Cellular Biology of the Lung

Edited by G. Cumming
and G. Bonsignore

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Cellular Biology of the Lung

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PREFACE

This volume records the proceedings at the Fifth School of Thoracic Medicine held at the Ettore Majorana School of International Scientific Culture in March 1981.

Foregathered there were a heterogeneous group comprising clinicians, pathologists, ultra-microscopists, biochemists, immunologists, cellular biologists and physiologists and they presented the twenty seven papers seen in the contents list. The discussion which followed each paper was faithfully recorded (and where necessary translated) and may be found after each author's presentation. This free discussion is perhaps the most valuable part of the School of Thoracic Medicine, and most clearly defines the present boundaries of knowledge, and the directions in which enquiry is being pursued.

The collaboration of many people made the production of this book possible - for the translation and the discussion typescript Miss Guiliana de Ferio, for the final typing and layout Miss Corinne Wade ably assisted by Miss Karen Wadey and Miss Kim Lekstrom. The illustrations have been dealt with where necessary by Mr. John Griffiths and the production of the book was done at The Midhurst Medical Research Institute prior to its delivery to Plenum Press.

CONTENTS

THE INNERVATION OF BRONCHIAL MUCOSA

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The airways which supply the respiratory portion of the lung receive both motor (efferent) and sensory (afferent) fibres. The efferent innervation has in general, excitatory and inhibitory components supplying smooth muscle, blood vessels and submucosal glands. Preganglionic parasympathetic fibres from vagal nuclei descend in the vagus nerve (cranial X) to ganglia located in the

Table 1. Sensory Innervation

Site	Type-Receptor Reflex		Consequence
Larynx	I — expiration	→	expiratory effort
Extrapulmonary airway	I — cough (ep)	→	inspiration followed by expiration blast
Intrapulmonary airway	I — irritant (ep)	→	bronchoconstriction hyperventilation unpleasant sensation
	II — stretch (subep) "Hering-Breuer"	→	increasing inhibition of inspiratory center
Lung	III—J-receptor	→	rapid shallow breathing bradycardia pulmonary hypotension

airway walls: arising from these ganglia are postganglionic fibres which innervate specific effector structures. Postganglionic fibres originating from paraverterbral sympathetic ganglia (stellate) innervate similar effectors. Afferent endings are of broadly three types based on their position, firing pattern, adaptation to maintained stimulus, and nerve supply (whether myelinated or not) (Widdicombe 1954 a & b; Widdicombe & Sterling 1970; Widdicombe 1974). The results of their stimulation are summarised in table 1.

The distribution of the gross nerve supply to the tracheobronchial tree is similar in many species and has been largely determined by silver staining methods (Berkley 1893; Spencer & Leof 1964; Nagaishi 1972). In the trachea and main bronchus nerve bundles and ganglia are found largely in the posterior membranous portion of the airway (Fisher 1964). On entering the lung the nerve bundles divide to form distinct peribronchial and perivascular plexi (species differences exist in the extent and immediacy with which this happens). The peribronchial plexus then further divides to form extra- and endochrondrial (Subepithelial) plexi: this division depending on the quantity and extension of supporting cartilage in succeeding generations of intrapulmonary airway (e.g. little in rat: more in man). In general, the more distal single bronchiolar plexus has fewer fibres and ganglia than plexi of airway generations of a higher order: nerve bundles are, however, found in the bronchiolar region and early light microscopic reports of alveolar innervation (Hirsch et al 1968) have now been confirmed by electron microscopy in both animals and man and show the depth to which nerve fibres penetrate the lung (Meyrich & Reid 1971; Fox, Bull & Guz 1980). The brief of the present paper is, however, to review the innervation of airway mucosa and the respiratory region will not be further considered. Similarly the innervation of bronchial and pulmonary vessels will be excluded from this review except to say that they receive a dual innervation also: i.e. rich adrenergic with species differences in the degree of cholinergic.

We are left therefore to consider the motor and sensory supply to the (i) epithelium lining the airways of the lower respiratory tract (with its numerous cell types), (ii) submucosal glands (with serous and mucous acini) and (iii) muscle responsible for airways calibre (fig. 1). Lastly a consideration of the distribution, structure and innervation of ganglia is of importance in the light of new findings and thoughts on the control of bronchial effectors. For clarity each section is broken into two areas: 1) an introductory light microscopic section dealing with the results of early silver strains, histochemical (acetylcholinesterase), fluorescence (for catecholamines) studies and the very recent results of immunocytchemistry (antibodies to specific transmitters): and 2) electron microscopic studies which are complementary to the above show the precise localization of nerve terminals (this will form the bulk of illustration due only to the author's own bias).

EPITHELIUM

Light microscopy

The early use of silver stains showed a rich epithelial innervation at all levels of airway in mouse (Honjin 1956), dog (Elftman 1943) rabbit, monkey and man (Fisher 1964), in each case this being derived from a subepithelial plexus. All but Fisher, who suggested a motor supply to epithelial goblet cells, believed the supply to be exclusively sensory. The results of the vital stains methylene blue (injected or perfused) give support to the presence of intraepithelial nerve fibres occurring as far peripherally as the respiratory bronchiole in a number of species (Larsell 1921; Fillenz and Woods 1970). Using histochemical techniques Fillenez and Woods (1970) failed to localize either acetylcholinesterase or catecholamine fluoresence to airway epithelia of guinea pigs, rabbits or dogs implying a lack of motor

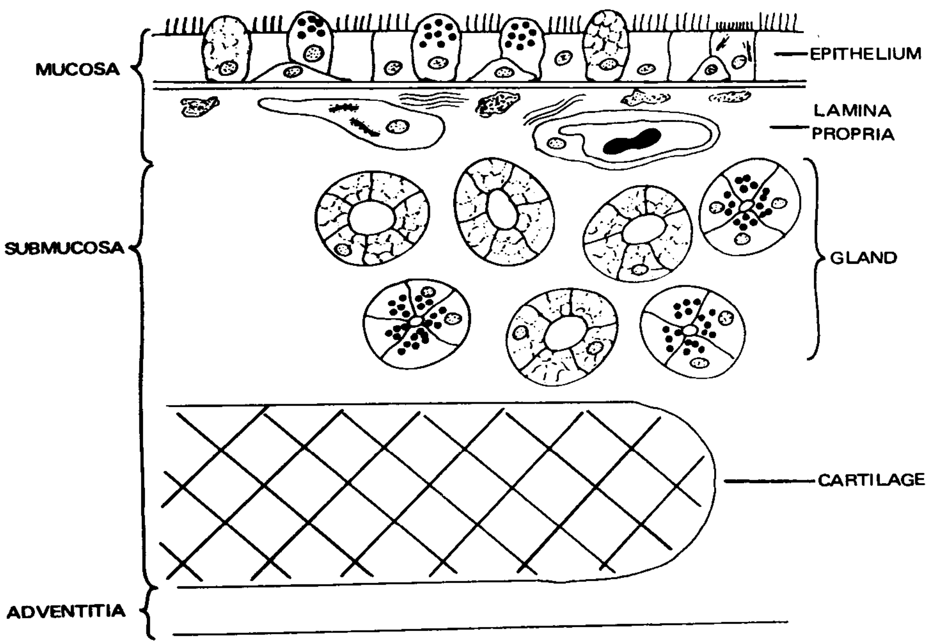

Fig. 1. Diagrammatic representation of airway wall.

innervation at this site. However, Ericson et al (1969) did describe occasional such fluorescent fibres within the epithelium of the upper trachea of mice.

The afferent nature of many of the intra-epithelial nerve fibres of rat has recently been shown using the techniques of cobalt chloride diffusion and precipitation following its 24 hr in vitro application to the distal cut ends of pulmonary vagi: swellings and hook-shaped terminations were found in bronchial and bronchiolar epithelium (Lacy 1980). As no evidence of transynaptic diffusion of cobalt was found the author considered all extra-ganglionic cobalt-filled nerve fibres as sensory.

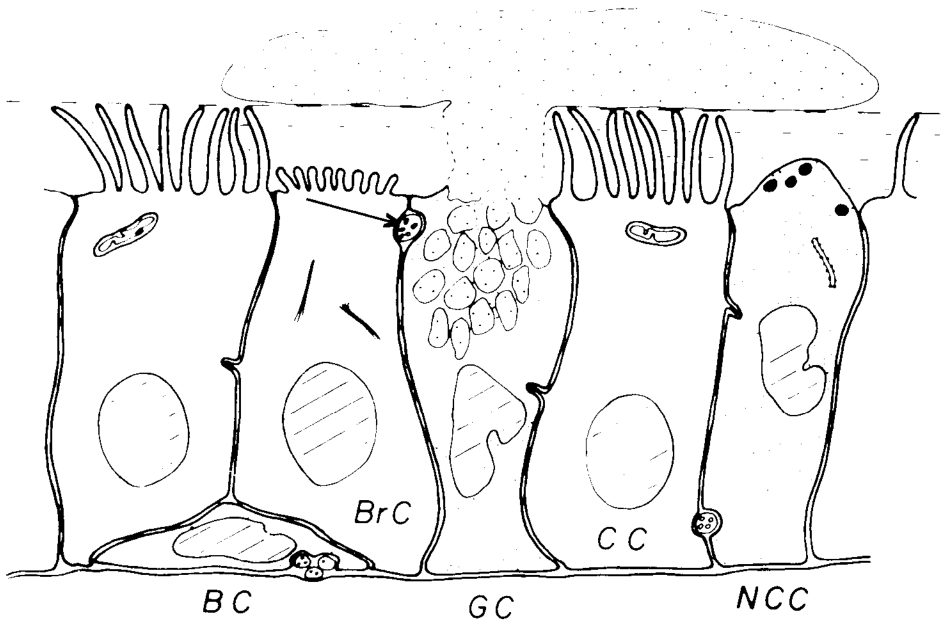

Fig. 2. Diagramatic representation of airway lining epithelium showing ciliated (CC), goblet (GC), serous (NCC), brush (BrC) and basal (BC) cells. Epithelial nerve fibres are seen either near to the lumen (arrow) or close to basement membrane (87%).

Electron microscopy

A number of electron-microscopic studies have now shown unequivocally that nerve fibres pierce the epithelial basement membrane and come to lie in apposition to a number of distinct epithelial cell types (see Jeffery & Reid 1973, Breeze & Wheeldon 1977; Richardson 1979). There appears, however, to be great species variation in their morphology, distribution and number at each airway level studied. For this reason a selection of well-studied species will now be considered:

Rat Few studies of epithelial innervation have been quantitative but in one such study of the rat lung by Jeffery and Reid (1973) intra-epithelial nerve fibres were found in extra- but not intra-pulmonary airway epithelium. There was a significantly higher concentration of intraepithelial nerve fibres in the upper (117 mm^{-1} epithelium) than in the lower (70 mm^{-1}) trachea or main bronchus (23 mm^{-1}). In each case more nerves were found in the anterior than in the posterior membranous wall. Most (87%) of the epithelial nerve fibres were close to the basement membrane and associated particularly with basal cells: 13% were superficial, many within 1 um of the airway lumen (fig. 2). All were without myelin, Schwann cell sheath or basement membrane and were surrounded by the plasma membranes of epithelial cells and separated from them by a gap of about 15 nm: no specialized synaptic complex was ever seen. The mean diameter of fibres was 0.4 μm and each was found either alone or in a group of up to seven fibres (fig. 3). Thirty three percent had dense-cored neurosecretory vesicles (fig. 4), most usually accompanied by a large number of agranular vesicles, 17% had agranular vesicles only, with the remaining 50% without vesicles but containing neurotubules and mitochondria. A few fibres larger than normal (1-2 μm) contained accumulations of β-glycogen and vesicles of both types (fig. 5). Nerve fibres were frequently associated with ciliated (fig. 4) mucous (fig. 6) and basal cells (fig. 3) but few were found with brush cells. Kultschitsky (syn-interalia Feyrter or granulated) cells were infrequently found: one showed a multiple innervation of nerve fibres some fibres with and others without vesicles (Jeffery & Reid 1973). The innervation of groups of such cells (called neuroepithelial bodies) has also been shown in a number of other species including man (Lauweryns et al 1970). These Kultschitsky cells are believed to be part of the APUD (amine precursor uptake and decarboxylation) system (Pearse 1969) and when grouped together as neuroepithelial bodies have (among many suggestions) thought to have a chemoreceptor function releasing their contents in response to hypoxia (interalia Cook and King 1969; Lauweryns et al 1973; Lauweryns & Cokelaere 1973; Moosavi et al 1973; see also Taylor of this volume).

The functional significance of neurosecretary vesicles in nerve fibres (fig. 4) is still controversial but if they do indicate motor function then the possibility that ciliated, mucous and Kultschitsky cells of the rat epithelium receive a motor innervation must be considered as likely. With regard to species variation in epithelial innervation, it is perhjaps somewhat surprising to see that mouse tracheal epithelium lacks an intra-epithelial nerve supply; with only the occasional nerve found in the epithelium of the larynx (Pack et al. 1980).

Goose Of relevance to motor innervation of airway epithelium is the finding of neurosecretory vesicles in intra-epithelial endings of goose tracheal epithelium, a species lacking submucosal glands but with abundant stimulation of the peripheral cut ends of the descending eosophageal nerves results in an outpouring of mucus suggesting a motor supply to an neural control of epithelial mucous cells at least in this species: 75% of the response was blocked by atropine with the mediator for the remaining atropine-resistant response not known.

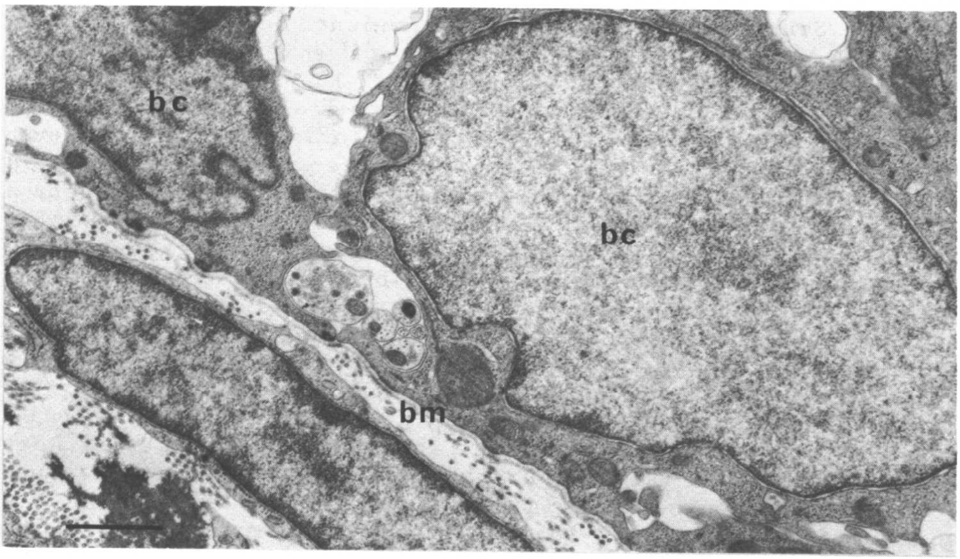

Fig. 3. Electronmicrograph (EM): a bundle of six intra-epithelial fibres in rat trachea. The largest fibre contains a single mitochondrion, four dense-cored vesicles and a large number of small agranular vesicles. Epithelial basement membrane (bm) and basal cells (bc). All electron micrographs are of tissue fixed by glutaraldehyde and osmium tetroxide: stained with uranyl acetate and lead citrate. 1 µm marker at bottom left corner.

Cat The results of another quantitative study in the cat showed similarities with rat in the epithelial distribution of nerve fibres (Das, Jeffery & Widdicombe 1978). Whilst intra-epithelial nerves were found at the hilum they were absent from a more peripheral intrapulmonary airway. Additionally the main carina was examined for intra-epithelial nerves and was found to have the highest concentration of nerves of all the airways examined. The nerve to cell ratios was 1:5, 1:3 and 1:12 for the lower trachea, carina and hilum respectively. In constrast to the rat most of the intra-epithelial fibres were without neurosecretory vesicles suggesting the majority had a sensory function.

In a further study these authors set out to assess the extent to which these intra-epithelial fibres were sensory by selective nerve section followed by an electron microscopic examination for evidence of nerve degeneration (Das, Jeffery & Widdicombe 1979). As physiological studies had shown that vagal block abolishes "irritant receptor" reflexes and that irritant receptor impulse

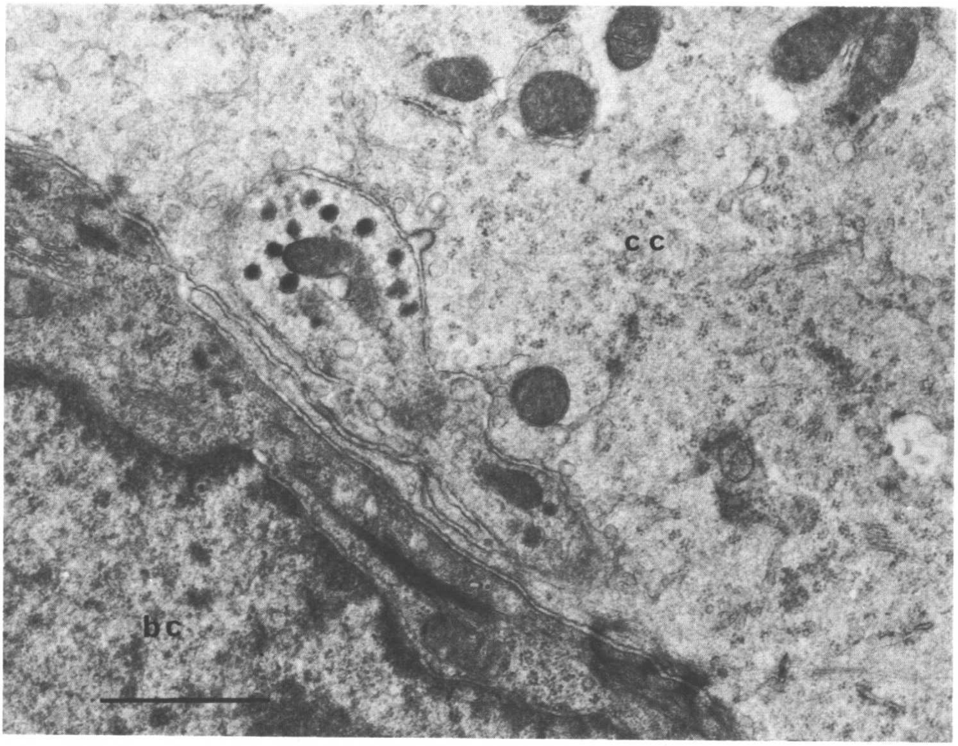

Fig. 4. EM of a single intraepithelial nerve fibre lying between a basal (bc) and ciliated (cc) cell. The fibre contains mitochondria and only vesicles of the dense-cored type. Rat trachea.

traffic was present in vagal fibres, infra-nodose unilateral cervical vagal section was carried out in a number of cats. This surgical intervention assumes that cervical vagotomy will cause degeneration of afferent fibres (whose cell bodies lie in the nodose ganglion) leaving intact the post-ganglionic parasympathetic motor fibres (whose cell bodies are in the airway wall).

Between 70 and 90% of the intraepithelial nerve fibres had degenerated five days following vagotomy, the degeneration being more complete in the hilum than in the trachea: this supports the conclusion of predominantly sensory epithelial innervation in the cat. As some degenerating nerve profiles were found on the contralateral (i.e. intact and innervated) side it is likely there is some "cross over" of nerve fibres, a suggestion which has recently received some support from studies in the dog (Dixon et al 1980).

Human Since Rhodin's (1966) study of the epithelium of human trachea in which he demonstrated a single intra-epithelial fibre (lacking neuro-secretory vesicles), little attention has been paid to this tissue. We have recently begun an examination of the innervation of human airways (4 levels) the epithelium being obtained by biopsy and from lung resection in a number of individuals. We have found that the lamina propria has a rich innervation with bundles of both myelinated and unmyelinated fibres. To date intra-epithelial nerves have been found in main stem extrapulmonary bronchus and trachea but not in any segmental airway examined. They are infrequently found, often close to basement membrane (fig. 7) but sometimes extremely close to the airway lumen (fig. 8). No fibre has been found to contain neurosecretory vesicles and they are thus likely to represent sensory fibres perhaps giving rise to "irritant" receptor discharge in response to inraluminal mechanical or chemical stimulation.

SUBMUCOSAL GLANDS

Light microscopy

The early studies of Larsell (1922) and Elftman (1943) showed, by silver staining techniques, that the submucosal glands of rabbit, dog and man were associated with nerve fibres derived from nearby autonomic ganglia, but the nature of such fibres was uncertain. Using histochemical methods (i.e. acetylcholinesterase) Wardell et al (1970) showed that these fibres were cholinergic in the dog: Falk-Hillarp fluorescent techniques indicative of catecholaminergic innervation were negative in this species. In contrast histochemical and fluorescence techniques applied to the tracheal airways of cat and monkey demonstrated the presence of both cholinergic and catecholaminergic innervation (Silva & Ross 1974, El-Bermani 1978). In cats fluorescent nerve fibres were seen passing close to and between glands and, by electron microscopy, their terminals were found to enter gland acini. Studies of human resection or post-mortem material have shown the presence of cholinergic fibres close to glands but there is a notable absence of catecholamine fluorescence in all but the most cranial aspect of the trachea. More recently Uddman et al (1978) have shown the presence of nerve cell bodies and fibres with vasoactive intestinal peptide (VIP)-like immuno-fluorescence close to submucosal glands.

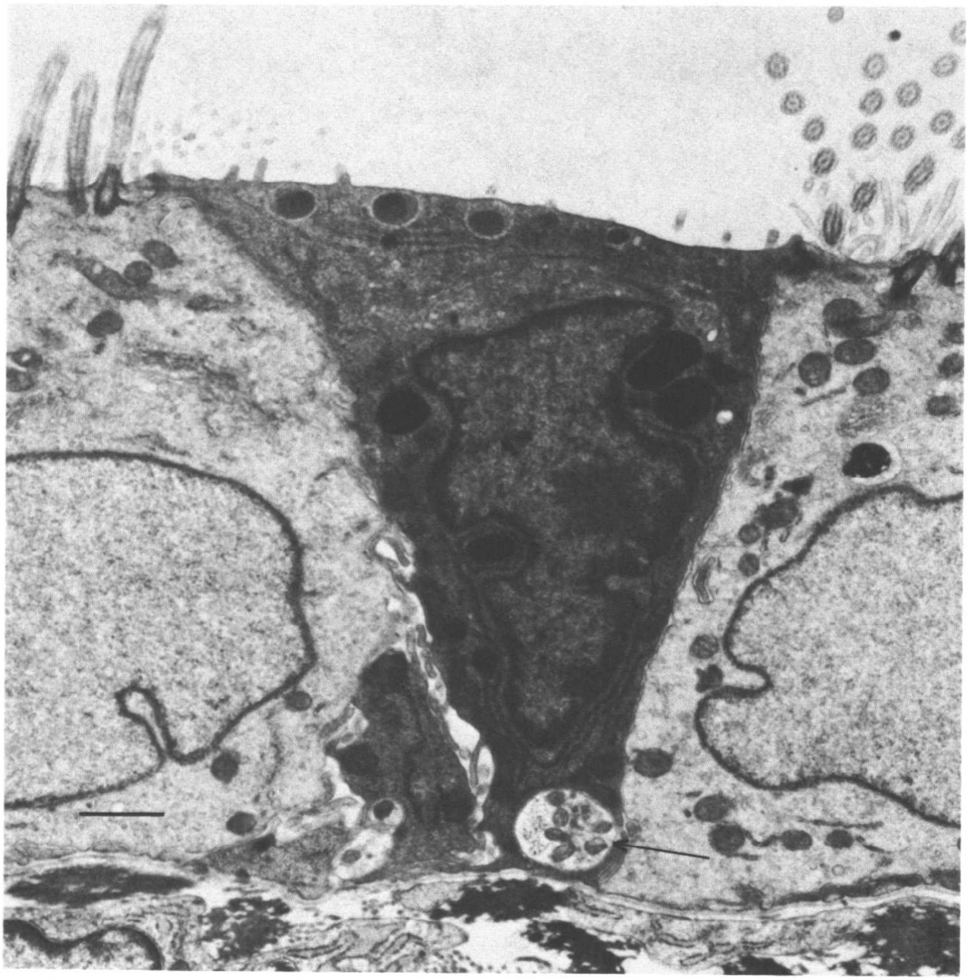

Fig. 5. EM of a single nerve ending close to epithelial basement membrane. The larger than usual profile has an accumulation of mitochondria and β-glycogen. Rat trachea.

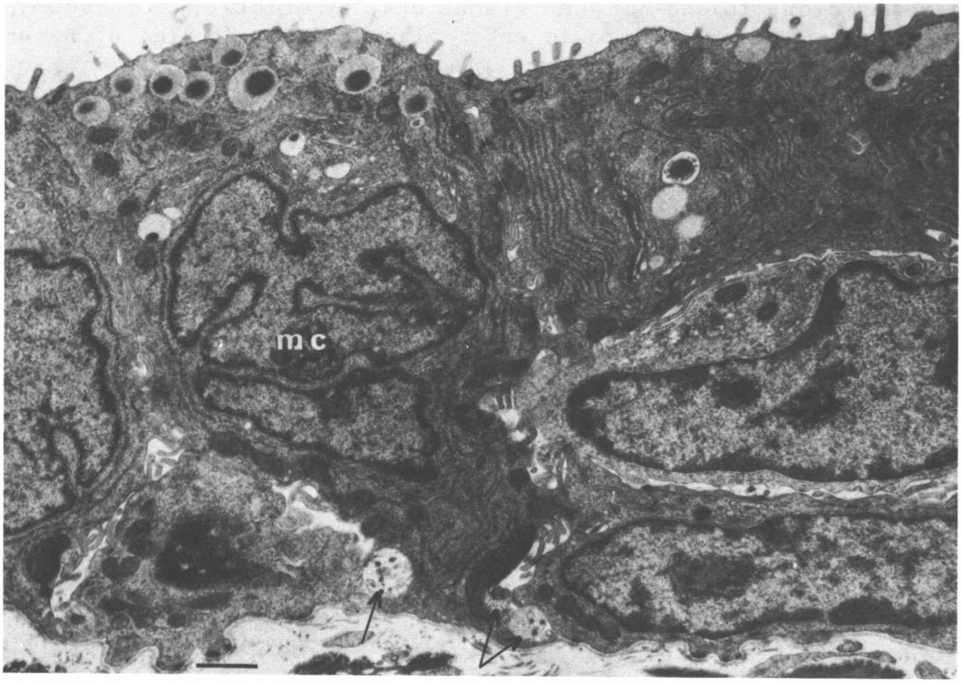

Fig. 6. EM of epithelial mucous cells (mc) with three nerve fibres at their bases (arrows). Rat trachea.

Electron microscopy

The innervation of submucosal glands has been examined also by electron microscopy. Their close proximity to gland acini is not disputed but the extent to which individual serous, mucous, or duct cells are innervated (i.e. intra-acinar/duct fibres) is not known. In man, Bensch et al (1965) and Meyrick & Reid (1970) showed intra-acinar varicose nerve endings with either agranular or granular vesicles, each structure indicative of motor function. Our studies have been concerned with the innervation of cat submucosal glands as this animal is much used to investigate the neural control of airway's secretion. In contrast to the human airway, cat submucosal glands occupy a larger portion of the airway wall: the Reid index for human is 0.33 and for cat is 0.75 (Jeffery 1978). Like the human they are comprised of both serous and mucous acini but the largest proportion (75%) of acini show a mixture of cell types with the serous cell most predominant.

In a recent study (Le Blond and Jeffery - in preparation) nerve fibre bundles were found surrounding cat gland acini (fig. 9) or as single fibres piercing the acinar basement membrane when they became closely invested by individual secretory cells (fig. 10). Whilst intra-acinar nerve fibres were found with all three types of acini, when counted, there were fewer intra-acinar nerves than expected (by random distribution) with serous and more with mixed acini. Conversely those nerve fibres outside but adjacent to acini were particularly associated with serous rather than with mixed acini. Seventy one percent of intra-acinar nerves contained neurosecretory vesicles of the agranular type suggestive of motor function. Nerve fibres with only dense-cored vesicles (5%) were found exclusively outside the acini: the transmitter associated with this latter type of vesicle is not yet known but it may well be a catecholamine or peptide (e.g. VIP). In summary the acini of cat sub-mucosal glands have been shown to be innervated by single fibres and surrounded by bundles or nerve fibres many of which would appear to have a motor function. The unequal distribution of nerves to distinct types of acini may well be important in influencing the volume and type of secretion which is discharged from airway submucosal glands in response to neural stimulation.

Our preliminary studies with human airways show that an intra-acinar innervation is lacking but that many nerve bundles lie outside and adjacent to acini (fig. 11). There is thus once again species variation in the innervation of secretory glands and care must be taken in extrapolating from animal studies to man.

Fig. 7. EM of human main stem bronchus showing an intraepithelial nerve fibre cut in longitudinal section. The neurotubules of the fibre are clearly seen and the fibre, orientated at right angles to the basement membrane (bm), is lying between a basal (bc) and non-ciliated (nc) cell.