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Globule leukocytes and mast cells in the rat trachea: their number, distribution, and response to compound 48/80 and dexamethasone*

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Summary. Globule leukocytes in the epithelium of the rat trachea may be counterparts of mucosal mast cells that are located in the gastrointestinal tract. If they are indeed similar to mucosal mast cells, globule leukocytes would be expected to decrease in number in rats treated with dexamethasone but not in rats treated with compound 48/80, an agent which causes non-antigenic degranulation of connective tissue mast cells. In this study, we determined the number and compared the distribution of globule leukocytes and connective tissue mast cells in the tracheas of pathogen-free rats. We then determined whether the number of these two types of cells changes in rats treated for 5 days with compound 48/80, dexamethasone, a combination of compound 48/80 and dexamethasone, or saline. We identified globule leukocytes and mast cells in whole mounts and histological sections of rat tracheas by using a histochemical reaction that demonstrates the chymotrypsin-like protease (chloroacetate esterase) present in mast cell granules. Using this method, we found that aproximately 225000 globule leukocytes were present in the epithelium of the trachea. These cells were most abundant in the rostral trachea. Rats treated with dexamethasone had a 91% reduction in the number of globule leukocytes with protease-containing granules, but rats treated with compound 48/80 had a normal number of these cells. We found some 55000 connective tissue mast cells in the same tracheas. Mast cells were most abundant in the posterior membrane of the caudal trachea and in the lamina propria between cartilaginous rings. Rats treated with compound 48/80 had a 96% reduction in mast cells with protease-containing granules, but rats treated with dexamethasone had a normal complement of mast cells. We conclude that globule leukocytes are abundant in the tracheas of healthy rats, are similar in morphology and pharmacological responses to mucosal mast cells located in other organs of rats, and are more numerous than and have a different distribution than connective tissue mast cells. Globule leukocytes in the tracheal epithelium may have a role in respiratory defenses similar to that of mucosal mast cells in other organs.

Key words: Mast cells – Globule leukocytes – Rats – Trachea – Compound 48/80 – Dexamethasone – Histochemistry – Naphthol AS-D esterase

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Introduction

Rodents are known to have at least two types of mast cells that can be distinguished by their histochemical staining characteristics, histamine content, and responses to various stimuli (Cantin and Veilleux 1972; Miller 1980; Bienenstock et al. 1982; Befus et al. 1982). One type of mast cell, the connective tissue mast cell, is abundant in the connective tissue of most organs, particularly near blood vessels and nerves (Selye 1965). Connective tissue mast cells are known to be present in the rat trachea (Breeze and Wheeldon 1977), but their number and distribution there have not been systematically described.

A second type of mast cell, the mucosal mast cell, is located in the mucosa of the gastrointestinal tract. The number of mucosal mast cells in the gut increases in rats infected by intestinal nematodes (Miller 1980; Whur 1966); thus, these cells are thought to play a role in immunological defenses against parasites. Although mucosal mast cells have not been specifically described in the respiratory tract of rodents, there exists in the tracheal epithelium of normal rats as well as in the mucosa of other organs in certain other species, the globule leukocyte (Weill 1919; Toner 1963; Kent 1952; Jeffery and Reid 1975), a cell type which may be related to or identical to mucosal mast cells (Kent 1966; Murray et al. 1968; Mayrhofer 1980; Huntley et al. 1984b). Although globule leukocytes can be detected by electron microscopy and, with appropriate stains, by light microscopy, their number and distribution in the rat trachea – as in the case of connective tissue mast cells – have not been determined.

In this investigation we studied the number and distribution of globule leukocytes and connective tissue mast cells (hereafter referred to simply as "mast cells") in the rat trachea. We also determined whether globule leukocytes in the rat trachea have the same response to pharmacologic stimuli as do mucosal mast cells in the rat gastrointestinal tract, which degranulate or decrease in number after treatment with corticosteroids or ACTH (Rasanen 1960; Heap and Kiernan 1973) but are not so affected by compound 48/80 (Rasanen 1960). This response of mucosal mast cells differs from the response of connective tissue mast cells, which degranulate after treatment with compound 48/80 (Hunt and Hunt 1956) but not after corticosteroids (Rasanen 1960; Heap and Kiernan 1973). If globule leukocytes in the trachea are indeed analogous to mucosal mast cells of the gastrointestinal tract, granulated globule leukocytes would be expected to decrease in number after treatment

with corticosteroids but not after treatment with compound 48/80. There is already evidence that globule leukocytes in the trachea and mucosal mast cells in the gastrointestinal tract have similar responses to dexamethasone (Kent et al. 1954), but it is not known how globule leukocytes respond to compound 48/80. We therefore determined the changes in the number of globule leukocytes and mast cells after treatment with compound 48/80 and after treatment with dexamethasone.

To circumvent the technical difficulties of obtaining this information from histological sections, we devised a method of staining tracheal whole mounts with a histochemical technique to detect mast cells and globule leukocytes. Because the granules of both mast cells and globule leukocytes contain a chymotrypsin-like serine protease (Gomori 1953; Benditt and Arase 1956; Huntley et al. 1984a), they can be distinguished from other cells in the trachea by the red reaction product produced by the protease's action on a chloroacetate substrate. The intense color of this reaction product enabled us to detect these cells both in histological sections and in tracheal whole mounts. Furthermore, detecting the esterase activity of the granule-associated protease, unlike detecting the metachromasia of granule-associated proteoglycans, is unaffected by aldehyde fixation (Wingren and Enerback 1982; Becker et al. 1985). Our study revealed that globule leukocytes differed from mast cells in their number and distribution in the trachea and in their response to dexamethasone and compound 48/80, and that the response of globule leukocytes in the trachea to dexamethasone and to 48/80 was indeed similar to that of mucosal mast cells in the gastrointestinal tract.

Materials and methods

Animals

The experimental procedure followed in this study adhered to the published *Guiding Principles in the Care and Use of Animals* approved by the Council of the American Physiological Society and specific protocols approved by the Committee on Animal Care of the University of California, San Francisco.

Experiments were performed on specific pathogen-free, female Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 200–220 g. These animals were documented by culture or serology to be free of intestinal cestodes, helminths, protozoa, and common respiratory bacteria and viruses, including *Mycoplasma* species, Sendai virus, sialodacryoadenitis virus, rat coronavirus, and pneumonia virus of mice. Their diet was also replete in magnesium, deficiencies of which are associated with an increase in the number of globule leukocytes in the genitourinary system (Cantin and Veilleux 1972).

Tissue fixation

Rats were anesthetized with methohexital sodium (6–10 mg/100 g weight) then perfused through the aorta with 1% paraformaldehyde in 0.05 M phosphate-buffered 0.85% NaCl (PBS), pH 7.4, at a pressure of 120 mm Hg for 2 min. Tracheas were excised and dissected free of adjacent tissues, then fixed at room temperature for an additional 24–48 h.

Tissue processing

Two or three rings cut from each end of the tracheas were dehydrated in ethanol and embedded in glycol methacrylate (JB-4, Polysciences, Warrington, PA). Transverse sections 2.5 μm in thickness were cut from each of these specimens. The remainder of each treachea was cut longitudinally along the ventral surface, pinned flat on a sheet of plastic elastomer (Sylgard, Dow Corning Corp, Midland, MI), and washed in distilled water for 2 h.

Enzyme Histochemistry

To detect chloroacetate esterase activity, tissue sections were incubated in chloroacetate substrate for 60 to 90 min at 30° C, washed in distilled water, counterstained with Gill's hematoxylin and mounted with Permount (Polysciences, Warrington, PA). Whole mounts were immersed in the chloroacetate ester substrate solution for 14 to 18 h at 4° C on a rotator, washed in distilled water for 2 h, then cleared and mounted in glycerol (mounting media containing organic solvents tended to dissolve the reaction product in whole mounts). The solution of the substrate naphthol AS-D chloroacetate (Sigma), with hexazotized pararosanilin as the chromogen, was prepared exactly as detailed by Beckstead et al. (1981) and used within 1 h.

Tissue processing for electron microscopy

The tracheas of 2 untreated rats were prepared for electron microscopy. The rats were perfused through the aorta sequentially with two fixatives. The first fixative contained 3% glutaraldehyde, 0.075% hydrogen peroxide, 0.05% calcium chloride, 1% sucrose and 4% polyvinylpyrrolidone (PVP, MW 40000) in 75 mM cacodylate buffer (pH 7.1). The second fixative was similar to the first except that it lacked hydrogen peroxide. Approximately 200 ml of each fixative was perfused over 5 min at a pressure of 120 mm Hg. The tracheas were removed and full thickness specimens of the tracheal wall were excised from between cartilaginous rings and from the posterior membrane. The specimens were placed in the second fixative for 3–6 h, treated with 1.5% osmium tetroxide in 14 mM veronal acetate-HCl buffer (pH 7.4) for 18 h on a rotator at 4° C and 1.5% uranyl acetate in 25 mM sodium maleate buffer (pH 6.0) for 24 h at 4° C, then dehydrated in acetone and embedded in Epon (Ted Pella, Inc., Tustin, California). Sections approximately 0.5 μm thick for light microscopy and sections approximately 50 nm thick for electron microscopy were cut in a plane perpendicular to the long axis of the trachea to reveal the full thickness of the trachea. The epithelium, lamina propria and adventitia were visible in specimens from between cartilaginous rings; epithelium, lamina propria, smooth muscle, and adventitia were visible in specimens from the posterior membrane. Sections were mounted on single slot (0.4 × 2 mm) Formvar- and carbon-coated grids, stained with lead citrate, and examined with a Zeiss EM-10 electron microscope.

Quantitation of globule leukocytes and mast cells

The two populations of cells were counted in both whole mounts and 2.5 μm sections. Whole mounts enabled us to study the entire trachea and thereby provided an overview

of the distribution of the cell types in the trachea. Histological sections enabled us to study cellular and nuclear detail by light microscopy (and thus to verify the specificity of the histochemical reaction, see discussion), to better localize the cells in different regions of the trachea, and to corroborate the cell counts done on whole mounts. Examination of histochemically stained histological sections verified that globule leukocytes were present only in the epithelium whereas mast cells were present only in the lamina propria, muscle, and connective tissue.

Whole mounts. Whole mounts were examined at 160× with a compound microscope fitted with a calibrated lattice eyepiece which defined a square region of tissue 620 μm on each side. With the abluminal surface of the trachea facing upward, mast cells were counted in two such regions of the posterior membrane, one region within 6 tracheal rings of the larynx (rostral) and one region within 6 tracheal rings of the carina (caudal). With the luminal surface of the trachea facing upward, globule leukocytes were counted in two such regions of epithelium overlying the posterior membrane, one rostral and one caudal.

The numerical density of mast cells per cubic millimeter of trachea was computed from the original counts, which were expressed as number of mast cells/mm^2, and the average thickness of the posterior membrane with its overlying adventitia, which was measured in tracheal cross sections (0.2 mm). The numerical density of globule leukocytes per cubic millimeter was calculated from the original counts and the average thickness of the epithelium (0.012 mm).

Histological sections. To estimate the numerical density of mast cells and globule leukocytes in tissue sections, we counted the number of cell profiles that had histochemical reactivity and were sectioned through the nucleus. Sections were examined at 630× with a microscope. To compensate for the effects of counting a nonuniform population of ellipsoids (the nuclei) whose centers are not in the plane of each section, we computed correction factors for the number of nuclear profiles by using the method of Hendry (1976) as modified by Smolen et al. (1983). For this calculation we computed the mean caliper diameter of the nuclear profiles of globule leukocytes and mast cells by measuring their major and minor axes (Elias and Hyde 1983). The calculation showed that for globule leukocytes and mast cells, the number of cells present was 41% and 40%, respectively, of the number of nuclear profiles counted in sections 2.5 μm thick. The similarity of these two values reflects the fact that the nuclei of globule leukocytes and mast cells are similar in size, even though mast cells are much larger than globule leukocytes.

We then determined the volume of the region of sections in which the cells were counted. Mast cells were counted in a region of the posterior membrane bounded by the epithelial basement membrane ventrally, the ends of the cartilaginous rings laterally, and the outer edge of adventitia dorsally. The area of this compartment was measured with a Talos Model 614B digitizer, using a light microscope with a drawing tube to visualize the digitizer's cursor. Globule leukocytes were counted in the tracheal epithelium, the area of which was calculated as the product of the circumference of the tracheal lumen (the mean of 3 measurements) and the height of the epithelium (the mean of 12 measurements of the distance between the basement membrane and the luminal surface of epithelial cells, excluding cilia). The volume of each compartment was the product of its area and the section thickness.

Total number of globule leukocytes and mast cells

To estimate the total number of mast cells and globule leukocytes in the rat trachea, we measured the areas of the posterior membrane, cartilaginous rings, and intercartilaginous spaces in 3 tracheal whole mounts taken from saline-treated rats. We then determined the number of mast cells and globule leukocytes in each of the regions using the methods described above for counting cells in the tracheal whole mounts and histological sections, and used the mean of the rostral and caudal cell counts in each region in our calculations. The number of cells in each region was computed as the product of the number of mast cells or globule leukocytes in that region and the area of the region. These values were multiplied by 1.25 to correct for the removal of 5 of the 25 tracheal rings to prepare histological sections. The total number of globule leukocytes or mast cells in each trachea was taken as the sum of the number of cells in each of the 3 regions.

Drug treatment

Each of the four treatment groups consisted of 6 rats. All injections were given intraperitoneally, twice daily, for five days. The first group received compound 48/80, 100 μg in the morning and evening of the first day increasing in 100 μg increments to 500 μg in the morning and evening of the fifth day. The second group received injections of 0.5 mg dexamethasone in 0.125 ml phosphate buffer. The third group received injections of 0.5 mg dexamethasone in combination with the increasing doses of compound 48/80. The fourth group (controls) received injections of 0.85% NaCl, 0.125 ml. Four to six hours after the final injection, rats were anesthetized and perfused with fixative, and the tracheas were removed and processed for histochemistry.

Tracheal histamine content

Tracheal histamine content was determined for the four treatment groups (3 rats in each group). These rats received the same treatment regimens and anesthesia described for the rats used for the morphological studies, but they were perfused through the aorta with PBS instead of paraformaldehyde. The tracheas were removed, weighed, placed in 2% perchloric acid on ice, and frozen at −70° C within 30 min of the injection of anesthetic. Histamine content was assayed with the *o*-phthalaldehyde spectrofluorometric procedure (Shore et al. 1959), modifed for an automated spectrofluorometric analyzer (Alpkem, Clakamus, OR). Protein content was determined by the method of Lowry et al. (1951).

Data analysis

All values are expressed as mean ± S.E. Data were analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple range test.

Results

Normal morphology, distribution, and density of mast cells and globule leukocytes in the rat trachea

Our study of sections verified that only mast cells and globule leukocytes contained detectable amounts of chloroacetate esterase reaction product. Goblet cells and other epithelial secretory cells contained no detectable enzymatic activity. Polymorphonuclear leukocytes had sufficient reaction product to be detected in histological sections, but the nuclear detail of these cells distinguished them from mast cells and globule leukocytes. Polymorphonuclear leukocytes did not have enough enzymatic activity to be detected in tracheal whole mounts.

Mast cells

Mast cells seen by light microscopy in histological sections were 10–20 μm in diameter and contained homogeneously sized red granules and a central unindented nucleus (Fig. 1). Mast cells were most abundant in the connective tissue overlying the dorsal aspect of the trachealis muscle but were also present in the muscle itself and in the lamina propria between the cartilaginous rings. They were never seen in the epithelium. In whole mounts of rat trachea, mast cells appeared as large dark red cells with an oval nucleus (Figs. 3, 5). They were most numerous on the abluminal surface of the posterior membrane of the caudal trachea and in the intercartilaginous spaces (Figs. 5, 10).

We ascertained by electron microscopy that cells we identified as mast cells were ultrastructurally the same as mast cells described in the literature (Cantin and Veilleux 1972; Dvorak et al. 1983). Mast cells in the trachea were elongated cells that contained abundant electron-dense, membrane-bound cytoplasmic granules that were comparatively uniform in size, ranging from 0.3 to 1 μm in diameter (Fig. 7).

Mast cells were sparse rostrally and increased in number caudally. In whole mounts, their density on the rostral abluminal surface averaged 1400 cells per cubic millimeter whereas their density was more than twice this amount caudally (Table 1).

In tracheal whole mounts, there were 470 ± 70 mast cells/mm^2 in the posterior membrane, 10 ± 6 mast cells/mm^2 over cartilaginous rings, and 210 ± 125 mast cells/mm^2 between cartilaginous rings. The corresponding surface areas for each of these regions were 43 ± 4 mm^2, 109 ± 6 mm^2, and 23 ± 1 mm^2, with a total tracheal surface area of 175 ± 6 mm^2. These data indicate that there were approximately 25000 mast cells in each tracheal whole mount. Of this total, 80% of the mast cells were in the posterior membrane. Corresponding data derived from counts of mast cells in histological sections yielded a value of 55000 mast cells for the total number of mast cells present in the rat trachea. We believe this larger estimate more closely reflects the total number of mast cells (see discussion).

Globule leukocytes

Globule leukocytes were found only in the epithelium of the trachea. These cells, as seen by light microscopy in tracheal cross sections, were rounded cells that had a diameter of 5–10 μm, contained 1–6 pleomorphic bright red granules, and had an eccentric nucleus that was indented by the granules (Fig. 2). As seen by light or electron microscopy, the granules were variable in size but most were larger than the granules in mast cells. In whole mounts, globule leukocytes were visible as bright red cells near the luminal surface of the trachea (Figs. 4, 6, 10). The reaction product in globule leukocytes tended to diffuse beyond the limits of the granules, thereby making the cells viewed in the histochemical preparations appear somewhat larger than globule leukocytes seen by electron microscopy.

We confirmed by electron microscopy that the cells we identified by histochemical methods as globule leukocytes were the same as the globule leukocytes described by others (Cantin and Veilleux 1972; Jeffery and Reid 1975; Pearsall et al. 1984). Globule leukocytes in the tracheal epithelium were morphologically very distinctive cells. They contained prominent, membrane-bound, electron-dense granules that ranged in size from 1 to 2.5 μm (Figs. 8, 9). Globule leukocytes were easily distinguished from goblet and other epithelial secretory cells because globule leukocytes were rounded cells that had no attachments to epithelial cells and contained granules that were larger, more electron dense, and more heterogeneous in size than those seen in epithelial secretory cells (Figs. 9, 10).

Globule leukocytes were most abundant in the rostral

Figs. 1, 2. Histological sections 2.5 μm in thickness of rat trachea embedded in glycol methacrylate resin and treated according to the histochemical reaction for chloroacetate esterase. Sections were counterstained with hematoxylin. × 850

Fig. 1 shows mast cells in the connective tissue of the trachea's posterior membrane. The mast cells contain red reaction product in their homogeneously sized granules

Fig. 2 shows globule leukocytes in the tracheal epithelium that contain pleomorphic granules made visible by red reaction product

Figs. 3, 4. Whole mounts of rat tracheas treated according to the histochemical reaction for chloroacetate esterase

Fig. 3 shows ovoid mast cells in the connective tissue of the posterior membrane

Fig. 4 shows the smaller and more rounded globule leukocytes in the tracheal epithelium. Both types of cells are shown here at a magnification of × 850, which is the same as that of Figs. 1 and 2

Figs. 5, 6. The relative numbers and distributions of mast cells and globule leukocytes are shown in these micrographs of tracheal whole mounts stained for chloroacetate esterase. × 80

Fig. 5 shows mast cells that are most abundant on the abluminal surface of the trachea's posterior membrane and between cartilaginous rings

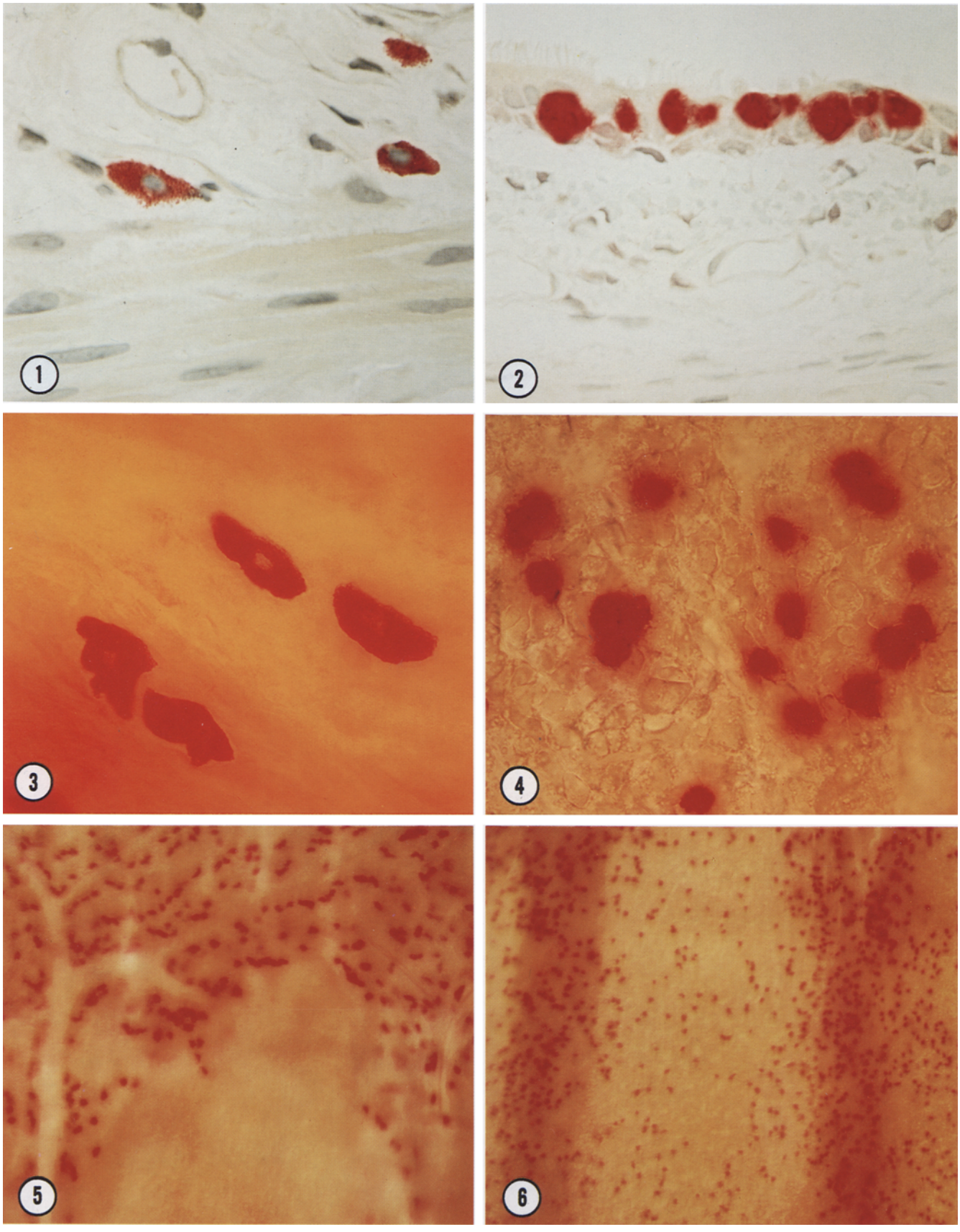
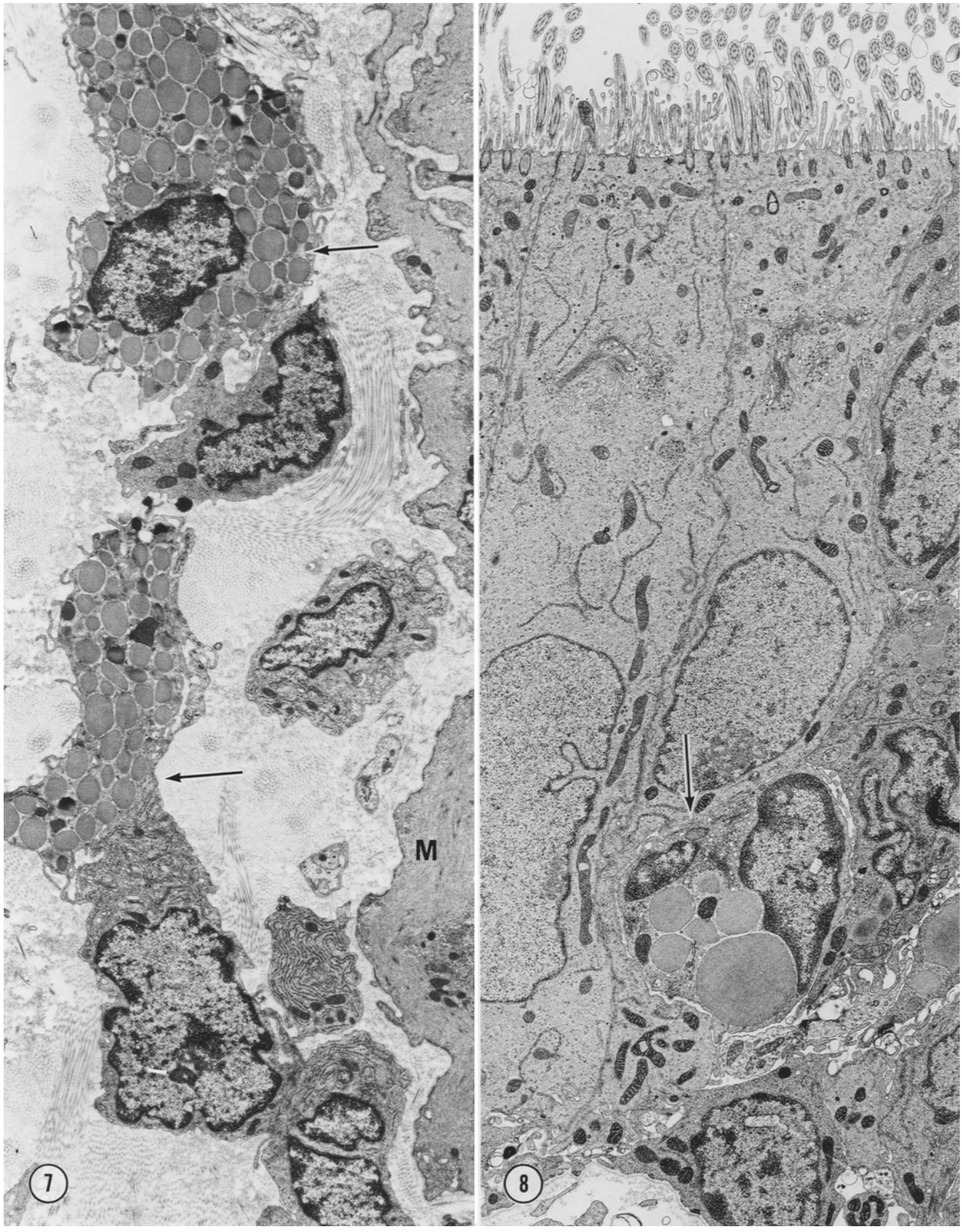


Fig. 6 illustrates the abundance of globule leukocytes visible in the tracheal epithelium, as viewed here from the luminal surface. The globule leukocytes are more abundant in the epithelium overlying the tissue between cartilaginous rings (two dark vertical bands) than in the epithelium over the cartilaginous rings themselves (pale vertical bands)



Figs. 7, 8. Electron micrographs comparing the ultrastructure of tracheal mast cells and globule leukocytes in the rat trachea. × 7000

Fig. 7 shows portions of 2 connective tissue mast cells (*arrows*) adjacent to smooth muscle cells (*M*) in the trachealis muscle. These mast cells contain electron-dense granules that are 0.3 to 1 μm in diameter

Fig. 8 shows a globule leukocyte (*arrow*) within the tracheal epithelium. The globule leukocyte contains five granules 1 to 2.5 μm in diameter, some of which indent the nucleus

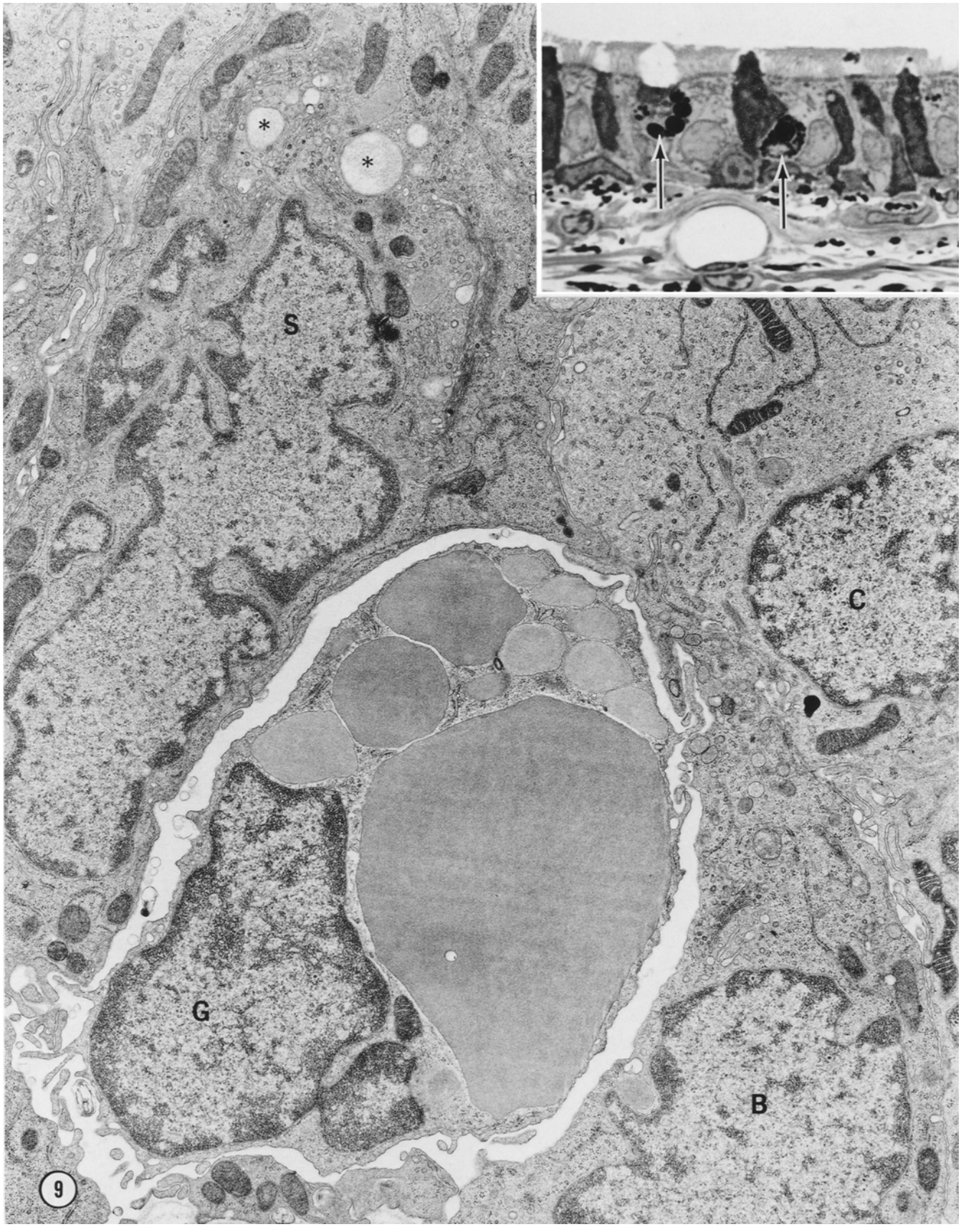


Fig. 9. Electron micrograph of the tracheal epithelium containing a globule leukocyte (*G*) adjacent to an epithelial secretory cell (*S*) and a ciliated epithelial cell (*C*). ×14500. The globule leukocyte can be readily distinguished from the secretory cell by the lack of attachment to neighboring cells and by its distinctive granules, which are more electron-dense and more heterogeneous in size and shape than those of the secretory cell (*asterisks*). The inset is a light micrograph showing the appearance, size, and distribution of globule leukocytes (*arrows*) in relation to epithelial cells. ×1300. In this 0.5 μm epoxy section stained with toluidine blue, globule leukocytes appear smaller than they do in 2.5 μm sections stained histochemically for chloroacetate esterase (Fig. 2), not only because the section is thinner, but also because toluidine blue does not diffuse as readily as the histochemical reaction product

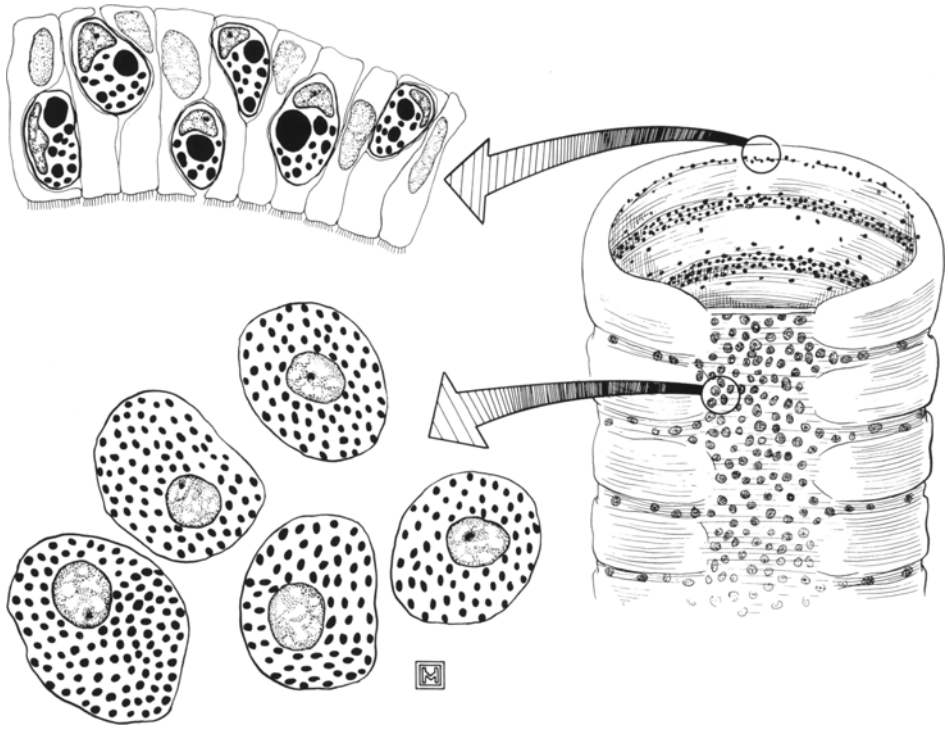


Fig. 10. Schematic diagram not drawn to scale showing the relative distributions of mast cells and globule leukocytes in the rat trachea. Mast cells are most abundant in the posterior membrane and in the lamina propria of the mucosa between cartilaginous rings of caudal trachea. Globule leukocytes, which are located only in the epithelium, are most abundant in regions between cartilaginous rings and over the posterior membrane of rostral trachea. Non-ciliated epithelial cells are excluded for simplicity

Table 1. Cell densities measured in whole mounts of rat trachea[a]

	Saline	40/80	Dexamethasone	48/80 and Dexamethasone
Mast cells				
Rostral				
Mean ± S.E.	1400 ± 400	30 ± 6[b]	2400 ± 1400	400 ± 300[b]
Range	300–2200	15–40	900–5200	200–1000
% change from saline	–	−98	+71	−71
Caudal				
Mean ± S.E.	3200 ± 200	140 ± 100[b]	3500 ± 400	900 ± 300[b]
Range	2800–3400	0–400	3000–4200	600–1700
% change from saline	–	−96	+9	−72
Average of rostral and caudal values	2300	85[b]	2950	650[b]
% change from saline	–	−96	+28	−72
Globule leukocytes				
Rostral				
Mean ± S.E.	180600 ± 24400	229000 ± 29500	15900 ± 7700[b]	95600 ± 13800[b]
Range	156000–229000	172000–269000	1300–27800	71600–119000
% change from saline	–	+27	−91	−47
Caudal				
Mean ± S.E.	71300 ± 30900	70400 ± 26300	5900 ± 2000[b]	10400 ± 3700[b]
Range	10000–108800	45500–104100	2600–9300	5700–17600
% change from saline	–	−1	−92	−85
Average of rostral and caudal values	126900	149700	10900[b]	53000[b]
% change from saline	–	+18	−91	−58

[a] Cells/mm^3, N = 3 for each group
[b] Significantly different from the saline-treated group, $p < 0.05$

portion of the trachea, where they tended to be more numerous in the epithelium over intercartilaginous regions than over the cartilaginous rings (Figs. 6, 10). Globule leukocytes appeared to spare regions of epithelium overlying mucosal lymphoid tissue. In whole mounts the density of globule leukocytes in rostral tracheal epithelium averaged about 181000 cells per cubic millimeter, whereas their density in caudal tracheal epithelium was less than half this number (Table 1).

Using the same methods we used for estimating the total number of mast cells in tracheal whole mounts, we calculated that some 230000 globule leukocytes were present in the trachea. This total was based on the densities of globule leukocytes in tracheal epithelium overlying the pos-

Table 2. Cell densities measured in histological sections[a]

	Saline	48/80	Dexamethasone	48/80 and Dexamethasone
Mast cells				
Rostral				
Mean ± S.E.	2700± 600	100±110[b,c]	1800±1000[d]	1500± 600[b,c]
Range	1700–5600	0–600	0–6000	0–4400
% change from saline	–	−96	−33	−44
Caudal				
Mean ± S.E.	7500± 1400	400± 300[b,e]	7400± 2200[d]	2100± 879[b]
Range	2400–11700	0–1300	0–12300	0–5200
% change from saline	–	−95	−1	−72
Average of rostral and caudal values	5100	250[b]	4600	1800[b]
% change from saline	–	−95	−10	−65
Globule leukocytes				
Rostral				
Mean ± S.E.	144400± 19500	171400± 28700[e]	29000± 4300[b,d]	78300± 14600[b,e]
Range	57000–188200	126300–284300	18400–42000	39300–133500
% change from saline	–	+19	−80	−45
Caudal				
Mean ± S.E.	82000±18300	73400± 17400[d]	9600± 4800[b,d]	4800± 2100[b]
Range	33400–147000	42700–121600	0–21400	0–12800
% change from saline	–	−10	−88	−94
Average of rostral and caudal values	113200	122400	19300[b]	41550[b]
% change from saline	–	+8	−83	−63

[a] N = 6 for each group, except where otherwise noted
[b] Significantly different from the saline-treated group, $p < 0.05$
[c] N = 5
[d] N = 4
[e] N = 3

terior membrane (1500 ± 300 cells/mm^2), cartilaginous rings (1200 ± 300 cells/mm^2), and intercartilaginous spaces (1400 ± 200 cells/mm^2). Thus, of the total number of globule leukocytes, some 28% were in the epithelium overlying the posterior membrane. Using the counts derived from histological sections, we estimated that a total of 220000 globule leukocytes were present in the trachea.

Effects of compound 48/80 and dexamethasone

Animals

Animals given compound 48/80 developed rapid shallow breathing and cyanosis within minutes of the injection, but appeared active and normal 8 h later. One animal died after the third of ten injections. The mean weight of these animals was not significantly different from the weight of control animals. Animals given dexamethasone had no apparent immediate effects, but after 2 to 4 days they ate and drank less, became agitated, and lost 20% of their body weight. Animals given both compound 48/80 and dexamethasone exhibited few of the immediate effects of compound 48/80, but after 2 to 4 days manifested the changes seen in rats treated with dexamethasone alone. No animal from the dexamethasone or combination treatment groups died.

Mast cells

The number of granule-containing mast cells in whole mounts of tracheas was reduced by 96% in animals treated with compound 48/80 ($p < 0.005$), whereas this number did not significantly change in rats treated with dexamethasone (Table 1). Tracheas in rats treated with the combination of compound 48/80 and dexamethasone had 72% fewer detectable mast cells than in saline-treated rats ($p < 0.05$).

Globule leukocytes

Rats treated with dexamethasone had a 91% reduction in granulated globule leukocytes ($p < 0.005$, Table 1), whereas we detected approximately the same number of globule leukocytes in tracheal whole mounts from rats treated with compound 48/80 as in controls. Rats treated with the combination of compound 48/80 and dexamethasone had 58% fewer detectable globule leukocytes than did saline-treated rats ($p < 0.05$).

Tracheal histamine content

Tracheas of control rats contained about 1500 ng of histamine. After 5 days of treatment with compound 48/80, alone or in combination with dexamethasone, the histamine content of the trachea was 67% less than that of controls. The histamine content per trachea did not change significantly after dexamethasone, but because the weight and protein content of such tracheas were significantly decreased (Table 3), the histamine content expressed per milligram of tracheal weight or per milligram tracheal protein was increased.

Histamine content of mast cells and globule leukocytes

We calculated that mast cells in rat tracheas contained 19 pg of histamine per cell. This calculation was based on our

Table 3. Histamine content, wet weights, and total protein in rat tracheas

	Saline[a]	48/80[b]	Dexamethasone[b]	48/80 and Dexamethasone[a]
Histamine content (ng)				
Mean ± S.E.	1500± 100	500± 60[c]	1300± 120	500± 50[c]
Range	1200–1700	500–600	1100–1500	400–600
% change from saline	–	−67	−13	−67
Histamine (ng) per mg protein				
Mean ± S.E.	360± 40	120± 10[c]	390± 10[c]	140± 20[c]
Range	220–360	370–390	100–140	90–190
Wet weight (mg)				
Mean ± S.E.	120± 10	90± 6	90± 12	80± 2
Range	90–130	90–100	80–110	80–84
Protein content (mg)				
Mean ± S.E.	6±0.5	4±0.2	3±0.3[c]	4±0.2
Range	5–7	4–5	3–4	3–4

[a] N=4 [b] N=3 [c] Significantly different from the saline-treated group, $p<0.05$

estimated total of 55000 mast cells per trachea and the observation that compound 48/80 caused a 95% decrease in the number of granule-containing tracheal mast cells (but no significant change in the number of globule leukocytes, Table 2), and a 67% decrease in the tracheal histamine content (Table 3). Thus the 52250 mast cells that were no longer visible contained a total of 1000 ng histamine or 19 pg histamine per cell. [The same calculations using the smaller number of mast cells (25000) found in tracheal whole mounts yielded a value of 44 pg histamine per mast cell.]

We similarly calculated that globule leukocytes in the rat trachea contained approximately 1 pg of histamine per cell. This was based on our estimate of 225000 globule leukocytes per trachea and the observation that dexamethasone caused a 91% reduction in the number of granule-containing globule leukocytes but no consistent reduction in the number of mast cells (Table 1) and a 13% decrease in the histamine content of the trachea (Table 3). Thus the 204750 globule leukocytes that were no longer detectable contained a total of 200 ng of histamine, or approximately 1 pg per cell.

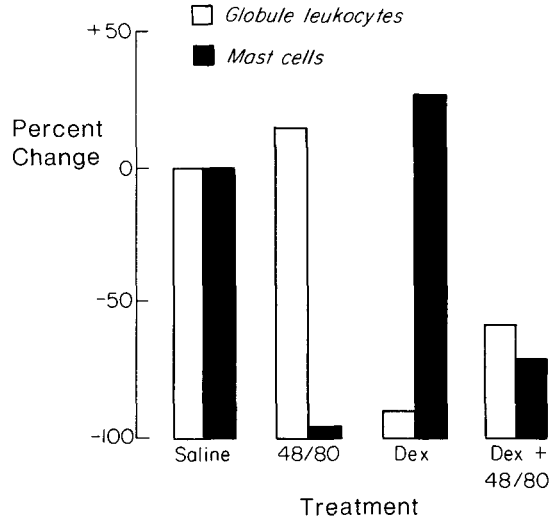


Fig. 11. Percentage change in the density of mast cells and globule leukocytes after saline, compound 48/80 (48/80), dexamethasone (Dex), and the combination of dexamethasone and compound 48/80 (Dex + 48/80). Globule leukocytes were counted in the epithelium overlying the posterior membrane of tracheal whole mounts. Mast cells were counted in the abluminal surface of the posterior membrane of the same whole mounts. The changes resulting from the various treatments were calculated from the average of the counts for 3 tracheas in each treatment group. The number of cells in each trachea was expressed as the mean of the counts for the rostral and caudal regions

Discussion

This study reveals that mast cells and globule leukocytes were numerous in the trachea of specific pathogen-free rats not infected with intestinal parasites. Mast cells in the rat trachea were similar to other connective tissue mast cells in their histochemical reactivity for chloroacetate esterase, ultrastructure, large degranulation response to compound 48/80, and little or no response to dexamethasone. Mast cells were most numerous in the trachealis muscle and its overlying adventitia and in the lamina propria of the mucosa between cartilaginous rings. Within the posterior membrane, mast cells were most abundant in caudal trachea.

Although the observed decrease in the number of mast cells detected in the trachea after treatment with compound 48/80 fits with the known effects of this substance on mast cells, we were surprised to find that dexamethasone tended to attenuate the effects of compound 48/80 on mast cells. Rats treated with the combination of dexamethasone and compound 48/80 had more granule-containing mast cells than rats treated with compound 48/80 alone. This difference, however, did not reach statistical significance.

Globule leukocytes – like connective tissue mast cells – contained chloroacetate esterase but – unlike mast cells – were located only in the epithelium and were most numerous in the rostral trachea. This distribution is consistent with the findings of others that globule leukocytes are most abundant in more proximal airways (Jeffery and Reid 1975; Breeze and Wheeldon 1977). Globule leukocytes have been found to be particularly numerous in regions above the vocal cords, laryngeal ventricles, and epiglottis (Kent 1966). Globule leukocytes also differed from mast cells in morphology and response to compound 48/80 and dexametha-

sone. Compound 48/80 did not decrease the number of globule leukocytes, whereas dexamethasone caused a 90% reduction in these cells. This finding suggests that dexamethasone had one or more effects on globule leukocytes: the drug may have (a) caused degranulation of globule leukocytes with loss of their chymotrypsin-like serine esterase, (b) stimulated the migration of these cells out of, or blocked their movement into the epithelium, or (c) modified the differentiation or maturation of globule leukocytes within the epithelium.

The total number of mast cells detected in whole mounts (25000) was less than one half of that estimated from counts in tissue sections (55000). Two technical factors may explain this difference. First, counts in whole mounts may underestimate the actual number of mast cells because the 0.2 mm thickness of tracheal whole mounts tends to impair the penetration of components of the histochemical reaction medium and thereby interfere with the staining of some mast cells. Second, the thickness of tracheal whole mounts may limit the visibility of some stained cells located deep in the tracheal wall. Despite these limitations, using whole mounts made it possible to count cells over large areas, which provided an overview of cellular distribution in the trachea and also decreased the sampling error.

By comparison, the density of globule leukocytes detected in the epithelium of whole mounts was similar to the number detected in cross sections. Because globule leukocytes are located in the epithelium, penetration of the histochemical reaction medium and visibility by light microscopy are not limited in whole mounts by the thickness of the trachea, and the counts of cells in tracheal whole mounts should therefore be comparatively accurate. There are, however, two potential problems associated with computing the number of globule leukocytes from counts made from histological sections. First, the counts must be corrected for the number of nuclear profiles not sectioned through the center of the nucleus, and second, a comparatively small number of cells are present in individual sections.

Based on counts done on histological sections of tracheas, we estimate that tracheal mast cells contain approximately 19 pg of histamine per cell. This is comparable to the corresponding value determined for rat peritoneal mast cells (10–20 pg per cell; Bienenstock et al. 1982).

By our determination, the histamine content of airway globule leukocytes is similar to that of mucosal mast cells (1 pg per cell; Bienenstock et al. 1982). The globule leukocyte of the rat trachea thus has several characteristics in common with mucosal mast cells of the rat gastrointestinal tract. The two cell types are similar in ultrastructure, are smaller than connective tissue mast cells, are located in the epithelium (although gut mucosal mast cells are also present in the lamina propria), contain a chymotrypsin-like protease with chloroacetate esterase activity, have less histamine per cell than connective tissue mast cells, and degranulate or decrease in number in response to corticosteroids but not to compound 48/80 (Rasanen 1960; Heap and Kiernan 1973; Bienenstock et al. 1982; Huntley et al. 1985).

Many of these features of globule leukocytes also apply to the bone marrow-derived mast cells of mice (Razin et al. 1982). The latter cell type is considered by some to be the same as or related to mucosal mast cells and, by inference, to the globule leukocyte. If globule leukocytes are indeed analogous to bone marrow-derived mast cells, then globule leukocytes may contribute to airway pathology by releasing mediators, such as leukotrienes C_4 and D_4 (Razin et al. 1982, 1983; Mencia-Huerta 1985), prostaglandins (Razin 1983), and platelet activating factor (Mencia-Huerta 1983), that are known to be released by cultured bone marrow-derived mast cells. The abundance of globule leukocytes in a sentinel position of the airway, even in specific pathogen-free rats, suggests their potential importance in mediating airway inflammatory and allergic responses.

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