A STUDY OF THE INNERVATION OF THE TAENIA COLI

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ABSTRACT

An electrophysiological and anatomical study of the guinea pig taenia coli is reported. Changing the membrane potential of single cells cannot modulate the rate of firing action potentials but does reveal electrical coupling between the cells during propagation. The amplitude of the junction potentials which occur during transmission from inhibitory nerves is unaffected in many cells during alteration of the membrane potential, indicating electrical coupling during transmission. The taenia coli is shown to consist of smooth muscle bundles which anastomose. There are tight junctions between the cells in the bundles, and these probably provide the pathway for the electrical coupling. The smooth muscle cells towards the serosal surface of the taenia coli are shown electrophysiologically to have an extensive intramural inhibitory innervation, but a sparse sympathetic inhibitory and cholinergic excitatory innervation. These results are in accordance with the distribution of these nerves as determined histochemically. As single axons are only rarely observed in the taenia coli, it is suggested that the only muscle cells which undergo permeability changes during transmission are those adjacent to varicosities in the nerve bundles. The remaining muscle cells then undergo potential changes during transmission because of electrical coupling through the tight junctions.

INTRODUCTION

Although extensive studies have been made of the spontaneous electrical activity (30, 12, 31) and of the innervation of the guinea pig taenia coli (3, 5, 9, 10) there have been few anatomical studies of this tissue (44, 52). Several investigations have indicated that the functional unit or effector in this tissue is the muscle cell bundle, within which there is electrical coupling between adjacent smooth muscle cells (12, 41, 51). The cells are mostly divided into those showing pacemaker-like spontaneous activity, consisting of slow diastolic depolarizations between successive action potentials, and those showing driven activity, in which the membrane potential is almost constant between action potentials (9). Part of the present study is concerned with providing further evidence that the smooth muscle cell bundle is the effector, showing the arrangement between the bundles, and describing the electrical and morphological coupling between the smooth muscle cells.

It seems likely that the junction potentials which occur in some of the smooth muscle cells of the guinea pig vas deferens during transmission from the hypogastric nerve are electrotonic potentials[1] (6, 14). These potentials have been interpreted as due to electrical coupling of these cells with those which undergo a permeability change during transmission (16). It has not previously been deter-

[1] The junction potentials in a large proportion of the smooth muscle cells are not affected by polarization of the smooth muscle cell membrane, even though normal action potentials can be obtained in these cells.

mined whether electrical coupling occurs between the smooth muscle cells of the taenia coli during transmission, although the ionic currents responsible for the potential changes during transmission have been studied (4, 8, 13). The extent of such coupling is examined in the present work.

Two different kinds of inhibitory innervation of the taenia coli have recently been described electrophysiologically: postganglionic sympathetic inhibitory nerves, which release noradrenaline (9), and intramural inhibitory nerves, which release an unidentified transmitter (3, 7, 10). The taenia coli also receives an innervation from cholinergic excitatory nerves (5). A comparison has been made in the present study between the distributions of these nerves as determined by electrophysiological and anatomical techniques. This has been done in the hope that a clearer understanding may emerge of what constitutes the autonomic neuromuscular junction in the intestine (7, 27, 45).

MATERIALS AND METHODS

Anatomy

Mature guinea pigs of either sex were used throughout this work. The animals were killed by cervical fracture followed by severing the carotid arteries.

Rectangular sections of the cecum wall, including approximately 2 cm long strips of taenia coli, were excised and stretched to their in vivo length on a wax plate.

NERVE FIBER STAINING: The preparations were immersed in an osmium tetroxide–zinc iodide solution (17) for 16–24 hr before being dehydrated in ethanol and embedded in paraffin wax. Four groups of transverse, horizontal, and longitudinal sections 7–10 μ thick were cut, each series covering a distance of 1.0 cm.

NUCLEAR STAINING: The preparations were fixed in Bouin's solution and embedded in paraffin wax. Transverse serial sections 8 μ thick were cut and stained with celestine blue–Van Gieson's double contrast stain. The disposition of small nerve bundles and of the nuclei was determined by photographing transverse sections of the taenia coli taken over a length of 1.0 cm, and projecting these onto a large grid where they were plotted and counted.

ELECTRON MICROSCOPY: The preparation was fixed by dripping the fixative directly on the tissue in situ for 2 min, after which a 1.0 cm length section of cecum wall, including taenia coli, was excised and pinned to a wax plate where fixation was continued for 5 min. After all spontaneous movement had ceased the material was trimmed, cut into 2 mm lengths, and refixed for 1–3 hr.

The following fixatives were used: (*a*) ice-cold Palade's solution (40), (*b*) ice-cold 1% potassium

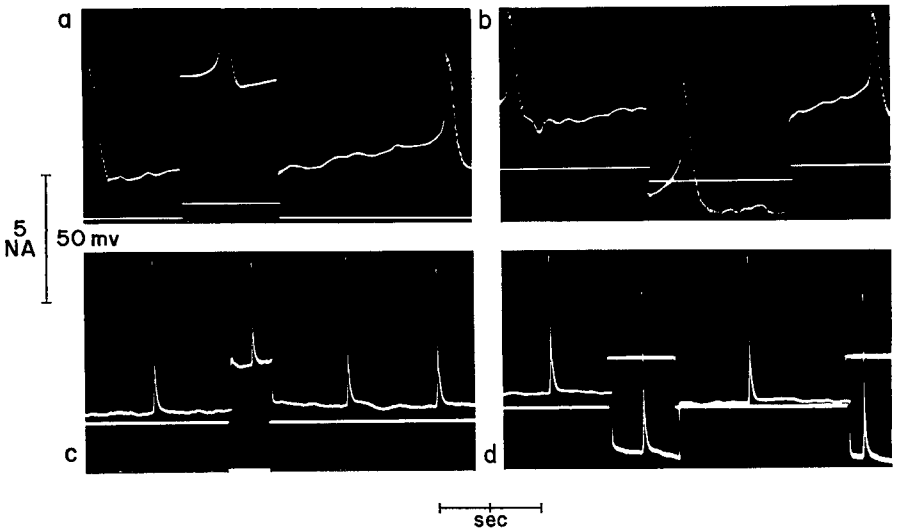


FIGURE 1 The effect of membrane potential changes due to passing current across the smooth muscle cell membrane during the spontaneous firing of action potentials. *a* and *b*, pacemaker cell. Depolarization of the membrane decreases the amplitude of the action potential; hyperpolarization increases the amplitude. *c* and *d*, driven cell. Change in membrane potential has the same effect on the action potential as in *a* and *b*. Note the difference in duration of pacemaker and driven action potentials. Rectangular pulses give the amplitude and duration of the current pulses.

permanganate in Veronal-acetate buffer (32), (*c*) ice-cold 2% osmium tetroxide in McEwen's buffer (35), and (*d*) 5% glutaraldehyde in 0.1 M sodium cacodylate or Millonig's buffer (36) and postfixation in ice-cold Millonig's osmium tetroxide fixative. Dehydration was carried out in either methanol or acetone and all material was embedded in Araldite. Silver-to-gold sections were cut and stained with either lead citrate (42) or lead citrate and 2% uranyl acetate. Serial sections were cut over a distance of 15 μ and mounted three per grid (200 mesh). Comparable areas were examined and photographed on at least one section of each grid.

Electrophysiology

In vitro preparations of the guinea pig taenia coli were prepared and studied in the same manner as that previously described (9). The same microelectrode was used for both recording membrane potentials and passing current. The potential recorded across the electrode resistance during current passing was balanced out using the Wheatstone bridge method of Araki and Otani (2). The bridge balance was checked for both depolarizing and hyperpolarizing currents before and after each impalement. The observations recorded when the bridge was considerably out of balance, as determined after the impalement, were discarded. The sympathetic nerves were excited by stimulating the perivascular nerves to the taenia coli through platinum wire electrodes (9). The intramural inhibitory nerves of the taenia coli were stimulated through two 1.5 mm diameter loops of platinum wire placed around the taenia coli and separated by 2 mm (10). Both sets of nerves were stimulated with current pulses of 200 μsec duration. Atropine sulfate (10^{-7} g/ml) was sometimes present in the solution bathing the preparations so as to eliminate the effects of the cholinergic nerves.

RESULTS

Electrophysiological Identification of the Effector

It seems likely that the effector responsible for propagation of the action potential in the taenia coli is not a single cell, but a group of smooth muscle cells (7, 51). The rate of firing of spontaneous action potentials in either pacemaker or driven cells was not altered when the membrane potential was increased or decreased by passing current through an intracellular electrode (Fig. 1) (31). It may be suggested that a change in the membrane potential of driven cells (Fig. 1 *c*, *d*) would not be expected to modulate the rate of action potential firing, as the spikes only occur in these cells because of electrical coupling with

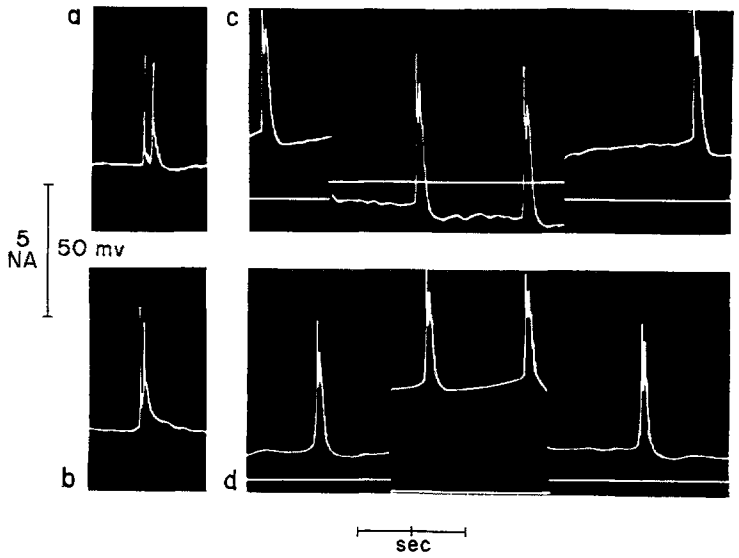


FIGURE 2 The formation of a double-spike action potential from two separate action potentials. *a* and *b*, two action potentials occurring at progressively closer intervals. *c* and *d*, hyperpolarization and depolarization of the membrane during the fully developed double-spike action potential, revealing the regenerative nature of both components of the action potential. Rectangular pulses give the amplitude and duration of the current pulses.

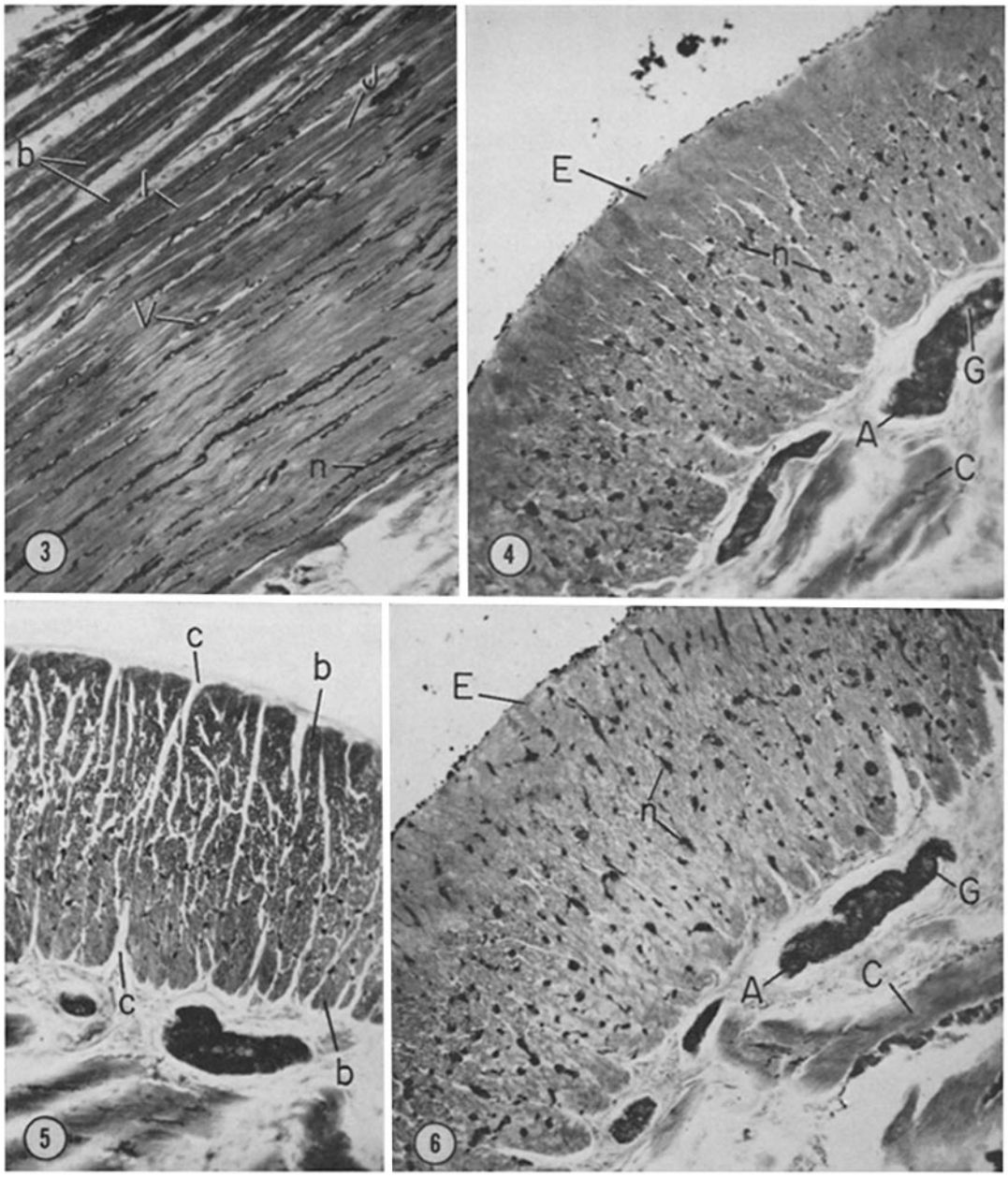


FIGURE 3 Horizontal section through the taenia coli (towards the serosal surface) showing the arrangement of the muscle cells into parallel aligned bundles (*b*). Nerve fibers (*n*) and capillaries (*V*) course through the connective tissue bands separating the muscle bundles. At irregular intervals adjacent bundles fuse (*J*) or slender interconnections (*I*) are apparent. Champy fixation and staining; paraffin wax–embedded. × 150.

FIGURES 4 and 6 The nerve fibers (*n*) but not the ganglion cells (*G*) are stained an intense black. In Fig. 4 there are comparatively few nerve fibers visible towards the serosal surface (*E*) of the preparation, in contrast with Fig. 6. Fig. 6 is sectioned in a region where numerous nerve fibers are passing towards the serosal surface; hence the orientation of the nerve bundles in the upper layers of the taenia coli differs from that in the more basal layers. *A*, Auerbach's plexus; *C*, circular smooth muscle layer of cecum. Champy fixation and staining; paraffin wax–embedded. × 150.

FIGURE 5 In this transverse section through the taenia coli the connective tissue bands (*c*) separating the muscle bundles (*b*) are exaggerated as a result of shrinkage during preparation. Numerous connections between adjacent bundles are evident. Champy fixation and staining; paraffin wax–embedded. × 150.

adjacent cells. However, as intracellular current pulses were unable to modulate the rate of firing in pacemaker cells, in which presumably there is a continual cycle of membrane permeability changes which should be modified by changes in membrane potential (28), other explanations for this effect must be sought. It was not possible to initiate propagating action potentials with an intracellular electrode as judged from the lack of contraction of the whole tissue during stimulation of single cells. These results can be explained by assuming that a propagating action potential occupies a membrane area much larger than that of a single cell, and therefore changing the membrane potential of a few cells is insufficient to initiate action potential propagation (37, 46) or modulate the rate of action potential firing. Further evidence which points to the fact that a single cell contributes little to the action currents which are responsible for the propagated action potential is indicated by the very different levels at which the action potential is initiated when the membrane potential is altered (Figs. 1, 2, and 8). It seems unlikely that the change in threshold could simply be due to inactivation or delayed rectification as described in the ionic theory (28); rather, it seems likely that most of the current producing the spike comes from sources other than the impaled cell.

In some smooth muscle cells of the taenia coli, double-spike action potentials were occasionally observed (Fig. 2). These double-spike action potentials were formed from two separate action potentials as shown in Fig. 2 *a* and *b*. Further evidence for this interpretation was obtained by changing the membrane potential of these cells during the double-spike action potential. Hyperpolarization of the membrane increased the amplitude of both spike components of the action potential (Fig. 2 *c*), while depolarization decreased both components (Fig. 2 *d*). The effect of membrane potential change on the amplitude of both components was therefore in the direction expected for a simple action potential or regenerative potential change (28). As the maximum rate of firing of action potentials in pacemaker cells of the taenia coli is about 1 cycle/sec (9) it would be expected that, unless there are several pacemaker areas close together which independently generate

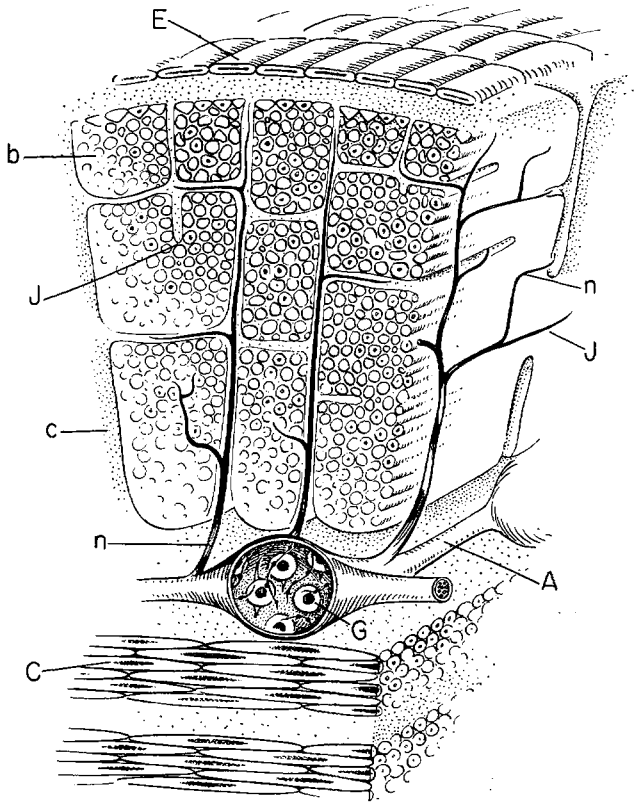


FIGURE 7. Simplified diagram of a section through the taenia coli and underlying tissue. *A*, Auerbach's plexus; *b*, smooth muscle cell bundle; *C*, circular smooth muscle layer; *c*, connective tissue; *E*, serosal epithelium; *G*, ganglion cell; *J*, junction of muscle bundles; *n*, nerve bundle.

action potentials in the same group of muscle cells, the rate of action potential firing in driven cells would be no greater than 1 cycle/sec. The double-spike action potentials may therefore represent two action potentials having their origin in two different but interconnected groups of muscle cells (12).

Structure of the Effector

Transverse, horizontal, and longitudinal sections show that the smooth muscle cells are grouped into bundles which are separated by bands of connective tissue (Figs. 3 and 5). The intervening connective tissue bands, which also serve as passageways for capillaries and nerve bundles, are widest at the basal surface of the taenia coli but tend to become thinner towards the serosal surface. The muscle bundles do not extend in an uninterrupted fashion from the basal to the serosal surface (Figs. 3, 6, and 7) but are divided at irregular intervals by horizontally disposed bands of connective tissue (Figs. 5 and 7). The bundles vary from 18 to 100 μ wide and from 20 to 300 μ high and have been observed in horizontal sections to extend along the length of the taenia coli for over 2.0 mm before losing their individual identity by fusion with neighboring bundles. Fusion of adjacent bundles occurs either by broad connections involving 40 or more cells or via narrow bands of cells (Fig. 3). It is suggested that the bundles are the structural equivalent of the electrophysiological effector. The connections between bundles would allow for electrical coupling and the passage of propagating action potentials from one effector to another.

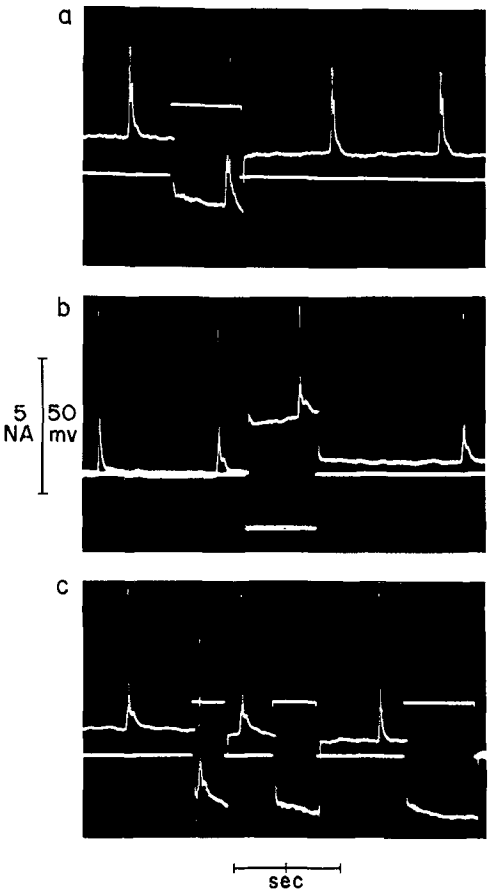


FIGURE 8. Action potential coupling between driven smooth muscle cells. The amplitude of the slow coupling potential at the foot of the spike is unchanged during a change in membrane potential, whereas the spike amplitude changes as for a regenerative response. The time course of the coupling potential is different in *a* and *b*. In *b* and *c*, a spike is shown in which no coupling potential is clearly evident, presumably because the spike was initiated from the peak of the coupling potential. Rectangular pulses give the amplitude and duration of the current pulses.

Action Potential Coupling between Smooth Muscle Cells in the Effector

There is evidence to suggest considerable electrical coupling between the smooth muscle cells of the taenia coli (12, 31, 51). In cells showing driven activity (Fig. 8) the slow depolarizations at the foot of the action potentials were not altered by changing the membrane potential with an internal electrode, although the fast component of the action potential was increased by hyperpolarizing currents and decreased by depolarizing currents (Fig. 8). At junctions where electrical coupling occurs, polarization of the membrane at one side of the junction with an intracellular electrode does not alter the amplitude of the coupling potential (24, 34) which is due to action potential firing on the other side of the junction. As the slow depolarizations at the foot of the action potentials are unaltered by membrane polarization they are probably coupling potentials due to action potential firing in adjacent cells. Polarization of the membrane during an action potential or regenerative potential change should alter the amplitude of the action potential (28) and, as this occurred to the spike during current passing (Fig. 8), this fast component is probably due to a regenerative permeability change in the impaled cell.

Junction Potential Coupling between Smooth Muscle Cells in the Effector

Stimulation of the intramural inhibitory nerves to the taenia coli with single pulses results in a transient hyperpolarization of the smooth muscle cell membrane of about 20 mv (the inhibitory junction potential, IJP) which interrupts the spontaneous firing of action potentials (Fig. 9 *a*) and lasts for about 1 sec (10). Repetitive stimulation of these nerves leads to a summation of IJP's and hyperpolarizations of 30–40 mv (Fig. 10 *a*), followed by a period in which the rate of action potential firing is enhanced (1, 3). As it has been shown that the IJP is probably due to an increase in membrane permeability of potassium ions (8) the amplitude of the IJP should change with a change in the membrane potential (18, 48), for the potential difference between the membrane potential and the equilibrium potential for potassium ions provides the driving force for the currents which pass across the membrane during transmission and give rise to the junction potential. In many cells the amplitude of the IJP remained unaltered when the membrane potential was changed with an intracellular electrode (Fig. 9). In some cells a change in the membrane potential of up to 40 mv did not alter the amplitude of the IJP because of stimulation at 30 cycle/sec (Fig. 10).

The hyperpolarization which occurs in some smooth muscle cells of the taenia coli during sympathetic inhibitory transmission (9) was generally unaffected by a change in the membrane potential. As electrotonic coupling potentials are not affected by a change in membrane potential it seems likely that the hyperpolarizations in some cells of the taenia coli during inhibitory transmission are only due to electrical coupling with cells which undergo a permeability change during transmission. This coupling of the smooth muscle cells during transmission probably occurs across the same cell contacts which are responsible for electrical coupling during conduction.

Relationship between Smooth Muscle Cells in the Effector

The smooth muscle cells are approximately 200 μ long and 2–4 μ in diameter in the relaxed state. The nucleus is situated towards the center of the cell, and consequently the approximate arrangement of the cells can be ascertained from the disposition of the nuclei within a series of sections. The disposition of the nuclei indicates that the muscle cells have a staggered arrangement and that adjacent cells within a bundle overlap, so that each cell is surrounded by 10–12 other cells (Fig. 11). Projecting from the surfaces of the muscle cells are numerous finger-like processes which, at the highest resolution of the light microscope, appear to bridge the intercellular spaces and connect adjacent cells with each other.

The electron microscope reveals that the muscle cells which are situated towards the serosal surface of the preparation have a somewhat flattened V-shaped profile in transverse section. The flat-

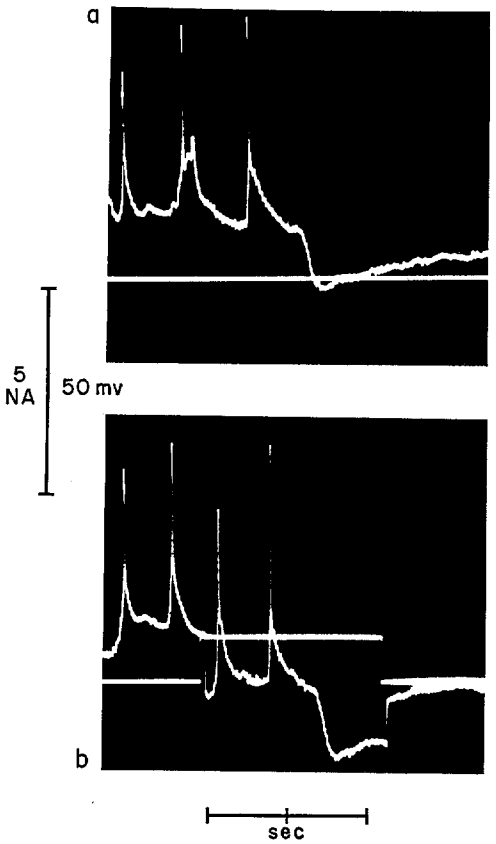


FIGURE 9. Effect of changing the membrane potential of a smooth muscle cell in the taenia coli during stimulation of the intramural inhibitory nerves with single pulses. *a*, hyperpolarization during the IJP in one cell due to stimulation of the intramural inhibitory nerves. *b*, change of the membrane potential of the same cell as in *a*, with an intracellular electrode, during the IJP. There is no change in amplitude of the IJP in *b*. Rectangular pulses give the amplitude and duration of the current pulses.

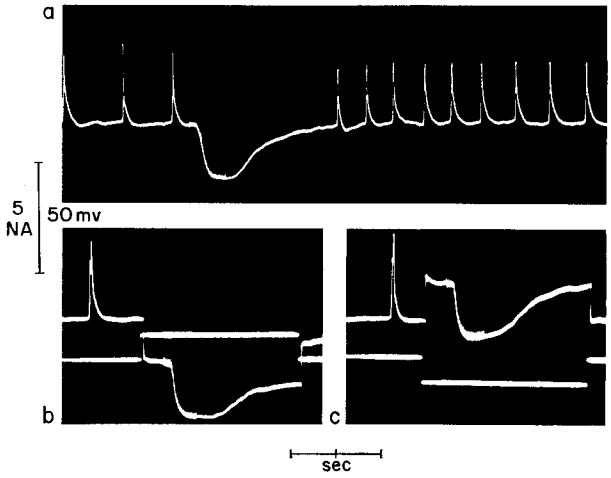


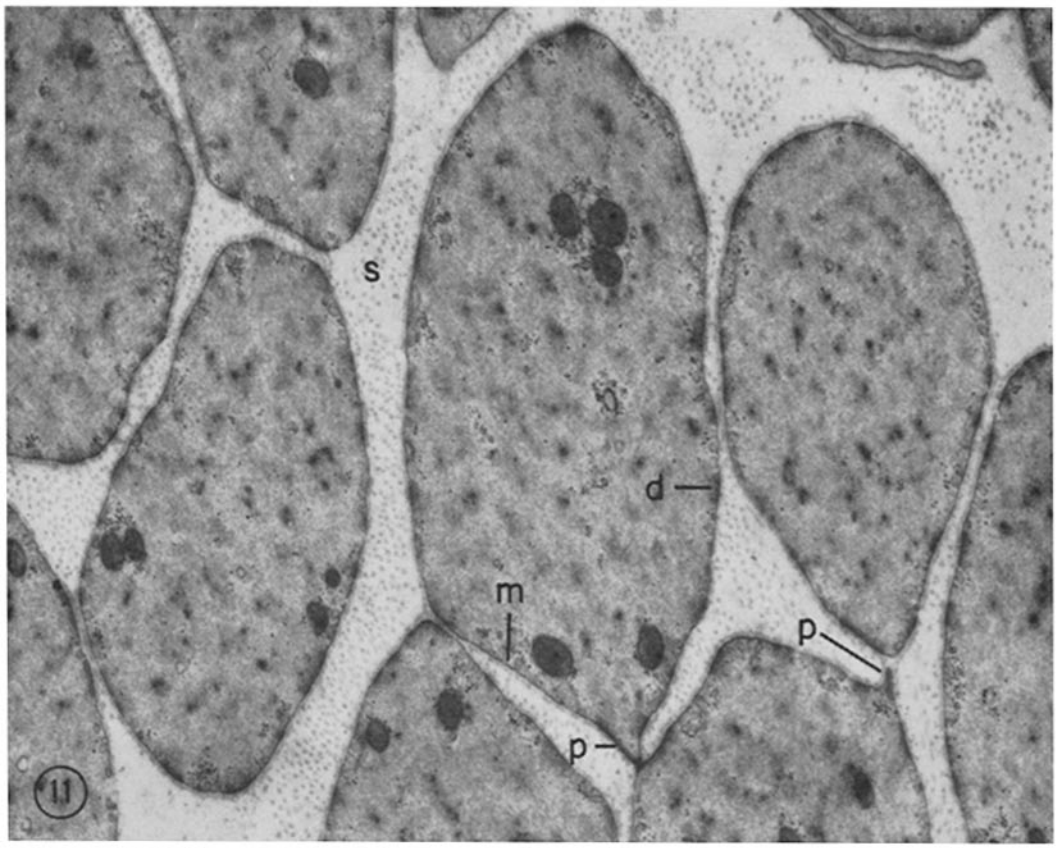
FIGURE 10. Effect of changing the membrane potential of a smooth muscle cell in the taenia coli during repetitive stimulation of the intramural inhibitory nerves, at 30 c/sec. *a*, hyperpolarization during the IJP in one cell due to repetitive stimulation of the inhibitory nerves. Note the increase of rate of action potential firing at the end of the IJP. *b* and *c*, alteration of the membrane potential of the same cell as in *a*, with an intracellular electrode, has little effect on the amplitude of the IJP. Rectangular pulses give the amplitude and duration of the current pulses.

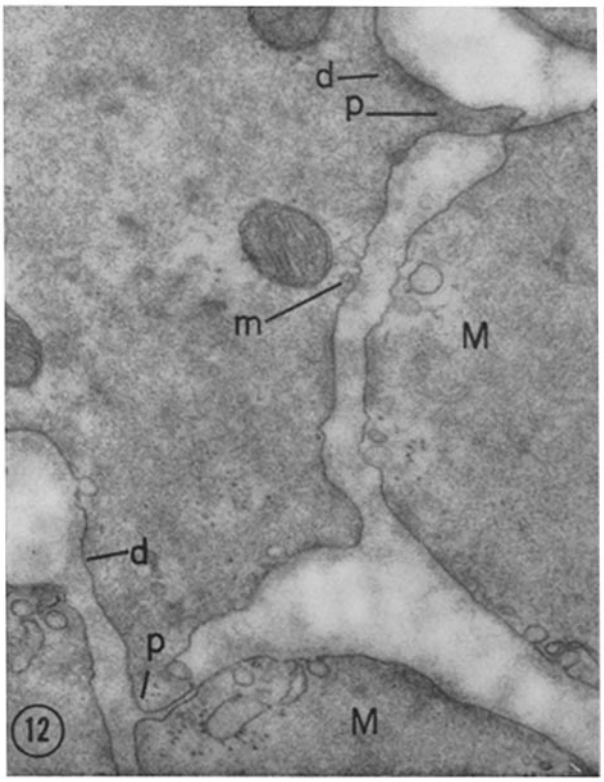
tened cell surfaces and particularly the angles of the cell profiles come into extremely close apposition with neighboring cells. More deeply situated cells usually are more widely separated but make contact with adjacent cells by distinct protrusions. The morphology of these protrusions depends on the method of fixation. Preparations fixed in osmium tetroxide–McEwen's buffer are composed of cells from the surfaces of which project numerous flanges (Figs. 11, 12, and 13). In transverse section these structures appear finger-like; however, serial sections demonstrate that they are approximately 0.1 μ wide, 0.4 μ high, and 1–2 μ long. An intercellular space of 50–150 A has always been observed between the summits of the flanges and the adjacent plasma membranes (Fig. 14). These projections are usually derived from the "dark areas" (Figs. 11–14) on the plasma membrane and contain the same inclusions as comparable regions which are not developed as intercellular bridges. Preparations fixed in Palade's solution or potassium permanganate are composed of cells which show bulbous surface projections (Figs. 16, 17, and 18). Often the cytoplasmic bulges are of comparatively low electron density and are attached to the parent cell by "necks" of various dimensions (Fig. 18). These bulges intrude into the intercellular space or into invaginations of neighboring muscle cells. Where the bulbous processes intrude into an adjacent cell the apposing plasma membranes form a nexus or tight junction (Figs. 16 and 17). Fusion of the membranes is usually not complete over the entire area of the intrusion (Figs. 16 and 17). In view of the generally low electron density of these bulbous protrusions and the enclosure of the neck by dark areas on the parent cell (Fig. 18) it is considered that these bulges are derived from those areas on the plasma membrane in which microvesicles occur.

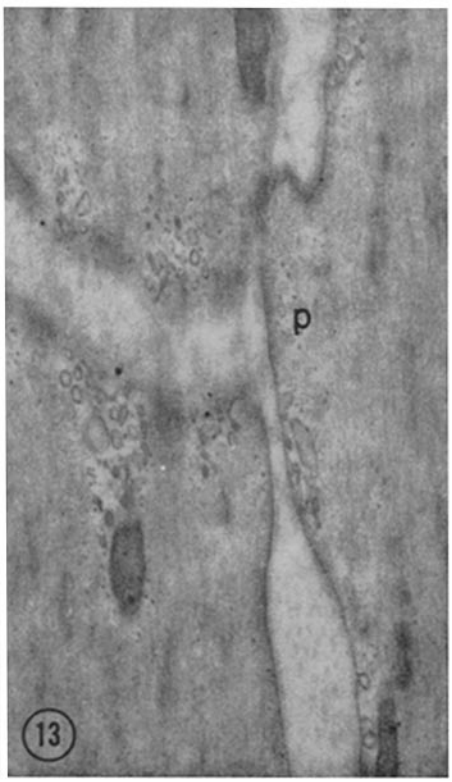
FIGURE 11 Transverse section through basally situated layers of taenia coli. Note wide intercellular spaces (*s*) and finger-like projections (*p*) from the surfaces of the muscle cells. Each of the three centrally situated cells is adjacent to five to six muscle cells. *m*, microvesicles; *d*, dark area. OsO_4-McEwen's buffer–fixed; section stained with lead citrate. × 14,000.

FIGURE 12 Finger-like projection (*p*) from one cell to two neighboring muscle cells (*M*). *d*, dark areas; *m*, microvesicles. OSO_4-McEwen's buffer–fixed; section stained with lead citrate. × 32,000.

FIGURE 13 In longitudinal section the flangelike appearance of the muscle cell projections (*p*) shown in Figs. 11 and 12 is apparent. OsO_4-McEwen's buffer–fixed; section stained with lead citrate. × 20,000.







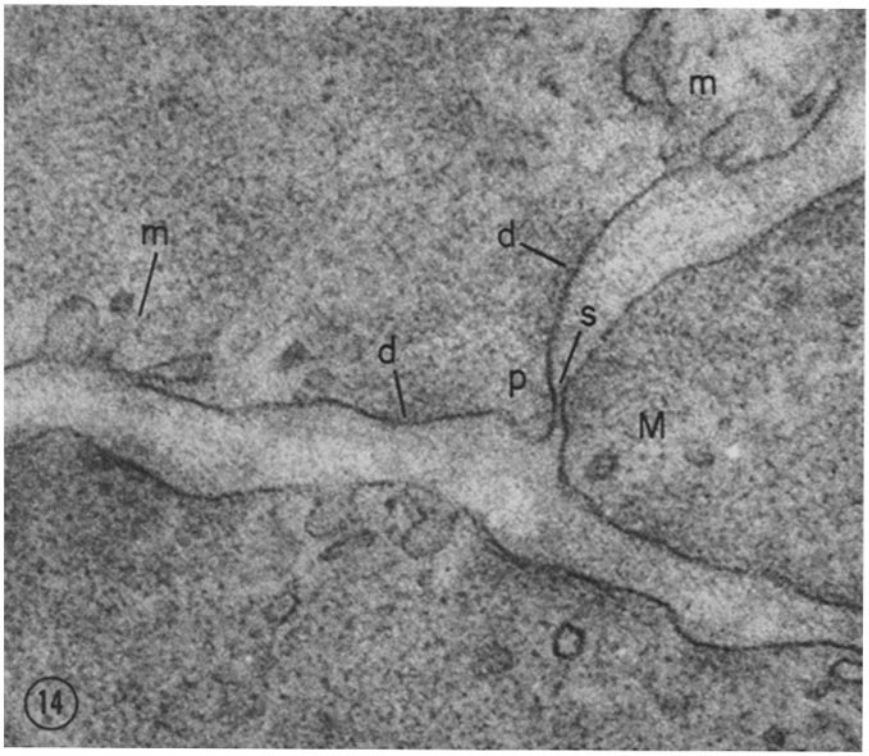


FIGURE 14 High magnifications reveal that a definite intercellular space (*s*) exists between the summit of the muscle cell projection (*p*) and the adjacent cell (*M*). Areas of microvesicles (*m*) lie on either side of the protrusion. Note alternation of microvesicles and dark areas (*d*) on the plasma membrane. OsO_4-McEwen's buffer–fixed; section stained with lead citrate. × 56,000.

Electrophysiological Study of the Innervation of the Effector

Microelectrodes were inserted into the serosal surface of the taenia coli, and the membrane potential changes of smooth muscle cells due to stimulation of inhibitory nerves were observed. Maximal stimulation of the intramural inhibitory nerves at frequencies as small as 1 cycle/sec blocked action potential firing in pacemaker cells. In driven cells neither action potential firing nor coupling potentials occurred during stimulation of these nerves (Figs. 9 *a* and 10 *a*). Action potential firing must therefore be blocked in pacemaker cells throughout the taenia coli as well as in those cells which receive a predominantly excitatory innervation (5), indicating that the smooth muscle cells receive an extensive intramural inhibitory innervation.

The sympathetic inhibitory innervation of the smooth muscle cells seems to be much less extensive than that of the intramural inhibitory nerves. Stimulation of these nerves at high frequencies sometimes left the membrane potential of driven cells unaltered, although there was a cessation of spontaneous action potential firing (Fig. 19 *c*), indicating an inhibition of pacemaker activity elsewhere (Fig. 19 *a*, *b*). Thus despite the electrical coupling of cells during transmission the membrane potential of some cells remains unchanged during sympathetic nerve stimulation, indicating that there is a sparse sympathetic innervation of the effectors towards the serosal surface. This interpretation is supported by the observation that single sympathetic junction potentials were never observed, membrane potential changes occurring only at high rates of nerve stimulation after a latency of hundreds of milliseconds (9, 25). Transmitter must therefore diffuse over large distances from sympathetic nerves to the smooth muscle cells.

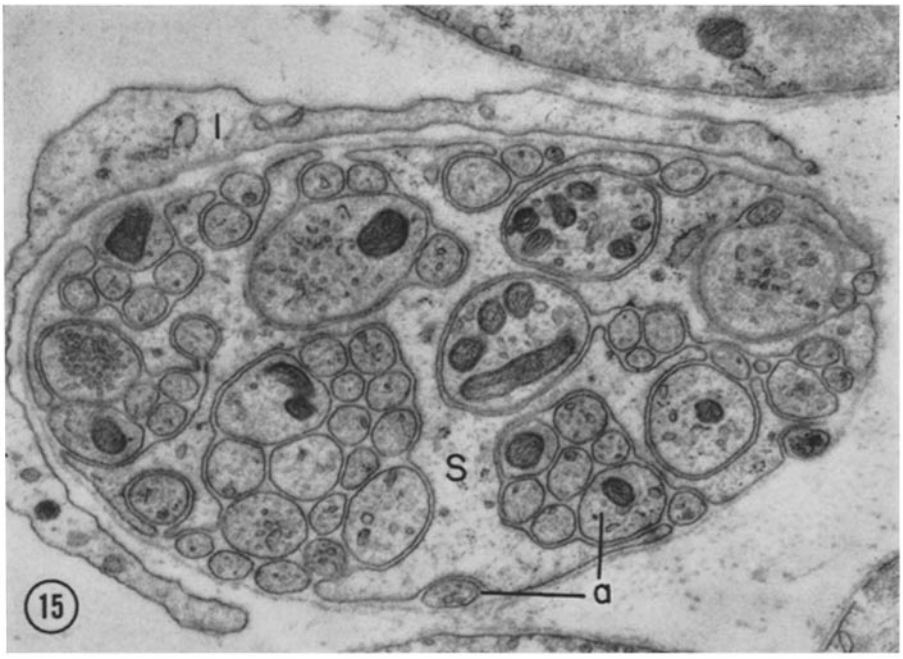


FIGURE 15 Transverse section through medium-to-large nerve bundle. Most of the axon profiles (*a*) are grouped in separate folds of the Schwann cell (*S*); a few single axon profiles lie in shallow grooves on the periphery of the bundle. Basement membrane separates the Schwann cell and axons from the adjacent interstitial cell sheath (*I*). OsO_4-McEwen's buffer–fixed; section stained with lead citrate. × 16,000.

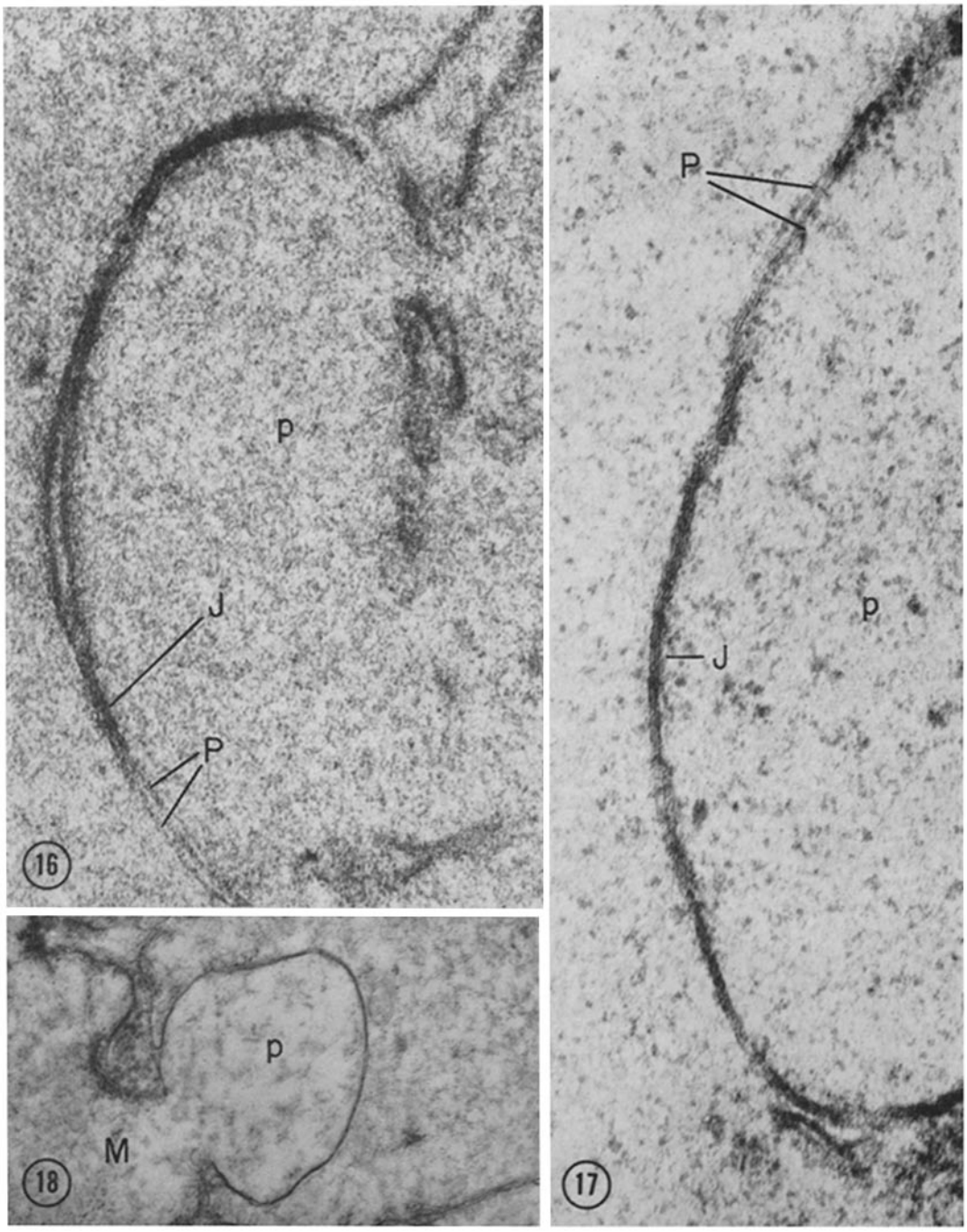
Anatomical Study of the Innervation of the Effector

The neurons of Auerbach's plexus are grouped into ganglia in the connective tissue separating the taenia coli from the circular muscle layer of the cecum (Figs. 4–6). Ganglia are also distributed over the remainder of the surface of the circular muscle layer beneath a subserosal layer of connective tissue. Passing from one ganglion mass to another are thick (15–30 μ diameter) strands of nerve fibers forming a rather open two-dimensional network. Reconstructions of Auerbach's plexus from serial sections have shown that the ganglia are distributed at irregular intervals beneath the taenia coli. Each neuron is enmeshed in a dense plexus of fine varicose nerve fibers, and many of these varicosities when examined with the electron microscope contain granular vesicles. Fluorescent histochemical studies show that many of the axon varicosities on the surface of the ganglion cells contain primary catecholamines (Fig. 20).

Nerve bundles (10–15 μ diameter) leave Auerbach's plexus and pass into the connective tissue bands between the muscle bundles. Occasionally medium-sized nerve bundles (6 μ diameter) pass directly between the circular and longitudinal muscle of the cecum without forming part of Auerbach's plexus.

Arterioles traverse the connective tissue between the longitudinal and circular muscle coats of the cecum. At the point where the arterioles pass into the connective tissue bands at the base of the taenia coli they lose their medial muscle sheath and become confluent with capillaries. The capillaries run with nerve fibers in the connective tissue between the muscle bundles (Fig. 21). The capillary endothelium is nonfenestrated and is frequently in close relation to the processes of fibroblasts (44) and pericytes (Fig. 21).

The density and distribution of nerve fibers in and between the muscle bundles varies both along the length and across the transverse face of the taenia coli. Generally only small nerve bundles (2 μ diameter, 3–5 axons) are found towards the serosal surface. Small, medium (2–10 μ diameter, 20–40 axons), and large (10–15 μ diameter, about



FIGURES 16 and 17 Bulbous muscle cell protrusions (*p*) forming intermittent nexus arrangements (tight junctions) with adjacent cells. *J*, fusion of adjacent plasma membranes; *P*, plasma membrane. Permanganate-fixed; section stained with lead citrate. × 100,000.

FIGURE 18 Bulbous protrusion (*p*) attached by relatively narrow neck to parent cell (*M*). OsO_4-Michaelis' buffer–fixed; section stained with lead citrate. × 16 000.

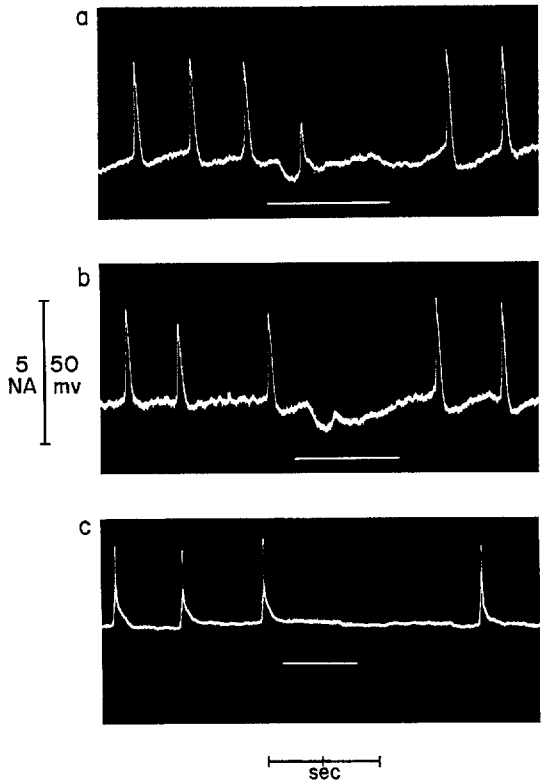


FIGURE 19. Effect of repetitively stimulating the sympathetic nerves on pacemaker and driven smooth muscle cells. *a* and *b*, the diastolic depolarization in a pacemaker cell is not sufficient to fire an action potential during the hyperpolarization due to stimulation of the sympathetic nerves at 30 c/sec. *c*, action potential firing ceases without any hyperpolarization of the membrane of a driven cell during stimulation of the sympathetic nerves at 30 c/sec. The white bars give the duration of stimulation of the nerves.

70 axons) nerve bundles are found in the connective tissue bands in the underlying muscle of the taenia coli, but only the medium and small nerve bundles enter the muscle bundles (Figs. 22, 23, and 24). At intervals of about 1 mm along the taenia coli there is a marked decrease in the number of small nerve bundles (Figs. 4 and 6). As only one example of a single axon (Fig. 22) has been observed (also compare Yamauchi, 52) it seems likely that all the axons in a small nerve bundle terminate within a short distance of each other (Fig. 25).

The large nerve bundles are more or less completely surrounded by the processes of fibroblasts (Figs. 26 and 27), whereas the smallest nerve bundles frequently do not have an investing connective tissue cell sheath (Figs. 21–24). The axon profiles in transverse sections of the nerve bundles are enclosed partially or completely by processes of Schwann cells (Figs. 15, 26, and 29). The axons are arranged within the Schwann sheath either as groups of 2–30 fibers or as individual fibers usually situated on the periphery of the bundle (Fig. 15). Serial sections show that the number of axons in each group can change within 10–15 μ, either as a result of a change in the disposition of the Schwann sheath or as a result of collateral sprouts leaving one group of axons and entering another in the same bundle.

The diameter of the axon profiles varies from 0.08 μ to 4 μ (Fig. 29). The large axon profiles correspond to the varicosities seen with the light microscope. Evidence for this view has been obtained from longitudinal and serial sections through the nerve bundles (Fig. 30). Conversely the smallest axon profiles correspond to the intervaricosities (Fig. 30). Varicosities vary in their dimensions but generally have a fusiform appearance (Fig. 30) so that axon profiles with a wide range of diameters are always observed in a transverse section through a nerve bundle. The relative proportion of axon varicosity profiles to axon intervaricosity profiles in transverse sections is 2:3.

The varicosities contain widely dispersed neurotubules, clumps of neurofibrils, and agranular and granular vesicles (Figs. 22–24, 26–30). In some axons the agranular vesicles are evenly distributed throughout the varicosities (Fig. 27); in others the vesicles are clumped together either in the center (Fig. 30) or against the outer membrane, facing a smooth muscle cell (Figs. 24, 26, and 28). In order to detect the granular vesicles easily, a combination of glutaraldehyde fixation and double staining has been found to be most satisfactory. Approximately 15% of the axons contain granular vesicles and, although in any one varicosity up to 12 of these vesicles have been observed, the vesicles are never clumped together or show any preferential arrangement with regard to the axon membrane.

Longitudinal and serial sections show that because of the interweaving of the axons within the nerve bundles most of the varicosities which contain agranular and granular vesicles occur on the periphery of the bundles, where often no Schwann cytoplasm intervenes between them and the adjacent muscle cells (Figs. 15, 26, and 30). These varicosities are sometimes as close as 1000 A to the nearest muscle cell profile. In only a few in-

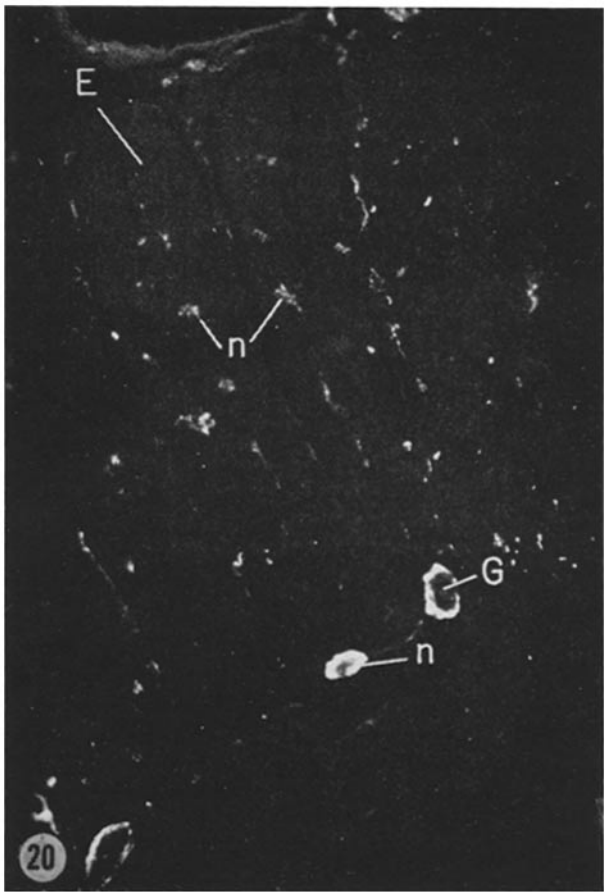


FIGURE 20. Transverse section through the taenia coli and Auerbach's plexus. The nerve fibers (*n*) on the surface of the ganglion cells (*G*) are intensely fluorescent. Fluorescent nerve fibers (*n*) are sparsely scattered throughout the muscle bundles and in the connective tissue bands between the bundles. *E*, serosal surface of taenia coli. Formaldehyde condensation method for monoamines; 1-hr incubation. × 150. (Courtesy of J. R. McLean and G. Burnstock.)

stances has fusion of the adjacent basement membranes of the nerve bundle and muscle cell been observed (Fig. 28).

DISCUSSION

The Effector

The autonomic effector in the muscle layers of the intestine has been identified as a bundle of smooth muscle cells (7). Tomita (51) has shown that the taenia coli consists of cable units which respond in much the same way as an axon to passive electrotonic currents (29). It has also been shown that when the diameter of the taenia coli is reduced to less than about 100 μ, action potential propagation fails (15, 51). These results, together with the inability of single cells to set up propagating action potentials or modulate the rate of action potential firing, indicate that the effector is not a single cell, but a group of cells. As the taenia coli has been shown to consist of muscle bundles it seems likely that these are the functional units. The anastomosing of muscle bundles provides the anatomical basis for the probable electrical coupling between the effectors.

Electrical Coupling of Smooth Muscle Cells in the Effector

Electrical coupling has been shown in some cells during the propagation of the action potential. The tight junctions observed by Dewey and Barr (22) in this tissue and by Oosaki and Ishi (39) and Taxi (49) in other intestinal tissues may provide the anatomical connection for this electrical coupling (24). In this study both tight junctions and areas of very close apposition between adjacent cell membranes have been observed. It is not possible to determine on the basis of the morphological data whether either or both of these anatomical connections are present in vivo. How-

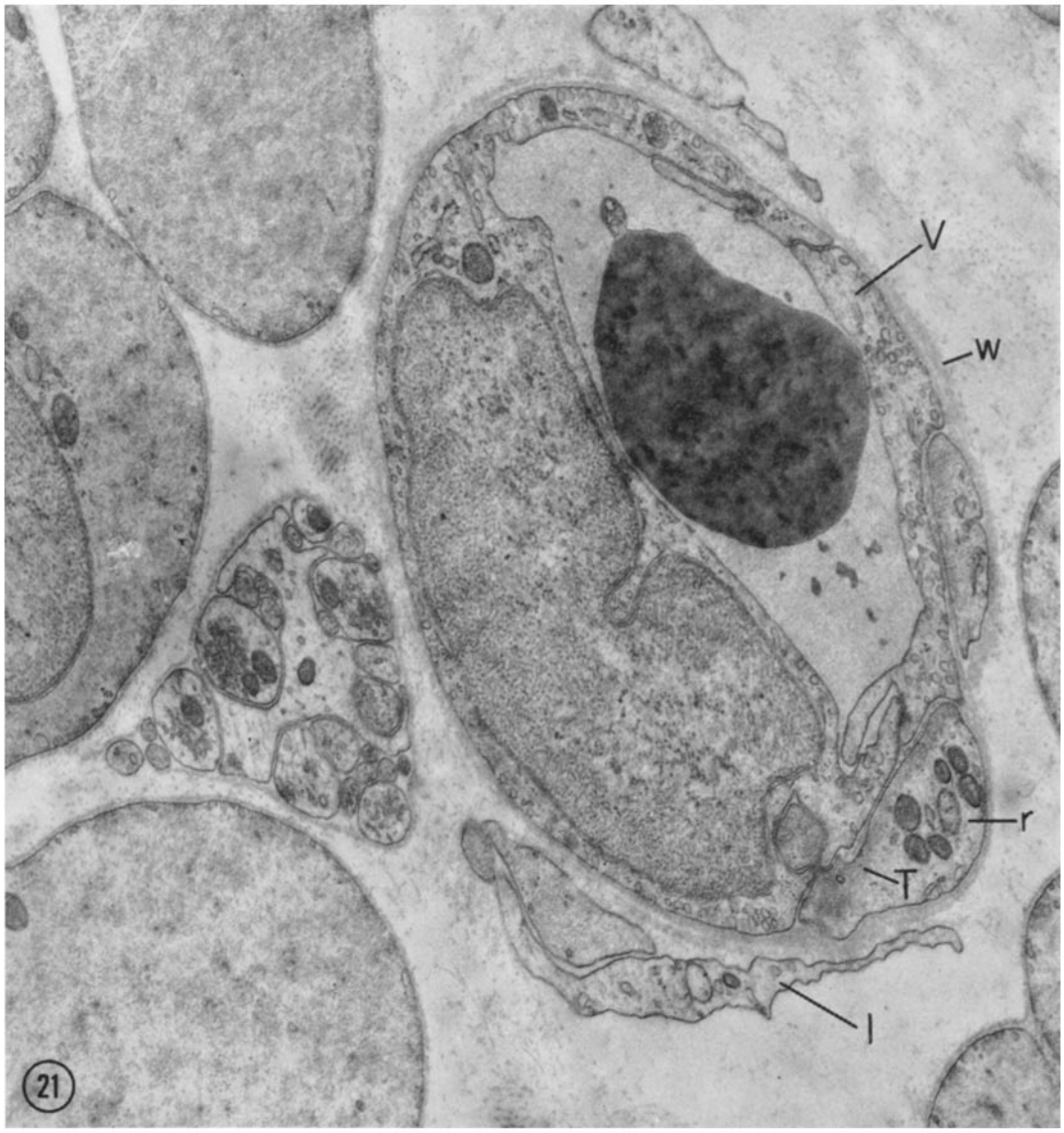


FIGURE 21 A section through a connective tissue band separating two muscle bundles. A capillary and a nerve bundle lie in the connective tissue. The capillary endothelium (*V*) is nonfenestrated and is enclosed in a well-defined basement membrane substance (*w*). Cell profiles external to the endothelium and surrounded by the basement membrane substance are tentatively identified as pericytes (*r*). Cell profiles external to the basement membrane substance but still associated with the capillary are considered to be interstitial cells (*I*). Note dense web (*T*) on endothelial margin of pericyte, not seen in endothelial or interstitial cell. OSO_4-McEwen's buffer–fixed; section stained with lead citrate. × 10,000.

ever, the area of the close appositions as revealed in serial sections is probably insufficient to provide an alternative to the tight junctions for electrical transmission (34). The electrical coupling between the smooth muscle cells during transmission from both the intramural and sympathetic inhibitory nerves probably also occurs as a result of the tight junctions.

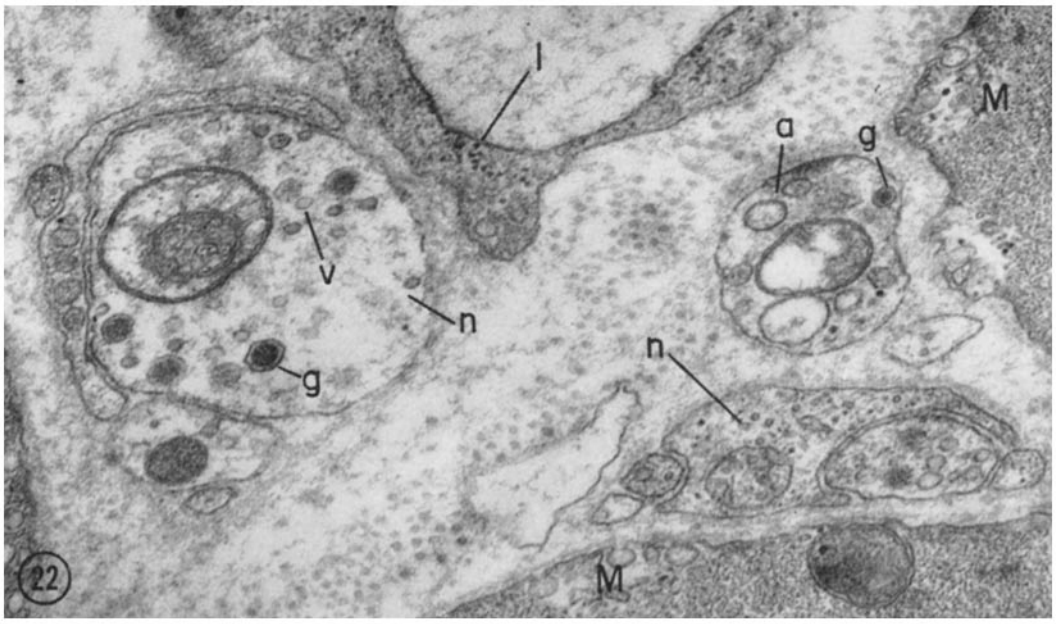


FIGURE 22 Small nerve fascicles (*n*) and a single axon (*a*) lying in close proximity to muscle cells (*M*). The large axon profile in the left fascicle contains four granular vesicles (*g*) as well as scattered agranular vesicles (*v*). The profile on the right is considered to be a single axon because it contains a granular vesicle (*g*); no other cell in the taenia coli contains vesicles of this type. *M*, muscle cell; *I*, interstitial cell. Glutaraldehyde-Millonig's buffer–fixed; OsO_4-postfixation section stained with lead citrate and uranyl acetate. × 55,000.

Innervation of the Effector

As an IJP can be recorded in the smooth muscle cells and its amplitude can be reduced by decreasing the strength of stimulation (10), most of the smooth muscle cells are affected by a number of intramural inhibitory nerves. This effect may be due to the direct action of transmitter on the cell membrane or to the electrical coupling between the cells. Because of the extensive coupling of the IJP between the cells during transmission, all cells do not have to be directly affected by the transmitter and undergo a permeability change. The smooth muscle within a length constant (51) of these cells may then receive an electrotonic potential resembling the IJP through the tight junctions. There is also evidence for such coupling between smooth muscle cells in the vas deferens during excitatory transmission (6, 11).

Since there are only small numbers of nerve bundles towards the serosal surface of the taenia coli very few muscle fibers are directly adjacent to an axon. However, the twisting of axons within the Schwann sheath would permit those muscle cells which are adjacent to a nerve bundle to be influenced by different axons (or collaterals) at different points along their length. A similar arrangement has already been demonstrated by Thaemert (50) in the frog intestine. It is likely that transmitter is released from the varicosities along the length of an axon as well as at the axon termination (7, 11, 33, 38). The smooth muscle cells which are next to the small nerve bundles probably receive a multiple intramural inhibitory innervation from the few axons in these bundles. Electrophysiological studies indicate that about six intramural inhibitory nerves have a substantial effect on each smooth muscle cell (10). If it is assumed that any one smooth muscle cell is affected by only one nerve bundle during transmission, then most of the nerves in the small nerve bundles towards the serosal surface must be of the intramural inhibitory type. This conclusion is supported by the very sparse distribution of adrenergic (sympathetic) fibers as revealed by the fluorescent

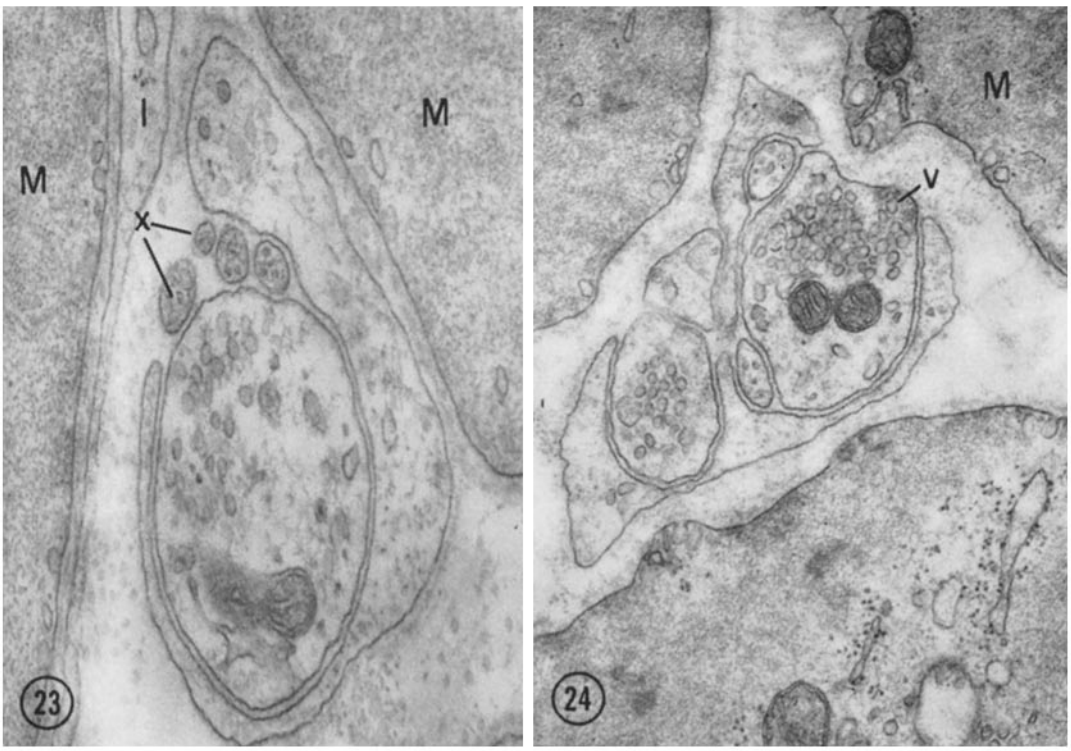


FIGURE 23 Small nerve vascicle traversing a narrow cleft between two muscle cells (*M*). The nerve fascicle is losing contact with the interstitial cell sheath (*I*). *x*, axon intervaricosities. OSO_4-McEwen's buffer-fixed; section stained with lead citrate. × 25,000.

FIGURE 24 Small nerve fascicle which has lost contact with the interstitial cell sheath as it has passed deep into a muscle bundle. Note agranular vesicles (*v*); in one axon profile these are bunched together towards the surface of the axon, which is free of Schwann cell and facing a muscle cell (*M*). OsO_4-McEwen's buffer-fixed; section stained with lead citrate. × 34,000.

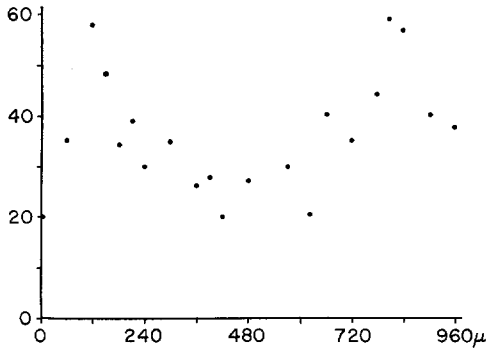


FIGURE 25 The number of small axon bundles (less than 4 μ diameter) observed in transverse sections of the taenia coli over a 1 mm length of tissue. Abscissa, length of taenia coli at which a transverse section was examined. Ordinate, number of axon bundles observed in a transverse section.

histochemical technique. It is also supported by the electrophysiological evidence which points to a large diffusion distance for the sympathetic transmitter, indicating a sparse sympathetic innervation, and to the existence of very few muscle cells receiving a predominantly excitatory innervation (5).

The periodicity observed in the number of small nerve bundles in the taenia coli implies that these nerve bundles either terminate within 1 mm in the muscle, pass out of the taenia coli altogether, or join up to make larger bundles. As the first alternative seems the most likely, all the axons within the small bundles must terminate within a very short distance of each other.

The granular vesicles found in some of the axon profiles of the taenia coli resemble those observed

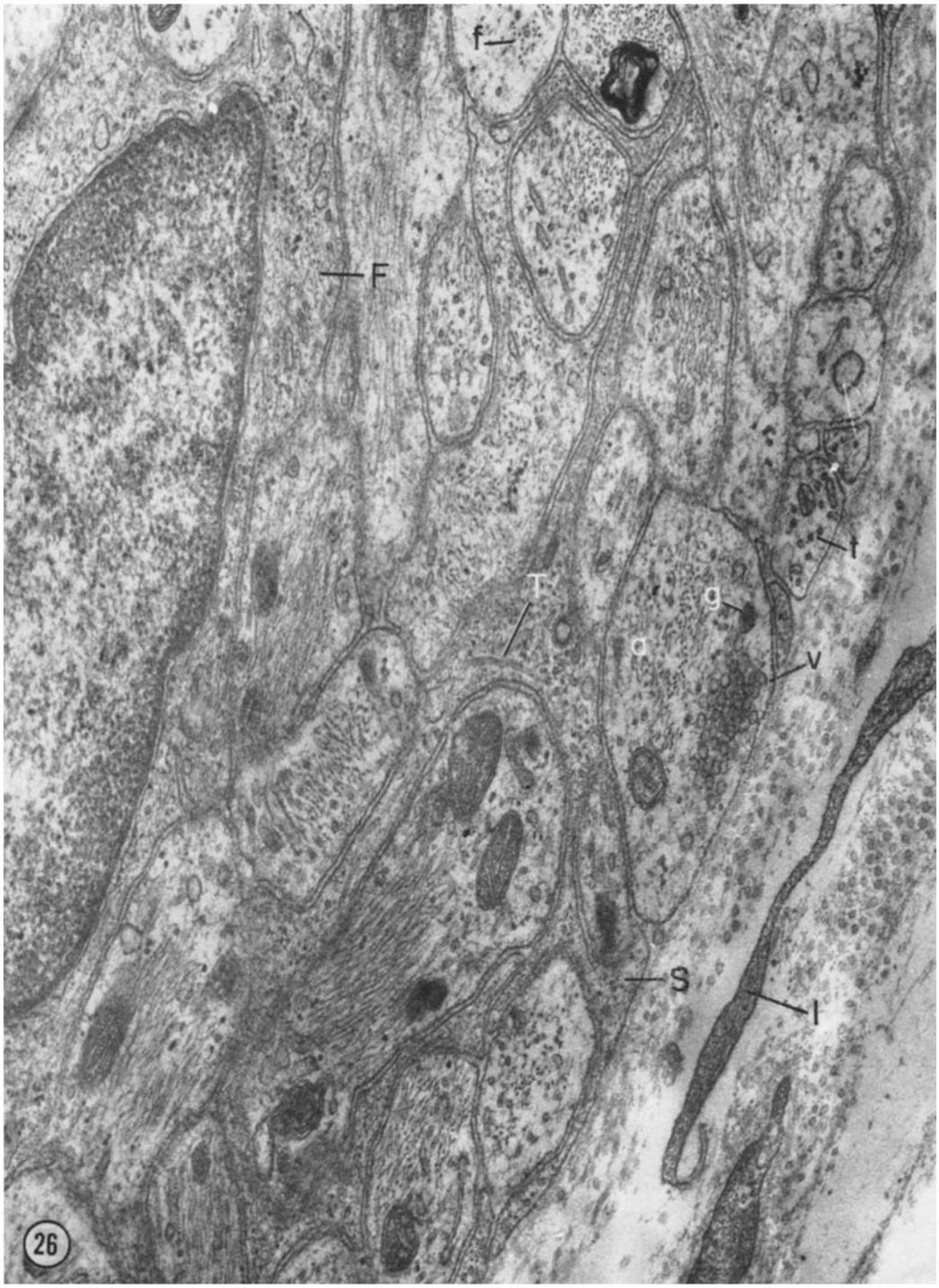


FIGURE 26 Oblique section through a large nerve bundle in the taenia coli. Although one of the axon varicosities (*a*) on the periphery of the bundle has one margin free of Schwann cell cytoplasm (*S*) it remains separated from the adjacent muscle bundle (not shown) by interstitial cell processes (*I*). Nevertheless agranular vesicles (*v*) are conspicuously clumped together against the free margin of the varicosity; a single granular vesicle (*g*) is also present. Note neurofilaments (*f*) and neurotubules (*t*). Tubules (*T*) and filaments (*F*) are also components of the Schwann cell cytoplasm. Glutaraldehyde-Millonig's buffer–fixed; OsO_4 postfixation; section stained with lead citrate and uranyl acetate. × 36,000.

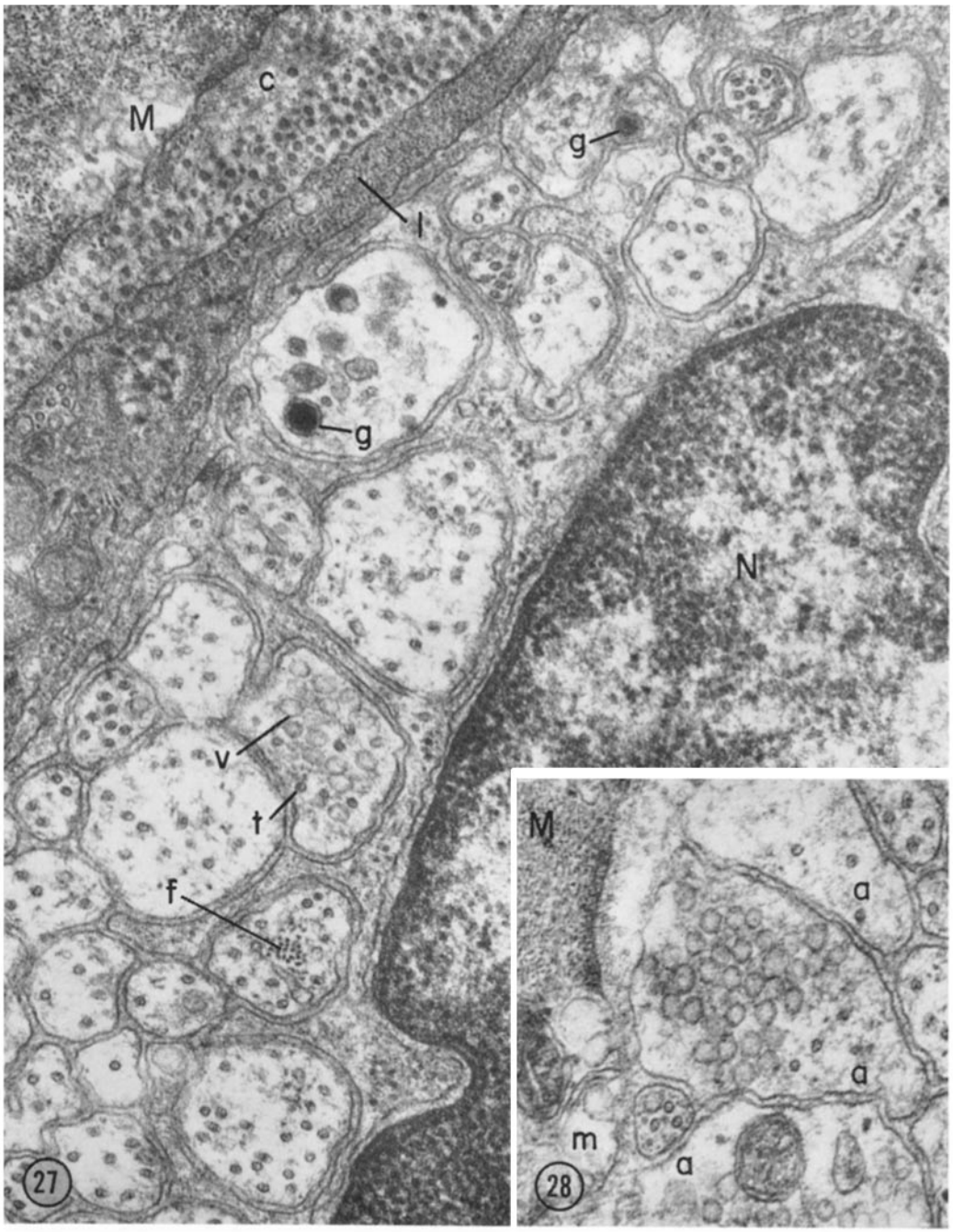


FIGURE 27 Transverse section through a very large nerve bundle. Note granular vesicles (*g*) in two peripherally placed axon profiles, and one profile packed with agranular vesicles (*v*) among which are a few neurotubules (*t*). Most axon profiles contain neurotubules only; neurofilaments (*f*) occur in groups. The interstitial cell sheath (*I*) forms a complete and intimate investment about the nerve bundle. *M*, muscle cell; *c*, connective tissue; *N*, Schwann cell nucleus. Glutaraldehyde-Millonig's buffer–fixed; OsO_4 postfixation; section stained with lead citrate and uranyl acetate. × 62,000.

FIGURE 28 Peripherally placed axon profiles (*a*) in close apposition with an adjacent muscle cell (*M*). *m*, patch of microvesicles on muscle cell membrane. Glutaraldehyde-Millonig's buffer–fixed; OsO_4 post-fixation; section stained with lead citrate and uranyl acetate. × 65,000.

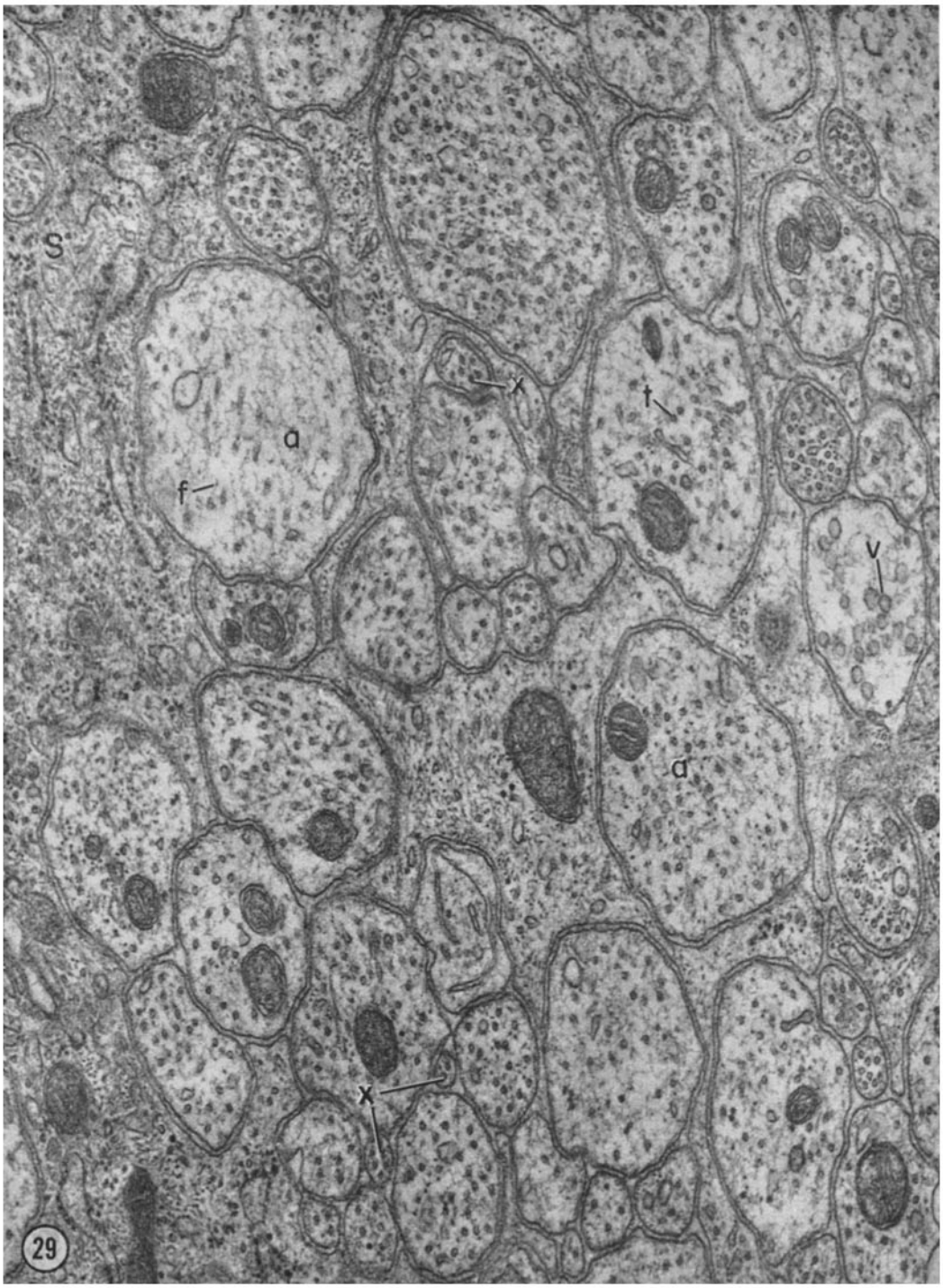


FIGURE 29 Transverse section through a portion of a very large nerve bundle. All axon profiles shown in this figure are situated at some distance from the periphery of the bundle. Despite the deep location of the axons, typical varicosities (*a*) and intervaricosities (*x*) are present but the majority of varicosities are devoid of granular or agranular vesicles (*v*). Instead they are packed with neurotubules (*t*) and neurofilaments (*f*). *S*, Schwann cell cytoplasm. Glutaraldehyde-Millonig's buffer–fixed; OsO_4-postfixation section stained with lead citrate and uranyl acetate. × 38,000.

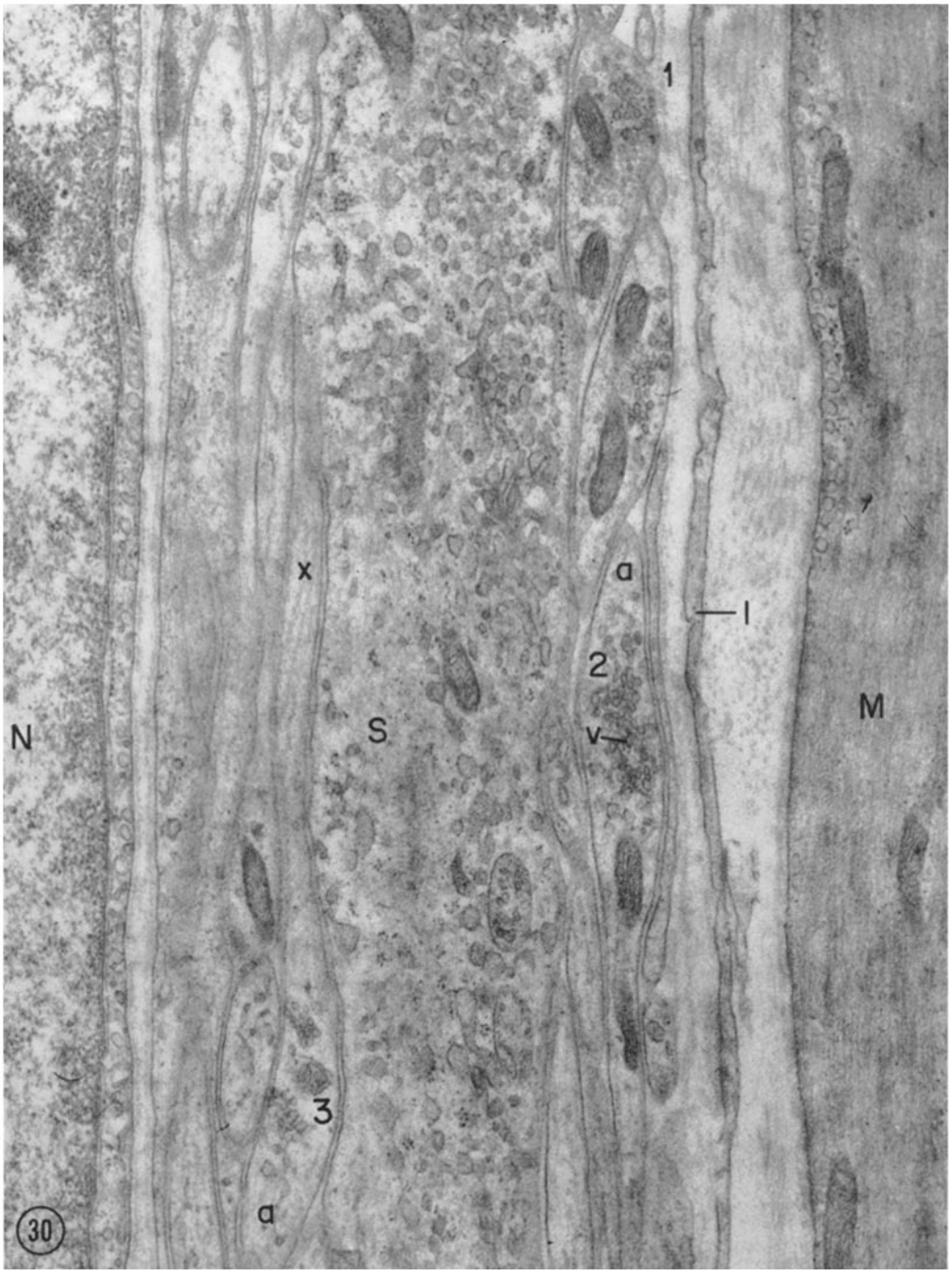


FIGURE 30 Longitudinal section through a nerve bundle. Numerous axon profiles (*a*) are cut in longitudinal and oblique section on either side of a large mass of Schwann cell cytoplasm (*S*), which forms the core of the bundle. The wide varicosity and narrow intervaricosity (*x*) components of the axons are evident. The oblique alignment and overlapping of the varicosities result from the interweaving of the axons within the bundle. Note the grouping of the agranular vesicles (*v*), in some axons against the outer margin of the varicosities (*1*), in others as a single clump (*2*) or as small groups (*3*) towards the center of the varicosity. *M*, smooth muscle cell; *I*, interstitial cell; *N*, nucleus of smooth muscle cell. OsO_4-McEwen's buffer–fixed; section stained with lead citrate. × 22,000.

in the autonomic nerve fibers of other tissues (19, 26, 47, 49). Glutaraldehyde fixation and postfixation in osmium tetroxide do not affect the structure of the granular vesicles in the taenia coli; therefore it is unlikely that they contain noradrenaline (19). Richardson (43) has pointed out that it is perhaps significant that type I vesicles (26) appear to be a characteristic feature of cholinergic axons (autonomic). This suggestion is supported by the cell fractionation studies of cholinergic nerves in the brain (21). Coupland's (19) investigations on the adrenal medulla, however, indicate that granular vesicles of this type could represent adrenaline-binding substance. Present data are insufficient to enable a correlation to be made between the inclusions found in the axons of the taenia coli and the transmitter substances released.

The question arises as to the maximum distance between an axon and smooth muscle cell in the taenia coli at which the transmitter can still produce a substantial permeability change in the cell membrane. This distance has been estimated for the sympathetic innervation of the vas deferens as about 1000 A (11). In the taenia coli the closest approach of the intramural nerves to the muscle cell is about 1000 A. If it is assumed that each varicosity releases about the same amount of transmitter per impulse (23, 38), then the concentration of transmitter at the membrane due to release from a varicosity at 1000 A will be about 30 times the concentration due to release at 3000 A (16). If the concentration of transmitter at the muscle cell determines the magnitude of the permeability changes in the membrane (20), then an axon at 3000 A from a muscle cell would require about 30 varicosities in order to produce the same effect as a single varicosity at 1000 A. For a muscle cell 200 μ long it seems likely that the maximum number of varicosities of a single axon at 3000 A would be about 30 (38). Varicosities may have to be within 3000 A of a smooth muscle cell in the taenia coli if they are going to produce a detectable permeability change in the membrane during transmission.

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