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The epithelial innervation of the lower respiratory tract of the cat†

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INTRODUCTION

Intra-epithelial axons have been identified by electron microscopy in a variety of species, including man, in both extra- and intrapulmonary airways (Altenähr, 1965; Rhodin, 1966; Luciano, Reale & Ruska, 1968; Cook & King, 1969; Lauweryns, Cockelaere & Theunynck, 1972; Jeffery & Reid, 1973; Hung *et al.* 1973; King *et al.* 1974; Walsh & McLelland, 1974). There has been no previous report on the ultrastructural features of these axons in the cat.

The present study of intra-epithelial axons was undertaken to determine their ultrastructure, their concentration at different levels of the extra- and intrapulmonary airways, and their association with various types of epithelial cell. Physiological studies have demonstrated the presence of 'irritant' receptors in mammalian airways and have indicated their close association with airway epithelium (Mills, Sellick & Widdicombe, 1970; Armstrong & Luck, 1974; Sampson & Vidruk, 1975). Hence a knowledge of the ultrastructure of intra-epithelial axons in the cat is relevant to our understanding of the afferent and motor functions of the airway epithelium.

MATERIALS AND METHODS

Eight adult cats of 'clean' stock were used after acute physiological experiments not expected to change the appearance of the structures being studied.

The following airway levels were chosen: (1) lower trachea, (2) carina, (3) hilum (axial pathway of left lower lobe) and (4) distal airway (small lateral bronchus 0·5–0·7 mm in diameter). After removal from the anaesthetized animal the airways were first fixed in 3 % glutaraldehyde (0·1 M-cacodylate at pH 7·4), post-fixed in 1 % osmium tetroxide (0·1 M-cacodylate at pH 7·4), and then dehydrated in graded methanols and epoxy-propane. The tissue was embedded in Araldite (Ciba), and ultrathin sections were cut using an LKB ultratome III and picked up on uncoated grids (LKB 200 mesh copper with 85 μm square holes). Sections were orientated so that the epithelium was parallel to a grid bar, thus enabling 85 μm lengths of epithelium to be examined by electron microscopy (Siemens Elmiskop 101), as described by Jeffery & Reid (1973). Ten such sections, representing a total length of 0·85 mm epithelium, were examined for each airway level taken from each animal. At least three animals were used for each airway level and, for each level, the results are based on a count of thirty 85 μm lengths of epithelium.

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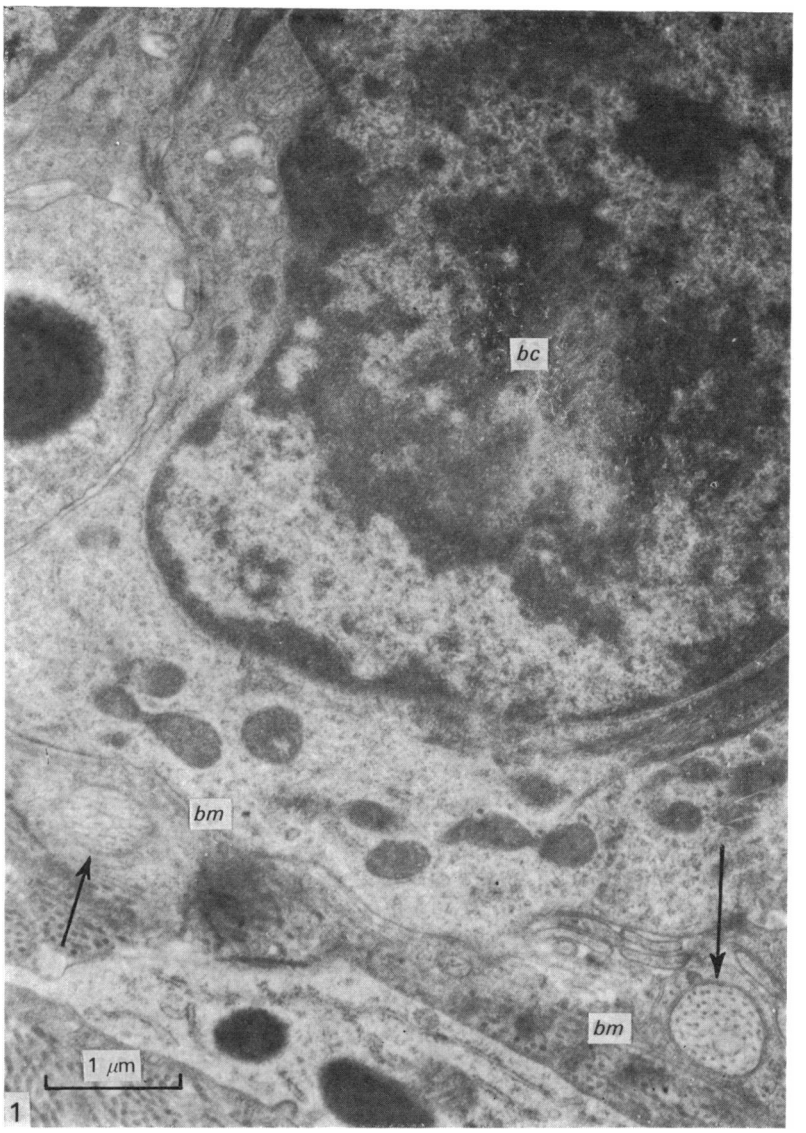

Fig. 1. Electron micrograph of cat trachea showing two axons with neurotubules (arrows) one intra-epithelial, and one below the basement membrane (*bm*). Basal cell (*bc*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. × 18000.

Epithelium was examined and the numbers of axons and epithelial cells, and the numbers of axons associated with each epithelial cell type were recorded. Axons were divided into three categories on the basis of their ultrastructural appearance (see Results).

According to their location within the epithelium, axons were assigned to one of the three following zones: (*a*) a zone of 4·5 μm depth of which the lower limit was the epithelial basement membrane, (*b*) another of similar depth of which the upper limit was the luminal edge and (*c*) a mid region between (*a*) and (*b*) with limits less well defined, but of approximately 8 μm depth. Three epithelial cell types were

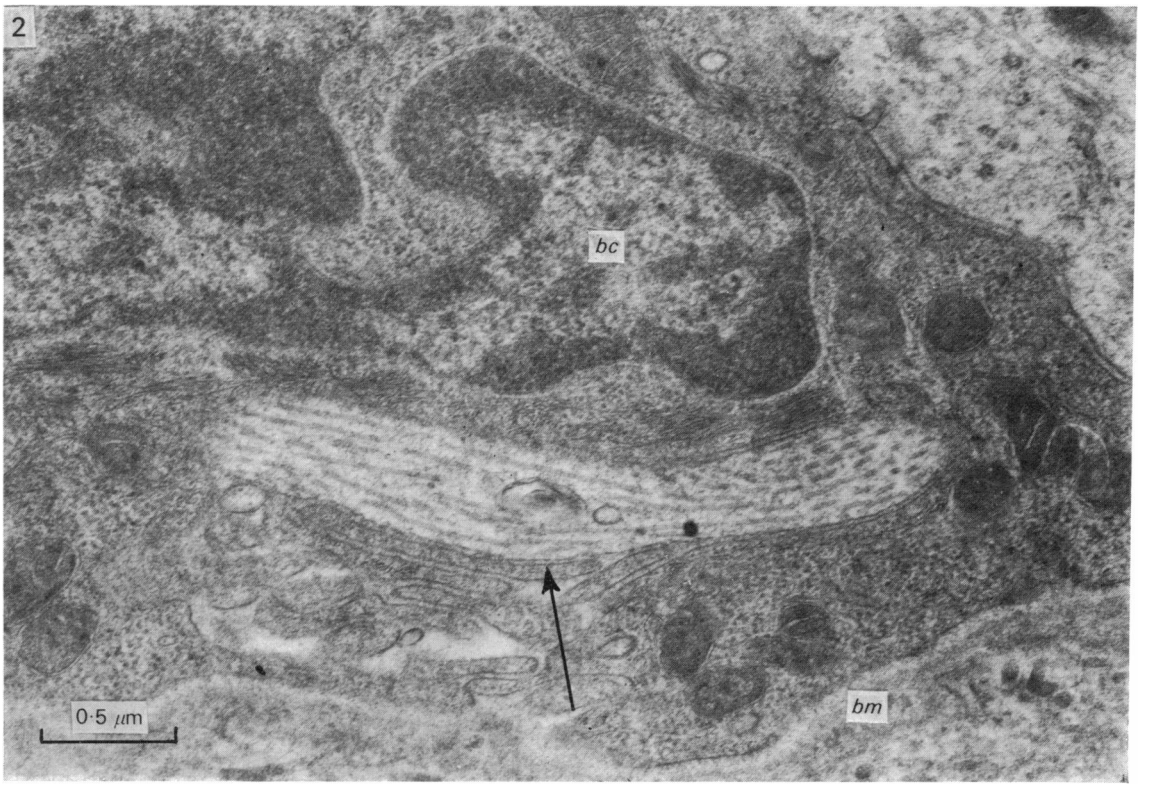

Fig. 2. An intra-epithelial axon (arrow) found at the carina containing neurotubules in longitudinal section and associated with a basal cell (*bc*). Basement membrane (*bm*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. × 36000.

clearly recognized: (1) ciliated, (2) goblet and (3) basal. A ciliated or goblet cell, each of which reached the airway lumen, was counted only if it showed a nucleus in section. Basal cells were recognized by their position, sparse cytoplasm and characteristic nuclei.

RESULTS

Morphology

Ultrastructurally, intra-epithelial axons appeared as electron-lucent profiles with neurotubules and, often, mitochondria (Figs. 1, 2, 3). Three types of axon profile were identified: (1) containing only clear vesicles; (2) containing dense-cored vesicles, often with clear vesicles as well, and (3) without any vesicles. Axonal diameters were usually 0·5 μm or less. Some axons, especially those occurring at the luminal edge of the epithelium, were larger (0·6 μm–1·1 μm in diameter), with varying numbers of mitochondria (0·1 μm–0·2 μm in diameter) and agranular vesicles (33–66 nm in diameter) (Fig. 3). Occasionally dense-cored vesicles (40–80 nm in diameter) were present also. On one occasion an axon containing only dense-cored vesicles was observed (see Fig. 4).

Neither myelin, Schwann cell sheath nor basement membrane were found surrounding intra-epithelial axons. Instead, the axons were surrounded by the cell membranes of the adjacent epithelial cells. The membranes of axon and epithelial cell were never

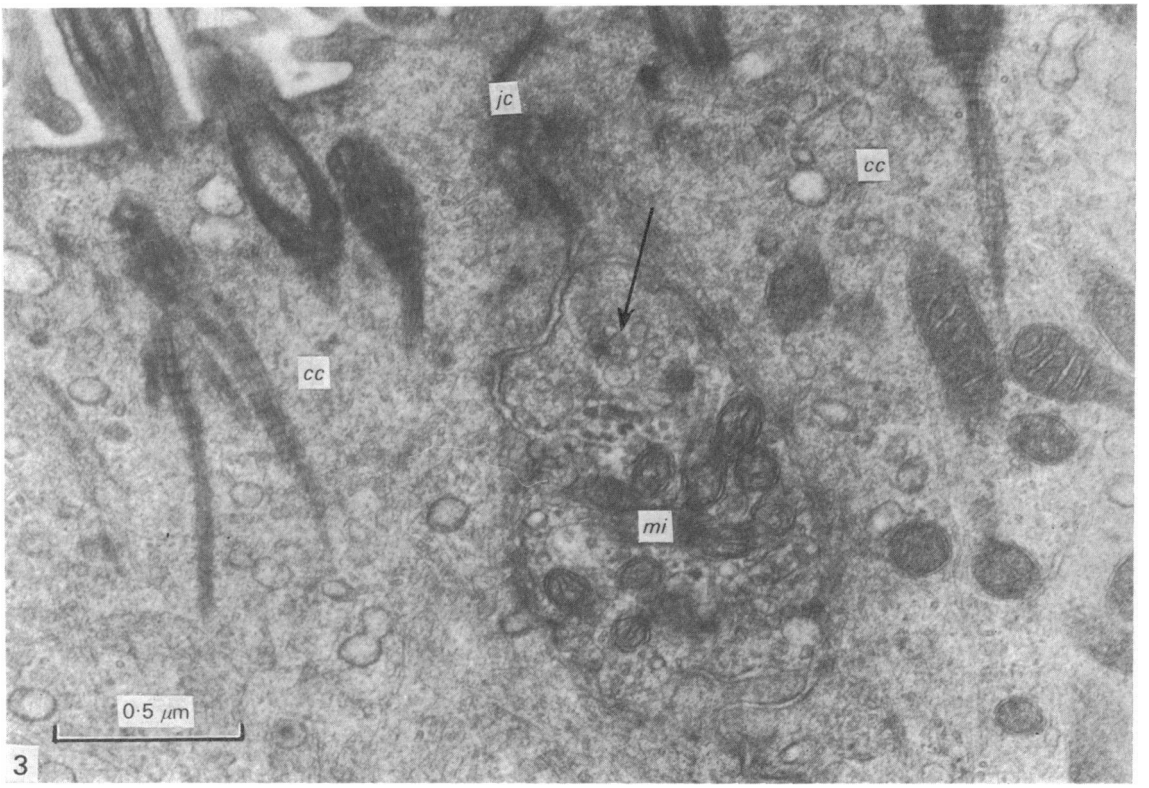

Fig. 3. Luminal edge of carina showing an axon terminal containing numerous mitochondria (*mi*) and clear vesicles (arrow). Two ciliated cells (*cc*) are joined by a junctional complex (*jc*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. × 50000.

fused; a gap of about 16 nm was always present. Nor was there evidence of localized thickenings suggesting a synaptic complex.

Concentration

The concentrations of axons at four airway levels were assessed (see Table 1). It was highest at the carina, being significantly greater than at the hilum or in the trachea ($P < 0{\cdot}001$). No intra-epithelial axons were found in distal airways. Axons were, however, observed in distal airways, in the lamina propria, in groups surrounded by Schwann cells, their basement membranes and collagen (Fig. 5).

Distribution

At each airway level the numbers of axons located in three epithelial zones, namely basal, mid and luminal (see Material and Methods), were recorded (Table 2). In the trachea and carina significantly more axons were found in the basal zone than in the other two zones ($P < 0{\cdot}001$). About 85 % of axons were found in the basal zone. The concentrations in the mid and luminal zones were similar. At the hilum, axons were only observed in the basal and luminal zones, where their numbers were similar. It was characteristic of the axons in the luminal zone that they were located about 1 μm from the surface, just deep to the junctional complex.

Most intra-epithelial axons were without vesicles (see Table 1). In the trachea and

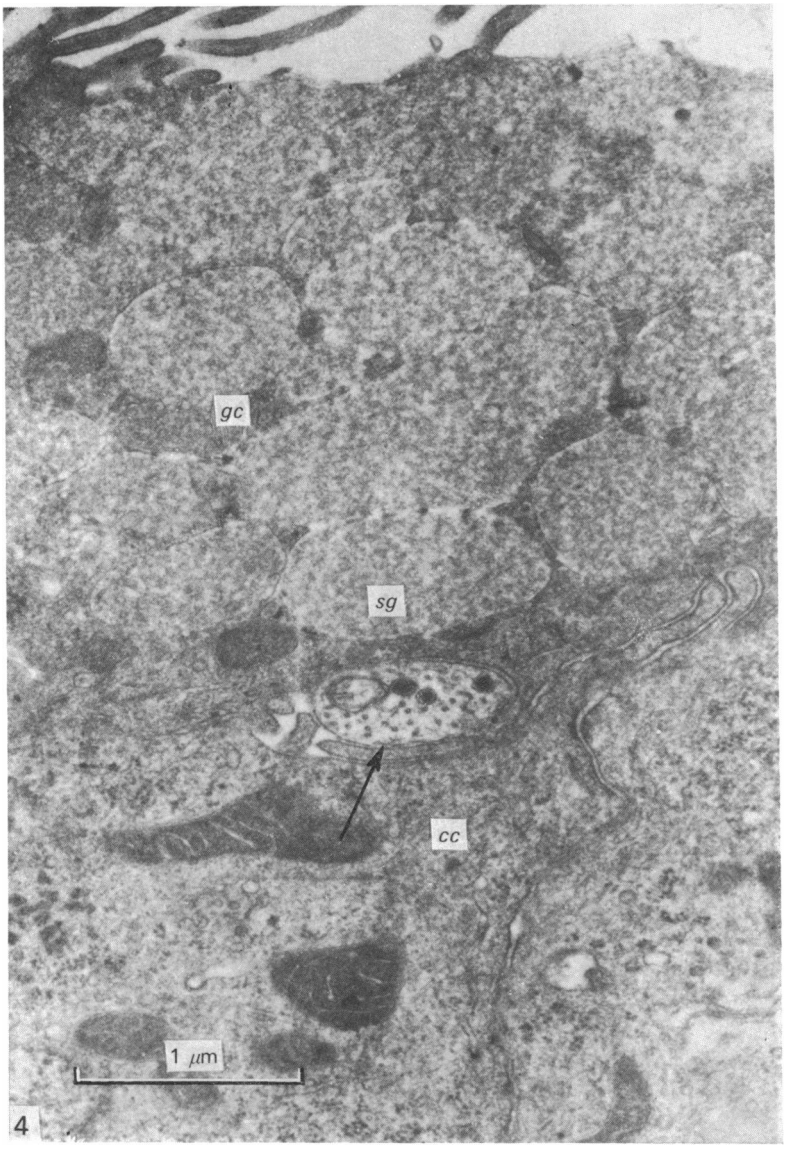

Fig. 4. Hilar epithelium with an axon (arrow) containing three dense-cored vesicles and neurotubules. Adjacent goblet (*gc*) and ciliated cell (*cc*). Secretory granules (*sg*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. × 30 000.

at the carina the majority of axons containing vesicles were located in the basal zone, while at the hilum most were close to the lumen.

The frequency of occurrence of axons with epithelial cells was determined. For the trachea, the mean and standard error of the number of epithelial cells in 0·85 mm epithelium was 160 ± 26, for the carina 163 ± 13, for the hilum 162 ± 4, and for the distal airway 137 ± 10. The values gave axon-to-cell ratios of 1:5, 1:3, 1:12 and zero in the lower trachea, carina, hilum and distal airway respectively.

The percentage of each cell type associated with axons is shown in Table 3. A Chi square test applied to all the airway levels comparing axon-to-cell ratios showed no

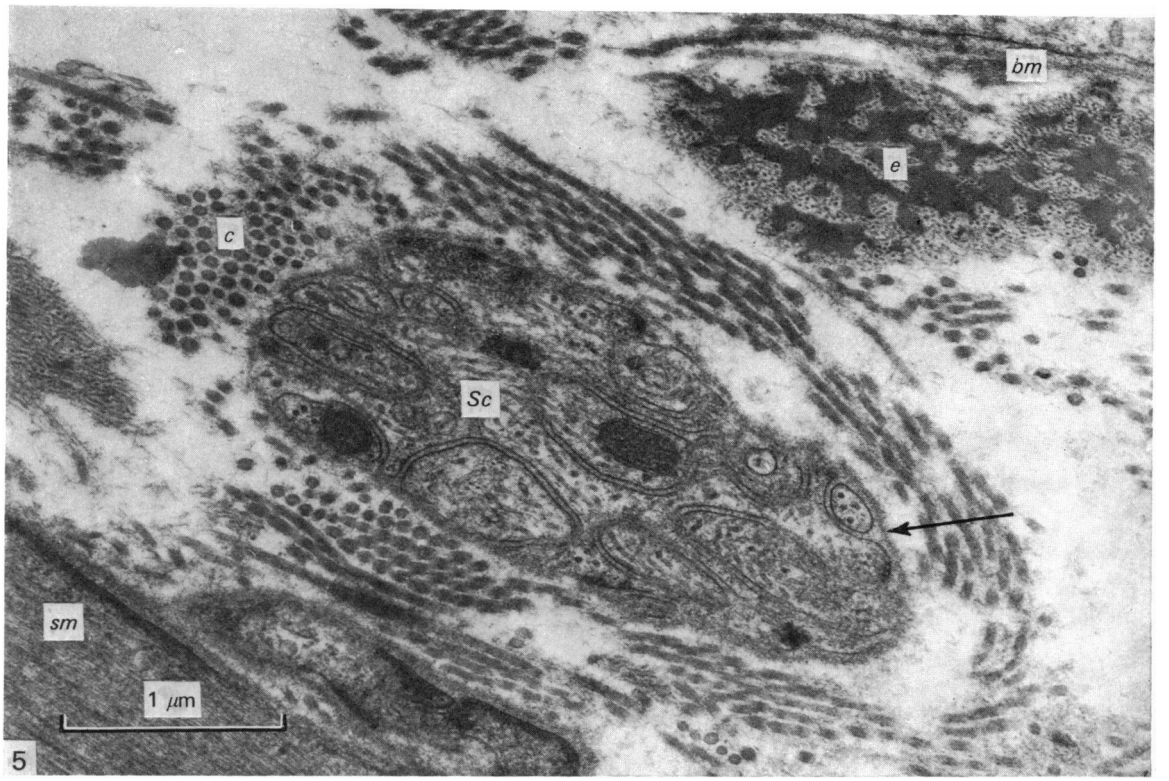

Fig. 5. A section through distal airway illustrating a nerve bundle in the lamina propria. Axons are surrounded by Schwann cell (*Sc*) and an outer sheath of basement membrane (arrow). Epithelial basement membrane (*bm*), smooth muscle (*sm*), and elastin (*e*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. ×30000.

Table 1. *Numbers of axons in 85 μm epithelium, and their vesicles*

	Number	% with vesicles		
Airway level		Clear	Dense-cored	None
Lower trachea	3·5±0·4	16	1	83
Carina	6·4±0·5	12	0	88
Hilum	1·4±0·2	29	5	66
Distal airway	None	—	—	—

Values are means±S.E.

significant departure from a random distribution of nerves within the epithelium, indicating that no one cell type was particularly associated with axons.

DISCUSSION

The cat resembles a variety of other vertebrate species (man, mouse, rabbit and chicken) in that intra-epithelial axons are present in both extra- and intrapulmonary airways (Rhodin, 1966: Cook & King, 1969; Lauweryns *et al.* 1972: Lauweryns & Peuskens, 1972; Hung *et al.* 1973; King *et al.* 1974; Walsh & McLelland, 1974).

Table 2. *Axon concentration in three zones of epithelium*

Zone	Trachea	Airway level: Carina	Airway level: Hilum
Basal	2·8±0·4	5·8±0·5	0·8±0·2
Mid	0·3±0·1	0·3±0·1	0
Luminal	0·4±0·1	0·3±0·1	0·6±0·2

Values are means±S.E., for axons per 85 μm epithelium.

Table 3. *Percentage of epithelial cell types associated with axons*

Airway	Cell type: Ciliated	Cell type: Goblet	Cell type: Basal
Trachea	19	39	23
Carina	37	8	48
Hilum	12	3	7
Distal airway	0	0	0

In the rat, intra-epithelial axons were not observed in intrapulmonary airways: intrapulmonary airways distal to the first generation lack cartilage in this species (Jeffery & Reid, 1973). In the distal airways which were studied in the cat (a small lateral bronchus), cartilage was sparse and intra-epithelial axons were not observed. However, in the mouse, Hung *et al.* (1973) found intra-epithelial axons as far distally as the bronchioli.

The axons found in the present study, and particularly those near the airway lumen, were often large, had many mitochondria, agranular vesicles of variable diameter, neurotubules, and lacked Schwann cells (see Results). These characteristics conform to the criteria described by King *et al.* (1974) for afferent endings. Neuroepithelial bodies as described by Lauweryns *et al.* (1972) for other species were not found in the present study. On only one occasion was a similar granulated cell found, but it was not innervated.

For some time it has been thought that axon terminals or varicosities rich in vesicles (clear, granular or both), but not mitochondria, are motor in function (De Robertis & Bennett, 1955; Palay, 1956; De Robertis, 1966; Richardson, 1966; Tranzer & Thoenen, 1968). With one exception such axons were not observed within the airway epithelium of the cats used in this study. However, in the few submucosal glands observed, of three axon profiles seen, two were packed with neurosecretory vesicles; these axons were associated with serous acini (see Fig. 6).

Physiological studies have demonstrated that the 'irritant' receptors responsible for reflex hyperpnoea and bronchoconstriction, on inhalation of irritant gas, dust, smoke or aerosol, are closely associated with airway epithelium (Mills *et al.* 1970; Fillenz & Widdicombe, 1971; Sampson & Vidruk, 1975). It is possible that the intra-epithelial axon profiles, particularly those of the luminal edge, represent irritant receptors, or at least their nerve supply.

It is presumed that intra-epithelial axons derive from nerves in the lamina propria, but no actual penetration of the epithelial basement membrane by axons was observed. The point at which the axons enter the epithelium is thus unknown.

The random distribution of axons with respect to different types of cell in the

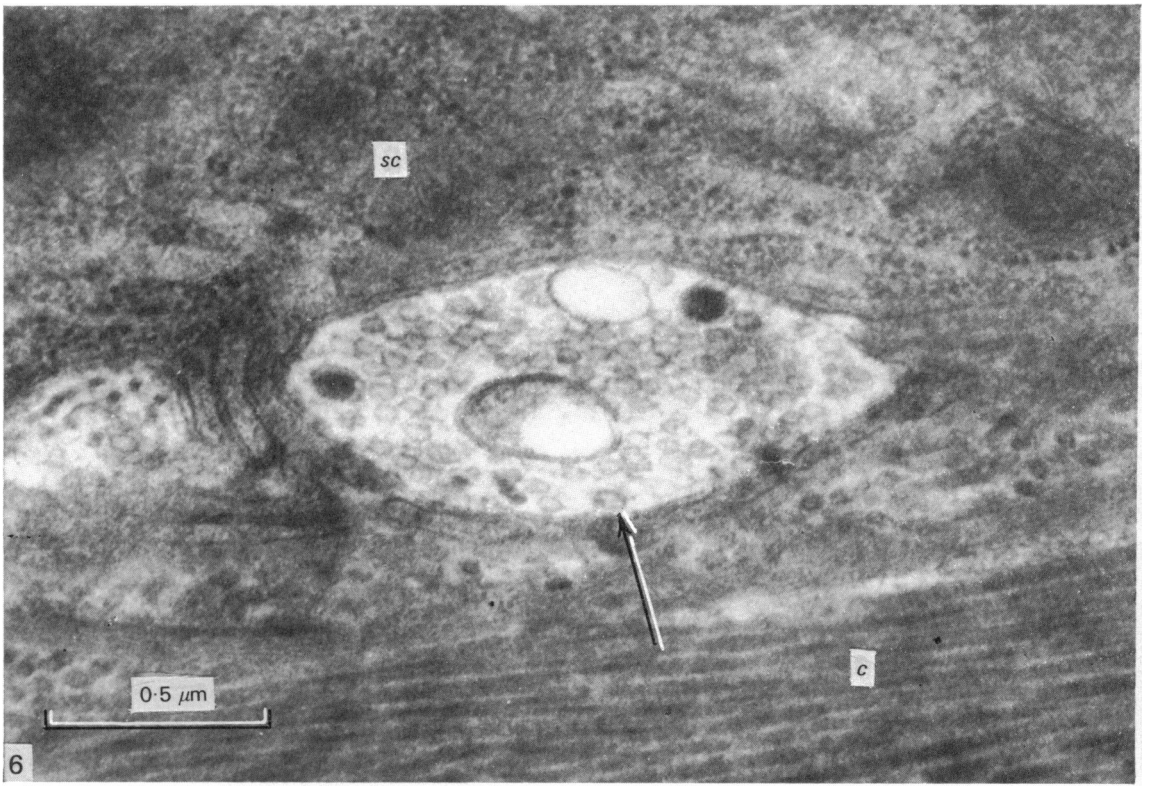

Fig. 6. Part of submucosal gland with an axon (arrow) adjacent to a serous cell (*sc*), probably motor in function as it is packed with clear vesicles and also contains two dense-cored vesicles. Collagen (*c*). Glutaraldehyde and osmium tetroxide: uranyl acetate and lead citrate. × 60000.

airway epithelium of the cat is interesting in the light of a similar study in the rat where axons were found to be particularly associated with basal cells (Jeffery & Reid, 1973).

It is clear from the literature that morphological studies alone cannot establish unequivocally the functional nature (whether afferent or motor) of these intra-epithelial axons. Physiological studies are now in progress to resolve this issue.

SUMMARY

A quantitative ultrastructural study of intra-epithelial axons in the lower respiratory tract of the cat has compared the innervation at four airway levels, two extra- and two intrapulmonary. The morphology of intra-epithelial axons has been described, and their association with different epithelial cell types recorded. Their morphology suggests that most are afferent in function.

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