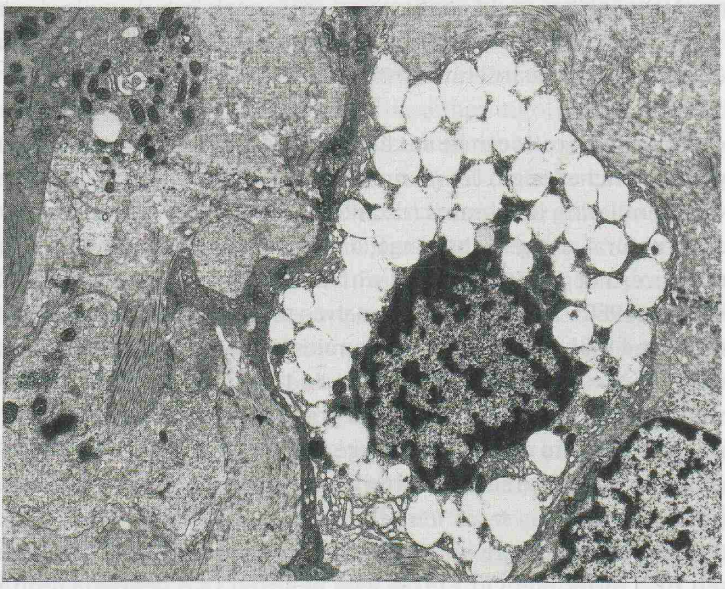

Figure 3. This cell is almost degranulated, but the overall morphology is suspect for a mast cell (reproduced with permission from The Journal of Laryngology and Otology).

- swelling of the area inside the perigranular membrane;
- granule fusion with the cell membrane;
- the formation of intracellular channels;
- extrusion of granules.

They found that very few of the cells are degranulated. *In situ* degranulation was also studied by Friedman and Kaliner (1988). These authors found resting cells in the two patients studied and did not observe a microtubular system, nor signs of exocytosis. They suggested that alveolar and nasal mast cells possess differences in degranulation. Nasal polyps are probably not atopic in origin, and mast cell degranulation has been found (Cauna et al., 1972; Busutill et al., 1986; Drake-Lee et al., 1984). These changes involve the inferior turbinate in some cases as well (Drake-Lee et al., 1987).

Kawabori and co-workers performed *in vivo* allergen challenge in five patients, who were sensitive to house dust mite, and studied the mast cells by curetting the surface of the inferior turbinate. There was an increase of mast cells following challenge, but they found no basophils. This is contradictory to the findings of Okuda c.s, who have written extensively on the topic and reviewed their work in 1983 and 1985, combining both light- and electron-microscopical findings. They noticed an increase in basophilic cells 30 min after *in vivo* challenge, and believed that it is possible to differentiate two types of mast cells as well as basophils. Basophils and mucosal mast cells occurred near the surface and in the epithelium, connective-tissue mast cells were situated deeper. They stated that surface mast cells were the trigger to the reaction in pollinosis.

We studied the ultrastructure of 46 mast cells taken from 9 normal inferior turbinates; they varied considerably in size, shape and distribution, but were found mainly in the submucosa. There were no obvious differences in the morphology of the mast cells of different sizes, and they could not be subgrouped into either connective-tissue- or mucosal mast cells, on the basis of the number or morphology of the granules. Three-quarters of the cells had electron-dense granules only, and the remaining 10 cells had scrolls in some of the granules. Patients tended to have cells with similar granule morphology. An intact-granule index was determined to assess the degree of degranulation and a few cells were found to be degranulated in the normal nose.

Normal morphology was compared with 80 cells from patients with perennial rhinitis due to dust mite sensitivity. There was an increase in the number of mast cells, but there was no basophilia. Extensive degranulation was seen, but there was no evidence for intraepithelial reaction (Figures 2 and 3; cf. Drake-Lee and Price, 1991).

CONCLUSIONS

The morphological classification of mast cells is fraught with difficulties, but it is possible to draw some conclusions on human nasal mast cells. We believe that it is impossible to classify mast cells by staining and fixation alone, until all the changes that occur during maturation and the differences found at different sites have been evaluated. Morphology shows them to be either spindle-shaped or spherical. Also, more mast cells are present in the superficial glandular layer as compared to the epithelium, in which only very few are present. Epithelial mast cells may increase in seasonal allergic rhinitis, but conclusions drawn from studies that use the chloroacetate esterase method to demonstrate mast cells, are limited because the stain is not cell specific. Electron microscopy confirms the findings obtained with light microscopy and, in addition, shows that a few of the cells are degranulated in the normal nasal mucosa. Degranulation in perennial allergic rhinitis affects all cells, irrespective of size. Human nasal mast cells are pleomorphic and cannot be divided into two morphologically distinct groups.

REFERENCES

1. Busuttil A, More I, McGeveny D. Ultrastructure of the stroma of nasal polyps. Arch Otolaryngol 1976; 102: 589-595.
2. Caulfield J, Lewis R, Hein A, Austin K. Secretions in dissociated human pulmonary mast cells. J Cell Biol 1980; 85: 299-311.
3. Cauna N, Hindover K, Manzethi G, Swanson E. Fine structure of nasal polyps. Ann Otolaryngol 1972; 81: 41-58.
4. Combs J, Lagunoff D, Denditt E. Differentiation and proliferation of embryonic mast cells of the rat. J Cell Biol 1966; 25: 577-592.
5. Craig S, Schechter N, Schwartz L. Ultrastructural analysis of human T and T/C mast cells identified by immunoelectron microscopy. Lab Invest 1988; 58: 682-691.
6. Drake-Lee A, Barker T. Free and cell-bound IgE in nasal polyps. J Laryngol Otol 1984; 98: 795-801.
7. Drake-Lee A, Barker T, Thurley K. Nasal polyps. II. Fine structure of mast cells. J Laryngol Otol 1984; 98: 282-292.
8. Drake-Lee A, Chevreton E, Lowe D. The effects of different fixation on the distribution and numbers of mast cells in patients with nasal polyps. J Laryngol Otol 1988; 102: 1099-1101.
9. Drake-Lee A, Price J. The ultrastructure of nasal mast cells in normal subjects and patients with perennial allergic rhinitis. J Laryngol Otol 1991; 105: 1006-1013.
10. Drake-Lee A, Price J, Milford C, Bickerton R. Nasal mast cells: A preliminary report on their ultrastructure. J Laryngol Otol 1987; Supplement 13.
11. Ehrlich P. Beiträge zur Kenntnis der granulierten Bindegewebszellen und der Eosinophilen Leukozyten. Anatomy und Physiology (Leipzig) 1879; 3: 166-169.
12. Enerback L. The gut mucosal mast cells. Monogr Allergy 1981; 17: 222-232.
13. Enerback L, Pipkorn U, Granurus G. Intraepithelial mast cells in hay fever. Int Arch Allergy Appl Immunol 1986; 80: 44-51.
14. Fox B, Bull T, Guz A. Mast cells in the human alveolar wall: An electron microscopy study. J Clin Pathol 1981; 34: 133-1342.
15. Friedman M, Kaliner M. In situ degranulation of human nasal mucosal mast cells: Ultrastructural features in cell associations. J Allergy Clin Immunol 1985; 76: 70-82.
16. Fujita H, Asagami C, Murozumi S, Yamamoto K, Kinoshita K. Electron microscopic studies of mast cells of human skin. J Ultrastr Res 1969; 28: 353-370.

17. Galli S, Dvorak A, Dvorak H. Basophils and mast cells: Morphological insights into their biology, secretory patterns and function. Progr Allergy 1984; 34: 50–141.
18. Garrett J, Osman I, Smith R. Selection of a simple protease procedure for identifying mast cells in routinely fixed human tissues. J Histochem Cytochem 1987; 35: 541–547.
19. Gomori G. Chloroacyl esters as histochemical substrates. J Histochem Cytochem 1953; 1: 469–479.
20. Greenwood B, Orr T, Coleman H. The ultrastructure of mucosal mast cells. Eur J Resp Dis 1986; Supplement 147: 210–216.
21. Hardy W, Wesbrook F. The wandering cells of the alimentary tract. J Physiol 1895; 18: 490–524.
22. Hopwood D. Fixation and fixatives. In: Bancroft J, Stevens A, Eds. Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh, 1982, pp. 20–40.
23. Kawabori S, Okuda M, Unno T. Mast cells in allergic nasal epithelium and lamina propria before and after provocation. Clin Allergy 1983; 13: 181–189.
24. Li C, Lam K, Yam L. Esterases in human leukocytes. J Histochem Cytochem 1973; 21: 1–12.
25. Michels N. The mast cells. In: Downey H, Ed. Handbook of Haematology. Hamish Hamilton Medical Books, London, 1938, pp. 232–273.
26. Mygind N. Nasal Allergy. Blackwell Scientific Publications, Oxford, 1978.
27. Naclerio R, Togias A, Proud D, Kagey-Sobotka A, Adkinson N, Norman P, Lichenstein L. The measurement of inflammatory mediators in nasa secretions. In: Veldman JE, Ed. Immunobiology, Autoimmunity and Transplantation in Otorhinolaryngology. Kugler Publications, Amsterdam, 1985, pp. 273–278.
28. Okuda M, Otsuka H. Heterogeneity of the human nasal mast cell. In: Veldman JE, Ed. Immunobiology, Histopathology, Tumor Immunology in Otorhinolarngology. Kugler Publications, Amsterdam, 1986, pp. 317–323.
29. Okuda M, Ohtsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. Eur J Res Dis 1985; Supplement 128: 7–14.
30. Otsuka H, Denburg J, Dolovich J, Hitch D, Lapp P, Rajan et al. Heterogeneity of metachromatic cells in human nose: Significance of mucosal mast cells. J Allergy Clin Immunol 1985; 76: 695–702.
31. Pearce F. On the heterogeneity of mast cells. Pharmacology 1986; 32: 61–71.
32. Pipkorn U, Karlsson G, Enerback L. Phenotypic expression of proteoglycan in mast cells of the human nasal mucosa. Histochem J 1988; 20: 519–525.
33. Schwartz L, Irani A, Roller K, Castells M, Schechter N. Quantitation of histamine, tryptase and chymase in dispersed human T and T/C mast cells. J Immunol 1987; 138: 2611–2615.
34. Stobel S, Miller H, Ferguson A. Human intestinal mast cells: Evaluation of fixation and staining techniques. J Clin Pathol 1981; 34: 851–858.
35. Trotter C, Orr T. A fine structure study of some cellular components in allergic reactions. Clin Allergy 1973; 3: 411–425.
36 Trotter C, Salter D, Wilson J, Hall G. A comparison of methods for nasal mast cell demonstration. Rhinology 1990; 28: 17–23.
37. Ts'ao C, Patterson R, McKenna J, Suszko I. Ultrastructural identification of mast cells obtained from human bronchial lumens. J Allergy Clin Immunol 1977; 59: 320–326.
38. Viegas M, Gomes E, Brooks J, Gatland D, Davies R. Effect of the pollen season in nasal mast cells. Br Med J 1987; 414.

A.B. Drake-Lee, FRCS
Queen Elizabeth Hospital
Edgbaston
Birmingham B15 2TH
UNITED KINGDOM