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Experimental degeneration of intra-epithelial nerve fibres in cat airways*

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INTRODUCTION

A previous quantitative and ultrastructural study of intra-epithelial nerves in cat airways (Das, Jeffery & Widdicombe, 1978) led to the conclusion that most are likely to be afferent (sensory) and, as such, probably represent the 'irritant receptors' described in physiological studies (Mills, Sellick & Widdicombe, 1969; Koller, 1973; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Mortola, Sant'Ambrogio & Clement, 1975). However, direct evidence as to whether the observed fibres are afferent or motor (or both) is lacking.

Histological changes in the lungs have been reported following surgical vagotomy or chemical blockade of the vagus nerves in rabbit, cat, dog and rat (Nigro *et al.* 1968; Hirsch *et al.* 1968; Blumcke & Schmidt, 1969; Fillenz & Woods, 1970; Cottle & Nash, 1974;). None of these studies dealt with the effects of denervation on airway intra-epithelial nerves. However, physiological studies have shown that vagal block abolishes 'irritant receptor' reflexes in cats (Widdicombe, 1954*a*), implying that most of all irritant receptors are supplied by fibres which run in the vagus nerves; this conclusion is supported by recordings of nerve impulse traffic in vagal fibres coming from airway irritant receptors of cats (Widdicombe, 1954*b*; Armstrong & Luck, 1974), and several other species (Mills *et al.* 1969; Koller, 1973; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Mortola *et al.* 1975).

We have now performed unilateral vagal nerve section in cats, followed by examination of the respiratory epithelium for evidence of nerve degeneration. The method assumes that cervical vagotomy will cause degeneration of afferent fibres (whose cell bodies are in the nodose ganglion), leaving intact the postganglionic parasympathetic motor fibres (whose cell bodies are in the airways and lungs).

MATERIAL AND METHODS

The experiments were performed on four adult cats of 'clean' stock, anaesthetized with pentobarbitone sodium (30 mg/kg).

The right vagus nerve was exposed and cut at the level of the cricoid cartilage. A short length (about 1 cm) was then removed from the peripheral stump to delay any post-operative nerve regeneration. On the same side the superior laryngeal nerve was

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also cut to exclude fibres entering the trachea from the cranial aspect. The cats were allowed to recover, and on either the fifth or twelfth post-operative days were anaesthetized with pentobarbitone sodium (100 mg/kg). As the animals became deeply anaesthetized, a tracheal cannula was inserted and the lungs were inflated with 3% glutaraldehyde (0·1 M-cacodylate buffer, pH 7·2) via the cannula. The following two airway levels were then dissected and removed for subsequent post-fixation: (1) lower intrathoracic trachea and (2) the hilus, i.e. the main axial pathway entering the lower lobes of the right and left lungs. The tracheal segment was cut sagitally into two halves, each to be embedded separately, the left half to be used as a control.

The left hilar airway also served as a control. The tissues were post-fixed in 1% osmium tetroxide (0·1 M-cacodylate, pH 7·2). Thin sections were stained with uranyl acetate and lead citrate (Stempak & Ward, 1964), and from each tissue block ten sections, each with an 85 μm length of epithelium, were examined. Each section was studied first along the luminal, and then along the basal, zone of the epithelium; each of the selected zones was 4·5 μm wide (see Das *et al.* 1978).

The number of nerve fibres was counted in each 85 μm length of epithelium; the mean for the left or right half of each airway level was the result of twenty such counts (ten in each animal). The sections were examined 'blind' to exclude observer bias. Since any nerve might have appeared in several serial sections, the counts do not represent actual numbers of fibres, but only appearances in sections.

RESULTS

Nerve profiles of normal appearance were present in all the sections examined, whether from the vagotomized or the control side. They were recognized by the following features: (*a*) electron-lucent cytoplasm, (*b*) slender mitochondria, (*c*) neurotubules, and (*d*) clear and, occasionally, dense-cored vesicles (Fig. 1). These features were similar to those of intra-epithelial nerve fibres found in our previous study in normal cats (Das *et al.* 1978). In contrast, and particularly on the vagotomized side, a second group of profiles was found which had only one or two of the above features and, while resembling nerve fibres, they could not be considered normal in structure. Many of their features indicated nerve fibre degeneration – e.g. (*a*) axonal enlargement, (*b*) loss and disruption of axoplasmic organelles (Fig. 2), (*c*) accumulation of osmiophilic bodies (Fig. 3), and (*d*) a disrupted plasma membrane (Babel, 1970; Garrett, 1971; Nishi & Stensaas, 1974). Furthermore, these profiles were quite distinct from such organelles as lamellar bodies, multivesicular bodies and other kinds of lysosome. In contrast to normal nerve fibres, most of the degenerating fibres were intracellular, and therefore not surrounded by epithelial cell processes. Both normal and degenerating nerve profiles were counted and recorded separately.

Normal nerve profiles

After unilateral vagotomy there was a reduction in the number of normal nerve profiles on the ipsilateral side in both epithelial zones, and at both airway levels. The reduction was most marked in the basal zone. On the vagotomized side, the numbers of nerves remaining after 5 and 12 days were similar. The pooled luminal and basal counts showed the reduction to be statistically significant on both days (Table 1).

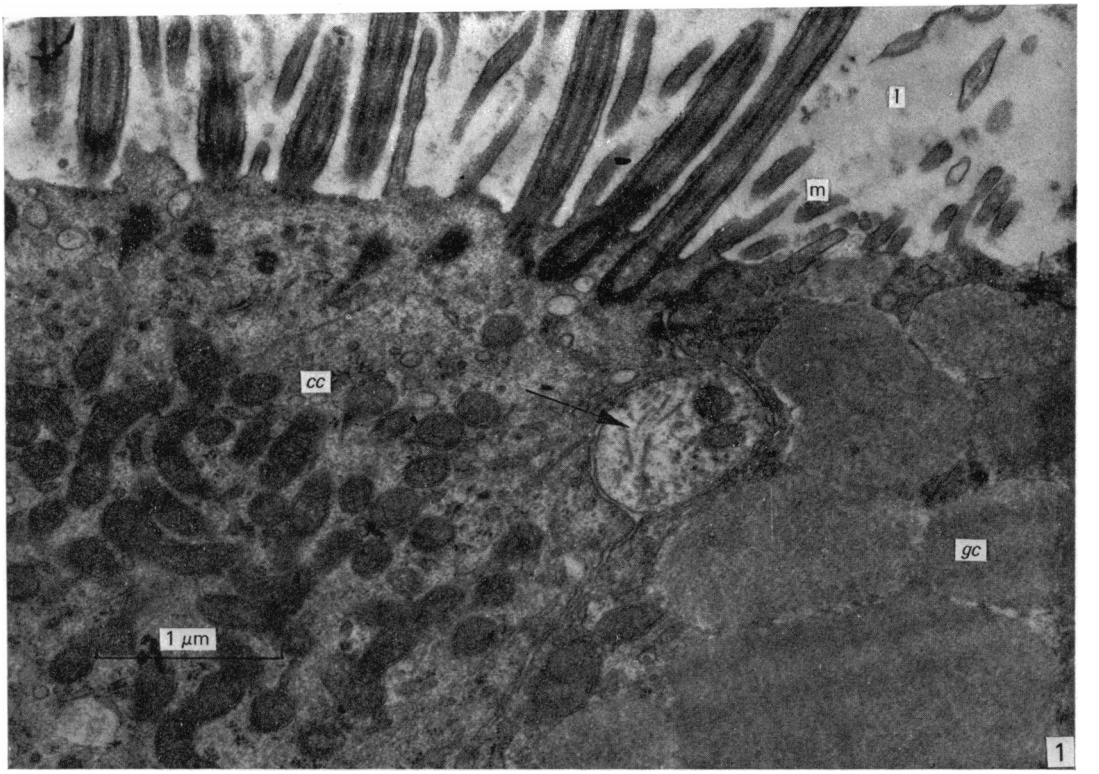

Fig. 1. An intra-epithelial nerve fibre of normal appearance in the left hilar bronchus. The fibre has electron-lucent cytoplasm, neurotubules (arrow) and two slender mitochondria cut in transverse section. Ciliated cell (*cc*); goblet cell (*gc*); microvilli (*m*); airway lumen (*l*). ×24000.

Table 1. *Numbers (means ± s.e.m.) of intra-epithelial nerve fibres per 85 μm length of epithelium and the percentage of nerve fibres occurring along the luminal edge (zone of 4·5 μm)*

	Time after vagotomy			
	5 days		12 days	
Airway level	Left side (control)	Right side (vagotomized)	Left side (control)	Right side (vagotomized)
Trachea	2.9±0·4	0·7±0·2**	2·4±0·3	0·8±0·2**
(% at lumen)	(14)	(29)	(8)	(25)
Hilum	1·1±0·3	0·2±0·1**	2·2±0·3	0·1±0·1**
(% at lumen)	(18)	(50)	(23)	(0)

Means are of 20 sections; ** $P < 0·001$.

Degenerating nerve profiles

The vagotomized side, especially, showed many structures suggestive of degenerating nerves. These profiles could be divided broadly into two types: (*a*) those clearly extracellular, being surrounded by epithelial cells but showing a loss of axoplasmic organelles and evidence of oedema (Fig. 2); and (*b*) those which were intracellular,

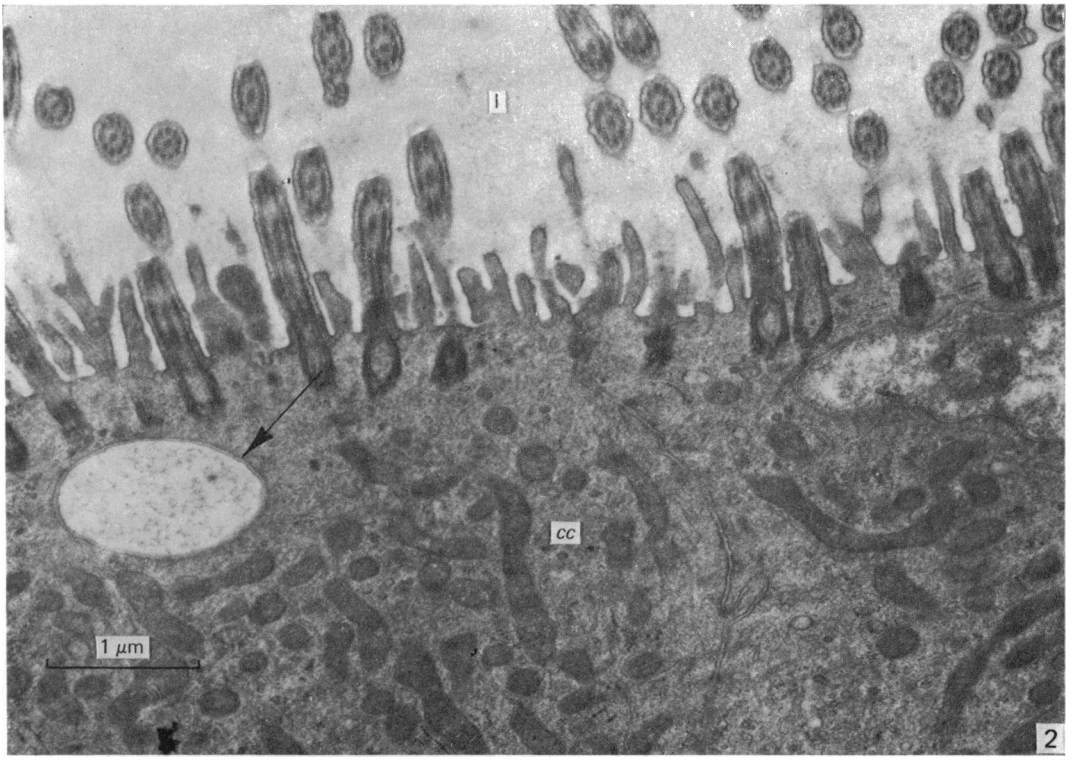

Fig. 2. Two intra-epithelial profiles from the right side of the trachea 12 days after vagotomy. The one marked with an arrow is probably a nerve fibre in the process of degeneration; it has a double unit membrane and an electron-lucent matrix, with loss of axoplasmic organelles. Ciliated cell (*cc*); tracheal lumen (*l*). ×19200.

similar to (*a*) but which, in addition, contained electron-dense bodies and vesicular structures about 25 nm in diameter (Fig. 3).

The number of all such degenerating profiles was recorded for each of the left and right sides at each airway level. Compared with the control side there were three times as many such profiles present on the vagotomized side. It was also noted that epithelial cell lysosomes appeared more frequently on the vagotomized side.

Nerves of the lamina propria

While the majority of the intra-epithelial nerves of the vagotomized side were in various stages of degeneration, most but not all of those axons located in the lamina propria beneath the epithelial basement membrane were of normal structure. Occasionally, however, groups of axons were found in which some members showed degenerative changes while others were normal (Fig. 4). No such group was found on the control side.

At one site nerve fibres from the lamina propria appeared to pierce the epithelial basement membrane (Fig. 5). Rarely, an intra-epithelial nerve was surrounded by a cell resembling in structure a Schwann cell, a feature not previously reported.

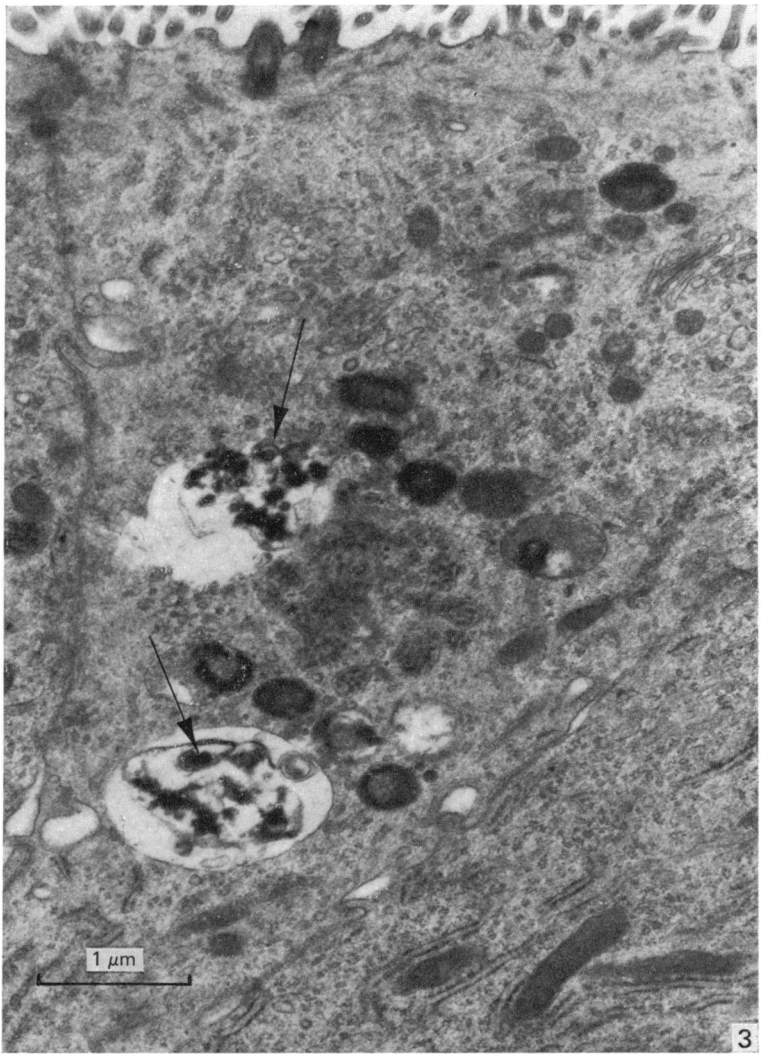

Fig. 3. Two intra-epithelial profiles which may represent a further stage in nerve fibre degeneration, or fusion with cell lyosomes to form phagosomes. Each has an electron-lucent matrix with an accumulation of osmiophilic material (arrows) and there is loss of axolemma. Right hilar bronchus 12 days after right vagotomy. × 18600.

DISCUSSION

The results show an overall reduction of 73% and 90% of intra-epithelial nerve fibres on the vagotomized sides of the trachea and hilar bronchi respectively. Most of the nerves (77%) had degenerated by five days following vagotomy, with little further degeneration after twelve days. At each time interval a greater percentage of nerves had degenerated in the hilar bronchi than in the lower trachea.

Total nerve fibre degeneration did not result from this procedure. The fact that nerve fibres remained might be explained in several ways: (1) They may be afferent fibres from the contralateral intact vagus which cross the midline (see below). (2) They may be afferent fibres which need longer than twelve days for complete

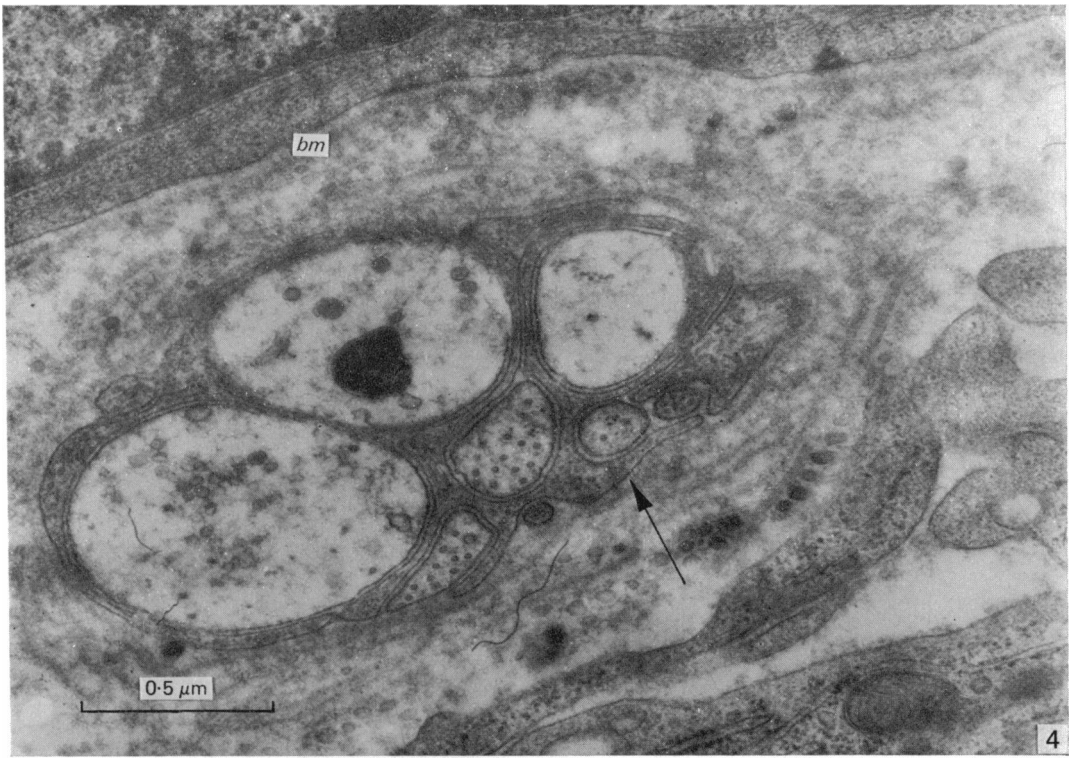

Fig. 4. A group of axons in the lamina propria just external to the epithelial basement membrane (*bm*), surrounded by a single Schwann cell. Four of the axons show neurotubules and can be considered normal, while three are enlarged and have lost axoplasmic organelles. Schwann cell basement membrane (arrow). × 51000.

degeneration. However, allowing for normal variation between individuals, the percentage of lost or degenerating axons after twelve days was not greater than after five days, suggesting that the degeneration due to the vagotomy was complete by five days. (3) They may be postganglionic parasympathetic efferent fibres and therefore not affected by infra-nodose cervical vagotomy. Previous morphological results, based on ultrastructural appearances, indicated that there might be a very small number of efferent fibres within the epithelium (Das *et al.* 1978). (4) They may be sympathetic afferent or efferent fibres not running in the cervical vagus (Widdicombe, 1954*b*; Holmes & Torrance, 1959; Phipps & Richardson, 1976).

It seems to us most likely that the nerves remaining after vagotomy are from fibres crossing to the contralateral vagus. Firstly, one would expect greater crossover for the trachea compared with the hilar bronchi, and more nerve fibres remained on the ipsilateral side in the trachea than in the hilar bronchi. Secondly, there were occasional 'degenerating profiles' on the contralateral side. Thirdly, the contralateral (innervated) side showed some loss of epithelial nerves as compared with non-operated cats; the average fibre concentration for the basal and luminal zones of non-operated cats was 32 nerve fibres/0·85 mm for trachea, and 14 nerve fibres/0·85 mm for hilar bronchi (Das *et al.* 1978), compared with 17 nerves/0·85 mm for trachea, and 9 nerves/0·85 mm for hilar bronchi for both sides together for the operated animals (this paper). Thus, a total loss of about 44% of fibres, for both

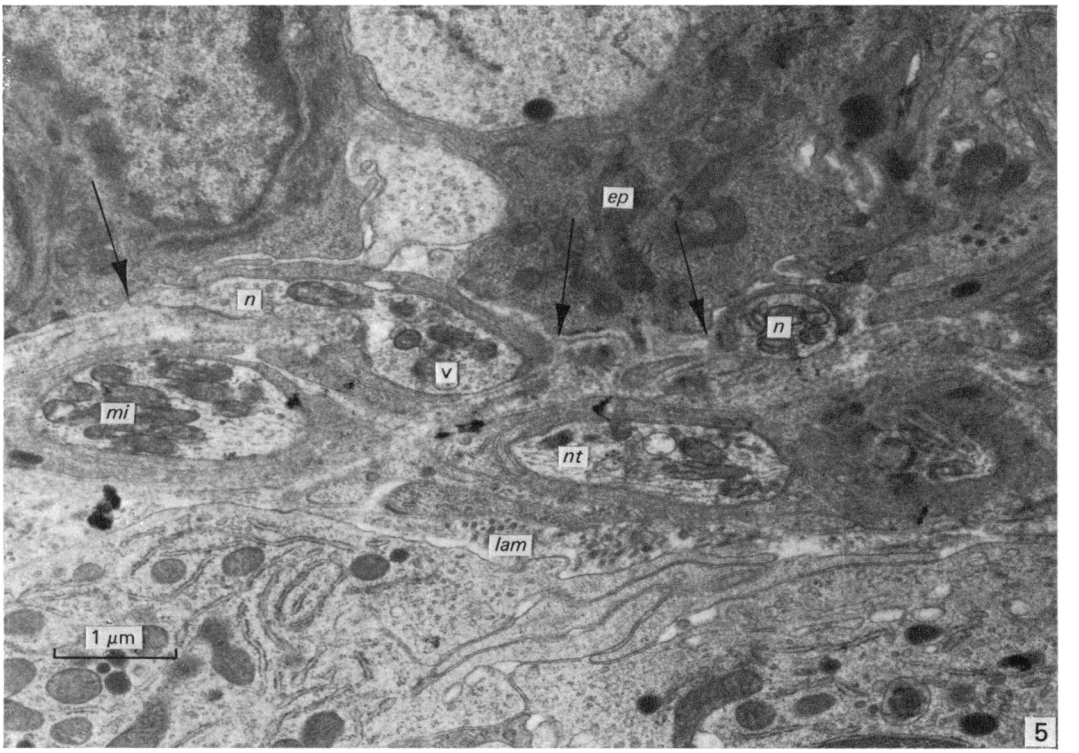

Fig. 5. Oblique section through the right (vagotomized) side of the trachea showing normal nerve fibres (*n*) piercing the epithelial basement membrane (arrows). Three fibres show typical mitochondria (*mi*) and neurotubules (*nt*), while a fourth shows agranular vesicles (*v*). Epithelium (*ep*); lamina propria (*lam*). × 16000.

sides together, occurred after unilateral vagotomy, supporting the view that the great majority of the epithelial fibres are afferent.

If the conclusion is accepted that the majority at least of the observed epithelial nerve fibres are afferent, the question arises as to their function. Three types of afferent end organ in the trachea and lungs of the cat have been described; irritant (or rapidly adapting) receptors, pulmonary stretch receptors, and type-J receptors. It is probable that many or most of the nerves seen in the epithelium are terminals of, or fibres connected to, irritant receptors, since physiological results indicate that they lie within the epithelium (Mills *et al.* 1970; Fillenz & Widdicombe, 1971; Mortola *et al.* 1975; Sampson & Vidruk, 1975). In addition, their distribution conforms closely with the results of physiological experiments where receptors were found to be concentrated at the carina and rare in the walls of smaller airways (Widdicombe, 1954*b*; Fillenz & Widdicombe, 1971; Mortola *et al.* 1975). Receptors with non-myelinated vagal fibres have been identified in the airway walls of the cat (Coleridge & Coleridge, 1977), but their precise locations and concentrations are unknown; they are assumed to be analogous to the J-receptors described in the alveolar wall (Meyrick & Reid, 1971).

The increase in the number of cellular inclusions resembling lysosomes, autosomes and phagosomes on the vagotomized side is of interest. It is possible that epithelial cells may act as phagocytic cells, ingesting degenerating nerves which would then

become intracellular. Epithelial cells are known to be capable of phagocytosis under certain conditions (Richardson, Hogg, Bouchard & Hall, 1973; Yardley & Brown, 1973). An increased number of lysosomes would be necessary to digest the intracellular nerve fragments, either for expulsion from the cells or for retention as residual bodies.

SUMMARY

This paper describes a quantitative and ultrastructural study of the degeneration of the intra-epithelial nerves in the epithelium of the lower respiratory tract of the cat following unilateral cervical infra-nodose vagotomy and section of the superior laryngeal nerve. A significant reduction in the number of intra-epithelial axons was found on the denervated side, degeneration being more complete at the hilus than in the trachea. Cellular inclusions resembling degenerating axons were observed especially on the denervated side, and their ultrastructural morphology is described. The functions of nerves which degenerated, and of those which remained unaffected, are discussed.

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