**Original**

# Spatial lay-out of various smooth muscles

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## Abstract

The characteristic mechanical activities of the smooth muscles found in all organs of the body are highly variable and depend mainly on the spatial arrangement of the muscle cells and the stroma: mass, orientation, relationships, links, constraints, which are deployed in various configurations. These structural features are examined here for their mechanical relevance, in light and electron microscopic views of several muscles of viscera and blood vessels, in a selection of mammalian species. Smooth muscles are incompressible and therefore maintain constant volume. They do not have available space and any movement of a part requires displacement of another part. Most of them have no terminations or points of attachment, and in hollow organs such as intestines, blood vessels and uro-genital tract they usually form structures closed onto themselves, such as rings or bag-like containers In these situations, changes in the size of the lumen is achieved very efficiently by a concentric inward enlargement that accompanies muscle contraction. The longitudinal arrangement of collagen blocks an elongation of small blood vessels upon contraction, further enhancing the efficiency of lumen reduction. In other muscles, links between layers and special arrangements of the stroma allow both shortening and elongation of a tubular organ to occur. The mechanics of smooth muscles has many characteristic features (some unique, some shared with those of hydrostats, some at variance with other muscles) and histological data are a contribution to our understanding of these properties.

**Key words:** smooth muscle mechanics, blood vessels, urinary bladder, intestines, hydrostats

## Introduction

Present, and active, in every organ of the body, the smooth musculature constitutes innumerable anatomical structures. In the wall of hollow organs smooth muscles are found in two distinct configurations. One is that of tubular organs, such as blood vessels and intestines, the other is that of bag-like cavities, such as the urinary bladder, here referred to as ampullary muscles. In the first case the mechanical role is a short-term storage and propulsion of a fluid or a semi-fluid or a hard content. In the other case there is storage of a fluid for some time and then a rapid expulsion. In both cases, the musculature is closed upon itself, forming rings or arrays of

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bundles without end points but with some kind of annular continuity around a cavity, the lumen.

Other smooth muscles are in the form of a single, straight, long bundle, or a cord or ribbon (as the tenia coli), or a laminar sheet (as in the musculature of the trachea), with or without mechanical external influence from surrounding structures. Other, more diffuse musculatures have roles that depend more basically from the physical environment of the tissue in which they are embedded. They include small groups of muscle cells (as those attached to hair follicles) to individual muscle cells (as in the acini of salivary glands).

The mechanics of the contractile activity of such an extreme variety of muscles is equally varied, in line with the complexity of the mammalian body physiology, and it is also remarkable given the relative uniformity (or the evident but limited variations) of the constitutive units of the muscle, the smooth muscle cells, and of the extracellular materials in which they are embedded, the muscle stroma. It seems that the variations and individual characteristic of these many muscles are due to a great extent to the spatial arrangement or architecture of muscle cells and stroma – the topic dealt with in this article.

The fundamental activity of smooth muscles is contraction, that is activation of an actin-and-myosin contractile apparatus. At a molecular level there is much to learn from some similarity with the skeletal muscles. At the cell and tissue level, however, the mechanics of smooth muscles has little in common with the skeletal musculature. First, smooth muscles have no skeleton and usually (not invariably) lack a firm point of attachment outside the muscle. The muscle itself, or a part of it, may act as a hard, rigid element for attachment of other parts of the musculature. Second, smooth muscles are composed of soft (at rest), semi-fluid components, embedded in a fibrillary frame that, although rigid, is pliable and deformable (but not plastic) while providing strong internal support; they usually lack a capsule or an enveloping sheet of connective tissue. Third, gravity is the largest force counteracted by a skeletal muscle and the internal resistance from body and organs is small, while by contrast in smooth muscle gravity has negligible direct effects. Lastly, the compactness of smooth muscles (layers, cords, bundles) is high, and often there is virtually no empty areas in a muscle, and the movement of a part involves the displacement of another part. Above all, muscle cells, stroma, and the muscle as a whole are all incompressible, that is, they have constant volume. This property, which is common in many living tissues, has a major effect in smooth muscle mechanics. What is reduced in one area (contraction) must be added in another area (fattening).

Both incompressibility and absence of skeleton are characteristic of hydrostats, structures that are frequently found in living organs and bodies. Hydrostats have been extensively investigated, especially in invertebrates (1), recently (2) and in classical studies (3), with a special interest in the motility of the Octopus arms (4, 5). Man-made hydrostats are key elements of the technology about soft robots (6, 7).

A survey of the field with such a wide brief over such a large range of structures, as is attempted here, is bound to be superficial and unsatisfactory, and is limited by the modest availability of data for a direct mechanical analysis. However, these matters have been touched on by all major studies on the mechanical physiology of gut, airways and uro-genital organs and of blood vessels. Relevant studies have been numerous. On blood vessels Wolinski and Glagov (8) initiated the mechanical investigations, and a good summary is in the book by Wainwright et al. (9) while there are continuous advances (e.g., 10). On the gut, the interest has been sustained for over a century, from the seminal studies of Bayliss and Starling (11) to recent advanced investigations (12). On the stroma, the notion of the collagen assisting in the force transference in smooth muscle was put forward long ago by Mullins and Guntheroth (13).

One can see general principles (as well as sets of local 'solutions') that constitute a common mechanics of soft muscular organs, principles that may deserve a more extensive exploration. Clearly, a strong tradition of similar studies exists, based on principles already outlined by D'Arcy Thompson (14), designed to explore the

significance of form in biology. Furthermore, the combined approach of microscopy and mechanical understanding is inspired by classical positions such as the one outlined by Konrad Lorenz (15) who emphasized the indispensable role of description.

## Material and Methods

The materials used for this study were obtained from mammals immediately after death. Organs were dissected out, measured, mounted on cork slabs, distended intraluminarly when appropriate, and fixed, after minimal trimming, by immersion in glutaraldehyde (3–5% in sodium cacodylate buffer, with or without the addition of 1–2% formaldehyde; or with 4% osmium as primary fixative). Then the specimens were post-fixed with osmium, contrasted with lead citrate, infiltrated with resin (Araldite) and cured for a few days at 60 °C until hard. By keeping the trimming of the preparations to a minimum, some specimens for embedding were up to several centimetre in size, in order to recognize all the axes and the orientation of the muscular structures investigated.

Sections were cut at 1–3 µm for light microscopy (examined unstained by phase contrast microscopy) and at ~100 nm for electron microscopy (mounted on copper grids and contrasted with lead citrate).

Segments of all regions of the gut from oesophagus to anal canal were collected from rats, guinea-pigs, rabbits and mice. Blood vessels at various levels of the entire vascular tree were obtained (after perfusion of saline and then by fixative through the heart) were obtained from rats and guinea-pigs. Trachea and bronchi, uro-genital organs, iris, skin samples, salivary glands, were also collected from all the species . Observations on other mammalian species (shrews, cats, sheep, pigs) are not presented here because of insufficient quantitative basis. However, they produced no results at variance from those reported here. Intraluminal perfusion, extensive pinning down of specimens on hard supports, limited dissection were employed to avoid or reduce muscle contraction as an effect of chemical fixation. Preparations with clear signs of contraction were not used.

Some of the materials were collected in the course of other investigations, quoted in the references from this author, which contain additional technical details.

The results are illustrated both with micrographs (which are inevitably single cases, but authentic and natural) and with schematic drawings (which illustrate common or frequent occurrences, but are affected by reflection, interpretation and averaging).

The illustrations were chosen from various animal species, to stress the general occurrence of the features outlined.

## Results and Discussion

### *Small muscular tubes*

Widespread examples of this kind are the arterioles, the smallest arterial vessels. At rest their media is about half the thickness of their wall and is only one muscle cell thick (Fig. 1). The muscle cells vary in length in different locations, and they make between 2 and 7 turns around the lumen. They are arranged circumferentially but perforce they are very slightly oblique to the length of the vessel (a helical shape, or that of a tight corkscrew). Mechanically, this is the equivalent of closed rings in a well aligned sequence.

The cells have dense bodies to which myofilaments (actin) and intermediate filaments are attached. However, most of the contractile apparatus is inserted into dense bodies attached to the cell membrane; these can be very large and can project deeply into the cell, thus concentrating much contractile force onto discrete
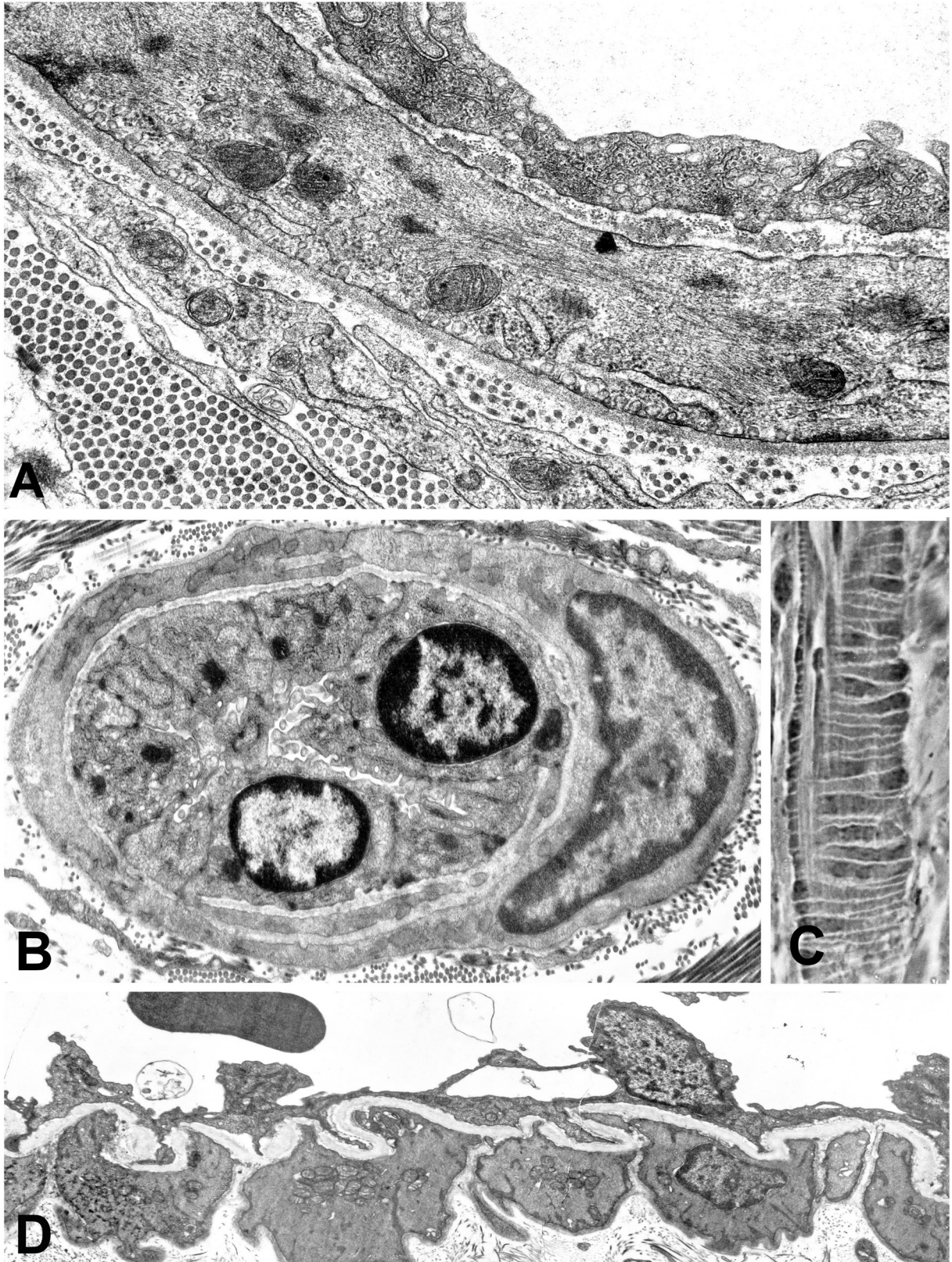
**Fig. 1.**

points at the cell surface. Dense bodies are far more numerous on the abluminal side of the cell than on the luminal side, sometimes by a factor of 10 to 1. Therefore, the pull of the muscle contraction is exerted mainly against the external aspect of the media. Lateral adhesion and adherens type junctions link adjacent coils of a single cell or of two cells in the sequence. All the elements of the contractile apparatus are parallel to each other and share the curvature of the entire cell. The nucleus is equally curved. Curvature of the cells is uncommon in smooth muscle, and is not found in isolated muscle cells in vitro. The tight curvature of the muscle cells of arterioles requires a strong and extensive adhesion of the abluminal surface of the muscle cells to the endothelium, presumably its basal lamina. The adventitia of arterioles is inconspicuous and merges with the surrounding connective tissue, where, in addition to some fibroblasts and their thin laminar processes, there is abundant collagen; there, the collagen fibrils run almost exclusively along the length of the vessel, making the vessel longitudinally inextensible.

Small arterioles in contraction are recognized in micrographs because of the obliteration of the lumen. The contraction is isotonic (reduction in length of the muscle cell and decrease of the vessel circumference) and reaches its maximum when no more space for shortening is available because of the occlusion of the lumen; any further contraction must be isotonic (hardening of the muscle without further shortening).

The contracted muscle cells in arterioles are shorter in length (smaller circumference of the vessel), and are enlarged in transverse sectional area by an equivalent amount (Fig. 2).The transverse profile of a contracted muscle cell is widened (fattened), although not uniformly: the expansion takes place almost entirely on the axis that is radial to the vessel and not on the longitudinal axis that is parallel to the vessel's length. If the transverse profile of the contracted muscle cells were isodiametric (uniform lateral expansion) the arteriole would grow in length; in fact, elongation does not occur because it is impeded by the collagen of the adventitia, which is longitudinally arranged, and is inextensible.

The mechanics of the arterioles and similar muscular tubes is thus characterized by the muscle forming closed rings, by the wall maintaining its volume, and by the vessel's elongation being blocked. The effect of these three factors is a maximizing of the process of closure of the lumen, which occurs with much less contraction than it would if shortening of the muscle cells were not accompanied by increase in their width or if the vessel were allowed to elongate.

## Larger muscular tubes

Examples of this kind, found again mainly in the vascular system, have a thicker layer of musculature, the vessel's media.

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**Fig. 1.**   A. Transverse section of an arteriole seen by electron microscopy in the oesophagus of a rat. From top right there is the lumen, then the lining made by two endothelial cells, then a muscle cells lying circumferentially, with a thick basal lamina on its abluminal surface. The space beyond the muscle is occupied by connective tissue, with fibroblast processes and longitudinally-oriented collagen fibrils. Width of field of view: 8.5 μm.

B. Transverse section of an arteriole of the mucosa of the urinary bladder of a rat, by electron microscopy. A single layered musculature in moderate contraction and a ring of compressed endothelial cells produce a complete occlusion of the lumen. Width of field of view: 31 μm.

C. Light micrograph of a grazing section along an arteriole of the submucosa in the ileum of a guinea-pig. The muscle cells of the media are arranged circumferentially and appear tightly aligned in a single layer. Width of field of view: 46 μm.

D. Longitudinal section of arteriole from the ileum of a mouse by electron microscopy. The lumen, at top, has a red blood cell and is lined by an endothelium showing a couple of nucleated endothelial cell profiles. The media consists of an array of muscle cell profiles (one nucleated) derived from circumferentially arranged muscle cells encircling the lumen. Width of field of view: 21 μm.
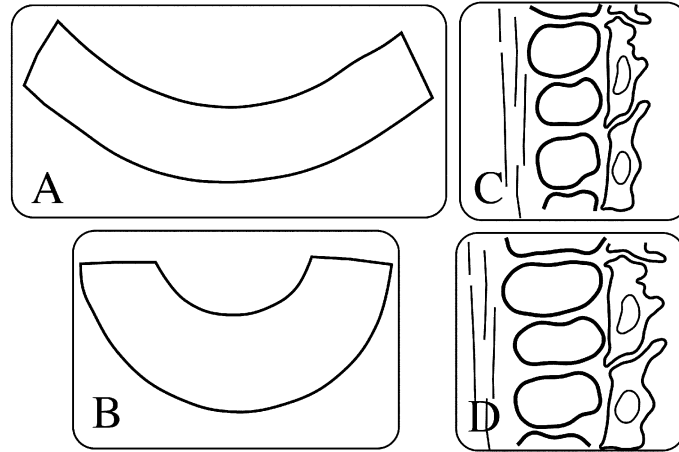
**Fig. 2.** Sketches representing a tube (like an arteriole as in the previous figure) seen in transverse section (half of the full profile), at rest (A) and when contracted (B). With contraction (shortening of the circular musculature) the circumference is reduced and the wall thickness is increased. In longitudinal section, the sketch (C) highlights a layer of endothelial cells facing the lumen (to the right), a mono-layer of muscle cells (in transverse section), and some fibrils (running longitudinally) near the outer surface. In contraction (D), the relations of the components are unchanged, and the thickness of the wall is increased. Due to the inextensible fibrils the tube does not elongate and the contracted muscle cells expand only in a radial direction.

Its thickness ranges from only 2 or 3 muscle cells to scores of them, all arranged circumferentially but never long enough to make a full turn around the tube, and less curved than in smaller vessels. The larger the vessel the thicker its wall is, but also the smaller the wall becomes by relation to the size of the vessel (that is, it constitutes a lesser percentage of the radial dimension of the vessel). Also, the larger the vessel is the more abundant in the media is the extracellular fibrous components, the stroma; at its largest it comprises fibres and laminae of elastic material, and large bundles of collagen fibrils, running approximately parallel to the muscle cells (and, in avian species, layers of fibroblasts can alternate with layers of muscle cells).

The adventitia too is thick and consists of collagen and elastic fibres, both far predominantly lying along the length of the vessel.

In the large blood vessels of many species (such as laboratory rodents) the population of muscle cells in the media is uniform in appearance. In various locations there are example of a strictly regular circumferential arrangement of the musculature, and examples where some muscle bundles run obliquely and less regularly, although in all cases each muscle cell lies parallel to the vessel's surface. In other species, especially among the larger ones, for example the sheep, the media of large arteries is made of distinct layers of musculature, each with a population of muscle cells of different appearance (including differences in cell size), while still all arranged circumferentially and roughly parallel to each other. The mechanical effect of a layered structure of the vascular media is unknown. Large body mass, high blood pressure, great mobility of the animal, adaptation to large variations in vascular dynamics may play a role.

The contraction of the large muscular tubes is an enlarged version of that described for small muscular tubes. Muscle contraction shortens the cells and this amounts to a decreased circumference of the tube with a reduction of the lumen. The simultaneous and equivalent increase in width of the media is non-uniform, since it occurs on a radial axis of the vessel and not in the longitudinal direction of the vessel. Shortening of the vessel circumference and thickening of the muscular media both contribute to reduction of the lumen, in effect adding their changes in shape. This geometrical relationship makes the effect of muscle contraction more ef-

**Vessels with different wall thickness, from zero to 50% of vessel's radius**

Ratio of external to internal diameter ( $D_{ext}$ / $D_{int}$ ) in the vessel at rest:

| 100/100 | 100/90 | 100/80 | 100/70 | 100/60 | 100/50 |

Size of vessel's wall, e.g., sectional area as percentage of vessel size:

| ~ 0 | 19% | 36% | 51% | 64% | 75% |

Contraction (shortening) by 10% / External diameter changes from 100 to 90 with elongation of the vessel:

Size of vessel's wall, e.g., sectional area as percentage of vessel size:

| ~ 0 | 19% | 36% | 51% | 64% | 75% |

without elongation of the vessel (physiologic occurrence):

Size of vessel's wall, e.g., sectional area as percentage of vessel size:
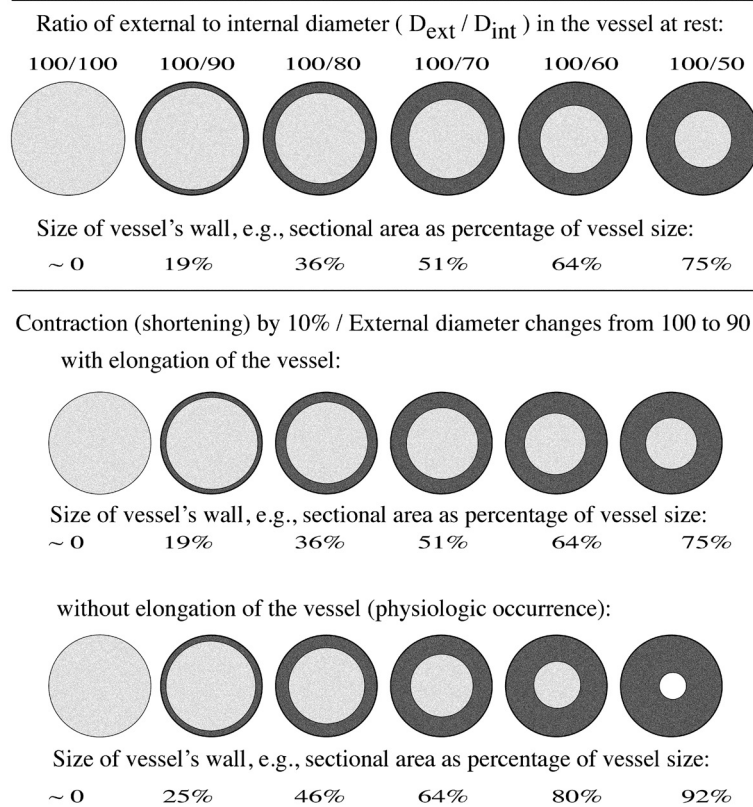
| ~ 0 | 25% | 46% | 64% | 80% | 92% |

**Fig. 3.** Geometrical interpretation of the effect of contraction in tubular structures, or vessels, of different wall thickness. In the first row are six transverse profiles of tubes with different wall thickness, from an imaginary tube (to the left) where inner and outer circumference are the same (dimensionless wall) to a tube in which the wall thickness is 50% of the tube radius (to the right). Within this series the wall occupies between 0% and 75% of the tube sectional area. With a circumferential constriction (contraction of circular musculature by 10%, or down to 90% of resting length) the external diameter changes from 100 to 90 and the circumference from 314 to 283. In the middle set the profiles are shown in the case when the 10%-contracted tube expands both radially and longitudinally. In the bottom set the tube expands only radially and does not change length, and a more substantial reduction of the lumen is obtained with the same amount of contraction. [Upon looking at these images, it should be noted how, for many observers, such as this author, visual perception of modest changes in dimensions, especially areas, are imprecise or vague for some human eye and would remain undetermined if they could not be measured.]

ficient, especially in tubes where the wall is thick by relation to the radius of the tube (Fig. 3).

Amongst organs with circularly arranged musculature, the iris of some species is a special case. It is a closed ring that, on contraction, does not encounter external resistance, both to the reduction in circumference and to the increase in thickness.

## Tubular organs with less circumferential musculature

Some tubular organs have a muscular coat in which the cells are arranged in bundles, and these run circumferentially or obliquely at a small angle to each other. This arrangement is found in the ureter and in the bile duct, both characterized by slow transport and low intraluminal pressure. Their musculature is not as tightly packed as in the majority of visceral muscles, and there is abundant connective tissue between the muscle bundles. Most bundles eventually merge with each other, providing anatomical and mechanical continuity.

## *Tubular organs with orthogonal layers of muscle*

Intestine

An arrangement of the musculature in distinct layers is common in the walls of the intestine, and in other visceral organs. Two or more layers of musculature lie orthogonal to each other, an arrangement found both at rest and in contraction and also in organs that are not strictly cylindrical, as is the case of the stomach. In a pair, the layer closer to the lumen has the muscle cells arranged circularly, while in the layer external they are longitudinal.

The thickness of the muscle coat varies along the length of the gut and so does the proportion of circular versus longitudinal musculature, the former being the thicker (a rare exception being the layers of the muscularis mucosae in the terminal colon).

The mechanical relationship between a circular and a longitudinal muscle layer is in best evidence in the wall of the ileum. In vivo, unlike the preparations in vitro, both main muscles are at work together. They act on the content of the lumen and on the shape of the wall having also a strong effect on each other. In a schematic representation, contraction of the circular muscle (which maintains a constant volume) shortens the length of that muscle and the circumference of the gut at that level is reduced (Fig. 4). At the same time the contraction is accompanied by two additional effects. First, the shortened circular muscle has increased thickness (with an
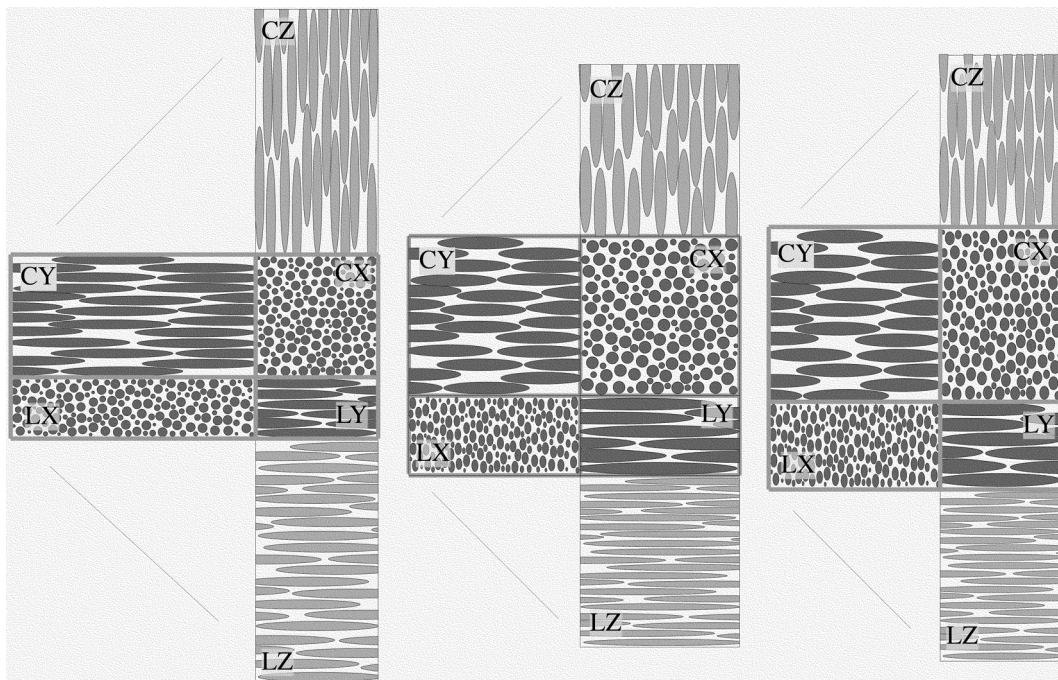


**Fig. 4.** Schematic representation of the arrangement of the ileal musculature at rest and on contraction, in the form of a rectangular block of tissue seen in all three perspectives. Each of the three sets has a central part made of four rectangles, representing, at top right, the circular muscle in transverse section (CX), then at top left the circular muscle seen sideways (CY), then at bottom left the longitudinal muscle in transverse section (LX), and then at bottom right the longitudinal muscle seen sideways (LY). Above the set of four is another rectangle showing the circular muscle seen from above, that is from the luminal side (CZ). Below is a rectangle showing the longitudinal muscle seen from the serosal surface (LZ). The first set, to the left, show the various views when the intestine is at rest. The middle set show the changed when the circular muscle is contracted. The third, to the right, presents the appearance of the two layers under various views when the longitudinal muscle is contracted. According to this geometrical analysis, contraction of one element affects the entire musculature when seen in all of its three axes.
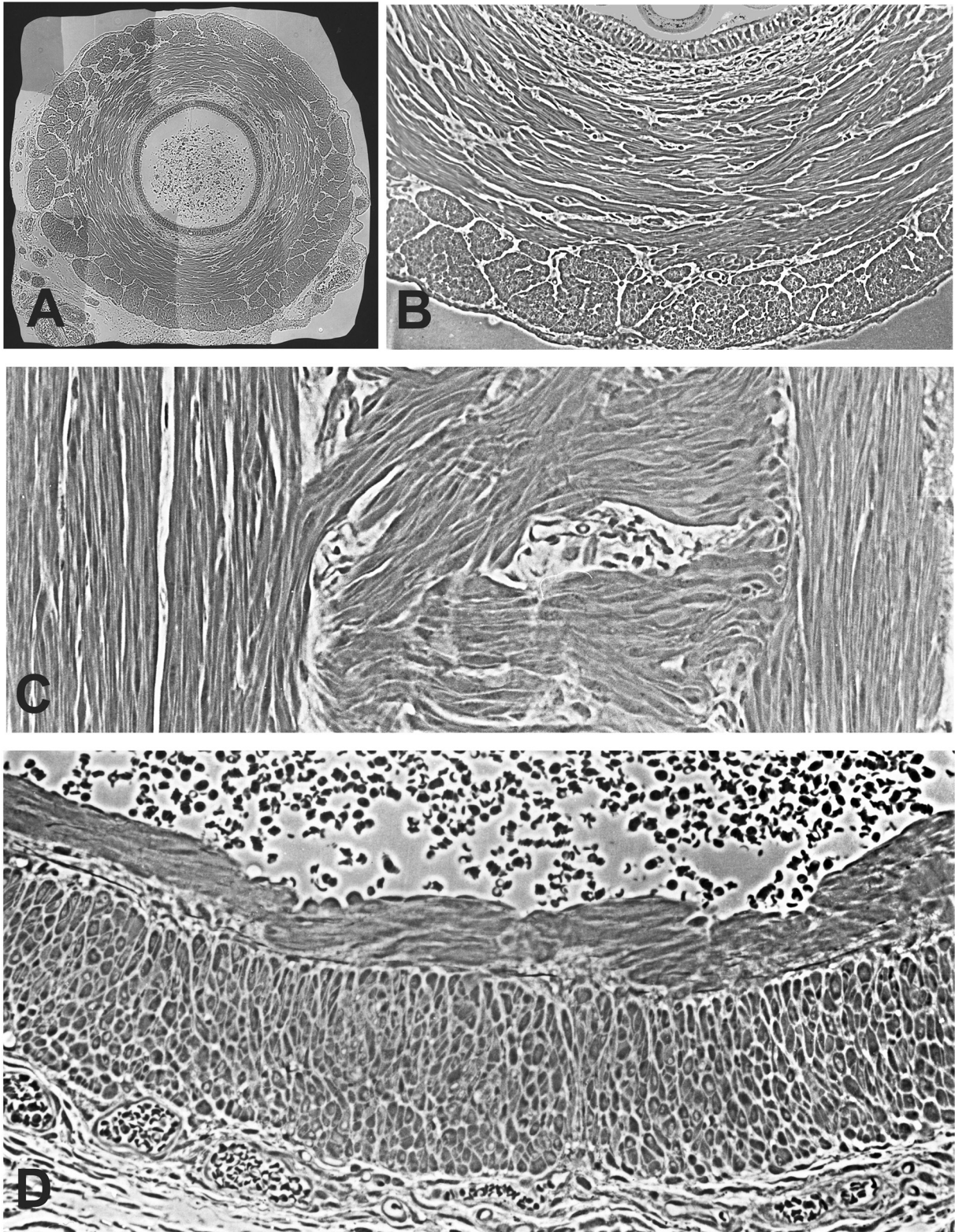
**Fig. 5.**

increased radial dimension within the wall). Second, the shortening of the circular muscle produces a sideways compression of the adjacent longitudinal muscle. Similarly, in the hypothetical case of a pure contraction of the longitudinal muscle, there would be again a triple effect: shortening of the longitudinal muscle (decrease of the length of the intestine), thickening of the longitudinal muscle (increase in its radial dimension) and lateral compression of the adjacent circular muscle. In practice one can assume that any change in shape of on element or a layer of the wall affects the form of the other layers. The muscles work together, assisted by the special arrangement of the collagen in the muscle coat and in the submucosa (16, 17). In contrast with what is found in blood vessels, thanks to the collagen distribution, a segment of intestine can both shorten or elongate.

Vas deferens

In the vas deferens (of mice, rats and guinea-pigs) there is a conspicuous muscle coat. Even at rest its thickness is up to half the radius of the duct. There is an inner circular layer, thicker, and an outer longitudinal layer, firmly adhering to each other and occasionally exchanging bundle of muscle cells (Fig. 5A–C).

Activation of the musculature reduces the circumference of the vas up to the point when there is occlusion of the lumen and full ejection of its content. In the process, the vas does not shorten or elongates. Any of the force generated by the circular muscle that would elongate the vas is counteracted by the powerful longitudinal musculature (and by the longitudinally arranged collagen). Given the constant volume of the tissue, and the considerable thickness of the circular layer, occlusion of the lumen is achieved with a modest shortening of the musculature, hence in a relatively short time. As a rule based on this geometry, the thicker the musculature (relative to the diameter of the organ) the faster the emptying of the lumen.

Blood vessels

Within the range of structural patterns in the musculature of blood vessels, there are examples of a media with an inner circular layer and an outer longitudinal layer (both of them with some irregularity in the orientation of the bundles). This is common in large veins and in some cases, such as the portal vein (of laboratory animals), the longitudinal layer is much thicker than the circular one (Fig. 5D). The mechanical significance of the muscle arrangement in veins is unclear, and the morphogenetic effect of the very low pressure of blood in veins remains to be explored.

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**Fig. 5.**   A. Transverse section of the vas deferens of a guinea-pig (photographic montage) showing the regular distribution of the musculature and its remarkable thickness (more than half the radial dimension of the duct). The lumen is filled with semen. Width of field of view: 540 μm

B. Transverse section of the vas deferens of a guinea-pig. The epithelium lining the lumen is visible at the top, over a thin lamina propria. The musculature is conspicuous; an inner layer, larger, comprises bundles of muscle lying approximately circumferentially. An external layer, thinner, is made of longitudinally oriented musculature. Width of field of view: 1.15 mm.

C. Longitudinal section of the vas deferens of a guinea-pig. The external layer of the musculature, to the right and to the left, has a regular longitudinal arrangement. The internal layer has a more irregular pattern with a predominantly circular arrangement. To the left a bundle of musculature links the two layers. Width of field of view: 490 μm.

D. Light micrograph of the portal vein of a rat in near transverse section. Near the lumen (filled with blood cells) is a layer of approximately circular musculature. The bulk of the musculature of the media is very thick, compact and longitudinal. Several venules full of blood cells are present in the adventitia, at bottom. Width of field of view: 225 μm.

### Cord- or ribbon-like muscles

In muscle such as the ano-coccygeus or the costo-uterine muscle, upon contraction *in situ*, there is hardening and an increased resistance to stretch. However, in vitro these muscles can undergo extensive isotonic contraction, not unlike the tenia. They are also rare examples of muscles with a discrete insertion and some kind of tendinous apparatus.

In the intestine, the tenia coli (three large cord-like bands of musculature running longitudinally along the caecum, in guinea pigs, or along the colon, in humans) is more complex and the most widely investigated. Teniae derive from the longitudinal musculature that initially is spread around the organ and then become condensed into three parallel massive bands. Their length is extensive, without discontinuities, approaching a meter each in humans and up to 20 cm in guinea-pigs (18).

Their combined width covers less than a tenth of the circumference of the organ, but their thickness far surpasses that of the subjacent circular muscle layer (with which there is an occasional exchange of a muscle bundle).

At rest the muscle bundles are oriented strictly longitudinally and they are inserted on laminae of connective tissue arranged radially to the length of the organ. The straight longitudinal arrangement of the tenia muscle does not reduce the mechanical role of the fattening of the contracting muscle bundles; upon contraction the thickness of the tenia increases and the intramuscular septa become shorter along the length of the muscle and longer across the thickness of the muscle. *In situ* teniae are rather flat (more than ten time wider than they are thick). Isolated in vitro, a tenia acquires a roundish profile, narrower and thicker. In situ, or when it is mounted in vitro together with the rest of the wall, the transverse profile of the tenia is much affected by the underlying circular muscle, and is quite deformable (Fig. 6). These changes are mirrored by the individual muscle cells which undergo a substantial (and reversible) change in shape and remain capable of contracting in a similar way. The cell profiles of the muscle cells mirror the change in the profile of the tenia.

### Ampullary smooth muscles

A good example of this type of muscular architecture is in the urinary bladder (Fig. 7A, B), contrasting with that of the ureter (Fig. 7C). The detrusor muscle of the bladder is made up by muscle bundles (except for some limited regions where the musculature forms a lamina parallel to the long axis of the organ, caudal to cranial). Bundles are ill defined (or difficult to define), of various sizes and running seemingly in all directions, although always parallel to the outer surface of the wall, as recently described (19). The bundles seem to be arranged randomly, which cannot be true in view of the mechanical efficiency, the uniform distribution of strain, the reversibility of the extreme changes in wall dimensions, and the smoothness of its surfaces.

The musculature is closed concentrically onto itself, without terminal points or discontinuities. In contraction the outer surface of the detrusor (and to a great extent the inner surface too) remains utterly smooth and the mucosa (urothelium, tunica propria and in some species a muscularis mucosae) is thrown into tall and regular folds projecting into the lumen. As with the previous examples, contraction shortens the bundles, which also fatten, increasing the thickness of the wall as it expands in the direction of the lumen. The muscle being incompressible (and equally so the urinary mucosa) occlusion of the lumen is achieved quite efficiently.

The myometrium is another ampullary smooth muscle, of great anatomical complexity, including signs of the presence of layers, variability and wide changes during its physiologic cycles.
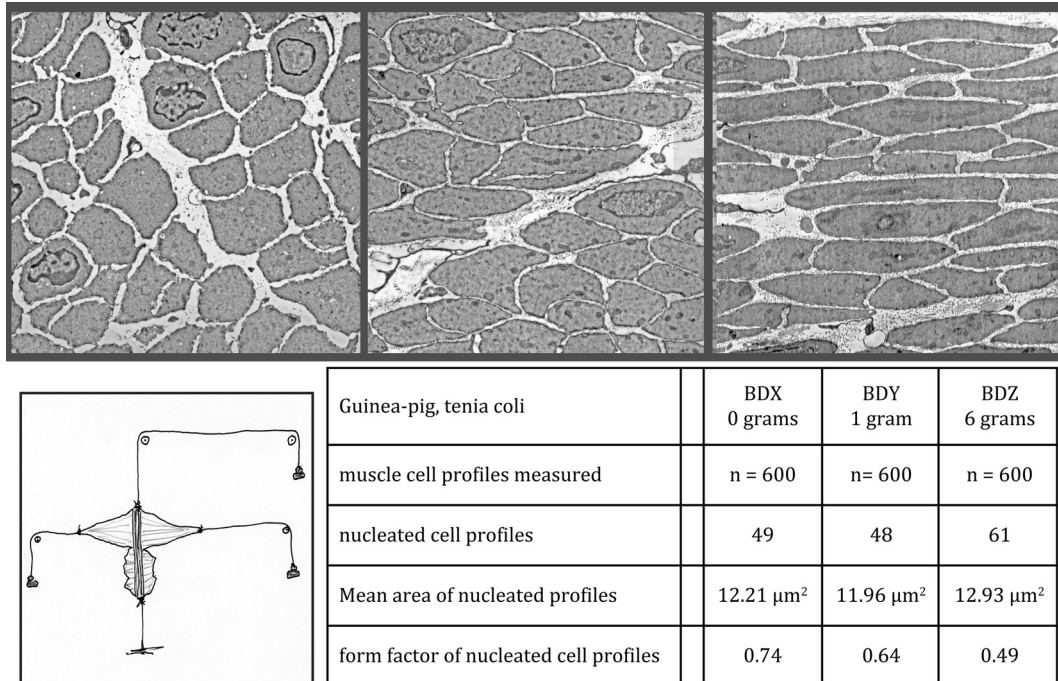
| Guinea-pig, tenia coli | | BDX 0 grams | BDY 1 gram | BDZ 6 grams |
|---|---|---|---|---|
| muscle cell profiles measured | | n = 600 | n= 600 | n = 600 |
| nucleated cell profiles | | 49 | 48 | 61 |
| Mean area of nucleated profiles | | 12.21 μm² | 11.96 μm² | 12.93 μm² |
| form factor of nucleated cell profiles | | 0.74 | 0.64 | 0.49 |

**Fig. 6.** The sketch at bottom left shows a strip of tenia coli from a guinea-pig set up vertically *in vitro*, with bands of circular musculature set up to allow monitoring of its length and degree of distension. Micrographs of transverse section of the tenia were taken when the circular musculature was under maximal distension (with a load of 6 grams), to the right, or under moderate load (1 gram), in the middle, or un-distended (0 grams load), to the left. The muscle cells profiles are markedly altered depending on the lateral distension imposed by the circular musculature onto the tenia. In numerical terms the relevant parameter is the form factor (which is 1 in a circle and decreases towards 0 as a profile becomes flatter).
Field of view of each picture: 20 μm.

## *Other muscular arrangements*

The are many additional arrangements of smooth musculature, including the dilator of the pupil (with radially oriented muscle cells), or the small, short and straight bundles of muscle cells associated with hairs. The two examples provide a mechanical effect mainly through shortening.

Short bundles of musculature are found in bronchi, usually inside cartilage plates on the outer side. The effects of contraction are more complex because the bundles are located inside a rigid wall and changes in shape due to contraction are as important as the elongation in itself.

Lastly, smooth muscle can form laminar structures, where their role is mainly a passive one. They may complete, with a relatively soft and resilient material, the circumference of a more rigid tube (as in the trachea), or they may provide a barrier that reduces mechanical stress on soft tissues (as in the lamina of smooth musculature lying longitudinally in the connective tissue between urethra and vagina).

Other complex arrangements are found for example in the oesophagus and the oviduct; here too the mechanics is founded on the layering and the distribution of muscle cells and stroma.

Of mechanical interest are sites where striated and smooth musculature lie very close to each other (without any morphologic suggestion of the existence of cells with mixed characters). Areas with both types of musculature exist in the oesophagus and in the urethra (Fig. 7D, E).
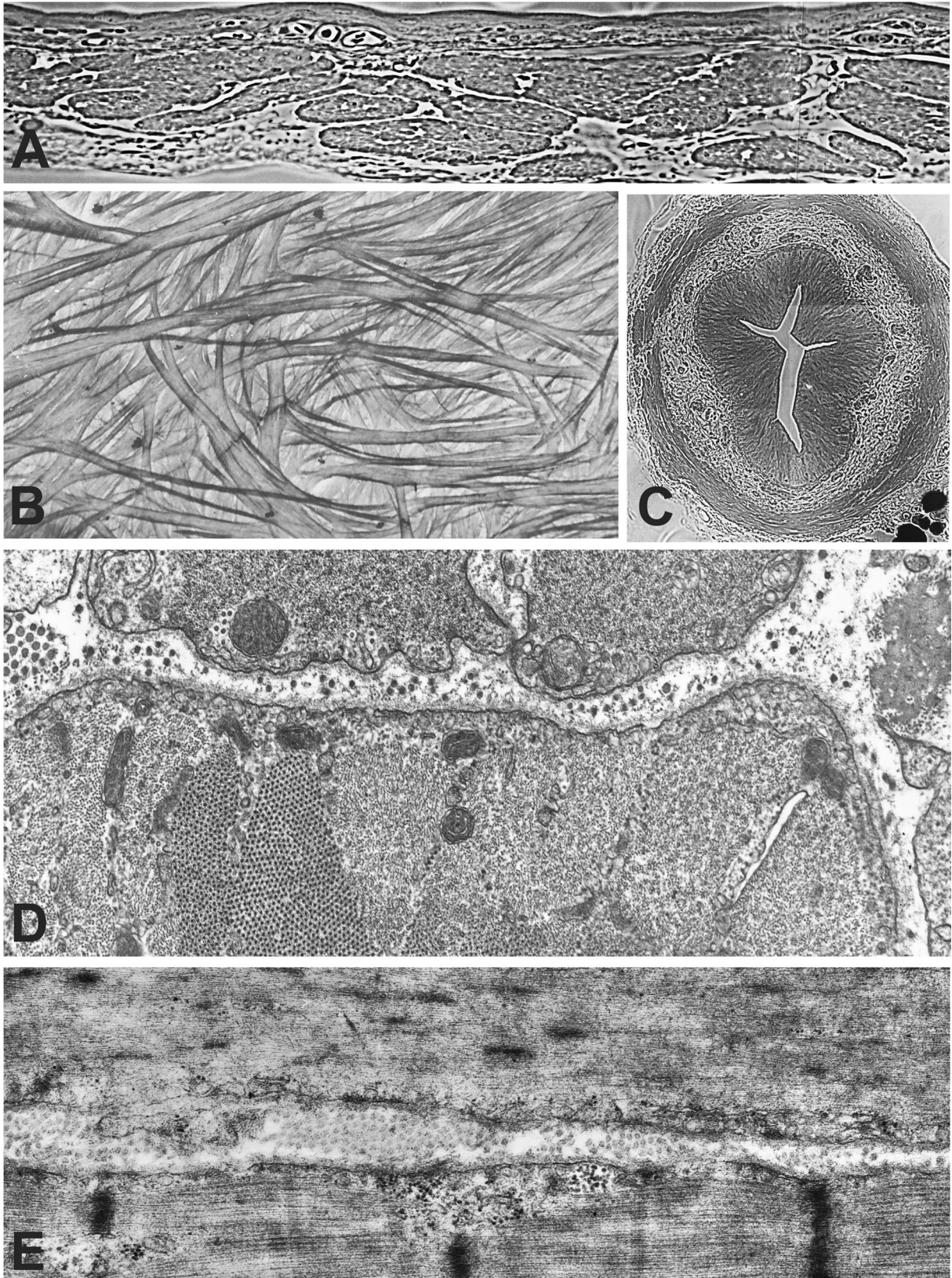
**Fig. 7.**

## *Single muscle cells*

Single, isolated muscle cells are found in many locations. On contraction they displace materials around them without exerting much force directly on any of them, as suggested by the absence of point of attachment at their surface.

The adhesion of myo-epithelial cells to the secretory cells and the basal lamina is strong enough that even in contraction the muscle cell does not project out of the acinus or become separate from it.

Muscle cells in vitro, normally single cells, are outside the interest of this study because they are reduced to single isolated cells, they have lost even their basal lamina, and they are embedded in an alien environment (or are in a suspension without an environment). Structural variation of smooth muscles extends to non-spindle shaped cells, which are rare in mammals but not altogether absent.

From a long perspective, smooth muscles, irrespective of organ or animal species, appear constituted by relatively similar elements that are structurally rather uniform: small spindle-shaped cells (a few micrometer wide at their widest and well below a millimetre in length, with a volume not larger than a monocyte) and a stroma with collagen fibrils and elastic fibres (both rigid and pliable). In a great measure, therefore, the wide range of mechanical activities of these muscles is founded on how cells and stroma are arranged in space.

While the structural unit is the muscle cell, it is the bundles of muscle cells that are the basic units in a mechanical context (without ignoring that single muscle cells too have specific mechanical properties, already highlighted by the work of Fred Fay (20, 21). Within a bundle the muscle cells have a compact assembly. The membrane-based junctions linking the cells contribute to transmission of the contractile pull along the bundle.

Junctions and adhesion between the cells have also an effect on the shape of the bundles. They contribute to the cohesion (and usually a round outline) of a bundle. The compactness of smooth muscle even if they do not have a fascia or an enveloping sheet highlights the importance of adhesion between cells. In small blood vessels the marked curvature of the muscle cells requires their adhesion to elements of the intima or the endothelium. It follows that histological studies, which are not new in the literature but far from exhaustive, can be important for understanding smooth muscle mechanics.

There are innumerable configurations of the musculature, most notably in the wall of hollow organs, which comprise tubular structures of all sizes. Within the various ranges of structures there are contrasting

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**Fig. 7.**   A. Transverse section of the wall of the bladder of a rabbit, photographed unstained by light microscopy. At the top the wall is lined by the urothelium, at the bottom by the peritoneal adventitia. Bundles of musculature, in various oblique sections are separated by connective tissue. Width of field of view: 580 μm.

B. Whole-mount preparation of the detrusor muscle of a rat bladder (apical region), imaged by direct trans-illumination in a darkroom enlarger onto photographic paper. The numerous muscle bundles have a wide range of orientations, without an apparent order. Some small black areas (two of them are near the upper edge) are intramural nerve ganglia. Width of field of view: approx. 2.6 mm.

C. Transverse section of the ureter in condition of near full contraction. The lining epithelium is thick and compressed sideway almost occluding the lumen. A tunica propria comprising connective tissue and blood vessels, separates it from a ring of musculature made of bundles lying approximately circumferentially. Width of field of view: 1.1 mm.

D. Electron micrograph of two smooth muscle cells, at top, and a striated muscle fibre in the wall of the oesophagus of a rat, all in transverse section, with the characteristic pattern of myofilaments and a few mitochondria. Width of field of view: 9 μm.

E. Similar preparation to D., with a smooth muscle cell, at top, and a striated muscle fibre, both in longitudinal section in the wall of the oesophagus of a rat. The striated fibre shows to sarcomeres in the middle, and two half sarcomeres on each side; Z lines and M,A and I bands are visible. The muscle cell is studded with dense bodies to which filaments are attached. A minimal space, with some collagen fibrils, separated the two muscle elements. Width of field of view: 11 μm.

configurations. For example, some muscles are very compact (tenia coli) while some have a looser arrangement (the ureter). In some organs the disposition of muscle layers is strictly orthogonal (typically in the intestine) while in other organs oblique or less geometric orientation of the muscle bundles is common, or there is no prevalent orientation.

The challenge here would be to cast light on the morphogenetic processes behind the different outcomes, the growth and development programs (in development, but also in evolution) accounting for the emergence of this variation in biological forms. It may be relevant, for example, that in the ileum the circular musculature develops first and the longitudinal one only afterwards (22). Similarly interesting is the evidence that small arteries, which are usually straight, become slightly more tortuous in old age. This was already noted by Leonardo (23) in about 1508 when he illustrated a vessel in a centenarian (who had died in his presence) and in a young boy.

Morphogenesis also includes processes that are not direct expression of the genome, but are due to the mechanical environment of a muscle or an organ in real time. Part of the environment are elements that are uniform for a given species (from gravity to other physical conditions) and part are elements that are related to the life of an individual.

Many smooth muscles form closed rings or near-spherical structures; they contrast with those that are straight and cord-like. Because the muscular tissue is incompressible, that is of constant volume, any shortening occurring with contraction is matched by an equal increase in width. With muscles that have no terminals but are closed onto themselves, as in rings, contraction reduces the circumference of the organ but it also increases the thickness of the wall.

Additionally, in many small tubular organs, such as arterioles, rigid collagen fibrils block an increase in length of the organ, and the thickening of muscle cells on contraction is also an enlargement (fattening) entirely in the radial direction.

Three structural features are combined in every vessel, first, the tissue being incompressible, second, the musculature forming a ring closed onto itself, and, third, the collagen being an obstacle to an increase in length of the vessel. The consequent mechanical effect is an enhanced efficiency, that is, the lumen of the vessel narrows or even becomes occluded by means of only a limited muscle contraction, the more so the greater the thickness of the muscle as a percentage of the radius of the vessel. Assuming a similar speed of muscle contraction, tubular organs with thick musculature, such as the vas, empty their lumen with less contraction than organ with a thinner musculature would and therefore in a shorter time.

Since smooth muscles behave as hydrostats, the precise orientation of the muscle bundles is mechanically less important than in muscles that have discrete insertion points and a linear mechanical lay-out. In different words, a change in shape of muscle cells and bundles is a better account of their mechanical activity, than simply shortening (24). In addition, contraction hardens the tissue and thus can provide support for other mechanical events around them.

## Conflict of Interest

The author declares that there are no conflicts of interest.

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