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THE FORCE GENERATED BY A VISCERAL SMOOTH MUSCLE

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SUMMARY

1. Strips of taenia coli from the caecum of the guinea-pig were mounted in an organ bath at 37° C; isometric contractions were elicited with 10^{-5} M carbachol. Each taenia was stretched to the length at which it produced the maximum active tension; it was then fixed and embedded for measurement of the transverse sectional area.

2. The maximal force produced ranged between 96·1 and 138·3 mN. This corresponded to a force of between 251 and 513 mN.mm^{-2} (mean: 416 ± 28 [n = 10]). Temperature changes in the range 23–38° C had little effect on the maximal force output.

3. When allowance is made for the extracellular space (about 32 % of the transverse sectional area), for the non-muscular cells present in the taenia (about 5 %), and for the non-contractile material present in the muscle cells (about 10 %), the maximal force generated was about 734 mN.mm^{-2} of contractile material (or almost twice as large as in skeletal muscle).

4. Electron microscopy revealed terminal apparatuses at the ends of muscle cells, anchoring the cells to the connective tissue, and cell-to-cell junctions (attachment plaques). In addition, many dense patches or dense bands, sites near the cell surface where filaments are seen to end, were scattered along the entire length of the muscle cell and lay close to bundles of collagen fibrils.

5. It is suggested that the production of such a large force by this smooth muscle is partly explained by the lateral attachment of some contractile units to sites along the entire cell length, which in their turn are anchored to the collagen network; the latter may be considered a sort of intramuscular tendon.

INTRODUCTION

The maximum isometric tension developed by a vertebrate skeletal muscle is generally considered to be about 350 mN.mm^{-2} (Close, 1972) (see some experimental values in Table 2). Striated muscles which have

longer sarcomeres, such as the extensor carpopoditi of the crayfish, produce a greater tension (Zachar & Zacharová, 1966). The maximal tension developed by arterial smooth muscles is about 220 $mN.mm^{-2}$ (Lundholm & Mohme-Lundholm, 1966; Herlihy & Murphy, 1973; Murphy, Herlihy & Megerman, 1974); it becomes about 370 $mN.mm^{-2}$ when allowance is made for the large amount of extracellular space (Murphy *et al.* 1974; Mulvany & Halpern, 1976). In the case of intestinal smooth muscles the maximal tension developed ranges between 70 and 200 $mN.mm^{-2}$ (Åberg & Axelsson, 1965; Meiss, 1971; Gordon & Siegman, 1971; Lowy & Mulvany, 1973) (see Table 2). In the case of visceral smooth muscle, precise estimates of the transverse sectional area are required and the problem was, therefore, re-investigated on the taenia coli of the guinea-pig caecum, using a histological method to estimate the transverse sectional area. Further electron microscopic obervations were carried out, extending a quantitative study of the taenia coli (Gabella, 1976), in order to cast some light on the mechanism of force transmission in this smooth muscle.

METHODS

Guinea-pigs of either sex, weighing between 415 and 660 g, were stunned and exsanguinated. Lengths of taenia of 5–15 mm with some of the underlying circular muscle and the intervening myenteric plexus were mounted vertically in an organ bath (20 ml.) in an oxygenated (95 % O_2 + 5 % CO_2) Krebs solution at 37° C. The Krebs solution had the following composition (mM): Na^+ 137·4; K^+ 5·9; Ca^{2+} 2·5; Mg^{2+} 1·2; Cl^- 134; HCO_3^- 15·5; $H_2PO_2^-$ 1·2; glucose 11·5 (Bülbring & Tomita, 1969).

The muscle strip was tied with a cotton thread to a light metal chain connected to a lever which was used for applying the various loads. The lever had an isotonic (displacement) transducer connected to the pivot, and could be locked at any one position to a force transducer (strain gauge). Changes in length were recorded electronically on a two-channel MX 212 Pen recorder (Devices, U.K.). For measurement of isometric tension, a UF 1 force transducer (Devices) was locked to the lever; the lever was then unloaded and the tension recorded on the pen recorder. Muscle strip, lever, and strain gauge were mounted on three separate micromanipulators.

After a 30 min recovery period, a rough length–tension curve (usually only four points) was obtained by stimulating the muscle with 10^{-5} M carbachol for 3 min each time. A period of 20 min was allowed between successive stimulations. Changes in length were obtained by adding (or taking off) weights to the lever (not more than 1 g every 30 sec). At the end of this part of the experiment, the muscle was returned to the length at which it had produced the greatest tension and made to contract twice or more. The largest of these contractions was taken as the maximum tension, but when the difference between successive contractions was greater than 10 % the experiment was discontinued. When the muscle tension failed to drop to less than 5 % of the active tension wihin 10 min of washing out the drug, the experiment was also discontinued.

Stimulation. Of the three methods of stimulation used (80 mM-K^+, electrical field stimulation, and carbachol), carbachol produced the most powerful contractions. Stimulation with a solution containing 80 mM-K^+ (NaCl partly substituted with KCl)

elicited contractions which were only 55–75 % of those obtained with carbachol. Higher concentrations of K^+ are known to produce swelling of the muscle cells (Jones, Somlyo & Somlyo, 1973) and they were not considered to be suitable for these experiments. Moreover, high K^+ solutions stimulate transmitter release, and this is likely to include an inhibitory effect on the taenia, in view of its heavy intrinsic inhibitory innervation (Burnstock, Campbell & Rand, 1966). Electrical stimulation (longitudinal field stimulation, with monophasic square pulses generated by an SD9 Grass stimulator, 1–4 msec duration, 5–50 Hz, supramaximal voltage, in trains of 5–30 sec) produced 50–70 % of the tension obtained with carbachol.

All the measurements reported here were carried out on taeniae stimulated with 10^{-5} M carbachol (carbamylcholine chloride, B.D.H.). The drug was added to the organ bath with a syringe from a freshly prepared 10^{-3} M stock solution, and it was washed out after 3 min. Changes in the concentration of carbachol, in the range 10^{-4} to 10^{-6}, had little effect on the tension produced (Text-fig. 1). With a concentration of 10^{-5} the tension was usually maintained throughout the stimulation period, or decreased slightly; with higher concentrations the tension fell dramatically before the end of stimulation; with lower concentrations the tension usually increased throughout the stimulation period.

Histology. The strips of taenia were fixed either 30 sec after the beginning of a contraction or when they had relaxed (zero tension) after several washes in Krebs solution. The fixative was 5 % glutaraldehyde buffered to pH 7·4 with 100 mM-Na cacodylate at room temperature. The fixation lasted 4–12 hr, during which time the muscles remained attached to the strain gauge in the same conditions used for the recording. The tissues were post-fixed in osmium, block-stained in aqueous uranyl acetate, dehydrated in ethanol and embedded in Araldite (Ciba).

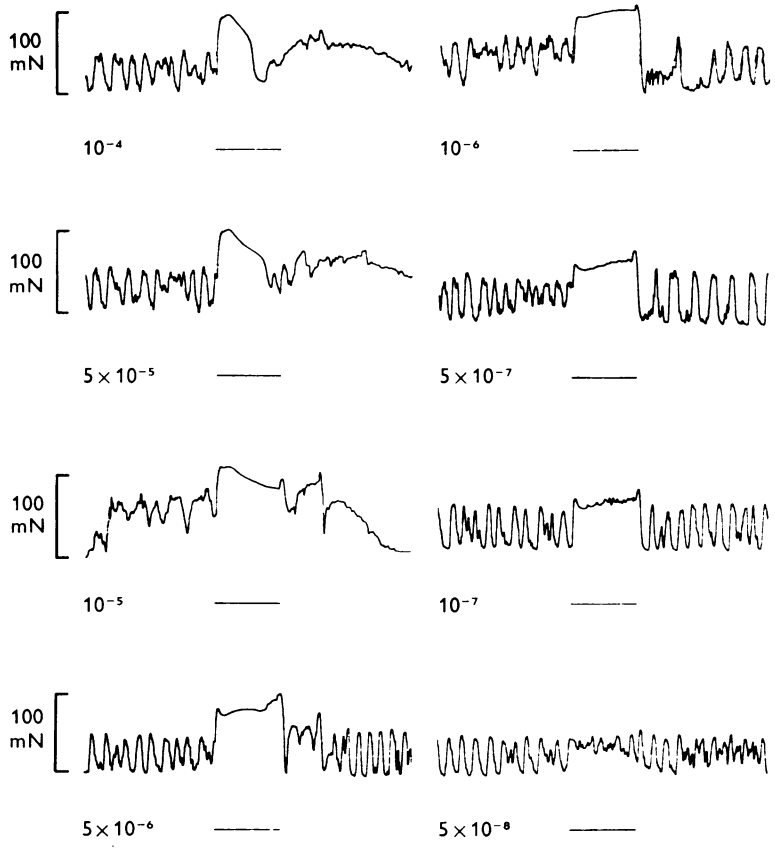
Measurement of the transverse sectional area. Sections 3 μm thick were cut with glass knives, mounted in D.P.X. and photographed in phase-contrast microscopy (at 63 ×). The area was measured with a planimeter on photographic montages (at 315 ×, Pl. 1). The entire area included within the outline of the taenia was taken as the transverse sectional area. Two sources of error in the determination of the sectional area were checked. (*a*) *Shrinkage during dehydration, resin infiltration and curing.* Experiments were carried out in which the lengths of strips of taenia were measured under a stereomicroscope during the various stages of the embedding. The over-all shrinkage ranged between 2·0 % (in strips which had been fixed under a load of 12 to 16 g weight) and 'non-measurable', i.e. less than 0·5 % (in strips fixed under a load of 1 g). In strips fixed under a load of 5–8 g, i.e. in conditions similar to those used for measuring the maximal tension, the shrinkage was about 1·5 %. Assuming (in the absence of direct experimental evidence) that a similar shrinkage occurs in the other two dimensions, the transverse area of the embedded taenia is about 97 % ($(100-1{\cdot}5)^2$ instead of 100^2) the area of the strip during the incubation. (*b*) *Compression of the sections.* Histological sections undergo some compression along the direction of cutting. To check this source of error a section of a taenia was cut and photographed; the next section was cut after rotating the block in the microtome chuck by 90° (around an axis perpendicular to the cutting face), and was photographed as usual. In this way, compression occurred in both sections but along orthogonal directions and could be directly measured by comparing the two sections. In sections 3·0 μm thick the compression was about 1·2 % (it varied slightly from one embedding to another). Therefore, the planimetric measurements on the photographic montages were increased by 1·2 %, and the new value was further increased by 3 %; this value was the transverse sectional area corrected for shrinkage and compression.

Each taenia used for area measurements was cut at two levels. When the two measurements differed by more than 10 % (this occurred in just over one third of the cases) the experiment was ignored.

RESULTS

Maximal tension

In ten experiments the maximum active tension developed by a strip of taenia, stimulated with 10^{-5} M carbachol at 37° C, ranged between 96·1 and 138·3 mN, mean $111 \pm 3{\cdot}8$ (S.E.). The transverse sectional area, corrected for shrinkage and compression, ranged between 0·193 and 0·451 mm^2.



Text-fig. 1. Isometric recording of a strip of taenia coli stimulated for 3 min at 37° C with various concentrations of carbachol. There is little difference in tension with concentrations of carbachol between 10^{-4} and 10^{-6} M. With a concentration of 10^{-5} M the tension falls slightly; with stronger concentrations the tension falls dramatically before the end of stimulation; with weaker concentrations the tension increases slightly throughout the period of stimulation. Top line: time marker in minutes.

The force developed per unit transverse sectional area ranged between 251 and 513 $mN.mm^{-2}$, mean 416 ± 28 (S.E.) (Table 1). The mean for male guinea-pigs (479 ± 28 ($n = 4$)) was slightly larger than that for female guinea-pigs (374 ± 34 ($n = 6$)) (Student's test, $0{\cdot}1 > P > 0{\cdot}05$).

TABLE 1. Maximum active tension developed by strips of taenia coli stimulated with 10^{-5} M carbachol at 37° C. The transverse sectional area was measured on histological sections, and is corrected for shrinkage and compression

Body wt. (g)	Sex	Maximum tension developed (mN)	Transverse sectional area (mm^2)	Tension per unit area ($mN.mm^{-2}$)
630	M	99·0	0·193	513
580	F	96·1	0·225	427
650	F	98·8	0·199	496
415	F	115·7	0·339	341
610	M	115·7	0·252	459
460	F	111·8	0·298	375
610	M	113·8	0·278	409
470	F	138·3	0·387	357
570	F	113·2	0·451	251
660	M	107·9	0·201	536
			Mean	416 ± 28 ($n = 10$)

Effect of temperature

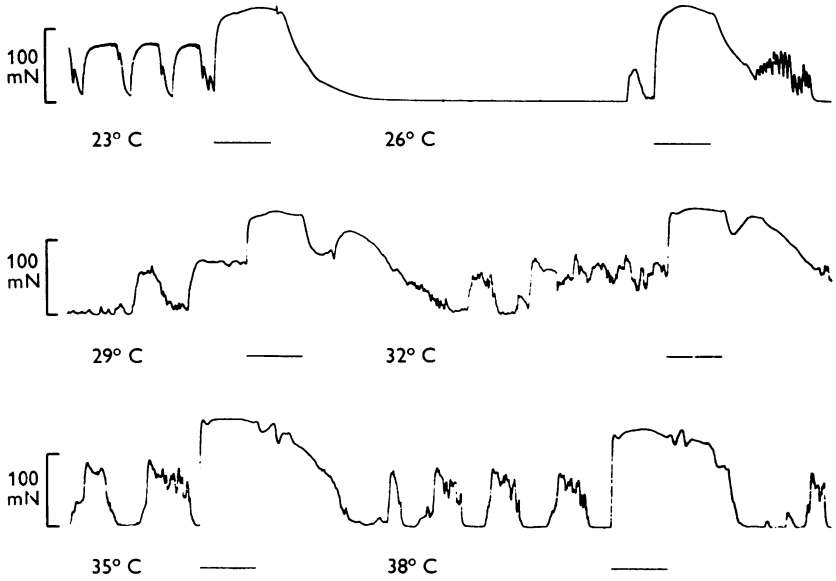
Changes in temperature in the range 23–38° C had little effect on the maximal output of tension (Text-fig. 2). The contraction at 29–32° C were slightly greater (approximately 10 % in six experiments) than at 23 or 37° C. No consistent difference was found between amount of tension developed at 23 and 37° C. The tension was about two thirds of maximal at 15° C, and was undetectable (i.e. less than 2 %) below 9° C.

Fine structure

Since an account of the fine structure of muscle cells of the taenia coli, stretched and contracted, has recently been published (Gabella, 1976), only the observations pertinent to the problem of transmission of force are reported here.

Near the surface of the smooth muscle cells there were numerous 'dense patches' (Pl. 2*A*), where thin (actin) filaments have been reported to enter (Pease & Molinari, 1960). The dense patches or, more accurately, dense bands were up to several microns long and parallel to the cell main axis. They were characterized by a layer of electron-dense material lying immediately underneath the cell membrane (Pls. 2*B* and 3*A*). In cells transversely cut at the level of the nucleus, the dense patches amounted to about 50 %

of the cell surface. In muscle cell profiles of smaller diameter (i.e. along the tapering ends of the cells) the percentage of the cell surface occupied by the dense patches increased, and some small cell profiles had the entire cell membrane incrustated with dense material.



Text-fig. 2. Isometric recording of a strip of taenia coli at various temperatures. The muscle was stimulated every 25 min with 10^{-5} M carbachol for 3 min (horizontal bars). In the 5 min after washing out the drug the temperature was increased by 3°. The effect of temperature in the range 23–38° C on the output of tension is small. The experiment was continued with a series of stimulations (not reproduced) during the reversed order of temperatures, with similar results. Top line: time marker in minutes.

Some dense patches or bands matched each other in adjacent cells, forming cell-to-cell contacts ('attachment plaques') (Pls. 2*B* and 3*A*). The intercellular gap varied between 10 and 80 nm; when it was wider than about 30 nm, the gap was occupied by electron-dense material, often displaying an ill-defined periodicity in direct continuation with the basal laminae investing the two muscle cells (Pl. 2*B*).

The majority of dense patches or bands, however, lay underneath bundles of collagen fibrils, a basal lamina more prominent than that over the rest of the cell surface intervening between the two structures. Only very rarely were collagen fibrils observed to contact the muscle cell membrane directly (i.e. without a gap occupied by a basal lamina), although this

is a common occurrence in the uterine muscle of this species (G. Gabella, unpublished). In the taeniae fixed at the length at which they produced maximal tension, the majority of collagen fibrils ran parallel or at a small angle to the longitudinal axis of the muscle (Pl. 4*C*).

Close to their ends many muscle cells acquired a stellate or convolute profile (Pls. 3*B* and 4*A*). Some finger-shaped invaginations of the cell membrane occasionally ran parallel to the cell length for a few microns. In transversely sectioned cells the invaginations appeared as oval or round profiles, 0·1–0·5 μm in diameter, coated on the inside by a thick basal lamina (Pl. 4*B*). They were reminiscent of the sarcolemmal invaginations observed at the ends of skeletal muscle fibres (Hanak & Böck, 1971). The terminal apparatuses at the ends of smooth muscle cells (changes in the shape of the cell profile and invaginations) effected a great increase of the cell surface, in spite of the reduction of the cell size at this level. In the vas deferens of the guinea-pig Merrillees, Burnstock & Holman (1963) have described tunnels, filled with basal lamina material, and deep grooves, which penetrate the tips of muscle cells, parallel to their long axes.

Arrangement of connective tissue

Transverse sections of the taenia coli showed prominent septa of connective tissue (Pl. 1). Some of the septa extended through the full thickness of the muscle, and were wider in its deeper portions and thinner towards the serosal surface. Thinner laminae of connective tissue were also seen, which were continuous with the septa but had a more irregular and ill-defined distribution (Pl. 1).

In serial transverse sections the position and pattern of the septa changed rapidly along the length of the taenia: they were found to disappear within few tens of microns or to merge with other septa.

In serial longitudinal sections, cut parallel to the serosal or the lumenal surface of the caecum, many septa of connective tissue appeared to extend only for a limited portion of the muscle length. The septum arrowed in Pl. 5*A* extends for about 150 μm. Moreover, many septa, although arranged longitudinally, were not parallel to the groups of muscle cells. In the example illustrated (Pl. 5*B* and *C*) the connective tissue septa form angles of between 4 and 5° with the groups of muscle cells. This arrangement was also borne out by reconstructions from serial transverse sections.

DISCUSSION

The maximal tension per unit surface of tranverse sectional area developed by the taenia coli of the guinea-pig was found to be greater than that reported by other authors (Table 2). The discrepancy may be partly due to

different methods of stimulation and different ways of estimating the transverse sectional area. Electrical field stimulation and high K^+ stimulation were preferred in the majority of previous experiments. Both methods however, were in the present experiments less effective and less consistent than carbachol. The measurement of the transverse sectional area on histological sections of plastic embedded taeniae was considered to give more reliable values than estimates based on length and weight of the muscle strip.

The taenia coli seems to be more effective, in terms of force developed per cross-sectional area, than other visceral smooth muscles, such as the duodenum of the cat (Meiss, 1968), the taenia coli of the rabbit (Gordon & Seigman, 1971), the tracheal muscle of the dog (Stephens, Kroeger & Mehta, 1969), or the uterus of the rabbit (Csapo, 1954). A discussion of these differences must, however, await further information on the structure of these various muscles. The very low value obtained for the uterus is no doubt partly due to the non-parallel arrangement of its muscle bundles, so that the tension developed in one direction is only part of the total force generated.

The influence of temperature, in the range 23–38° C, on the tension produced by the taenia coli was minimal. On the average the tension was slightly greater in the region 29–33° C. Similarly, Peiper, Laven & Ehl (1975) found that temperature changes in the range 25–37° C had no effect on the peak force generation of the portal vein of the rat (but had a dramatic effect on the rate of tension increase). Lowy & Mulvany (1973) found that the active tension of the guinea-pig taenia coli was 37% greater at 37° C than at 23° C (intermediate temperatures were not tested).

The force produced by the taenia coli of the guinea-pig was not only greater than that recorded from any other smooth muscle but also greater than the maximal force produced by vertebrate striated muscles (Table 2). This difference with striated muscle (416 $mN.mm^{-2}$ as against 350 $mN.mm^{-2}$) can be analysed by considering the amount of non-contractile material present in the respective transverse sectional areas.

The majority of the experiments on skeletal muscle (Table 2) were carried out on single isolated muscle fibres; therefore, the transverse sectional area included only the muscle fibre and no extracellular space. A recent electron microscopic study has shown that, in the frog, sartorius myofibrils comprise 83% of the cell volume (Mobley & Eisenberg, 1975). Therefore, the maximal tension of about 350 $mN.mm^{-2}$ fibre area (Ramsey & Street, 1940; Hodgkin & Horowicz, 1960) corresponds to a tension of 422 $mN.mm^{-2}$ of contractile material area.

The force measurements on taenia, on the other hand, were done on

TABLE 2. List of average maximal active tensions developed by striated muscles, vascular smooth muscles, and visceral smooth muscles, during isometric contractions and contractures, reported in the literature. Only some representative values are quoted for striated muscles. *A*, animal species and type of muscle; *B*, temperature in °C; *C*, kind of stimulation used; *D*, average maximal tension in (mN.mm^{-2}, ± S.E. number of cases in square brackets; in round brackets the highest value recorded); *E*, reference

A	*B*	*C*	*D*	*E*
		Striated muscle		
Frog semitendinosus[a]	12–14	tetanic stim.	346 ± 16 [n = 15] (433)	Ramsey & Street, 1940
Frog semitendinosus[a]	20	tetanic stim.	345 ± 49 [n = 5]	Hodgkin & Horowicz, 1960
	20	100 mM-K$^+$	363 ± 13 [n = 13] (440)	Hodgkin & Horowicz, 1960
Cat calf muscles[b]	*in vivo*	nerve stim.	127 ± 59 [n = 25] (215)	Helander & Thulin, 1962
Crayfish extensor carpopoditi[a]	18–23	173 mM-K$^+$	759 ± 39 [n = 17] (1134)	Zachar & Zacharová, 1966
		Vascular smooth muscle		
Cow mesenteric artery	37	Adrenaline	206[e]	Lundholm & Mohme-Lundholm, 1966
Hog carotid artery	—[d]	116 mM-K$^+$	220[e] [n = 11]	Herlihy & Murphy, 1973
Hog cartid artery	—[d]	155 mM-K$^+$	220[f]	Murphy *et al.* 1974
Rat mesenteric artery, ø 100 μm	36	155 mM-K$^+$	74[g, h]	Mulvany & Halpern, 1976
		Visceral smooth muscle		
Rabbit uterus	37	Field stim.	13[c, i]	Csapo, 1954
Guinea-pig taenia	37	High K$^+$ or carbachol	178 ± 51 [n = 19][e]	Åberg & Axelsson, 1965
Cat duodenum	30	Field stim.	73[e]	Meiss, 1971
Dog trachealis	37	Field stim.	108 ± 2·9[j]	Stephens *et al.* 1969
Guinea-pig taenia	36	—	147	Mashima, 1969[k]
Rabbit taenia	22	Field stim.	87 ± 11[l]	Gordon & Siegman, 1971
Guinea-pig taenia	23	Field stim.	200 ± 19 % [n = 35][e]	Lowy & Mulvany, 1973
Rabbit mesotubarium	25	Field stim.	25 ± 10 [n = 18][l]	Meiss, 1975

[a] Isolated muscle fibres.

[b] Whole muscle.

[c] Method for calculating the area not given.

[d] Temperature not given.

[e] Area calculated from length, weight and specific gravity of the muscle strip and by a histological method (no difference found).

[f] Since the tissue contained 40 % in volume of extracellular space, the maxium tension was 370 mN.mm^{-2} of smooth muscle cells.

[g] Area measured on electron micrographs of the tissue.

[h] Since the muscle cells represented 20 % in volume of the tissue the tension was 370 mN.mm^{-2} of smooth muscle cells.

[i] Calculated by Gordon & Siegman (1971).

[j] Area measured on histological sections (light microscopy).

[k] Quoted by Herlihy & Murphy (1974).

[l] Area calculated from length, weight and specific gravity of the muscle strip.

whole muscles. In order to find the value of the maximal tension per unit area of contractile material, allowance has to be made for intra- and extracellular non-contractile material. The extent of the extracellular space in smooth muscle varies greatly according to the method used for measuring it (Burnstock, Holman & Prosser, 1963). Among recent estimates of the extracellular space in the guinea-pig taenia coli are the following values: 44 % (^{60}CoEDTA method, Brading & Jones, 1969), 41 % (sorbitol method, Goodford, 1970), 48 % (^{60}CoEDTA method, Jones *et al.* 1973), 20 % (electron microscopy of selected, 'muscle cell rich' areas, Jones *et al.* 1973), In the present discussion an approximate value of 32 % will be used (this value was obtained from measurements of the extracellular space on low-power electron micrographs covering large parts of the transverse sectional area of the taenia). To this another 5 % should be added to account for the non-muscular cells present in the taenia (interstitial cells, endothelial cells, Schwann cells, axons). Therefore, only about 63 % of the transverse sectional area of the taenia is occupied by smooth muscle cells. But the transverse sectional area of the smooth muscle cells is made up by other components in addition to myofilaments. Caveolae (plasmalemmal vesicles) constitute 0·25 % of the cell volume; the estimate is made on the basis of 168,000 caveolae per cell, each 70×120 nm^2 in size, and a cell volume of 3500 μm^3 (Gabella, 1976). The sarcoplasmic reticulum represents about 2 % of the cell volume (Devine, Somlyo & Somlyo, 1972). Nuclei and mitochondria occupy 2 % and 3·5–4·0 % of the cell volume, respectively (Gabella, 1976). Altogether, these four structures take up about 8 % of the cell volume. Microtubules also consistently occur, but their quantitative distribution is unknown. Moreover, around each membrane-bounded intracellular structure there is a halo of several nanometers which is not penetrated by myofilaments. Therefore, the area occupied by contractile material does not exceed 90 % of the total cell area, and it is then about 57 % of the total transverse sectional area of the taenia. The average maximal force of 416 mN . mm^{-2} of muscle area corresponds to 734 nN . mm^{-2} of area occupied by contractile material.

Part of this large difference in the force developed between taenia coli and striated muscle may be explained by the difference in length of the myosin filaments: 1·6 μm in skeletal muscle fibres (Page & Huxley, 1963), 2·2 μm in smooth muscle cells of the portal vein of the rabbit (Ashton, Somlyo & Somlyo, 1975). On the other hand, the myosin content (in weight per unit weight of tissue) of smooth muscle is said to be only about 1/7 that of striated muscle (Helander, 1957; Marston & Tregear, 1972; Murphy *et al.* 1974).

Another explanation of the much greater tension produced by the smooth muscle cells of the taenia coli can be put forward by examining how

the force is transmitted along a smooth muscle. The force generated by the myofilaments is directly applied to the surface of the muscle cell (Pease & Molinari, 1960) or is transmitted there by the framework of intermediate filaments, or cytoskeleton (see Cooke, 1976). (The possibility that in smooth muscle cells the myofilaments are arranged in an oblique manner to allow numerous attachments along the cell length has been suggested by Rosenbluth, 1965, and by Fay & Delise, 1973). In both cases the dense bands or patches at the cell surface are the structures where the pull of the myofilaments is applied. Where dense patches match each other (attachment plaques) the force is transmitted from one cell to another. The same presumably occurs at the ends of a muscle cell, although in this case abundant extracellular space intervenes between the ends of two cells, apart from where there are direct end-to-end contacts. Because of intercellular connexions through terminal apparatuses (via connective tissue to other cells) and attachment plaques, the muscle cells are coupled in a series arrangement similar to that of the sarcomeres in skeletal muscle fibres, and transmit force chiefly in the longitudinal direction. However, many dense bands are scattered along the whole length of the muscle cell, and lie underneath the basal lamina and collagen fibrils. Therefore, the pull is not only transmitted from one end to the other, but also to many additional sites on the lateral surface of the muscle cell. With this arrangement a larger number of contractile units can work in parallel (as the skeletal muscle fibres of a pennate muscle), each transmitting its force directly to the collagen network, and the latter could be regarded as a kind of intramuscular tendon. This arrangement would necessitate a higher collagen content than required for skeletal muscle and it has been found that this is the case: in the guinea-pig the taenia coli has 3·4 times more collagen (4·25 mg hydroxyproline/g wet weight tissue) than the sartorius muscle (Gabella & Yamey, 1976).

Obviously, a crucial factor in the interpretation of smooth-muscle mechanics is the fine arrangement of the collagen fibrils, a factor about which little information is available. However, the present histological observations show that many of the numerous connective tissue septa present in the taenia extend only for a limited length of the muscle. Moreover, many connective tissue septa are not parallel to, but make a small angle with the longitudinal axis of the groups of muscle cells. Although it is not known how the force of the smooth muscle cells is transmitted to the collagen network, the morphological analysis suggests that these small, discrete connective tissue structures may provide attachment for the groups of smooth muscle cells, and may have an important role in maintaining mechanical tension along a length of taenia.

If correct, the hypothesis on the transmission of force outlined above

might mean that the force recorded between the two ends of a single isolated smooth muscle cell (as in the experiments of Canaday & Fay, 1976, and Fay, Cooke & Canaday, 1976) would be less than that of the same cell *in situ*. In fact, the force transmitted to many sites on the 'lateral' surface of the cell, and from them to the collagen network, would be fully effective only in an intact tissue.

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EXPLANATION OF PLATES

PLATE 1

Transverse section of a taenia coli, photographed in phase-contrast microscopy; montage. Ganglia of the myenteric plexus are visible between the circular muscle (in longitudinal section, to the left) and the taenia. Adipose cells, heavily osmicated, are present in the submucosa and at the attachment of the mesocolon (top). Calibration: 100 μm.

PLATE 2

A, a smooth muscle cell from a taenia fixed *in situ* during a moderate isotonic contraction. Numerous dense patches are visible at the cell surface. The cell protrusions, mainly displaying caveolae and sarcoplasmic reticulum, between the dense patches are characteristic of isotonically contracted muscle cells. *c*: collagen fibrils. Calibration: 1 μm.

B, an intermediate contact (attachment plaque) between two smooth muscle cells (arrow). Dense patches are present elsewhere at the cell surface. Thick, thin and intermediate filaments are visible in both cells. The arrowheads point to microtubules. Calibration: 0·5 μm.

PLATE 3

A, a contact between two muscle cells, with an incrustation of electron-dense material on the cytoplasmic side of the plasmalemma, and an intercellular gap of about 12 nm (unlabelled arrow). On either side of it there are cell-to-cell contacts where the intercellular gap is 45–55 nm and is occupied by amorphous electron-dense material continuous with the basal laminae of the two cells. Other dense patches (*p*) are situated beneath basal lamina and collagen fibrils. Thin (*a*), thick (*m*) and intermediate (*i*) filaments and microtubules are visible. *mi*, mitochondrion; *c*, collagen fibrils. Calibration: 0·5 μm.

B, terminal part of a muscle cell, characterized by an irregular shape and a high surface to volume ratio. The basal lamina is thickened and additional amorphous material is present within the hollow part of the cell profile. The dense dots in the muscle cells are glycogen granules. Calibration: 1 μm.

PLATE 4

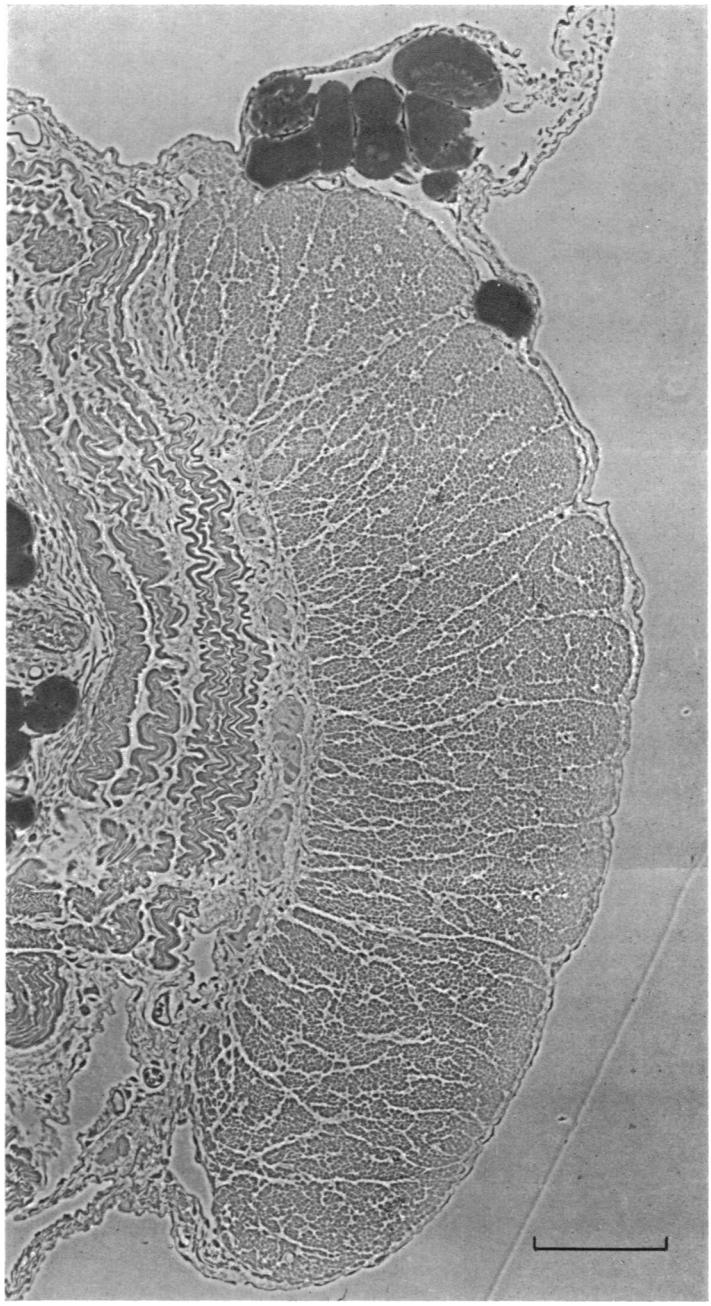
A, terminal part of a muscle cell where long, slender laminar protrusions realize a very large cell surface in spite of the reduction in volume. The dense dots in the muscle cells are glycogen granules. *f*, presumably an elastic fibre; *c*, collagen fibrils. Calibration: 0·5 μm.

B, transverse section of the terminal part of a muscle cell, cutting through longitudinal invaginations of the cell membrane. At the outside of the cell profile and at the inside of the invaginations a basal lamina is visible. Caveolae and sarcoplasmic reticulum are prominent in the neighbouring muscle cells. Calibration: 1 μm.

C, longitudinal section of a smooth muscle cell fixed during stretch. A band of dense material associated with the plasmalemma lies close to a bundle of collagen fibrils (*c*). Calibration: 1 μm.

PLATE 5

Longitudinal sections of a mildly stretched taenia. Araldite (Ciba) sections, 3 μm thick, parallel to the serosal or the lumenal surface (i.e. on a plane extending from top



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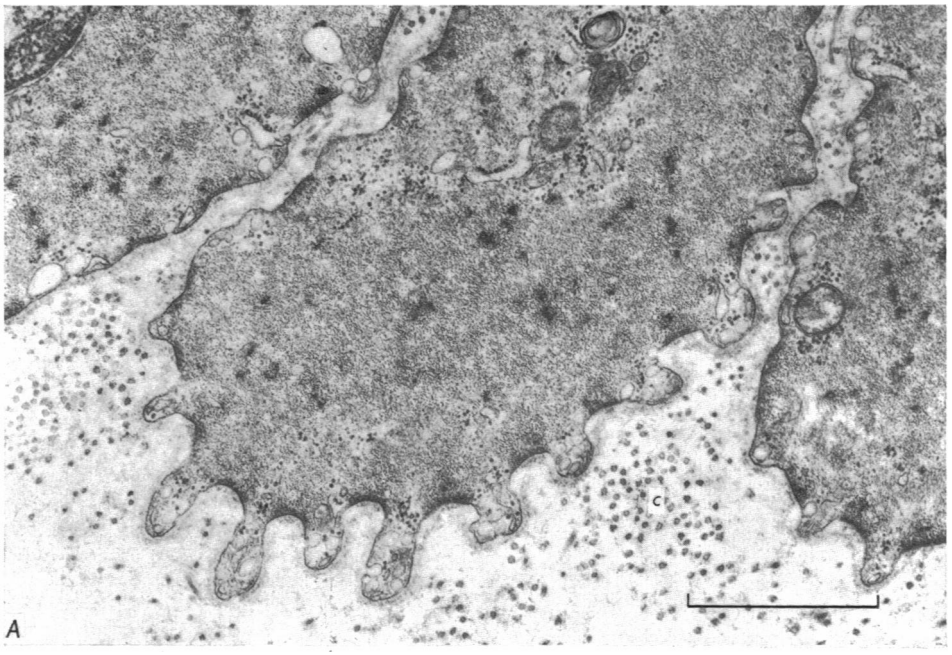
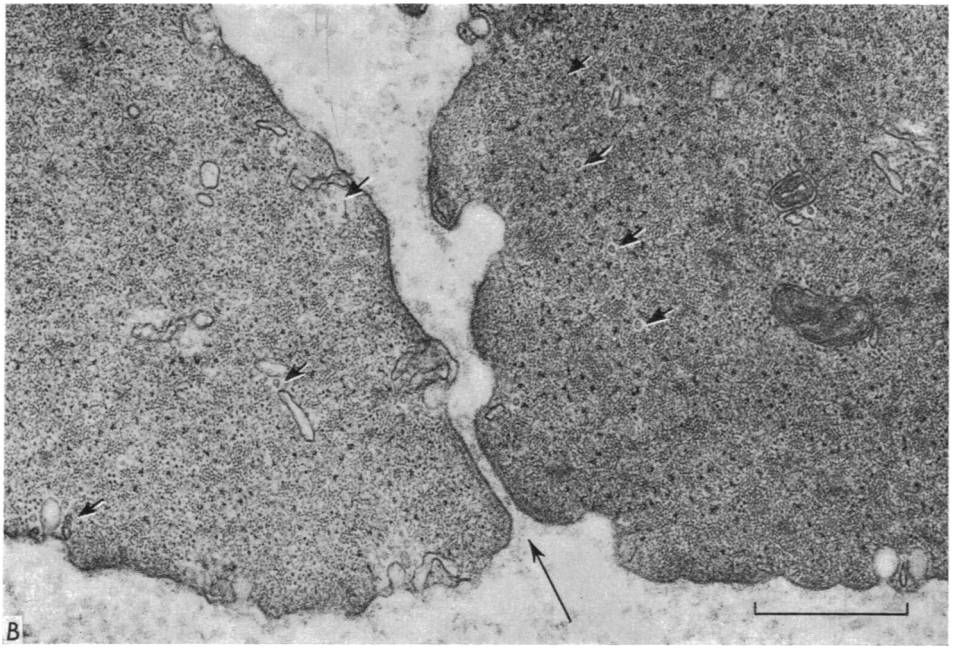
(*Facing p.* 212)





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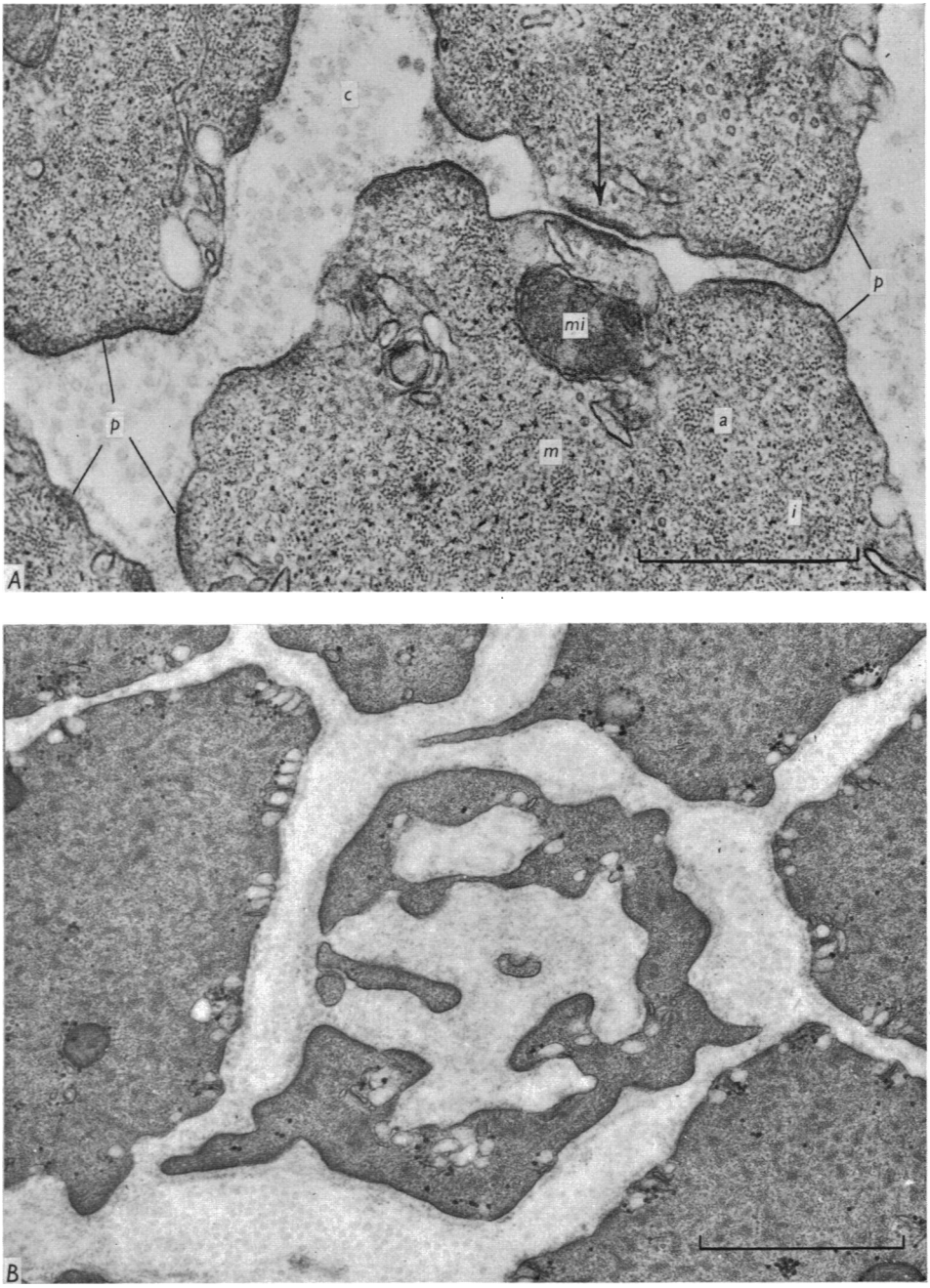

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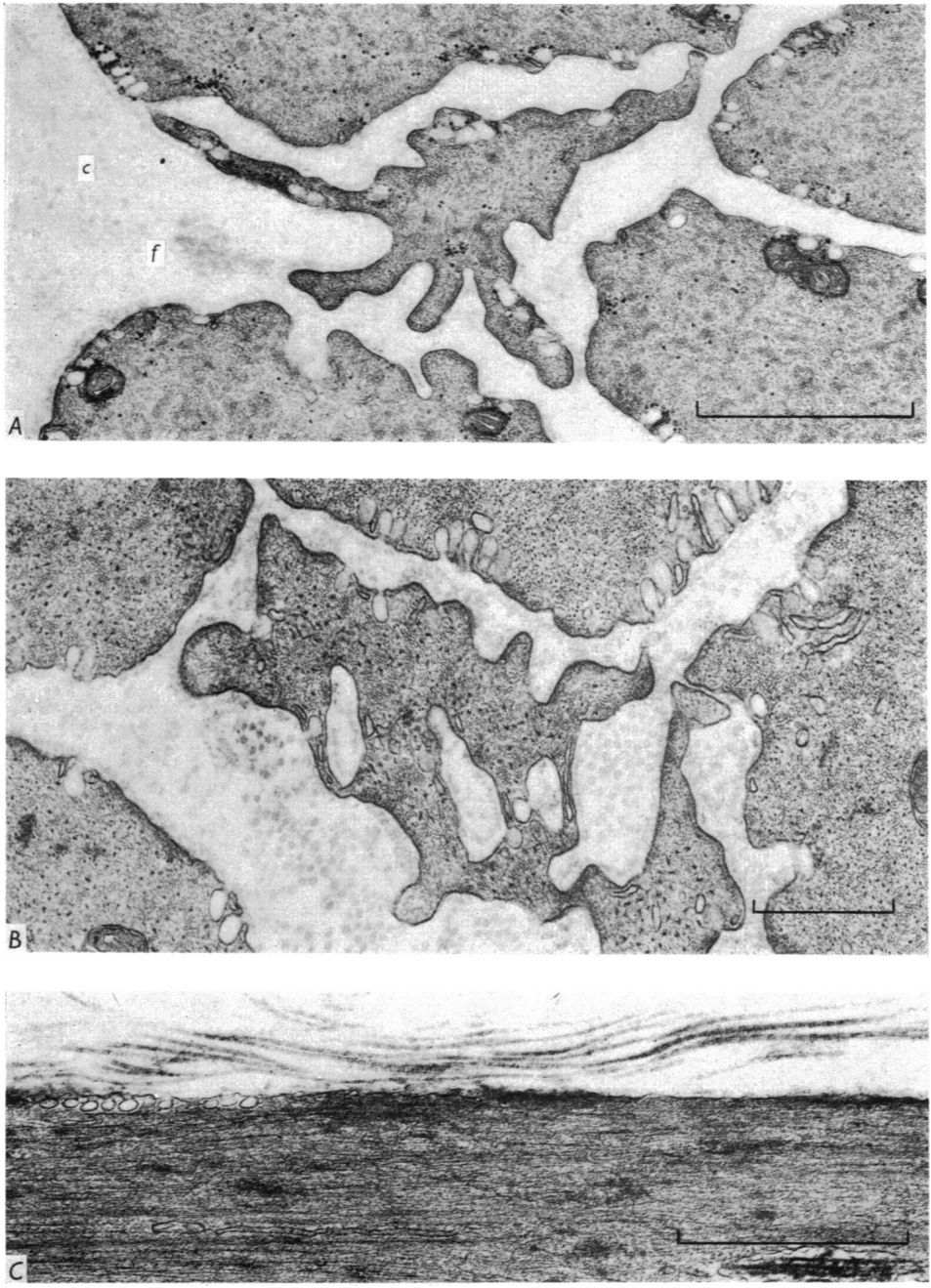

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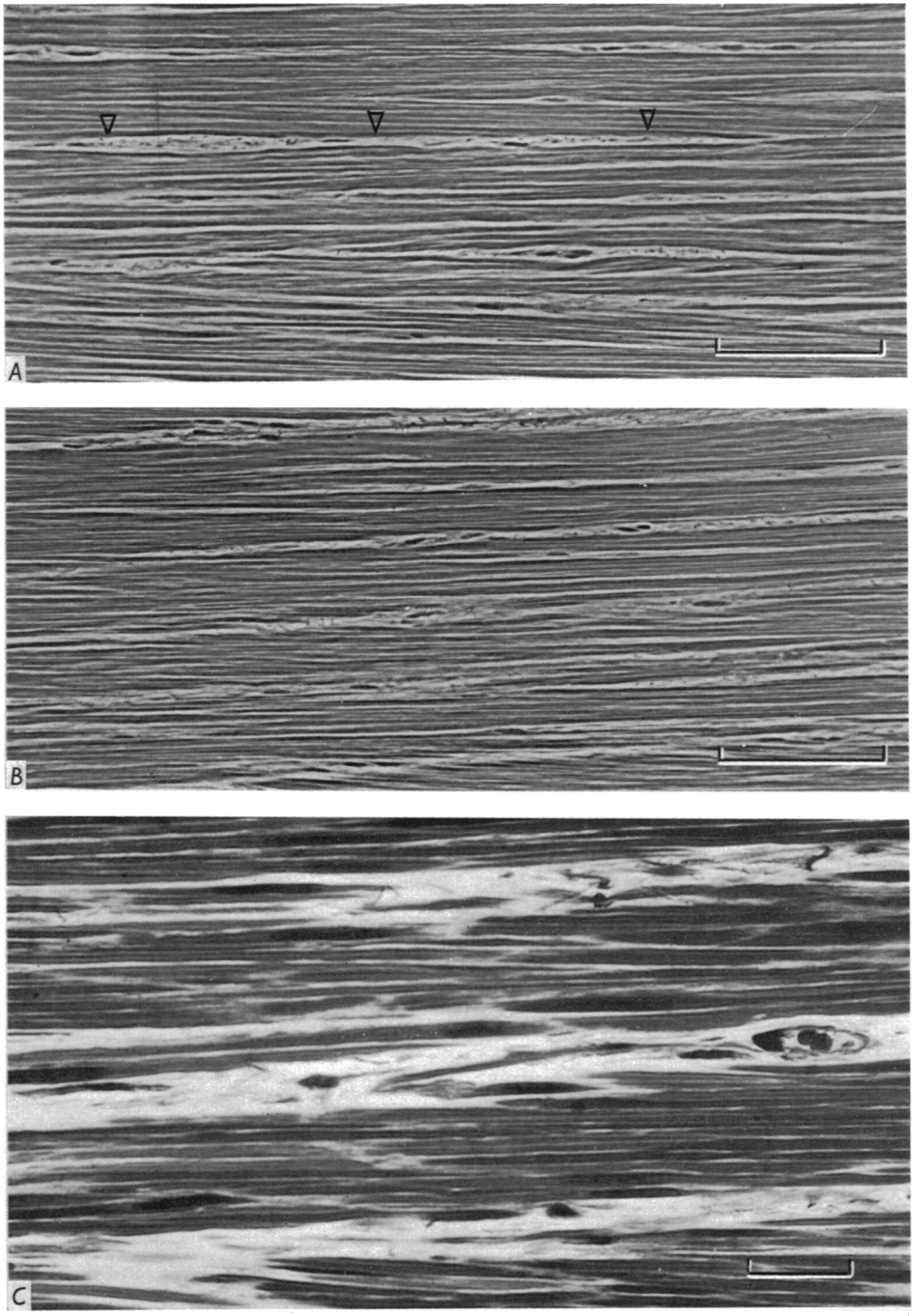

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to bottom in Pl. 1), were serially cut through the full thickness of a strip of taenia and examined in phase contrast microscopy.

A, this micrograph shows that each septum of connective tissue extends for only a limited length. For example the one indicated by arrowheads is confined to this microscopic field. Calibration: 50 μm.

B, groups of muscle cells are separated by septa of connective tissue (see Pl. 1 for a similar tissue in transverse section). A close inspection shows that the septa of connective tissue are not parallel to the group of muscle cells but form an angle of between 4 and 5° (in muscles mildly stretched). Calibration: 50 μm.

C, a micrograph of the same preparation as *B* at higher magnification showing that septa of connective tissue and groups of smooth muscle cells are not exactly parallel. Calibration: 25 μm.