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Distribution of tracheal and laryngeal mucous glands in some rodents and the rabbit

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ABSTRACT

We used scanning electron microscopy to count the number of mucous gland openings in the tracheae and lower portion of the larynges of the rat, guinea pig, hamster, mouse and rabbit. Cells of the airway surface epithelium were removed by protease digestion better to visualise the gland openings. The distribution of glands was further studied by conventional histology and by PAS/Alcian blue staining of whole mounts. In all rodent species, gland openings in the larynx occurred with a frequency of 1–2 per mm². Mice had no gland openings in their tracheae, and hamsters, only a handful. Rat tracheae contained 126 ± 42 gland openings ($\pm$S.D.; n = 6) at a frequency of ~ 0.6 per mm² at the top of the trachea and ~ 0.15 per mm² at the bottom. Guinea pig tracheae contained 153 ± 90 gland openings ($\pm$S.D.; n = 5), with 54% being in the top 40% of the trachea. In both rat and guinea pig, tracheal glands were found in the ventral aspect between the cartilaginous rings, and were absent from the dorsal membranous portion. Gland openings in most species were simple circles of ~ 50 μm diameter. However, glands in the rat trachea generally opened obliquely into shallow (~ 20 μm deep) oval troughs ($\sim 150 \times 75$ μm), which had their long axes oriented from head to tail. In the rabbit, there was no evidence of tracheal or laryngeal glands histologically. However, the tracheal and laryngeal surfaces contained numerous pits (~ 30 μm diameter) distributed evenly over and between cartilages at a frequency of ~ 4 per mm². These may correspond to the 'nests' of goblet cells described by others.

Key words: Mucus secretion; goblet cells; scanning electron microscopy.

INTRODUCTION

The tracheae of rat and guinea pig are used to study the neurohumoral control of mucus secretion (Adler et al. 1987; Savoie et al. 1995; Yeadon et al. 1995; Wagner et al. 1998). However, the mucus secreted comes from 2 main sources: goblet cells in the surface epithelium and submucosal glands. The relative contributions of these 2 are hard to estimate as there is no quantitative information in the literature on the numbers of tracheal glands in these species, though they are apparently commonest in the upper (cephalad) third of the trachea (Lamb & Reid, 1968; Jones et al. 1973; Spicer et al. 1982; Ohtsuka et al. 1997) and may increase in numbers near the carina (Steiger et al. 1995).

The trachea and airways of the Syrian golden hamster are used extensively in studies of tumorigenesis (Homberger, 1979). The tumours are generally assumed to come from surface epithelium, but glands are also present in hamster airways, where they are found predominantly adjacent to the larynx (Kleinerman, 1972; Emura & Mohr, 1975; Kennedy & Little, 1979), but also at the carina (Kennedy & Little, 1979). However, nothing precise is known as to their numbers, distribution or size.

Transgenic mice, lacking the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), are widely used to study the pathology of cystic fibrosis (Dorin et al. 1994). Interestingly, they show little airway disease, a finding that may reflect in part a dearth of airway glands in this species. Again,

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however, exact information on gland distribution or numbers is lacking. One report states that tracheal glands are present only within 1.5 mm of the larynx (Pack et al. 1981). Another claims that, though tracheal glands are essentially absent in young animals, they appear after 1 or 2 years of life (Nettesheim, 1970). A third finds them predominantly at the junction of the trachea and larynx, but also in small numbers in the upper third of the trachea (Borthwick et al. 1999).

Finally, it is generally stated that rabbit airways do not contain glands, and mucus release from pieces of rabbit tracheal wall is thought to reflect goblet cell degranulation (Conover & Conod, 1978; Adler et al. 1987). A single report, however, finds that the rabbit has 'only a few small tracheal glands', but less than the guinea pig and much less than rat, dog, rhesus monkey or man (Hughes, 1965). Konrádová et al. (1985, 1990) have reported that about 6% of the goblet cells are found in groups of 5–7. In response to irritation the numbers of goblet cells in such groups can increase to as much as 60% of the total, and the number of cells per cluster may also increase so as to form small 'intraepithelial' or 'endotracheal' glands (Konrádová et al. 1985, 1990).

In this paper, we describe the distribution, numbers and volume of glands in the trachea and subglottal region of the larynx in these commonly used experimental animals.

METHODS

Tracheae were obtained from tissue-sharing programs at the San Francisco or Berkeley campuses of the University of California or from Children's Hospital Oakland Research Institute. We used adult rats (Sprague-Dawley), mice (Black 6; Jackson Labs) and rabbits (New Zealand White). The guinea pigs (Hartley) were of various ages, but no age-related differences in the total number of gland openings per trachea were apparent. Larynges were cut just below the glottis.

To remove surface epithelial cells, whole tracheae or tracheal pieces were incubated with 0.4 mg/ml protease (Type IV; Sigma, St Louis, MO) at 4 °C for from 2 to 18 h, followed by vigorous shaking.

For scanning electron microscopy (SEM), tissues were fixed in 2.3% glutaraldehyde/0.05 M Na cacodylate, then rinsed in 0.05 M Na cacodylate/8.125% sucrose, and postfixed in 1% OsO_4/0.05 M Na cacodylate/3.14% sucrose (all at pH 7.4). The tissues were again rinsed in cacodylate/sucrose, and dehydrated by transfer through ethanol solutions of increasing strength. Next, they were critical point dried (Polaron, UK) and entire tracheae or segments of trachea were mounted on circular stubs of 12 mm diameter. They were then sputter-coated with platinum (Polaron, UK), and viewed in a conventional scanning electron microscope (AmRay, Bedford, MS). A photographic montage was made of the entire surface of each tissue piece at $\times 50$ magnification. All gland openings on these montages were identified, and the surface area of the tissue piece estimated gravimetrically from the montage.

To correct for shrinkage during processing for SEM, tissues were laid out on a grid and photographed under a dissecting microscope both when fresh and after critical point drying. Comparison of the 2 photographs showed that for all species tissues shrank on average 15% in the linear dimension during processing. Accordingly, estimates of linear dimensions from SEM specimens were corrected by dividing by 0.85, whereas the surface area of the dried tissue was converted to the surface area of the fresh tissue by dividing by $(0.85)^2$.

For light microscopy, tissues were fixed in 10% formaldehyde in phosphate-buffered saline, dehydrated in ethanols, embedded in paraffin, sectioned (5 μm), and stained with haematoxylin and eosin. For all quantitative analyses, sections were cut perpendicular to the surface epithelium. Gland volumes were estimated as described previously (Choi et al. 2000). In brief, longitudinal sections, cut through the entire length of the ventral aspect of the trachea, were displayed at $\times 15$ magnification on a video screen. The gland profiles and the surface epithelium were traced. The length of epithelium was determined with a cartographic measuring wheel, and the total area of gland profiles by gravimetry. The ratio of gland profile (in mm^2) to the length of surface epithelium (in mm) on the original section is equal to μl of gland per mm^2 of tracheal surface. Shrinkage of tissues during processing was estimated as 10% by comparing the length of fresh tracheae with that of the resulting sections. Therefore, as in our earlier study, the ratio of gland volume to surface area estimated from sections was converted to the ratio for fresh tissue by multiplying by 1.11 (Choi et al. 2000). In the results, maximal gland depth is given as the perpendicular distance between the surface epithelium and the outermost edge of gland tissue.

Staining of whole mounts with periodic acid Schiff (PAS)/Alcian blue was as in earlier studies (Tos, 1966; Choi et al. 2000).

Tests for statistically significant difference between

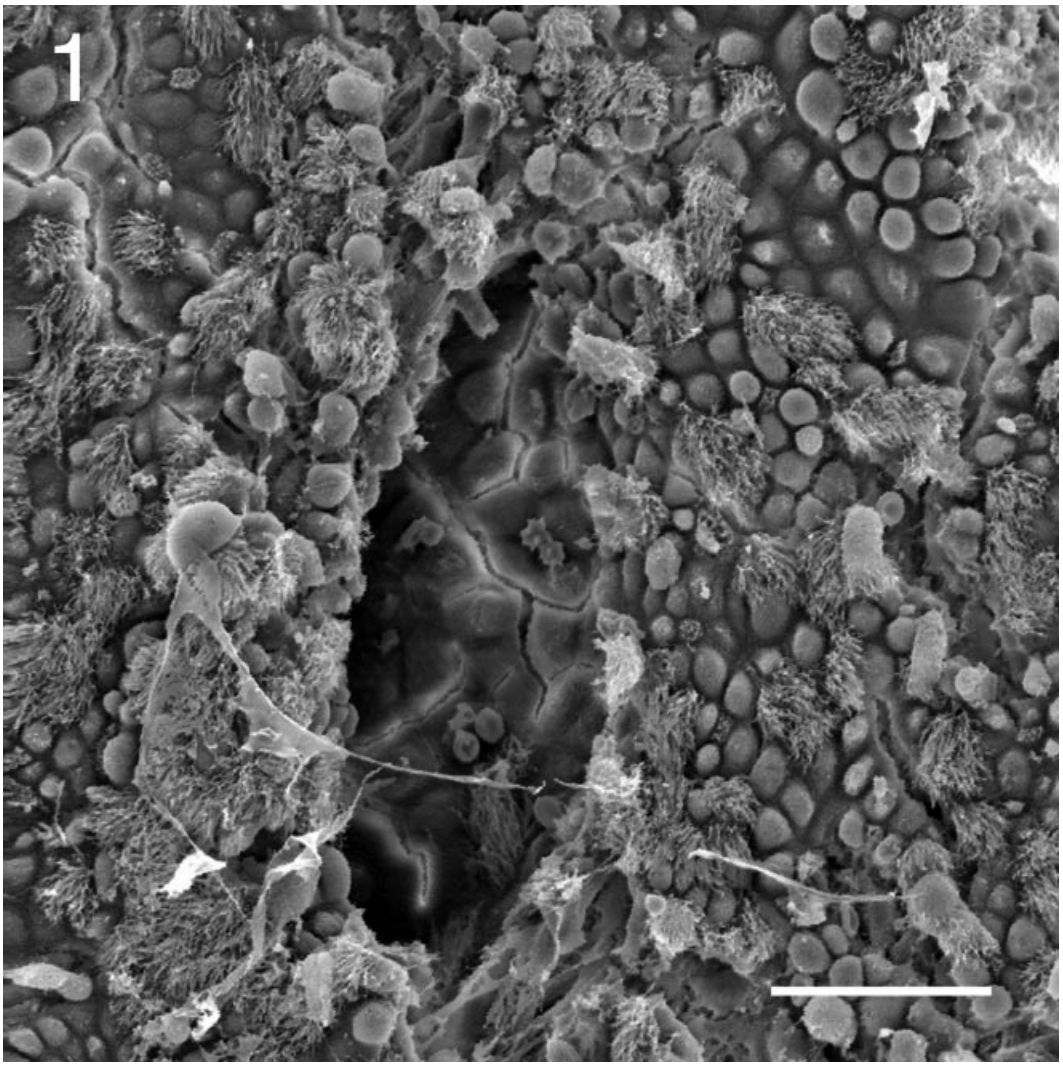

Fig. 1. Gland opening in rat trachea. Bar, 50 μm.

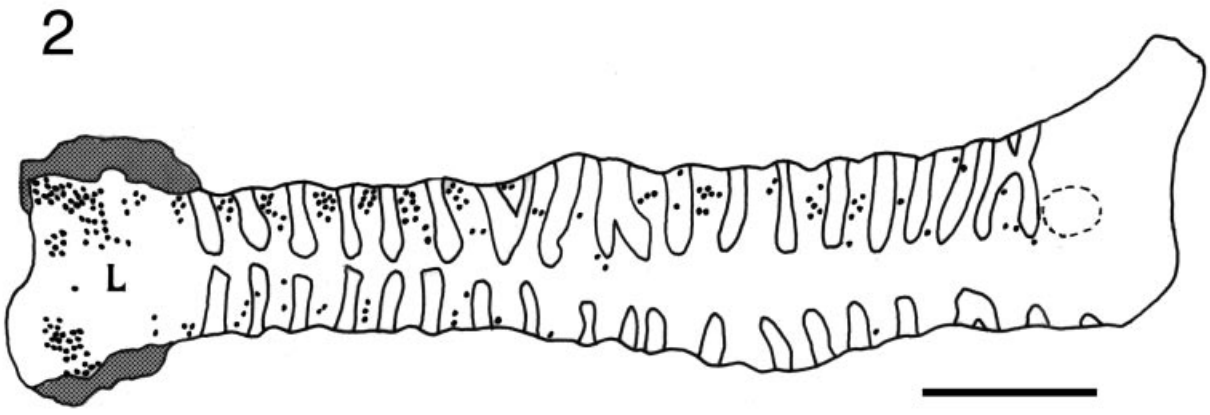

Fig. 2. Gland distribution in rat trachea and larynx. The trachea and larynx (*L*) were split along their ventral aspect and pinned out. The stippled areas at the sides of the larynx are striated muscle. The club-shaped structures poking in from the edges of the trachea are the cartilaginous rings cut in half. The hatched circle at the caudal end of the trachea is the opening of the first bronchus. Gland openings are shown as solid black dots (not to scale). Bar, 1 cm.

means were done by ANOVA, paired or unpaired *t* tests with $P < 0.05$ regarded as statistically significant.

RESULTS

In preliminary experiments on rat trachea, we found that in general gland ducts opened obliquely into shallow troughs ($\sim$ 20 μm deep) lined with squamous cells (see Fig. 1). However, at the magnification used for making montages ($\times$ 50), the openings were often hard to see. Therefore, better to visualise gland openings, all further studies were performed on tissues denuded of cells.

Rat

Figure 2 shows the distribution of gland openings in the lower larynx and along the trachea of one of the 6 rats studied. In the larynx, glands were found ventrolaterally at an average density of 1.80 ± 0.75 ($\pm$S.D., n = 5) per mm^2 of mucosal surface. Laryngeal gland openings were circular ranging from 40 to 100 μm in diameter. In the trachea, gland openings were found exclusively between the cartilaginous rings in the ventral aspect (Fig. 2). Glands opened into slit-shaped 'troughs' about 20 μm deep that had their long axes oriented longitudinally (Fig. 3*a*). Long axes

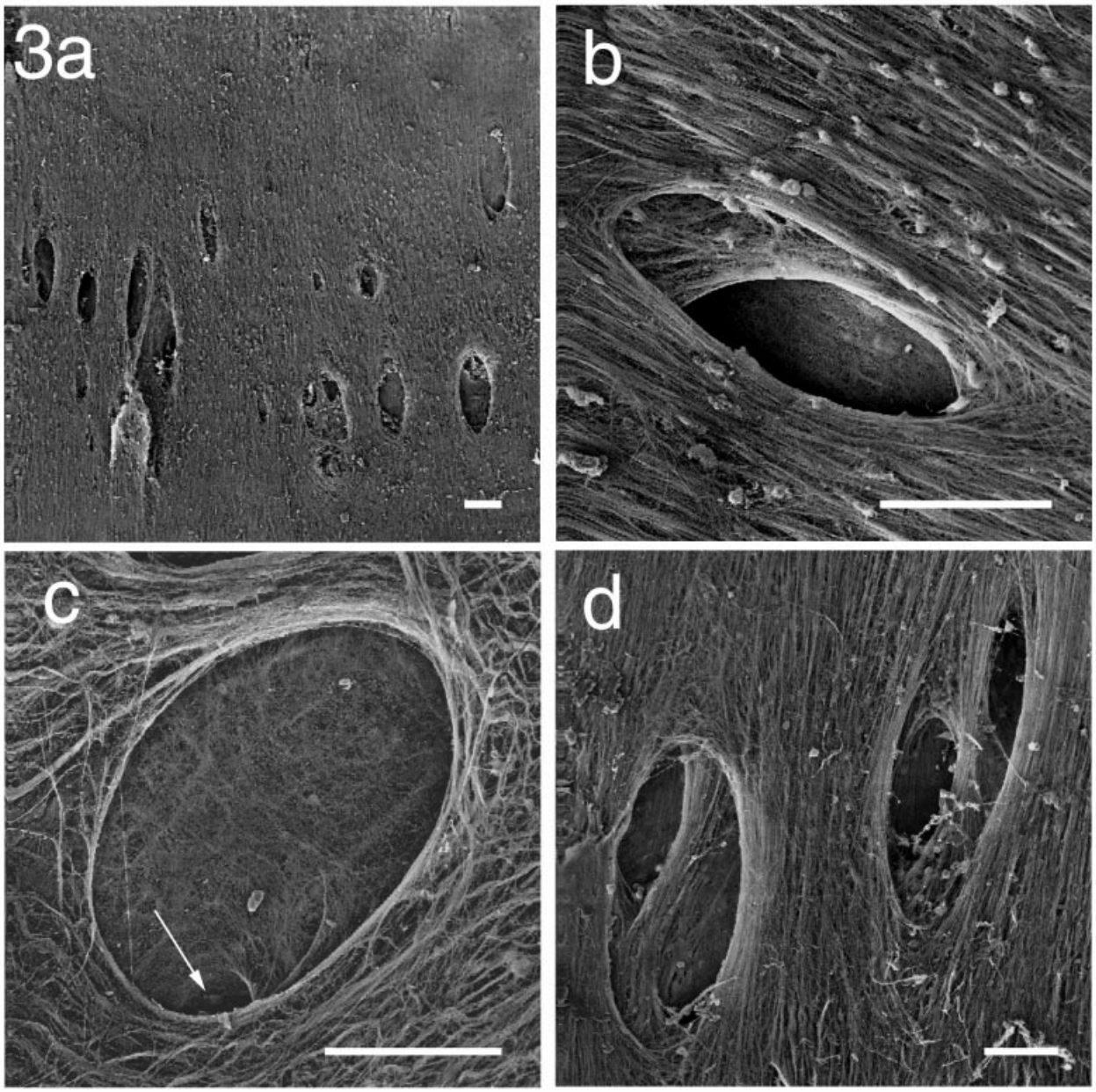

Fig. 3. Gland openings in rat trachea denuded of surface epithelium. (*a*) Group of 10 openings. (*b*) Single small opening. (*c*) Unusually circular 'trough'. Gland opening indicated by arrow. (*d*) Two troughs each receiving 2 gland openings. Bars, 100 μm.

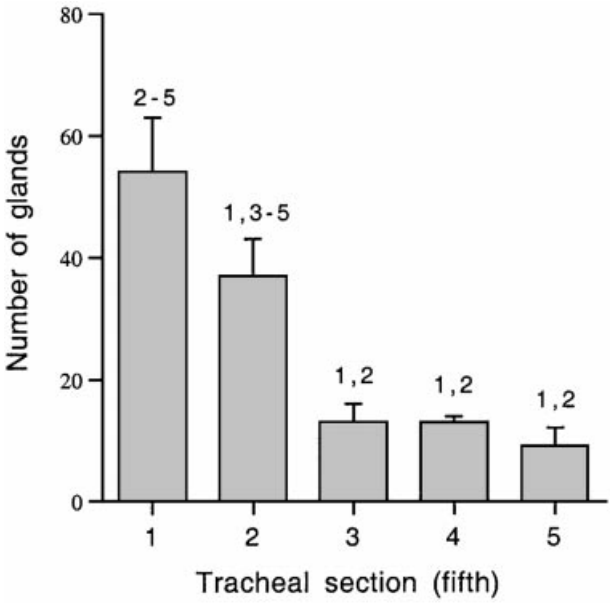

Fig. 4. Distribution of gland openings along rat trachea. Tracheae were divided into 5 equal lengths, with no. 1 being the most cephalad. Means ± S.E., n = 6. Numbers above columns show the tracheal sections from which that particular section is significantly different (ANOVA).

ranged from 50 to 300 μm, and the short axes were about half the length of the long axis. Gland ducts opened with approximately equal frequency into the caudal, medial or cephalad ends of the troughs. A typical opening is shown in Figure 3*b*. The circular nature of the trough shown in Figure 3*c* was highly unusual. In about 10% of cases, troughs contained 2 separate duct openings (Fig. 3*d*), though there were never more than 2 openings per trough. Figure 4 shows that the frequency of openings in the most cephalad fifth of the trachea was about 6 times that in the vicinity of the carina. The average of 54 openings in the most cephalad fifth corresponds to 0.63 openings per mm².

Light microscopy of longitudinal sections through the entire length of larynx and trachea confirmed the scanning electron microscopy. There were large masses of gland in the larynx (Fig. 5*a*) averaging 2.62 ± 1.01 μl per cm² (n = 3) and penetrating up to 300 μm from the surface. In the trachea, the glands were predominantly between the cartilaginous rings (Fig. 5*b*) up to a maximal depth of 200 μm. In 2 rats, there was marked dilation of the tracheal gland lumina (Fig. 5*c*). In transverse sections, glands were absent from the dorsal membranous portion. For 4 rat tracheae, longitudinal sections were cut through the first 16–21 intercartilaginous spaces (rat tracheae have 20–22 cartilaginous rings between the larynx and the carina). For all 4 tracheae, linear regressions showed significant declines in gland volume (μl per cm²) towards the caudal end of the trachea. One regression is shown in Figure 6. For all 4 regressions, the predicted gland volume in the 1st intercartilaginous space was 3.94 ± 1.45 μl/cm², declining to 0.19 ± 0.60 μl/cm² by the 20th space. Predicted values for the 10th space (i.e. halfway along the trachea) were 0.82, 0.94, 3.10 and 3.76 μl/cm². This rather large variability was caused by the dilation of the gland lumina in 2 animals; the volume of gland cells in all 4 animals was quite similar, with the values for each individual animal being within about 25% of the mean.

Guinea pig

There were 2.25 ± 0.85 glands per mm² in the guinea pig larynx (n = 5). As in the rat, they were circular with diameters of ~ 50 μm, but unlike the rat they were more evenly dispersed between dorsal and ventral aspects.

In the trachea, gland openings were predominantly between the cartilaginous rings in the ventral aspect of the trachea, but a few openings were also present along the edge of the dorsal membranous portion (Fig. 7*a*). The openings were circular, about 50 μm diameter, and lacked the trough seen in the rat (Fig. 7*b*). Gland openings were most frequent cranially (Fig. 8), though a modest increase in numbers occurred at the carina in about half the animals studied. There were 153 ± 90 glands per trachea (± S.D., n = 5), with considerably more variation between individuals in the guinea pig than the rat (range = 40–282 per trachea for the guinea pig and 89–157 for the rat). For an adult guinea pig, the tracheal surface area averages ~ 7 cm², and so the 51 openings in the top fifth of the trachea corresponds to ~ 0.35 openings per mm².

The light microscopic findings for the guinea pig resembled those for the rat. Figure 9*a* shows that there were large masses of glands in the larynx and at the border of the larynx and trachea. We had insufficient material to make an estimate of laryngeal gland volume, but the size and frequency of the glands were similar to those of the rat. Glands were also fairly common between the cartilaginous rings in the upper half of the trachea (Fig. 9*c*). Sections along the full length of the ventral aspect of 1 trachea revealed a significant decline in gland volume caudally. Least squares linear regression predicted gland volumes of 1.07 μl/cm² in the 1st intercartilaginous space, 0.57 in the 12th, and 0.06 in the 24th or last space. Whole mounts also showed that glands were abundant at the

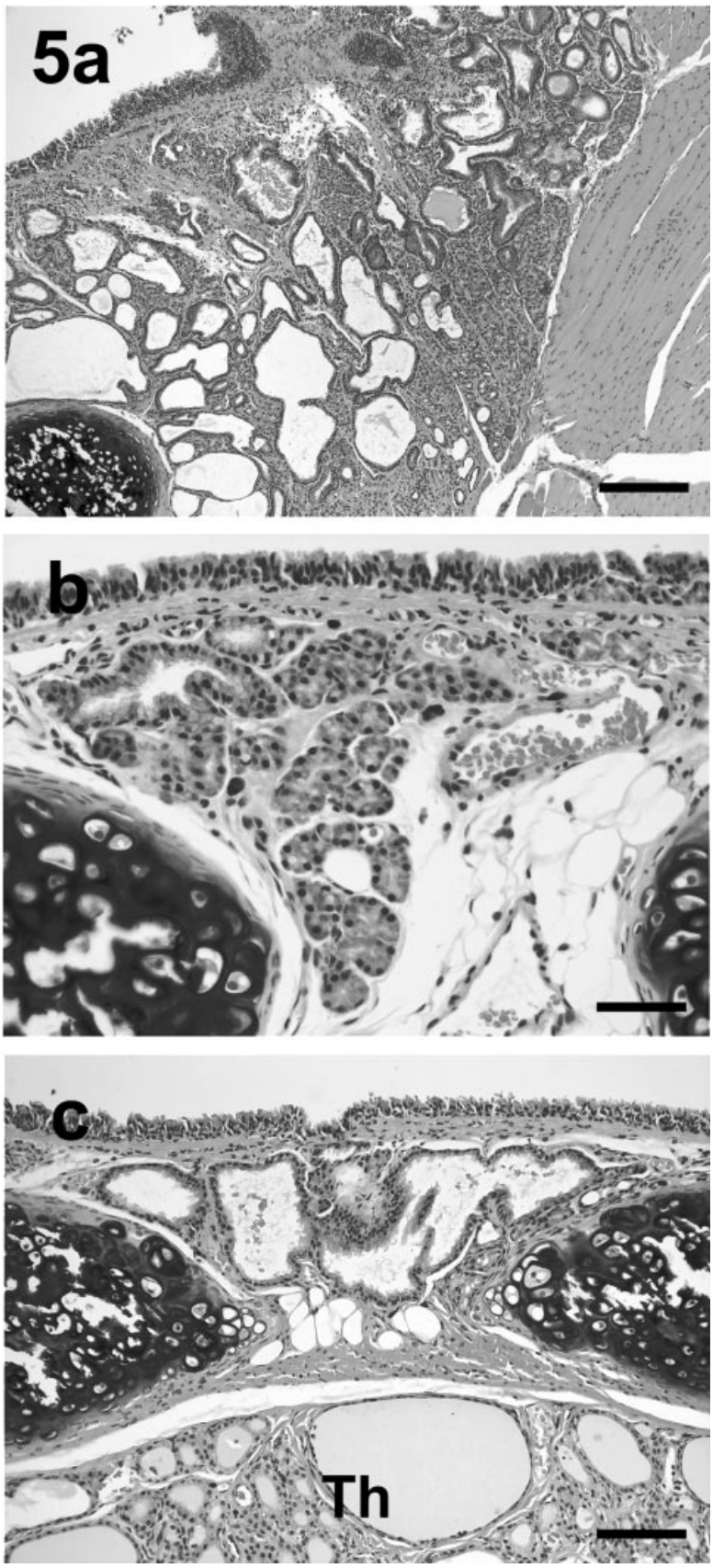

Fig. 5. Light microscopy of rat laryngeal and tracheal glands. (*a*) Laryngeal glands seen in a tangential section. Note that the lumina of many glands are markedly dilated. Bar, 200 μm. (*b*) Tracheal glands. Bar, 50 μm. (*c*) Dilated tracheal glands. Bar, 100 μm. Th, thyroid gland.

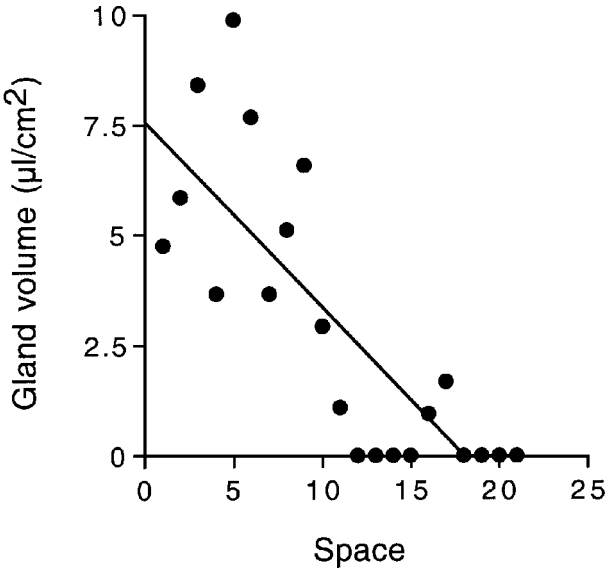

Fig. 6. Distribution of glands along a single rat trachea determined from light microscopy. The gland volume is plotted for each intercartilaginous space starting at the head end. The solid line is the best least squares linear regression. This trachea had markedly dilated gland lumina.

tracheolaryngeal border (Fig. 9*b*), and declined in number and size down the trachea (Fig. 9*d*).

Mouse

Small gland openings (~ 40 µm diameter) were present in the larynx at 0.67 ± 0.14 (S.D.) per mm² ($n = 4$). In light microscopical sections, glands were visible beneath the cricoid cartilage with a few in the space between the cricoid and the first tracheal cartilage, in agreement with our previous data (Choi et al. 2000). The volume of these laryngeal glands was ~ 3.0 µl per cm². No gland openings were found in the trachea ($n = 5$).

Hamster

Even with protease digestions of comparatively short duration (20–45 min), the connective tissue in some parts of the trachea was completely digested away so as to expose the surface of the cartilaginous rings, while surface epithelial cells remained in the regions between the rings. It was therefore impossible to obtain a precise estimate of the numbers of gland openings in the hamster trachea. However, based on data from 7 specimens we estimate that there are no more than about 5 glands per trachea.

PAS/Alcian blue staining of whole mounts confirmed these results; a large gland mass was clearly visible at the junction of the larynx and trachea (Fig. 10*a*), but no glands were detected elsewhere. Longitudinal sections were cut through the full lengths of the ventral portion of 3 hamster tracheae with attached larynges, and 1 isolated trachea. Glands were found abundantly at the junction of the larynx and trachea (Fig. 10*b*), with average laryngeal gland volume being 3.07 ± 0.85 µl/cm² ($n = 3$). In addition, occasional small gland profiles were found between the cartilages throughout the length of tracheae (Fig. 10*c*). The numbers of intercartilaginous spaces containing glands vs the total number of spaces were 4/16, 0/15, 1/16 and 0/16 for the 4 tracheae, with the spaces that contained glands being evenly distributed along the lengths of the tracheae. The average gland volume in the 4 tracheae was 0.13 ± 0.13 µl/cm² (range 0–0.52; $n = 4$).

Rabbit

Larynges and tracheae of 2 rabbits were studied by SEM. Very similar results were obtained in both animals. The laryngeal surface displayed small circular depressions at 4.10 per mm² in the first rabbit and 4.71 per mm² in the second (Fig. 11*a*). Their average diameter was 45.0 ± 21.4 (S.D.) µm in the first rabbit (range = 25–120 µm; $n = 391$) and 41.9 ± 20.5 (S.D.) µm ($n = 285$) in the second. Flat bottoms about 20 µm below the surface were clearly visible in some of the larger depressions. We therefore take these structures to be shallow pits rather than gland openings.

Pits in the rabbit trachea occurred at approximately the same frequency as in the larynx, but were of smaller diameter (Fig. 11*b*). A randomly selected 12 mm² area from the centre of each trachea had pits with mean diameters of 24.2 ± 15.6 µm (range = 10–80 µm; $n = 47$) and 34.8 ± 13.4 µm (range = 10–80 µm; $n = 42$); there was no apparent variation in diameter of the pits along the trachea. When numbers of pits were determined for each fifth of the length of the trachea, there were significant declines (linear regressions) in the numbers of pits caudally. In one trachea, there were 5.4 pits per mm² in the most cephalad fifth and 3.6 per mm² in the most caudal. The corresponding numbers in the other trachea were 4.4 per mm² and 0.8 per mm².

In longitudinal sections (2 tracheae), cross sections (3 tracheae), or whole mounts (2 tracheae), we saw nothing that could be positively identified as glands, supporting our conclusion that the surface structures seen by SEM were indeed shallow pits rather than gland openings. However, an interesting feature of the rabbit trachea, not seen in the other species studied, was the presence of large sinusoids approximately

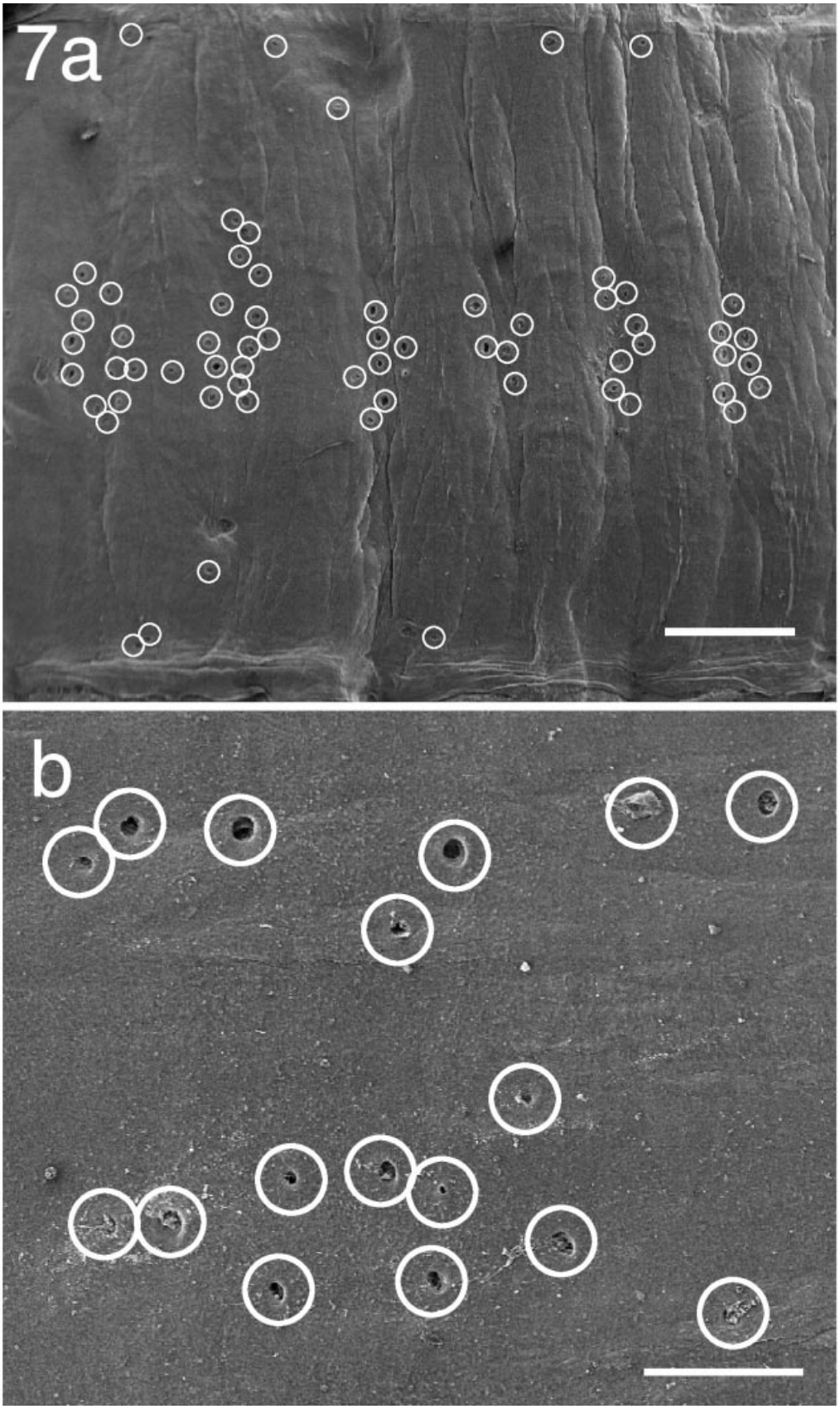

Fig. 7. Gland openings in guinea pig trachea. (*a*) Groups of glands between the cartilaginous rings, which run perpendicularly from top to bottom of the panel. The area between rings is approximately as wide as the rings themselves. Bar, 1 mm. (*b*) Two intercartilaginous groups of glands. In this panel a single ring runs from right to left between the two groups. Bar, 400 μm.

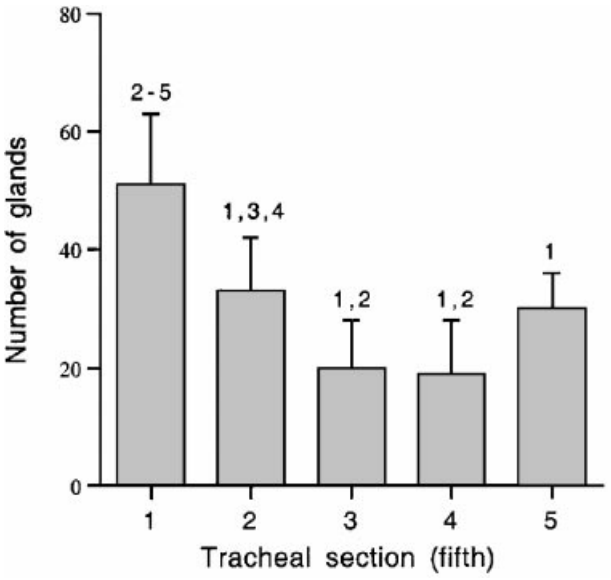

Fig. 8. Distribution of gland openings along guinea pig trachea. Means ± S.E., n = 5. Details as in legend to Fig. 4.

200 μm beneath the surface epithelium (Fig. 12). In cross sections at the level of either the cricoid or thyroid cartilage (1 larynx for each), we saw no glands, but sinusoids were present as in the trachea.

Interspecies comparison

The frequency and size of glands in the larynges and tracheae of the rodents studied is summarised in the Table, from which it is clear that gland openings are much more frequent in the larynx than the trachea. The Table also reveals that the average volume of individual tracheal glands is about 20 nl in all species studied, and that the volume of laryngeal glands is of the same order of magnitude.

DISCUSSION

The primary function of airway submucosal glands is to produce mucus. The primary function of airway mucus is to entrap inhaled particles and allow them to be removed by mucociliary clearance (Wanner, 1986). Previously, we reported a linear relationships between tracheal diameter and either gland volume or numbers of gland openings in 10 mammals ranging in size from mouse to ox (Choi et al. 2000). We explained this as follows: larger airways have larger velocities of air flow, and therefore deeper layers of turbulent flow at the surface and greater rates of particle deposition. The increase in the number of particles landing per unit area per unit time is compensated for by an increase in the numbers and overall volume of mucus-secreting glands.

In keeping with this relationship between airway size and gland numbers, in the rodents studied here the gland frequencies averaged over the tracheal surface as a whole ranged from 0 to 0.3 per mm², much less than for mammalian species the size of dog and larger where the frequency in the ventral wall ranges from 0.6 (pig) to 1.5 openings per mm² (ox) (Choi et al. 2000). Furthermore, within the 4 rodent species there was a rough correlation between airway diameter and gland numbers. Thus the mouse trachea with a diameter of ~1 mm had no glands. For hamster, the average tracheal diameter of ~1.5 mm corresponded to an average gland density of 0.07 openings per mm². The larger tracheae of guinea pig and rat (both ~2.5 mm diameter) had on average 0.2 and 0.3 openings per mm², respectively.

In a number of large mammalian species (ox, dog, sheep, goat, man), the volume of individual tracheal glands is constant at ~120 nl (Choi et al. 2000). However, in man there is a decrease in the volume of individual glands with decreasing airway diameter (Whimster, 1986). Glands develop as buds from the surface epithelium (Bucher & Reid, 1961; Smolich et al. 1976), and the thinner the airway wall, the less room there is for the bud to expand. Therefore, it was to be expected that the volume of tracheal and laryngeal glands in rodents (i.e. ~20 nl) would be much less than the volume of tracheal glands in larger mammalian species.

In agreement with previous workers (Jones et al. 1973; Hayashi et al. 1979; Spicer et al. 1982; Ohtsuka et al. 1997) we found glands to be commonest in the upper part of the rat trachea. However, at least in the Sprague-Dawley rats used here, glands were present throughout the trachea, though declining in frequency about 5-fold between the larynx and carina. Just how far down the trachea glands are present may depend on the strain used (Ohtsuka et al. 1997). The finding that glands were restricted to the intercartilaginous spaces of the ventral wall is also in agreement with previous studies (Lamb & Reid, 1968; Smolich et al. 1976; Hayashi et al. 1979).

Virtually nothing was known about glands in the trachea of the guinea pig. Some have reported that they are present (Adler et al. 1987; Okamura et al. 1996), others believe that there are few or none (Jeffery, 1983; Yeadon et al. 1995). Here we show that in numbers, distribution and volume they resemble quite closely the glands in the rat trachea. Some minor differences are that the guinea pig shows considerable individual variation in total number of glands, has a few glands adjacent to the dorsal trachealis muscle,

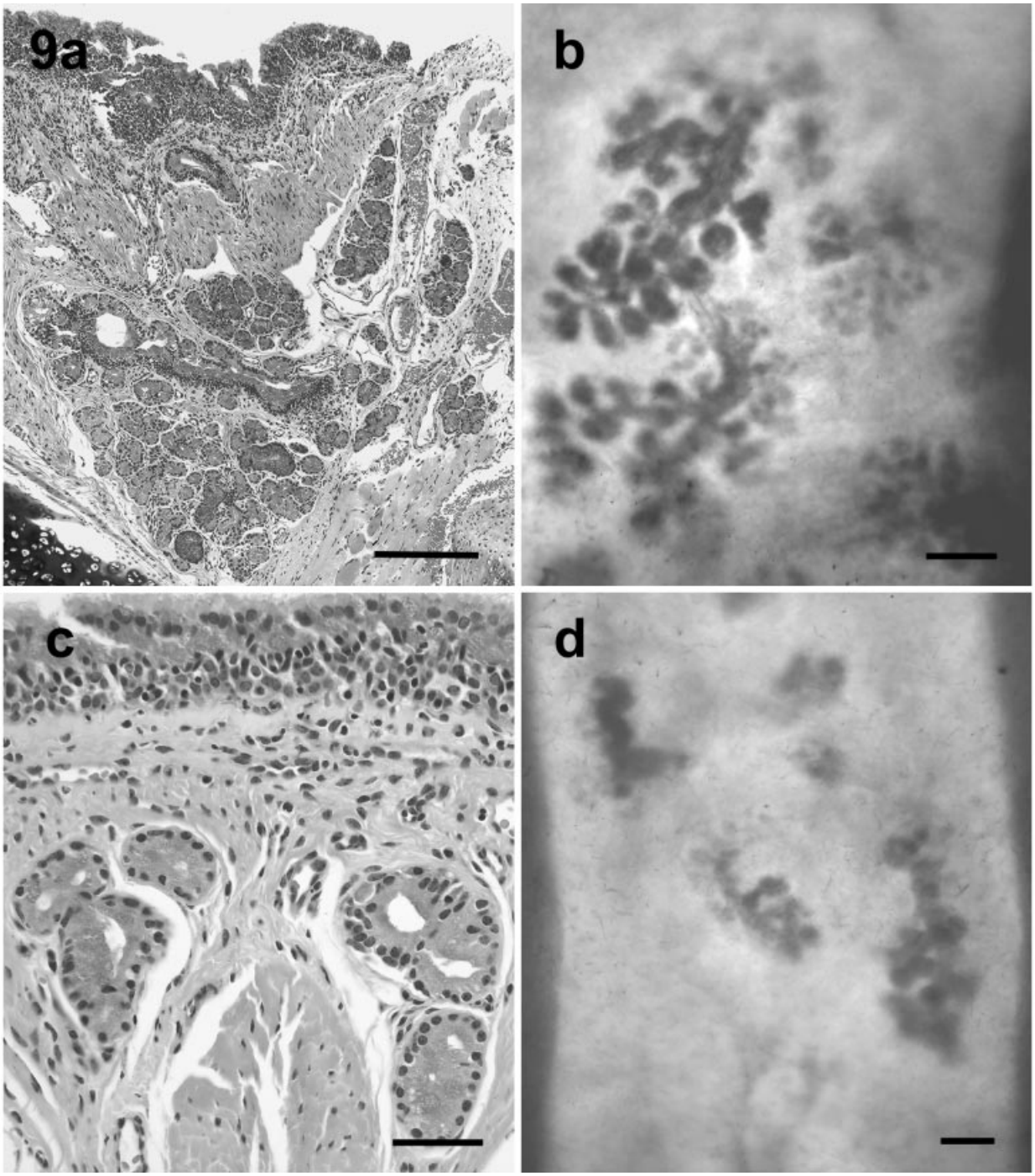

Fig. 9. Light microscopy of guinea pig laryngeal and tracheal glands. (*a*) Laryngeal glands. Bar, 200 μm. (*b*) Whole mount of tracheolaryngeal glands. Bar, 100 μm. (*c*) Tracheal glands. Bar, 50 μm. (*d*) Whole mount of tracheal glands. Dark areas at sides of panel are cartilaginous rings. Bar, 100 μm.

and shows a less marked decline in frequency of glands along the trachea.

Hamsters have airway glands, mainly at the tracheolaryngeal junction but also in the trachea itself (Kleinerman, 1972; Emura & Mohr, 1975; Kennedy & Little, 1979). However, 'they are so few as to be easily overlooked' (Kleinerman, 1972). In fact, we show here that the total numbers and volume of glands per unit luminal surface area in the trachea of hamsters is much less than in the rat or guinea pig.

In agreement with others (Pack et al. 1980), we found glands to be absent from mouse tracheae, except for a small number between the cricoid and 1st tracheal cartilages. However, depending on age and strain, this may not be a universal result. Thus, Nettesheim (1970) has stated that in mice aged 7–10

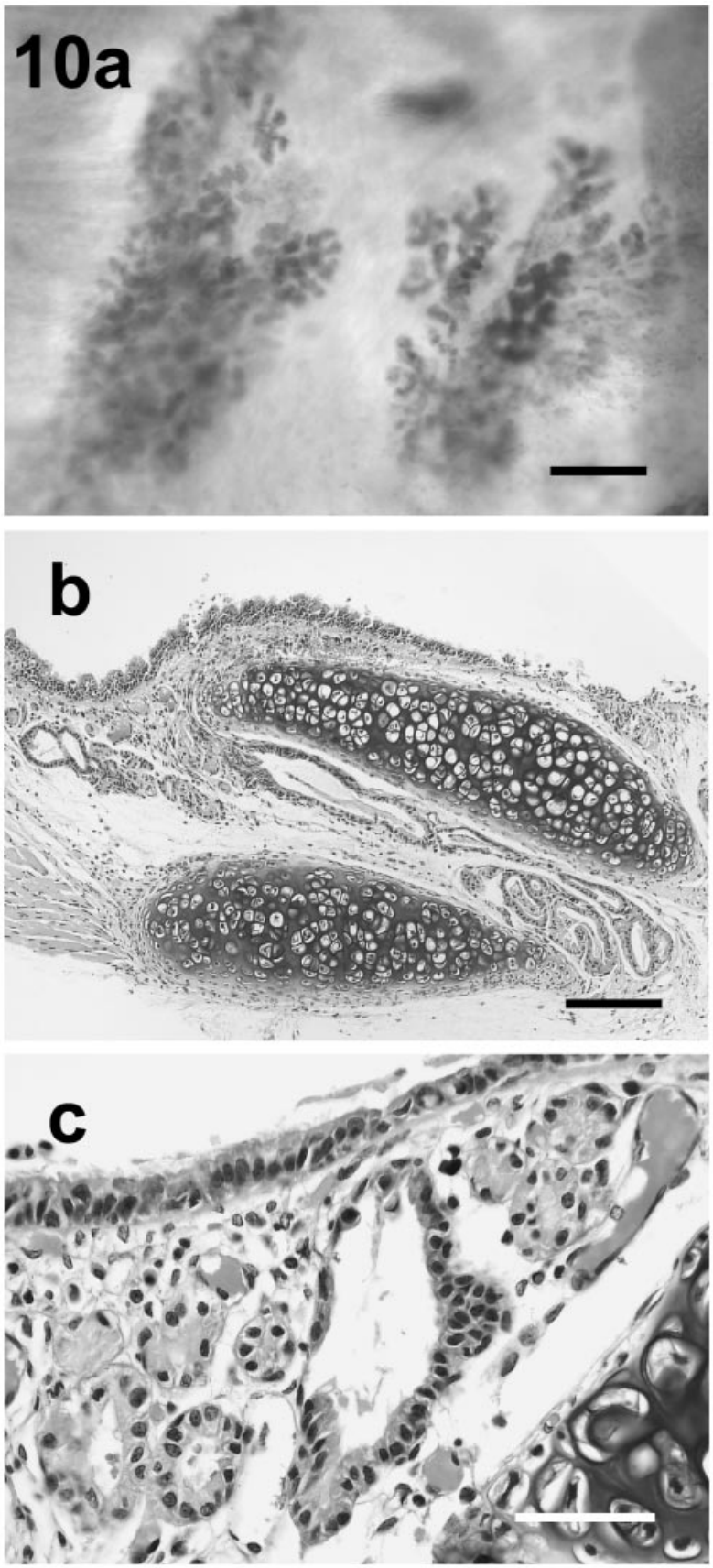

Fig. 10. Light microscopy of hamster laryngeal and tracheal glands. (*a*) Whole mount of tracheolaryngeal glands. Bar, 200 μm. (*b*) Tracheolaryngeal glands between cricoid cartilage below and 1st tracheal cartilage above. Bar, 100 μm. (*c*) Tracheal glands. Bar, 50 μm.

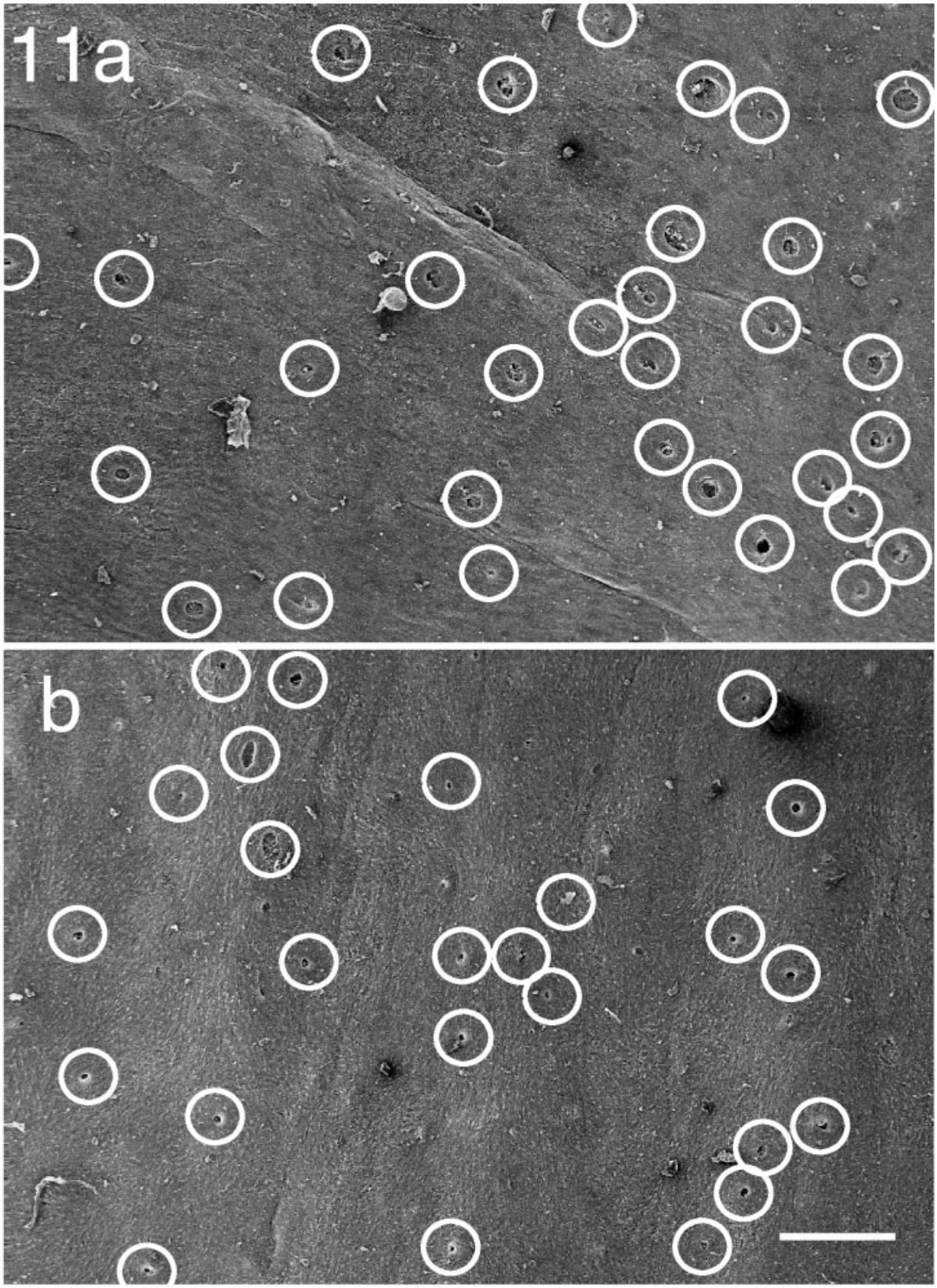

Fig. 11. Pits in the surface of rabbit larynx and trachea. (*a*) Larynx. (*b*) Trachea. Bar, 500 μm.

wk there are virtually no glands in the trachea, but that they are abundant at 1–2 y of age. In 7–8-wk old mice of mixed MF1/129 genetic background, Borthwick et al. (1999) stated that in all animals studied there were more than 10 glands in each of the first 2 intercartilaginous spaces. Less than half the animals had glands in the 3rd space. The average number in the 4th space was 0.9, and 0.3 in the 5th. No glands were

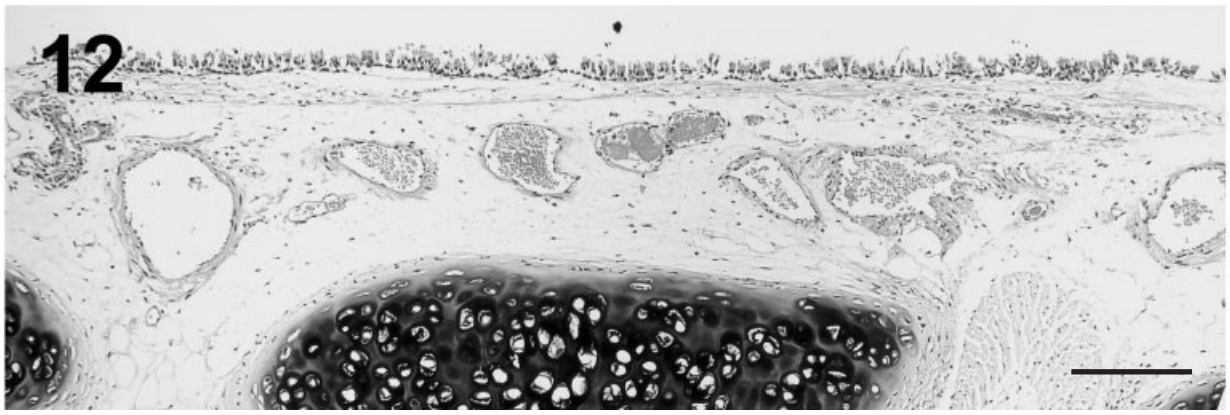

Fig. 12. Rabbit trachea showing sinusoids in the lamina propria. Bar, 200 μm.

Table. *Numbers and volume of tracheal and laryngeal glands in rodents*

	Larynx		Trachea*	
	Openings (per mm²)	Volume (μl per cm²)	Openings (per mm²)	Volume (μl per cm²)
Rat	1.8	2.6	0.3	0.5**
Guinea pig	2.3	ND	0.2	0.3
Hamster	ND	~ 3.0	0.03***	0.07
Mouse	0.7	3.1	0	0

* The numbers of openings and the volume of glands are the averages for the trachea as a whole. To obtain the estimates for gland volume in rat and guinea pig, the value for the ventral aspect at the centre of the trachea was halved, as was the value for the ventral aspect as a whole for the hamster.
** Data from the 2 animals with markedly dilated tracheal glands were not included.
*** Assumes tracheal surface area of 1.6 cm². ND, not determined.

found in the 6th to 10th spaces (a mouse has on average 10 spaces). Because groups of closely associated acini were counted as glands, it is not clear how these estimates of gland numbers correspond to the numbers of gland openings. However, it is clear that the mice used had a higher number of tracheal glands than those used by us (Black 6 strain). Interestingly Pack et al. (1981) using MFI mice (one of the parent strains of the mice used by Borthwick et al.) found submucosal glands to be 'restricted to the larynx and the most rostral trachea, extending to 1.6 mm down from the larynx'. This would correspond to the space between the cricoid and the 1st tracheal cartilage, the only place where we found tracheal glands. Presumably, 129 mice (the other parent strain of the hybrid mice used by Borthwick et al.) have unusually high numbers of tracheal glands.

The rabbit trachea has a larger diameter than that of any of the rodents studied here, but it is generally reported as having no glands (Adler et al. 1987). We were surprised, therefore, to find depressions in the surface of both the trachea and the larynx. However, in many aspects, these structures do not resemble the gland openings of other species. First, at up to 5 per mm², they are much more frequent than gland openings in even the largest species studied, such as 1.5 per mm² in the ox (Choi et al. 2000). Second, their average diameter of 20–40 μm is markedly less than that of typical gland openings (e.g. 50 μm in the guinea pig and 100 μm in the rat). Third, many of the depressions appeared to end in flat bottoms close to the airway surface. Fourth, frequency histograms of the diameter of the surface depressions showed normal distributions skewed towards larger diameters providing no evidence for 2 populations of openings. Thus it seems unlikely that the largest structures were gland openings whereas the smaller represented something else.

Two quantitative arguments suggest that the depressions in the surface of rabbit airways correspond to the small groups of goblet cells that Konrádová stated may form small 'intraepithelial' or 'endotracheal' glands in response to irritation (Konrádová et al. 1985, 1990). First, in the absence of surface irritation, 6% of goblet cells occur in groups of approximately 5–7 (Konrádová et al. 1985). Published light micrographs show apical membrane radii for rabbit tracheal goblet cells of ~ 3 μm (Adler et al. 1987). A pit of 20 μm diameter would contain 11 goblet cells of this size. Second, the frequency of the pits (i.e. ~ 5 per mm²) is also roughly consistent with their being goblet cell clusters. Thus, if goblet cells are 20% of the total columnar cells (Konrádová 1966), and the average apical membrane radius of ciliated cells is 7 μm (Adler et al. 1987), then there will be ~ 1500 goblet cells per mm². Of these, 6%, or 93 per mm², are in clusters. If each cluster has 11 cells, then there will be 9 clusters per mm². Of course, these calculations are rough, and the degree of irritation of the mucosal surface may have differed significantly

between studies. However, we feel that the agreement within an order of magnitude of our measurements of pit size and frequency with those predicted from Konrádová's data supports our hypothesis that the pits we saw do indeed correspond to goblet cell clusters.

The surface of the rabbit larynx contained the same sort of pits as in the trachea, slightly larger but at essentially the same frequency. In the larynx, as for the trachea, we did not see glands in cross-sections at the level of either the thyroid or the cricoid cartilages. However, the rabbit larynx does have glands (Bonte et al. 1969; Hallen et al. 1996), but they appear to be mainly around the epiglottis (Hallen et al. 1996), a region not sampled by us.

An unusual feature of the rabbit airways was a pronounced network of large sinusoids in the lamina propria (Fig. 12). This has also been reported by others (Sobin et al. 1963) using silicone rubber casts of the tracheal vasculature.

In general, in most species, gland openings were unremarkable circular openings of about 50 μm diameter. In the rat trachea, however, gland ducts emptied into flat-bottomed slits or 'troughs' that had their long axes oriented longitudinally. In early studies, in which we did not remove the surface epithelium, we found many of these troughs to be lined with nonciliated squamous epithelium (Fig. 1), suggesting that they serve as a temporary storage site for gland mucous secretions prior to their joining the mucociliary escalator. Why such troughs are present only in the rat trachea is unclear.

For the species studied, what are the relative amounts of mucus in goblet cells of the surface epithelium and mucous cells of the glands? We found that the average volume of glands in a rat or guinea pig trachea was ~ 0.5 μl per cm^2. Much of this gland volume will consist of mucous cells (Meyrick et al. 1969). If the surface epithelium is 30 μm in height, then its volume is 3 μl per cm^2, of which in health only a comparatively small percentage (say 10%) will be goblet cells (Lamb & Reid, 1968; Adler et al. 1990). These rough calculations suggest that the mucins in tracheal mucous secretions of the rat and guinea pig are derived approximately equally from surface epithelium and glands. In hamster, by contrast, the ratio of gland to surface epithelial volume is much less than in rat or guinea pig, and most mucins will be derived from the surface epithelium, which is probably also the source of most of the airway tumours developed in this species.

In conclusion, the larynges of all species studied contained glands, but the tracheae of mouse and rabbit were gland-free and that of the hamster virtually so. By contrast, glands were present along the full lengths of rat and guinea pig tracheae, almost exclusively between the cartilaginous rings in the ventral wall. The frequency of gland openings, however, was only one-half to one-tenth that of large mammalian species. The glands were also smaller. Thus whereas in mammals of 50 kg and over individual glands were ~ 120 nl in volume, rodent glands were ~ 20 nl. Although no glands were seen in the rabbit trachea, there were frequent small pits that may correspond to nests of goblet cells.

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