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Airways Smooth Muscle: Structure, Innervation and Neurotransmission

Edited by
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CHAPTER 1
Anatomy of Airways Smooth Muscle

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1. Introduction

The airways of mammals are endowed with smooth muscle. The main effects of the contractile activity of this musculature are: i. to alter the calibre of the airway, hence affecting air pressure and air flow; ii. to alter the rigidity of the airway wall. However, the physiological role of airways smooth muscle is still a matter of speculation [1]. Contractions are predominantly isotonic, i.e. they involve a reduction in length of the muscle and an increase in its thickness. On both accounts, muscle contraction reduces the calibre of the airway segment involved. An isometric component in the contraction is also present. This component is a modest one, on account of the small resistance offered by the content of the airways to compression; it is more substantial, however, when the musculature works against the elasticity of the surrounding

tissues, including elastic fibres and cartilages. Airways smooth muscle produces tonic contractions, since it is hardly possible that there are phasic contractions in train with the respiratory cycle. However, the discharge of nerve impulses – not only in sensory fibres but also in efferent fibres to smooth muscle – can be synchronous with specific phases of the respiratory cycle [2].

There are two basic configurations of airways musculature. The first is found in the trachea, where the muscle is inserted on the cartilages and is closely associated, both anatomically and functionally, with them (Figure 1). This musculature is by far the more extensively investigated and the better known of the two, although it seems to play a lesser role in the physiology and pathology of the airways. The second configuration occurs in the bronchi where the muscle is wholly internal to the cartilages, is not inserted onto them and lies close to the epithelium (Figure 2). In both cases the muscle is sharply outlined from the

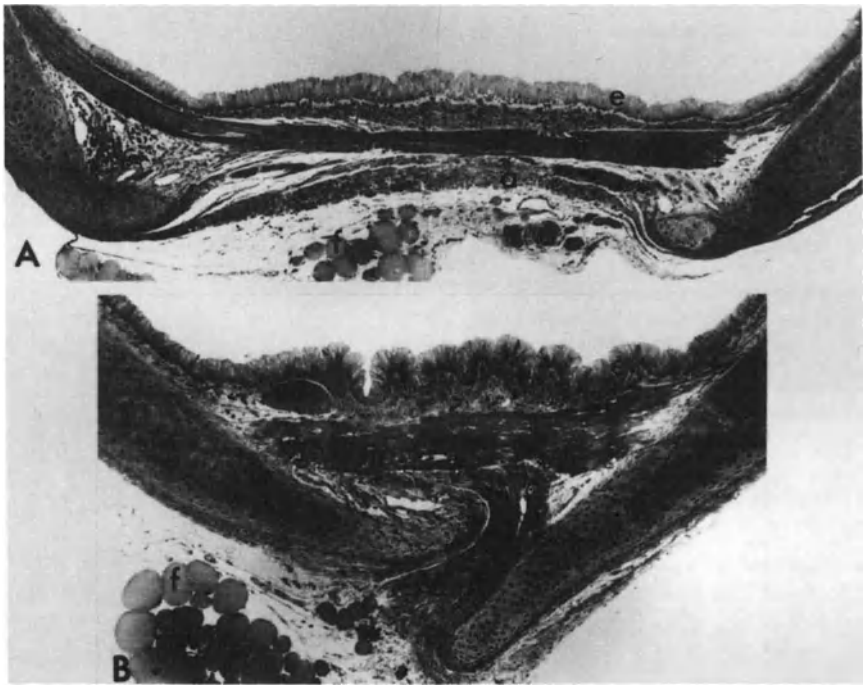

Figure 1. Light micrographs of the tracheal wall. Transverse sections of plastic embedded material. (From reference 82)
A. Posterior wall of the guinea-pig trachea, showing the ends of the cartilage (C), the tracheal muscle (m), the epithelium of the mucosa (e), connective tissue of the adventitia (a) and fat tissue (f). 62×
B. After pharmacological stimulation in vitro and maximal contraction of the muscle, the tracheal muscle is markedly shorter than at rest, the cartilage ends are brought together and partially overlap, the adventitia is squashed by the cartilage, and the mucosa is compressed sideways and develops prominent longitudinal folds. Symbols as in A. 62×

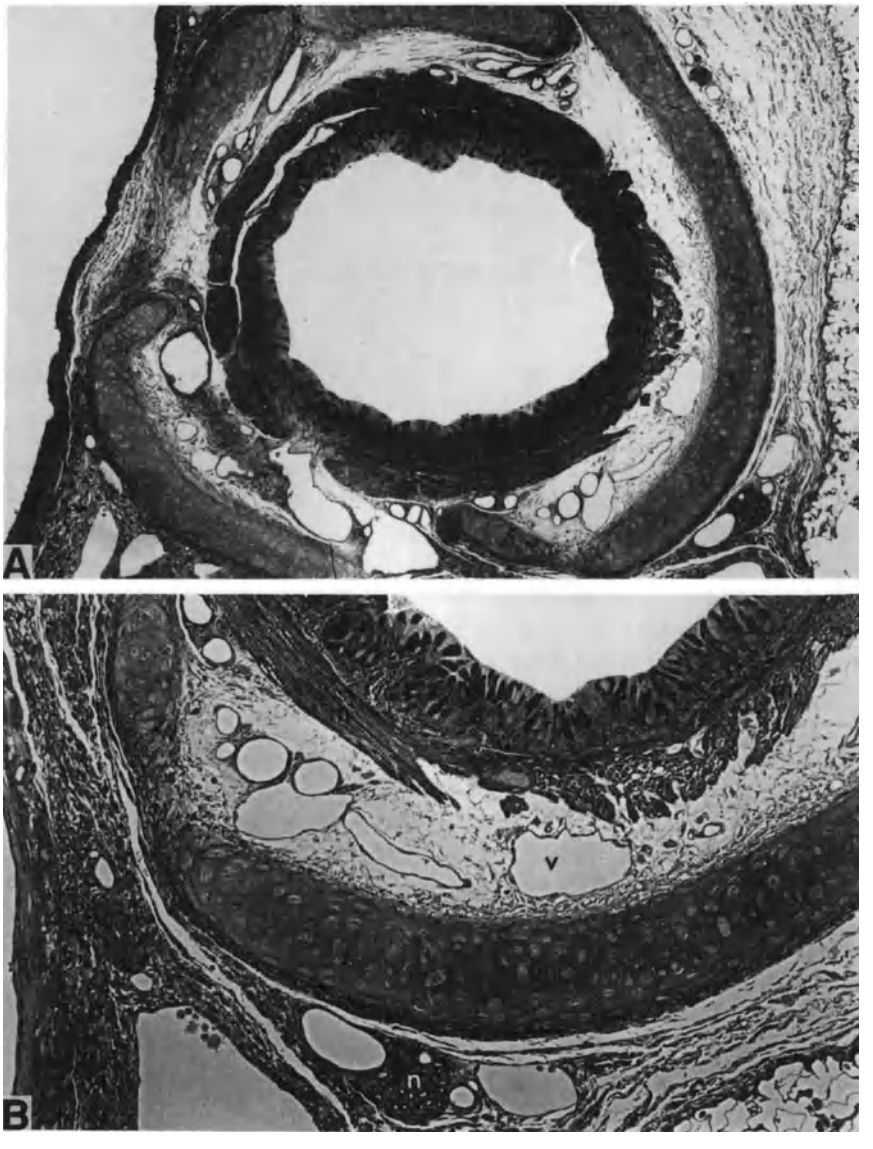

Figure 2. Light micrographs of the bronchial wall. Transverse sections of plastic embedded material.
A. Transverse section of a bronchus of a guinea-pig, showing the mucosa lining the lumen, the bronchial musculature immediately beneath, vessels and cartilages. 80 ×
B. Detail of A. Epithelium of the mucosa (e), smooth musculature in different orientations (m), cartilage (C), lymphatic vessels (v), a nerve trunk (n). 160 ×

adjacent tissue layers, although it has no perimysium or other sheathing structure.

2. Tracheal Musculature

In the trachea the muscle runs transversely and as a whole forms a band in the dorsal part of the organ, linking together the two ends of each cartilage (Figure 1a). The length of the muscle is the width of the band, and the muscle is the main component of the membranous portions of the tracheal wall. The muscle is divided into bundles, which are inserted onto a cartilage, including those mostly located level with the intercartilagenous space: these curve slightly near their end to reach the edges of a cartilage. The muscle is shortest in those animal species where the insertion is on the tips of a cartilage. In other species the insertion occurs on the outer surface (rat; dog; cat) or on the inner surface (guinea-pig; equine species; ruminants) of the cartilage, a small distance from the tip: in these conditions the muscle is somewhat longer and produces more marked effects on the diameter of the trachea. The mechanical advantage is enhanced by the fact that the tips of the cartilage can be pushed against each other until they overlap (Figure 1b). Furthermore, the contracting muscle may lift the mucosa and make it project into the lumen, an effect more marked with the insertion pattern found in the guinea-pig (Figure 1b). In certain large animal species, such as sheep or humans, some acini or ducts of tracheal glands are interspersed with the bundles of the tracheal muscle.

Bundles of longitudinally oriented musculature or a full longitudinal layer are occasionally found in the trachea. In the human the longitudinal musculature is more developed in the thoracic than in the cervical portion of the trachea [3] and its particularly well developed in the newborn [4]. Longitudinal bundles can have an asymmetric distribution, and they can be continuous with a transverse bundle [5]. In humans, according to Ferner and Müller [5], minute bundles of smooth muscle and elastic fibres (tunica musculo-elastica interna) run longitudinally in the lamina propria beneath the epithelium.

The mechanical activity of the tracheal muscle has been investigated mainly by experiments *in vitro*. When measurements of changes in length in the tracheal muscles are carried out *in vivo*, both the active shortening and the velocity of shortening are less than *in vivo* [6].

3. Bronchial Musculature

In the bronchi the musculature forms a thin layer located a short distance beneath the epithelium (Figure 2). Structural and mechanical

investigations of bronchial muscles are more difficult than those of tracheal muscle, because of their smaller size, their intramural location, their apposition to blood vessels of similar size, and their more irregular (or more complex) orientation. The orientation of this musculature remains somewhat uncertain and warrants further anatomical studies, taking advantage of modern techniques of staining *in toto* and optical sectioning. Classical studies have described helical arrangements in human bronchi [7], while others have seen annular structures and muscle bundles, with oblique orientation (some authors even described spiral arrangements!). In human bronchi a predominantly transverse arrangement of the musculature has been described, except in the smallest divisions where there are oblique muscle bundles [8]. It is possible that, as is the case in other visceral muscles, the precise orientation of the muscle bundles is not of crucial mechanical significance in a muscle where the cells are immersed in a tight connective tissue stroma and the effect of contraction is due to their change in shape rather than simply to their change in length [9]. The arrangement may not be specified in a geometric manner and the extensive branching of these airways would further interfere with a regular pattern. This anatomical aspect of bronchial muscles renders the interpretation of the contractile behavior in isolated strips (rings, longitudinal strips, helical strips) difficult, and may explain discrepancies between isometric and isotonic contractions [10].

On the basis of a helical arrangement of the bronchial musculature [7] it had been suggested that muscle contraction causes not only narrowing but also shortening of the bronchus. James et al. [11] have concluded that there is no shortening of the bronchus upon muscle contraction, on the basis of lack of changes in lumenal surface of the epithelium. It seems conceivable that extensive contraction, and concomitant increase of the width of the muscle, may cause elongation rather than shortening of the bronchus. With full isotonic contraction, shortening of bronchial muscle is accompanied by lifting of the epithelium into longitudinal folds which project into the lumen and further reduce its size [11].

There are large differences in the extent of bronchial musculature, not only between species but also between strains of the same species, as measured with morphometric techniques (one may point out the difficulty of doing morphometry on small smooth muscles, especially in material embedded in paraffin). In the lung of two strains of rat, Lewis and Fisher 344, the percentage volume occupied by bronchial smooth muscle is 3.2 and 2.5% respectively [12]. The larger amount of parenchyma musculature probably accounts in part for the airway responsiveness being greater in the Fisher than in the Lewis strain [13]. The amount of smooth muscle increases in bronchi of asthmatic pa-

tients [14–16] and this supports the notion that the increased amount of muscle contributes to the bronchial hyperresponsiveness.

4. Smooth Muscle Cells

The ultrastructural features of airways smooth muscle cells (of the trachea, since those of the bronchi have been investigated to a much lesser extent) are similar to those of other visceral muscles such as those of the alimentary and urinary tracts [17–20] (Figure 3). The muscle cells are short by comparison with the muscle length. They measure about 800 μm in length in the tracheal muscle of cattle [21] or about 1000 μm in the dog [22], and no more than 3–4 μm in diameter at their widest. The muscle cell volume is in the region of 3000 μm^3, a value close to those found in other visceral muscles. There is no evidence of the existence of different *types* of muscle cells within a muscle.

5. Muscle Bundles

The cells do not span the full length of the muscle, but they are linked to each other by cell-to-cell junctions, and to connective tissue by cell-to-stroma junctions, thus forming a compact mechanical unit. The division into bundles has a variable appearance, and even when distinctly seen in an individual transverse section of the muscle, it does not involve a proper subdivision of the muscle. Bundles merge and divide repeatedly along the length of the muscle.

6. Insertion onto Cartilage

In the trachea muscle, the bundles converge onto the cartilages and are inserted into them. The muscle cells that are involved in this process show specializations which amount to the formation of minute myotendinous junctions. These muscle cells have deep longitudinal invaginations of the cell membrane at the end nearest the cartillage, penetrated by bundles of collagen fibrils. The expanded amount of cell membrane provides a large surface for insertion of the contractile apparatus to the membrane and of the membrane to the connective tissue.

7. Muscle Cell Membrane

The cell surface, outlined by the cell membrane, is very extensive (7400 μm^2 in bovine trachea [21]). In consequence, the cells have an

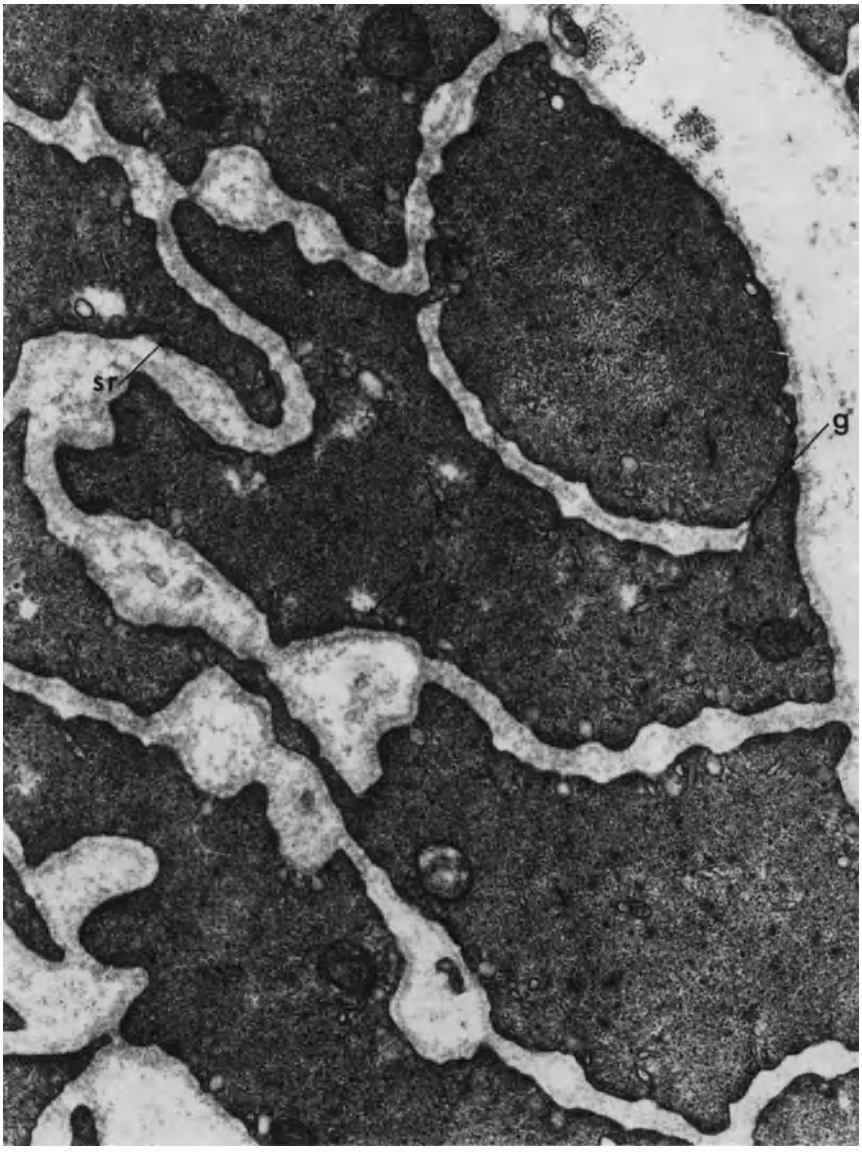

Figure 3. Electron micrograph of the tracheal muscle of a sheep. Transverse section. Muscle cell profiles of irregular outline interlock and project towards one another with laminar processes. The cells are packed with filaments: myosin filaments, actin filaments, intermediate filaments. The cell at top right of centre has a large central bundle of intermediate filaments (i). Other cellular structures include mitochondria, sarcoplasmic reticulum (sr), microtubules and caveolae. (g) points to a gap junction. 29 000 ×

exceptionally high surface to volume ratio, about 2.5 μm^2 of cell membrane per cubic micron of cell volume. The membrane is lined continuously by a basal lamina except at the sites of intimate cell-to-cell junctions.

The two main features of the cell membrane are the caveolae and the sites of insertion of the contractile apparatus (see following sections) (Figure 4). The two features occupy separate areas and probably represent distinct domains of the cell membrane. The intramembrane particles visualized by freeze-fracture (which mainly represent intrinsic

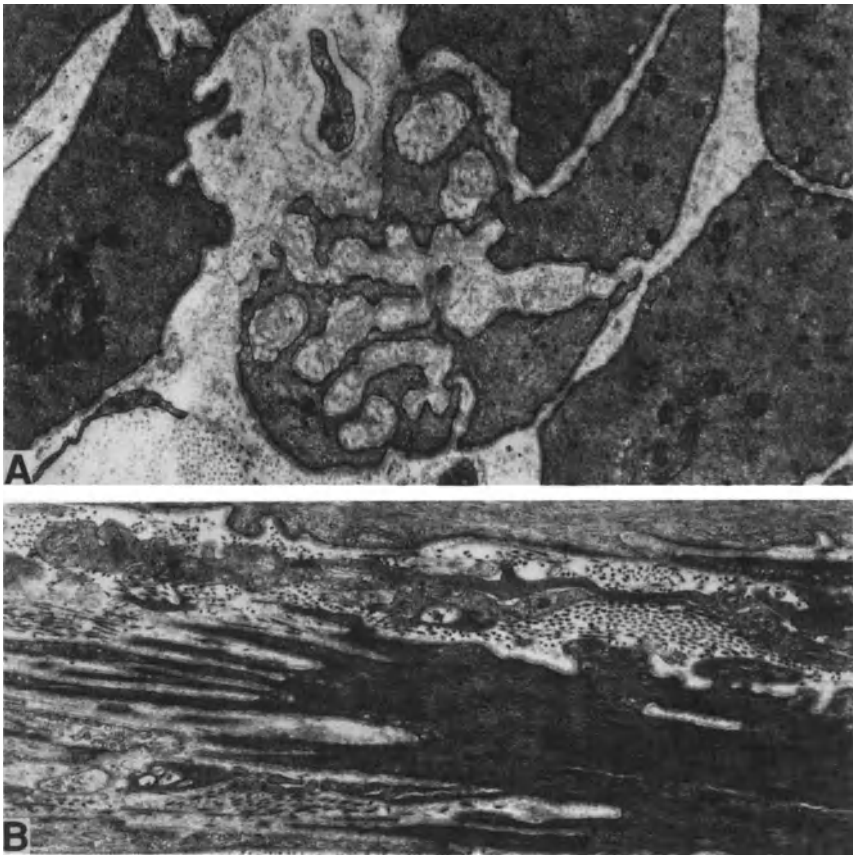

Figure 4. Terminal apparatus of muscle cells of the tracheal muscle of a rat.
A. In this transversely sectioned muscle the profile of a muscle cell (or possible two cells) becomes tortuous and compartmentalized, producing a large increase of the cell surface available for anchorage of the contractile apparatus to the stroma. Note the different appearance of the basal lamina and the intercellular material by comparison with the neighbouring regions. 11 500 ×
B. In longitudinal section the terminal part of the muscle cell appears to break into several elongated processes, an appearance due to tubular longitudinal invaginations of the cell membrane and basal lamina. 11 500 ×

membrane proteins) are predominantly concentrated in the membrane regions occupied by caveolae [23]. These areas are also characterized by the presence of dystrophin [24, 25] and of a calcium pump [26], whereas the regions where filaments are inserted are characterized by dense bands and, chemically, by the presence of vinculin and other cytoskeletal molecules.

Muscle cells taper markedly towards their ends and these parts of the cell often display deep longitudinal invaginations of the cell surface and elongated cytoplasmic processes (Figure 4). These specializations are accompanied by a thickening of the basal lamina, by a large amount of floccular extracellular material and by an increased development of the dense bands. The muscle cell's end is thus firmly anchored to the end of another muscle cell or to the stroma.

8. Caveolae

These small invaginations of the cell surface are uniform in size and shape (about 70×120 nm, with their long axis orthogonal to the cell surface, and a neck of about 35 nm) (Figure 3). They are arranged in rows of two or three, parallel to the cell's length. Their spatial density is over 30 per square micron and the total number of caveolae per cell is in the region of 170 000. On the whole they add about 70% to the amount of cell membrane present at the cell surface.

Caveolae of smooth muscle cells are stable structures, constant in number, not involved in pinocytosis; their cavity is accessible to extracellular space tracers such as ferritin [27] and their neck is covered but not penetrated by the basal lamina. High concentrations of calcium have been observed by electron probe inside caveolae [28], and the calcium pump ATPase has been localized in their membrane [26, 29].

The role of caveolae has not yet been firmly established, and the presence of similar organelles in other cell types (striated muscles, endothelial cells, pneumocytes) complicates the issue, because caveolae may not have the same function in all cell types. Structural characteristics of smooth muscle caveolae are their arrangement in rows (which may be due to the constraints imposed by the insertion of myofilaments in adjacent regions of the membrane), the presence of rings of intramembrane particles around their neck, and the frequent association with tubules and cisternae of sarcoplasmic reticulum. Caveolae create a space very near the cell surface, which may have a composition different from the extracellular space at large. Several authors have proposed a role in calcium transport across the membrane [26, 28, 30] or in the control of cell volume [31] or as miniature stretch receptors [32]. Caveolae may impart mechanical characteristics to the membrane of cells exposed to extensive stress and deformation.

9. Gap Junctions

These junctions are well characterized structurally, physiologically and biochemically [33], and are present in several tissues, including smooth and cardiac muscles and non-stratified epithelia. They are patches of the cell membrane of two cells where individual channels, formed by two connexons, one from each cell, allow diffusion of ions and small molecules between the cytoplasm of the two cells. The free diffusion of ions and of small metabolites provides electrical and metabolic coupling between the cells.

Gap junctions are consistently found between muscle cells of the tracheal muscle, although there is considerable variation in their frequency in different animal species [19] (Figure 3). They are rare and small in the guinea-pig [17, 19, 34] and the dog [22], whereas they are large and common in the mouse, rat and rabbit [19]. In humans, 2.7 gap junctions per 100 muscle cell profiles have been counted [35] and in the cow about 8 [21]. In the latter species there are about 145 gap junctions per muscle cell, occupying about 0.29% of the cell surface. A similar density of gap junctions has been found in human bronchial muscles, but the junctions are smaller in size than in the trachea [35].

There are, therefore, as in other smooth muscles, marked differences in the abundance of gap junctions in tracheal muscle of different species and they do not seem to correlate with the density of innervation. In the tracheal muscle of the dog gap junctions increase in number *in vitro* after treatment with potassium conductance blockers [36]. Great variability in the extent of gap junctions is found in most smooth muscles, which include cases where gap junctions cannot be found. The observations by thin section electron microscopy and by freeze-fracture have recently been confirmed by immunofluorescence studies with antibodies against the main gap juction protein, connexin [37, 38].

The flow of ions and small molecules through a gap junction is non-selective and bi-directional, and is limited to an upper size of 1.6–2.0 kDa [39]. The flow through the junctions is gated, i.e. can be regulated through a change of the number of channels that are open at any one time – for example by changes in calcium concentration or in pH or by antibodies, drugs, histological fixatives. When all the connexons are blocked the cells are uncoupled.

10. Adherens Junctions

Junctions of the adherens type, usually called intermediate junctions, are numerous in airways smooth muscles, as in other visceral muscles. They are circular or elongated patches and provide strong adhesion of the membranes of the cells across a gap of up to 60 nm occupied by

electron dense extracellular material which is in continuation with the basal laminae of the two cells. Each junction is formed by two dense bands (see below), one from each cell, and therefore it links bundles of myofilaments (and the contractile apparatus) of the two cells. Cell-to-cell adhesion is also provided by other junctions which are not linked to the myofilaments. There are also areas of direct apposition between two muscle cells, without apparent membrane specialization but with fusion of the two basal laminae. Lastly, airways muscle cells often have laminar or finger-like processes which abut, or penetrate into, an adjacent muscle cell.

11. Dense Bands and Dense Bodies

The dense bands are associated with the cell membrane and provide insertion to elements of the contractile apparatus (actin filaments) and the cytoskeleton (intermediate filaments) (Figure 4). Paired dense bands in adjacent cells are essentially intermediate junctions (adherens type junctions). The other dense bands are coupled extracellularly with collagen fibrils and other components of the stroma or matrix, and they should then be regarded as an element of cell-to-stroma junctions, or, in broader terms, as structures linking the contractile apparatus to the stroma. Dense bands appear as a felt of electron dense material, adhering to the cytoplasmic side of the cell membrane, about 30 nm thick and 0.2–0.4 μm wide; their length usually exceed 1 μm. The entire cell surface is studded with dense bands, which occupy between 30 and 50% of the cell profile, and an even greater percentage near the tapering ends of a cell. Bundles of actin filaments penetrate in the dense bands, approaching the cell membrane at a very small angle. The filaments are inserted with the same polarity of those inserted on dense bodies or on the Z-lines of skeletal muscles: when actin filaments are 'decorated' with myosin subfragment S1 the arrowheads are directed away from the point of insertion [40]. Alpha-actinin is present in dense bands (as in cytoplasmic dense bodies and in the Z-lines) [41]; unlike dense bodies and Z-lines, dense bands contain vinculin [42] and talin [43], molecules involved in linking the actin filaments to the cell membrane. Dense bands also receive insertion of intermediate filaments.

12. Contractile Apparatus and Cytoskeleton

The contractile apparatus is constituted by myosin and actin filaments, and the anchorage points of the latter, the dense bands and the cytoplasmic dense bodies. Actin filaments measure about 7 nm in di-

ameter, are usually assembled in cables rather than in rosettes, and are of undetermined length.

Myosin filaments measure about 15 nm in diameter and have an irregular transverse sectional profile. They are difficult to preserve in a consistent manner with the current electron microscope techniques, and therefore the spatial arrangement of these filaments in smooth muscle cells is not yet well understood.

Microtubules and intermediate filaments are the main components of the cytoskeleton in smooth muscle cells. Intermediate filaments are abundant in visceral muscle cells (Figure 3) and, chemically, they are of the desmin type. They measure about 10 nm in diameter, have a sharp profile; they are very long; their length, in fact, has not been determined, and points of termination are not seen. Intermediate filaments sometimes form a longitudinal bundle in the core of the muscle cell; commonly, they run into dense bands and around dense bodies, thus establishing a close anatomical and mechanical association with the contractile apparatus. Contractile apparatus and cytoskeleton are distinct but not separate.

13. Organelles

About 90% of the cytoplasm of smooth muscle cells is occupied by myofilaments. A volume of 5–7% is occupied by mitochondria and 1–2% by sarcoplasmic reticulum. The latter is, in mature muscle cells, predominantly of the smooth type: its cisternae and tubules often lie parallel and close to the cell membrane. A major role attributed to the sarcoplasmic reticulum of smooth muscle cells is that of intracellular storage of calcium, and calcium release upon excitation.

Mitochondria in canine tracheal muscle cells contain the same oxidative phosphorylation enzymes found in skeletal muscle mitochondria; however, the amount of mitochondrial protein in tracheal muscle is only about 10% of that in skeletal muscle [18], and the amount of ATP produced per unit volume of tissue is correspondingly smaller.

Often, tracheal muscle cells have accumulations of glycogen granules (glycogen "lakes") occupying areas up to 1.5 μm in diameter, partly outlined by sarcoplasmic reticulum [20]. These glycogen "lakes" appear by light microscopy as granules, very variable in size, scattered without apparent pattern along the length of the cells (Figure 5).

14. Stroma of the Muscle

Elastic fibres are very abundant in the trachea. They are immediately beneath the epithelium, predominantly in the membranous portion, and

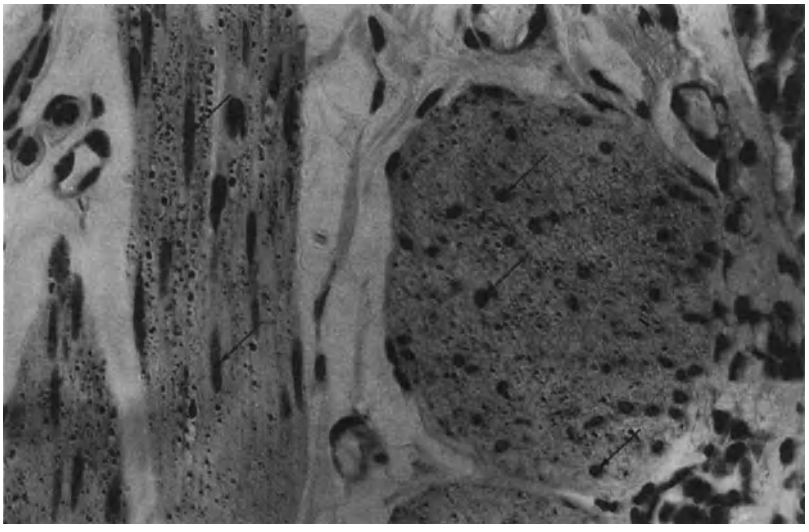

Figure 5. Light micrograph of a section of the guinea-pig tracheal muscle, stained with PAS for glycogen. Longitudinal (to the right) and transverse (to the left) muscle bundles. The dark structures (some indicated by arrows) are muscle cell nuclei, in longitudinal and transverse section. The smaller and more numerous dark particles are granules of glycogen. (From reference 20, modified.) 525 ×

run longitudinally. Additional elastic fibres, running approximately transversely, are found in the adventitia of the trachea. A few, small elastic fibres are found within the area of the muscle, and they run parallel to the muscle cells. The longitudinal elastic fibres are under considerable tension by the anchorage of the trachea to other organs at either end; when excised, the trachea shortens markedly. The transverse elastic fibres are under tension from the C-shaped cartilages; when the cartilages are cut, by a longitudinal slit through the ventral part of the trachea, the width of the membranous part of the trachea is reduced, even without muscle contraction. Since there is a substantial change of the physical properties of the cartilages with age (including hardening due to calcification), one would expect the contractile characteristics of the tracheal muscle to undergo very marked changes with age.

The morphological makeup of the elastic material in the trachea is complex; it includes elastic fibres (displaying a large amorphous core of elastin, surrounded by microfibrils), elaunin fibres (mainly composed of bundles of elastin microfibrils) and oxytalan fibres (composed of microfibrils only) [44]. Elastic material is very abundant in the lung, and many elastic fibres are associated with the bronchial musculature [45]. So extensive is the admixture of elastic fibres and smooth muscle in the bronchi that Macklin [45] suggested the term "myoelastic tissue".