THE ULTRASTRUCTURE AND DISPOSITION OF VESICULATED NERVE PROCESSES IN SMOOTH MUSCLE

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ABSTRACT

The walls of the gastrointestinal tract and urinary bladder of rats were fixed in osmium tetroxide, embedded in methacrylate, and sectioned for electron microscopy. The examination of sections of smooth muscle tissue with the electron microscope reveals the presence of bundles of unmyelinated nerve fibers within the intercellular spaces. In addition, vesiculated nerve processes, bounded on their outer surfaces by delicate plasma membranes and typically containing varying quantities of synaptic vesicles and mitochondria, make intimate contact with the surface of smooth muscle cells. These nerve processes are similar in structure and disposition to nerve endings previously described in skeletal muscle, in the central nervous system, in peripheral ganglia, in receptors, and in glands. It is concluded that the relationships existing between vesiculated nerve processes and the surface of smooth muscle cells constitute neuromuscular junctions. Profiles of protrusions of smooth muscle cells are often seen protruding into the intercellular spaces. Here they occur singly or in groups, originating from one or more cells. Because of the plane of section the protrusions may sometimes appear as individual entities between the muscle cells. In such cases care must be exercised in their identification because they have characteristics similar to sectioned nerve processes which also occur in the intercellular spaces.

INTRODUCTION

Since the middle of the last century (19), many investigators with the use of the light microscope have made attempts to identify the structural characteristics of the terminal ramifications of the autonomic nervous system in smooth muscle tissue and to determine their relationship to smooth muscle cells. Despite their many efforts, our present knowledge concerning these characteristics and relationships remains obscure. The difficulty encountered by these investigators, in adequately identifying the attenuated terminal nerve processes and their relationship to smooth muscle cells, may be attributed to the complexity of these nerve–smooth muscle relationships, to the unreliability of the silver and methylene blue staining techniques used, and to the relatively low resolving power of the light microscope. Extensive and critical reviews of the vast literature concerned with this problem can be found in the reports of Clara (8), Hill (25), and Hillarp (26, 27).

The electron microscope with its inherent

high resolving power has been used with success in determining the structural organization of a wide variety of tissues including smooth muscle tissue. With this fact in mind, it was considered highly desirable to restudy smooth muscle tissue with the electron microscope with the expectation of gaining further knowledge concerning the structural relationships existing between nerve processes and smooth muscle cells.

The identification of nerve–smooth muscle relationships should be based on previous descriptions of the structure of nerve endings in skeletal muscle (1, 9, 33, 35, 37–39, 43, 58), cardiac muscle (18, 56), the central nervous system (3, 13, 14, 21, 22, 24, 34), peripheral ganglia (12, 40, 49, 50), glandular tissue (2, 15, 45), and those structures which are thought to have primarily an afferent nerve supply (7, 11, 14, 17, 46, 47). From the foregoing studies it is apparent that a nerve ending which forms a synaptic junction with a pre- or post-synaptic structure possesses some or all the following characteristics: (*a*) it exists as the terminal portion of a nerve process which may or may not be swollen; (*b*) it is enclosed by a plasma membrane approximately 80 A in thickness; (*c*) a gap of 120 to 600 A separates the plasma membrane of the nerve ending from the plasma membrane of the pre- or post-synaptic structure, one or both of which may show increased density in places; (*d*) it contains a number of synaptic vesicles whose profiles are from 200 to 650 A in diameter and which are bounded by membranes 50 to 70 A thick. These vesicles contain a substance whose density is slightly greater than that of the substance surrounding the vesicles; (*e*) it usually contains mitochondria of a size, shape, and configuration similar to those found in nervous tissue in general; (*f*) it is usually surrounded by glial or Schwann cells except where it is in close apposition to the pre- or post-synaptic structure.

The typical mammalian motor end-plate of skeletal muscle, which has been frequently studied with the electron microscope (1, 9, 37, 39, 58), contains nerve endings which lie within depressions of the muscle fiber. The floor of these depressions is composed of the extensively folded plasma membrane of the muscle fiber. Only that portion of the plasma membrane between the openings of the folds is in close apposition to the surface of the nerve endings. An increased density of these apposed membranes is rarely seen. These findings differ from the findings with respect to most of the nerve endings encountered elsewhere, especially those found in the central nervous system and peripheral ganglia. However, those nerve endings synapsing on "tonus" skeletal muscle fibers (33, 38), on striated muscle of blood vessels (29), or on striated muscle of invertebrates (16, 36, 48) lack the folds in the plasma membrane of the muscle fibers. They appear to resemble more closely the nerve endings of the central nervous system and peripheral ganglia.

In recent years nerve-muscle relationships in smooth muscle tissue have been examined with the electron microscope (6, 20, 40, 52, 54, 57). The most significant result of these studies has been the demonstration that small bundles of unmyelinated nerve fibers, ensheathed in Schwann cells, are present in the smooth muscle coats of the gastrointestinal tract. Here they have been described as ramifying, in occasional company with interstitial cells of Cajal, histiocytes, and blood capillaries. In some instances, small nerve fibers containing synaptic vesicles have been observed to be near the surface of smooth muscle cells where they have, in some instances, been reported to form neuromuscular junctions. The present report supports these findings to some extent and defines in greater detail the ultrastructure of vesiculated nerve processes and their relationship to smooth muscle cells.

MATERIALS AND METHODS

During the course of this study, most of the observations were made from sections of the descending colon taken from 2-month-old female albino rats. In addition, sections of stomach, small intestine, and urinary bladder from the same animals were studied with comparable results. Each animal was anesthetized with Nembutal sodium and immersed in an ice water bath. Hypothermia reduces enzyme activity and therefore assists in the preservation of delicate structures in the tissues (32, 55). Temperatures of the rats were determined by placing a suitable thermometer within the descending colon. The animals' heads were kept above the water until visible breathing movements ceased. Thereafter, when a lower body temperature was desired, the animals were completely submerged to facilitate cooling. The colonic temperatures of the rats ranged from 5 to 35°C at the time of excision of portions of the walls of the gastrointestinal tract and urinary bladder which were immediately placed in a 1 or 2 per cent buffered osmium tetroxide solution (pH 7.7). These portions were then divided into smaller

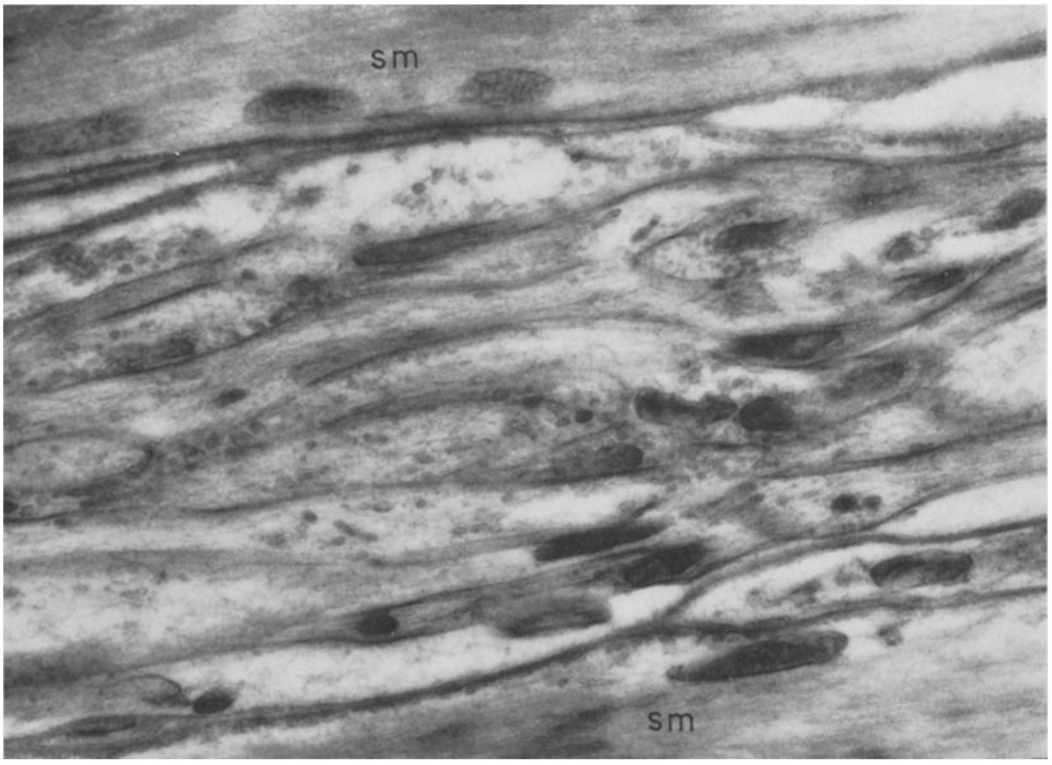

FIGURE 1

Electron micrograph of a longitudinal section through a portion of two smooth muscle cells (*sm*) and a relatively large nerve bundle. This bundle fills the space between the two muscle cells. No nerve endings are apparent. This tissue was taken from the inner circular layer of the muscularis externa of the pylorus of a rat. × 22,000.

pieces and left in the fixative for an additional 60 to 90 minutes. After fixation the tissues were dehydrated in methanol, embedded in methacrylate, sectioned with a Servall Porter-Blum microtome, and studied with an electron microscope (Philips EM 100-B and/or RCA EMU3-F).

OBSERVATIONS

In sections of both the outer longitudinal and the inner circular layers of smooth muscle of the muscularis externa of the gastrointestinal wall of rats, bundles of nerve fibers, such as those shown in Figs. 1 and 2, have been studied in order to determine their composition and distribution within the intercllular spaces of the muscle tissue. Nerve bundles are seen more frequently within the inner circular layer than in the outer longitudinal layer of smooth muscle. They are composed of unmyelinated nerve fibers of varying size and number. Some of the larger bundles (Fig. 1) may contain approximately thirty nerve fibers as they make their way between the muscle cells shortly after entering the muscle layers from the myenteric plexus. Some of the smallest bundles within the muscle contain only a few or sometimes only one nerve fiber. In the nerve bundles examined, all the nerve fibers are ensheathed by Schwann cells except at the periphery of the smaller bundles where some of the fibers relate themselves, or are about to relate themselves, to the outer surface of smooth muscle cells (Fig. 2). In those instances, in which the peripheral nerve fibers contain synaptic vesicles and mitochondria and lie closely applied to the surface of smooth muscle cells, they may be considered to form neuromuscular junctions. Cells, which have been tentatively identified as interstitial cells of Cajal, fibroblasts, and macrophages, accompany the nerve bundles in their journey within the muscle layers.

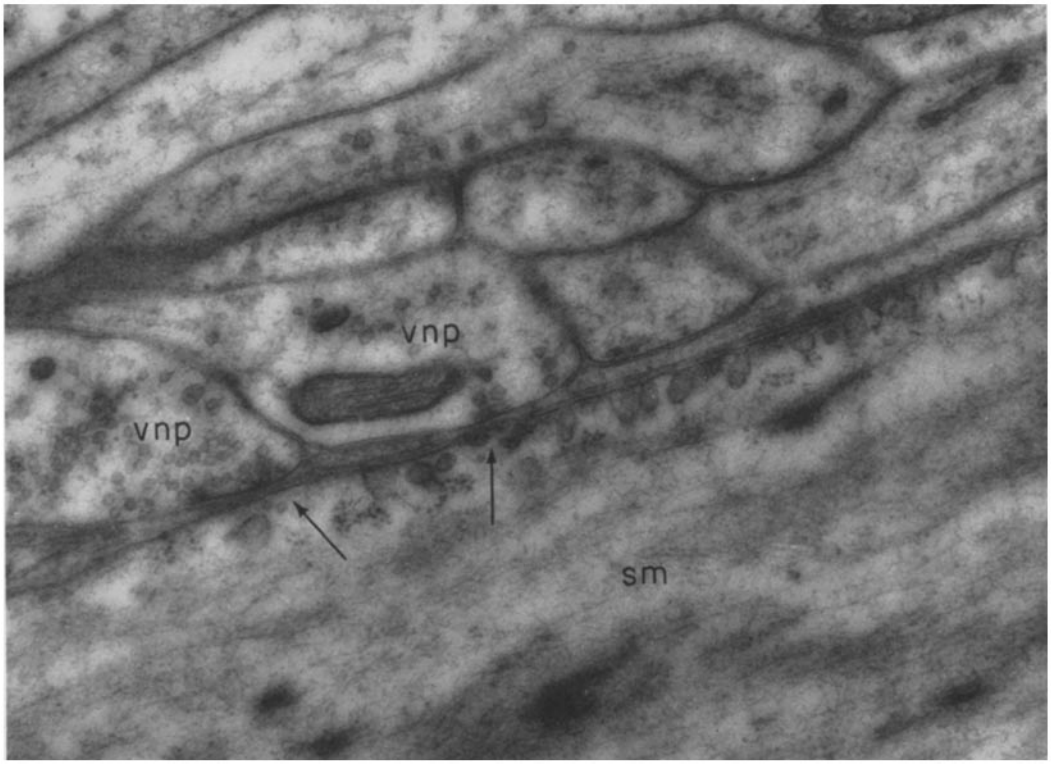

FIGURE 2

Electron micrograph of a longitudinal section through a portion of a smooth muscle cell (*sm*) and the peripheral portion of a small nerve bundle (upper half of field). Notice the close apposition of two emerging vesiculated nerve processes (*vnp*) with the surface of the muscle cell (arrows). An accumulation of synaptic vesicles within the nerve process and two vesicles of the same diameter beneath the plasma membrane of the muscle cell, are apparent at the junction indicated by the left arrow. The contiguous plasma membranes show increased density at this point. A similar situation can be seen at the junction indicated by the right arrow; however, the vesicles on either side of the plasma membranes are larger in their diameters. Notice the neurofilaments within the nerve fibers of the bundle and also the branching, thread-like myofilaments within the smooth muscle cell. This tissue was taken from the inner circular layer of the muscularis externa of the descending colon of a rat. × 40,000.

With regard to the number of vesiculated nerve processes which make intimate contact with smooth muscle cells, it appears from the examination of a great number of sections that these nerve processes are more numerous within the wall of the urinary bladder than in the walls of the gastrointestinal tract. Furthermore, the inner circular layer of the muscularis externa seems to contain more nerve-muscle contact points than the outer longitudinal layer. Nerve-muscle relationships in arterioles seem to be very rare. In consideration of the work of Richardson (42), however, it appears that the number of nerve-muscle contacts in the vas deferens of rats seems to greatly exceed the number within the smooth muscle tissue of the urinary bladder and gastrointestinal tract.

For comparative purposes and to bridge the gap of knowledge between what is now known concerning the structure of synapses in the central nervous system and peripheral ganglia and what will be subsequently described concerning the structure of vesiculated nerve processes making contact with smooth muscle cells, an electron micrograph of synapses within a ganglion of the myenteric plexus will now be considered. In Fig. 3, two typical nerve endings bounded by plasma membranes approximately 80 A in thick-

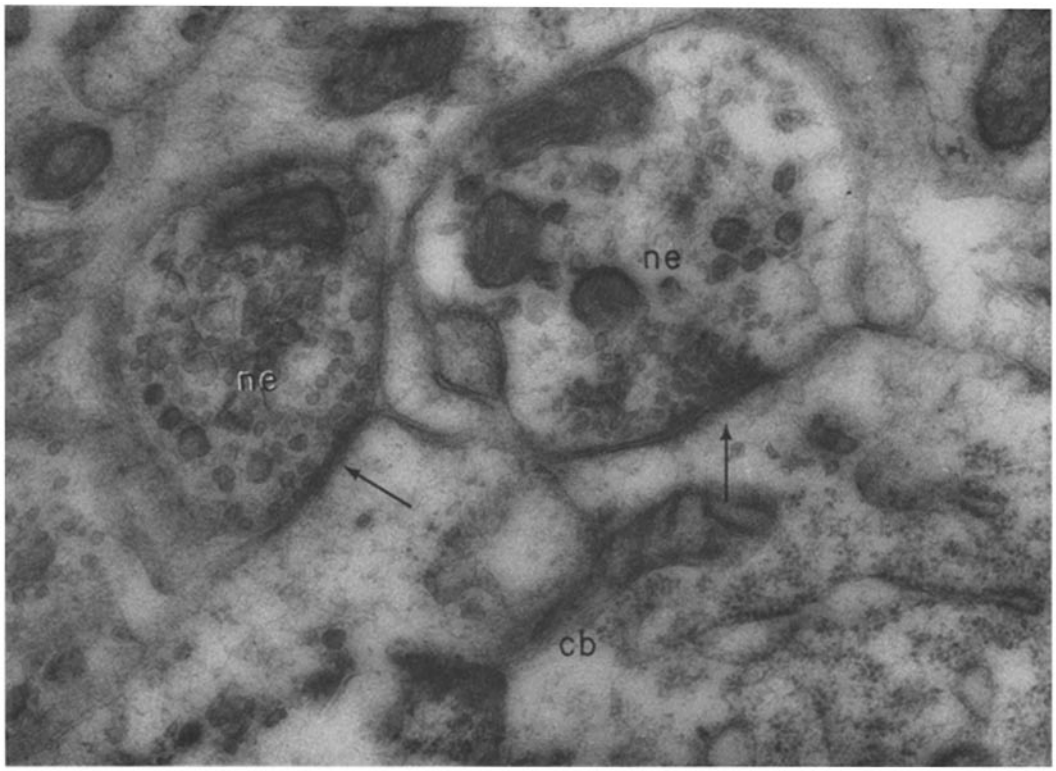

FIGURE 3

Electron micrograph of a section through a portion of the cell body of a neuron (*cb*) and two nerve endings (*ne*). The endings, containing large numbers of synaptic vesicles and several mitochondria with longitudinally oriented cristae, are in synaptic contact with the cell body. Notice the higher density of the pre- and post-synaptic membranes and the higher concentration of synaptic vesicles in these areas (arrows). This tissue was taken from a ganglion in the myenteric plexus within the muscularis externa of the descending colon of a rat. × 60,000.

ness and containing a multitude of synaptic vesicles and several mitochondria can be seen synapsing on the surface of the perikaryon of a neuron. These endings together with the plasma membrane of the perikaryon meet the previously mentioned required characteristics of synaptic junctions. The nerve ending on the right in the micrograph shows an accumulation of small vesicles in the vicinity of the synaptic junction. The diameters of these vesicles range from 160 to 300 A. Larger vesicles with an approximate diameter of 670 A are present deep within the ending. Mitochondria with an approximate diameter of 0.15 micron, showing longitudinally oriented cristae, can be seen within the ending. The contiguous membranes of both the ending and the perikaryon show increased density at the synaptic junction (arrows). The synaptic cleft or space between the two membranes is approximately 130 A across. The synaptic relationship of the nerve ending to the surface of the perikaryon in the left half of the micrograph is obscure due to the obliqueness of the plane of section through the contiguous membranes. However, small vesicles are apparent near the synaptic junction.

Generally, vesiculated nerve processes in smooth muscle tissue are bounded by plasma membranes approximately 80 A in thickness and contain synaptic vesicles and mitochondria. The diameters of these processes range from 0.2 micron to as much as 1.2 microns, the average diameter observed being approximately 0.5 micron. Vesiculated nerve processes have been observed in the muscularis externa of the stomach, pylorus, small intestine, and colon of the gastrointestinal

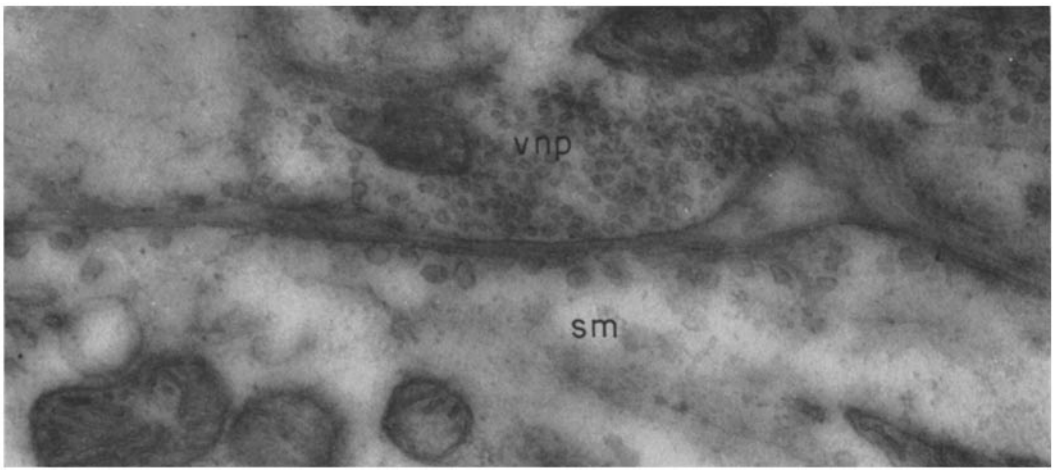

FIGURE 4

Electron micrograph of a section through a portion of a longitudinally oriented smooth muscle cell (*sm*) and a vesiculated nerve process (*vnp*) which is at the periphery of a small nerve bundle (not shown). Note the close apposition of the process with the surface of the muscle cell. Many synaptic vesicles and a mitochondrion are present in the nerve ending. A row of vesicles of larger size is present immediately beneath the plasma membrane of the muscle cell. This tissue was taken from the inner circular layer of the muscularis externa of the pylorus of a rat. × 50,000.

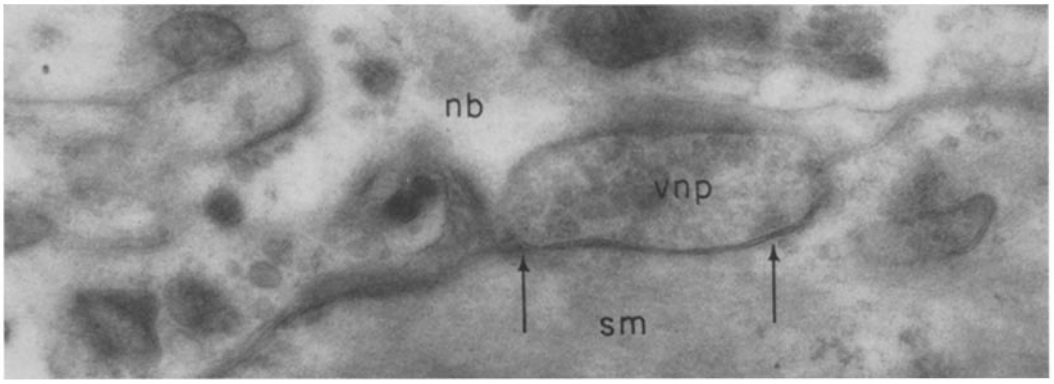

FIGURE 5

Electron micrograph of an oblique section through a portion of the periphery of a small nerve bundle (*nb*) and a smooth muscle cell (*sm*). A vesiculated nerve process (*vnp*), packed with synaptic vesicles, is in close relationship with the plasma membrane of the muscle cell. Notice the smaller vesicles and the increased density of the plasma membranes opposite the arrows. This tissue was taken from the inner circular layer of the muscularis externa of the descending colon of a rat. × 41,500.

tract, in the wall of the urinary bladder, and on the surface of the media of small arterioles. The processes shown in Fig. 2 and Figs. 4 to 11 represent the many observations made of vesiculated nerve processes in smooth muscle tissue during the course of this study and demonstrate their appearance when sectioned in various planes.

An attempt was made to determine the method of approach used by the nerve processes as they move in to make contact with the smooth muscle cells. Generally, one can say that the vesiculated nerve processes tend to orient themselves with the long axis of the smooth muscle cells. In Figs. 2, 4, 6, and 8, one finds that the smooth muscle

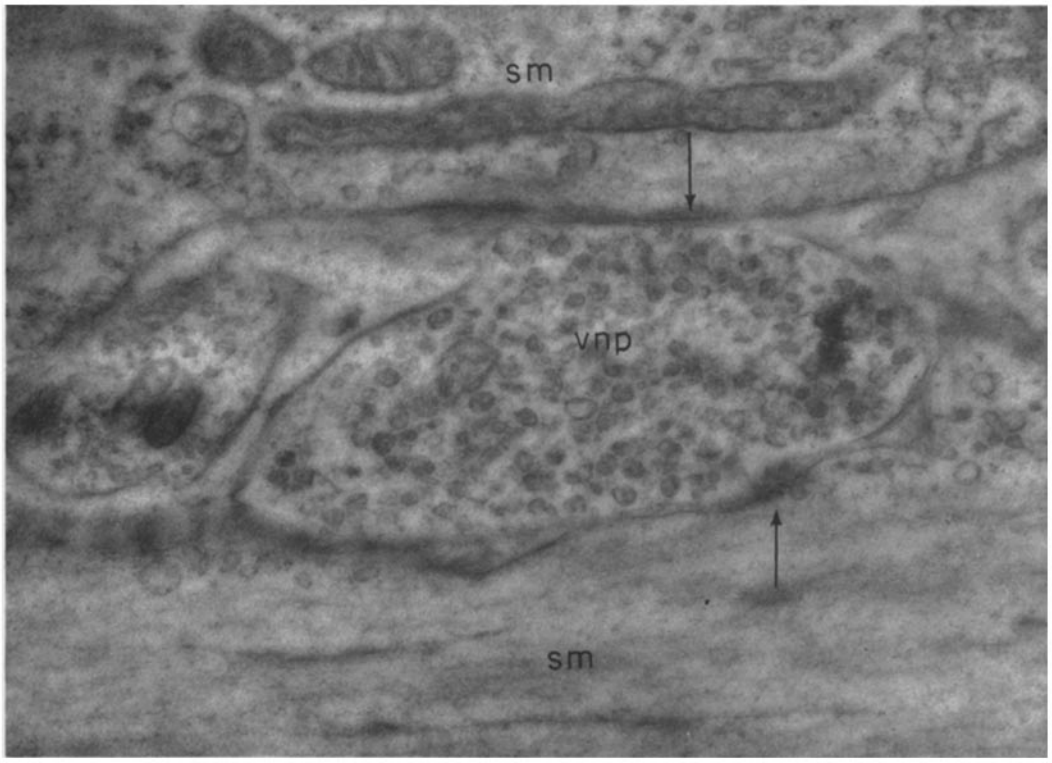

FIGURE 6

Electron micrograph of a section of a large vesiculated nerve process (*vnp*) lying between portions of two smooth muscle cells (*sm*) which were sectioned in a longitudinal plane. Notice the close apposition of the process with the surface of both muscle cells. An increased density of the plasma membranes of the nerve process and muscle cells is indicated by arrows. Take note of the very small vesicles in the nerve process and an invaginated caveolus of the plasma membrane of the muscle cell at the site indicated by the lower arrow. The process is completely filled with synaptic vesicles of various sizes. Several mitochondria are present in the peripheral sarcoplasm of the muscle cell in the upper part of the field. This tissue was taken from the inner circular layer of the muscularis externa of the descending colon of a rat. × 43,000.

cells are sectioned almost in a longitudinal plane and the vesiculated nerve processes, for the most part, have a longitudinal orientation. The vesiculated nerve process in Fig. 7 is sectioned in a transverse plane as is the smooth muscle cell of this figure. Occasionally these relationships do not follow the above stipulations. In Fig. 9, the vesiculated nerve process is sectioned transversely whereas the smooth muscle cell of this figure shows an oblique orientation. However, the obliquely sectioned smooth muscle cell in Fig. 5 also has an obliquely sectioned vesiculated nerve process making contact with its surface.

It has been reported (references 14 and 34) that the synaptic cleft or intercellular space between the pre- and post-synaptic membranes in synases in the central nervous system is approximately 120 to 200 A across, and in the motor end-plate of skeletal muscle 400 to 600 A (references 9 and 37). The synaptic clefts in Figs. 3 to 5 can be seen to fall within the range reported for synapses in the central nervous system. In Figs. 8 and 9, however, the synaptic clefts fall within the range reported for the synaptic clefts in the motor end-plate of skeletal muscle. The vesiculated nerve processes near the smooth muscle cell of the small arteriole in Fig. 10, however, are an exception, in that the space between the nerve process and the muscle cell is

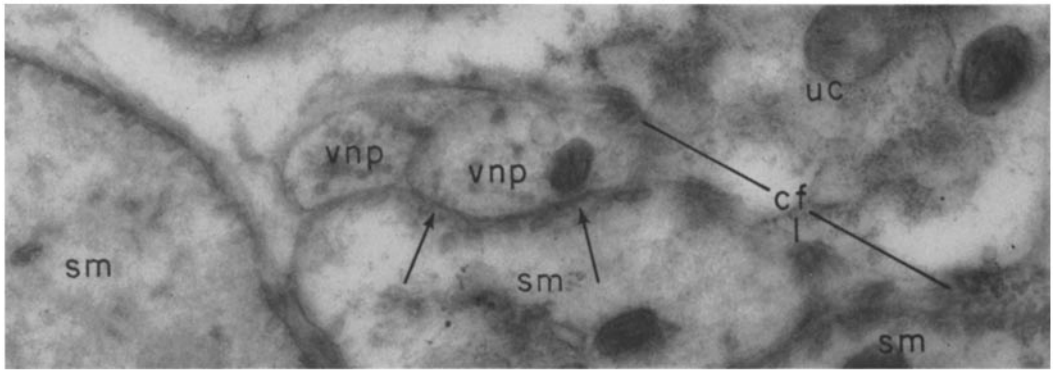

FIGURE 7

Electron micrograph of a transverse section through portions of smooth muscle cells (*sm*) and vesiculated nerve processes (*vnp*). Notice the close apposition of the processes with the surface of a muscle cell. Some of the smaller synaptic vesicles within the process are close to the surface opposite the arrows. Collagen fibrils (*cf*) can be seen between an unidentified cell (*uc*) and two of the muscle cells. Some fibrils are also apparent between the nerve processes and this cell. This tissue was taken from the outer longitudinal layer of the muscularis externa of the descending colon of a rat. × 40,000.

approximately 770 A across which seems wide when compared with neuromuscular junctions elsewhere and therefore may not constitute a functional neuromuscular junction. Synaptic clefts of small dimensions can be seen in Figs. 5 to 7. Here they range from 70 A in Fig. 6 to 160 A in Fig. 4. Fig. 2 shows synaptic clefts which measure 300 A across.

A homogeneous material similar to the basement membrane of other cells is present within the synaptic clefts of some neuromuscular junctions. There is an indication of layering of this material in Figs. 2, 4, 8, and 10. Layering of this material has been reported by Reger (37), Robertson (43), and Andersson-Cedergren (1) in the synaptic cleft in motor end-plates of skeletal muscle.

One can say that the plasma membranes of the vesiculated nerve processes and the smooth muscle cells show very little specialization. In Fig. 2 the synaptic membrane of the vesiculated nerve process shows an area of increased density. However, areas of increased density of both the pre- and post-synaptic membranes can be seen in Figs. 5 and 6. In addition to the aforementioned specializations the plasma membranes of some of the smooth muscle cells, such as those shown in Figs. 4 and 6, display "pinocytotic" vesicles which are in communication with the intercellular space in the area of the synaptic junction. However, only occasionally do these open vesicles occur in the area of increased density of the membranes, as seen in Fig. 6, even though they do occur elsewhere in the smooth muscle cell membranes. Pinocytotic vesicles which are not open to the intercellular space are regularly seen beneath the plasma membranes of smooth muscle cells. Their frequent occurrence is, in fact, an identifying feature of smooth muscle cells. Their diameters range from 400 to 700 A. An indentation of the surface of smooth muscle cells is frequently seen in the formation of synaptic junctions, which receives the vesiculated nerve process. In some contact regions, however, as indicated in Fig. 2 and portions of the neuromuscular junctions in Figs. 6 and 7, there is no depression.

As indicated previously, the presence of synaptic vesicles is an identifying characteristic of nerve endings. It will be noted that in Figs. 4, 8, and 10 the synaptic vesicles within the nerve processes possess diameters of from 200 to 400 A, and are fairly evenly distributed within the process. Synaptic vesicles of this same approximate diameter are present in the processes shown in Figs. 2, 7, and 9. They are not evenly distributed, however. In Figs. 2 and 7 some of the synaptic vesicles are concentrated near the synaptic membrane. Another frequent finding is represented in the nerve processes in Figs. 5 and 6 where there is a mixture of large and small vesicles. The larger ones in Fig 6 may reach a diameter of 840 A and seem to

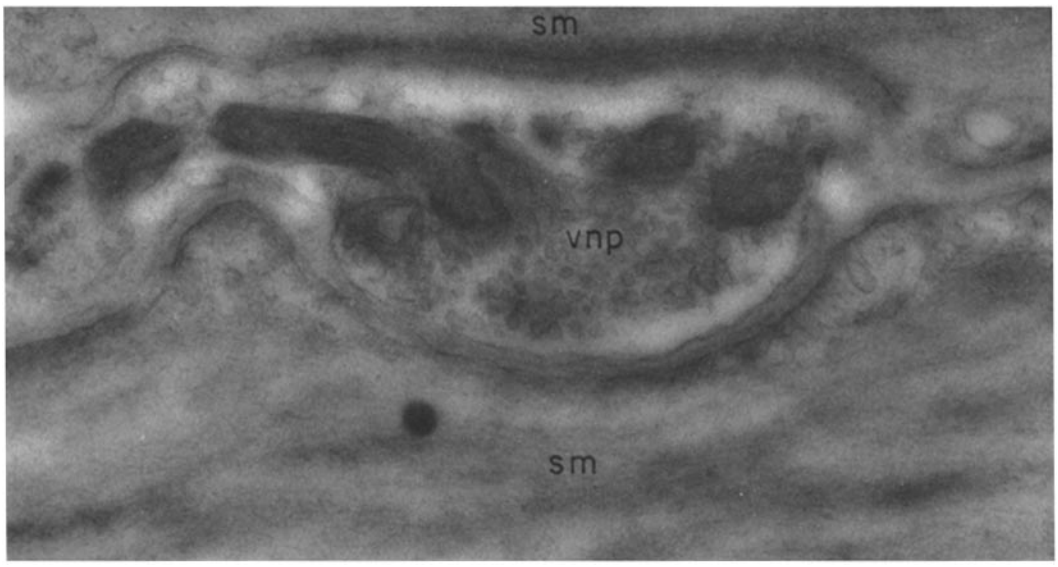

FIGURE 8

Electron micrograph of a longitudinal section through a portion of two smooth muscle cells (*sm*) and a vesiculated nerve process (*vnp*) which is lying within a depression of the surface of the muscle cell. Many synaptic vesicles and several mitochondria are present within the process. A close relationship exists between the plasma membranes of the nerve process and both muscle cells. The association of the nerve process with the muscle cell in the upper part of the field is somewhat obscure due to the plane of section of the plasma membranes. This tissue was taken from the wall of the urinary bladder of a rat. × 45,000.

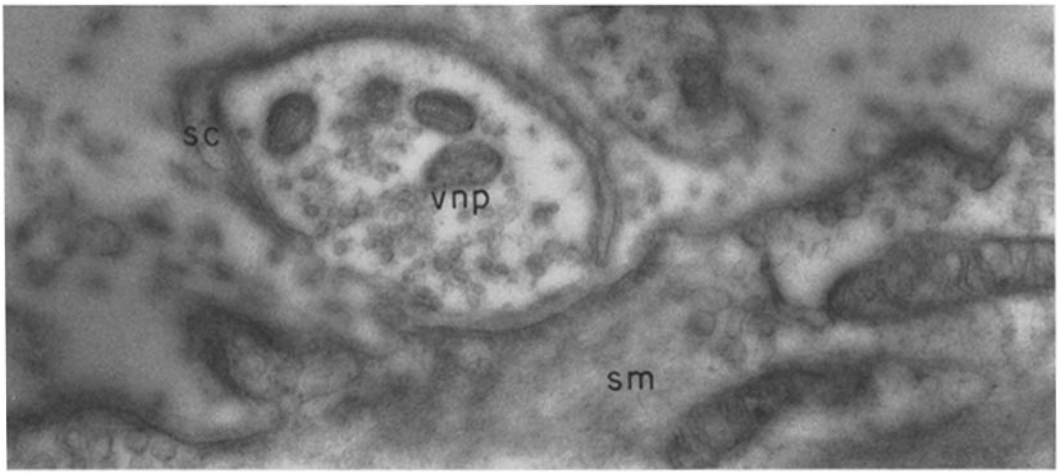

FIGURE 9

Electron micrograph of an oblique section through a portion of a smooth muscle cell (*sm*) and a transverse section through a portion of a vesiculated nerve process (*vnp*). Notice the close apposition of the nerve process to the surface of the muscle cell. The nerve process which is capped by a portion of a Schwann cell (*sc*) contains some synaptic vesicles and mitochondria. This tissue was taken from the wall of the urinary bladder of a rat. × 40,000.

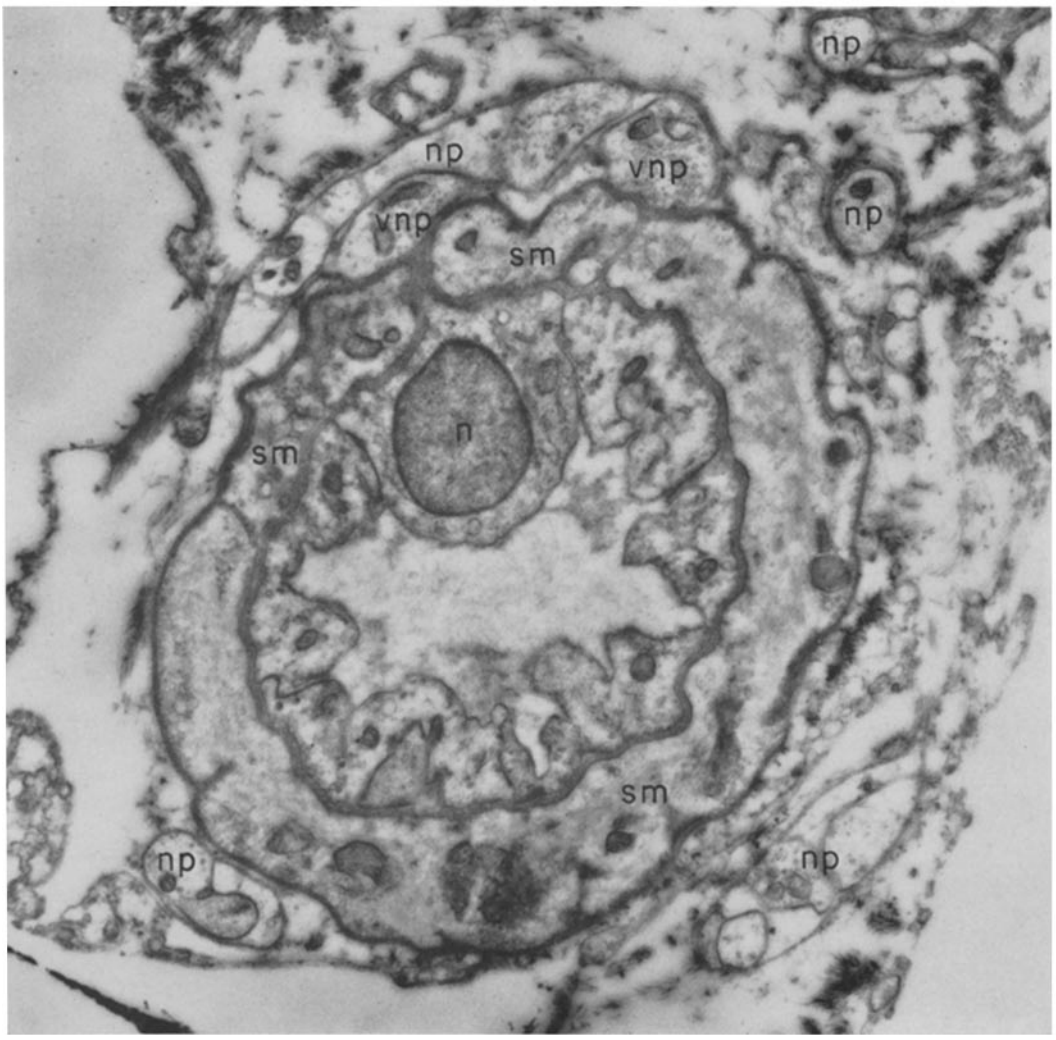

FIGURE 10

Electron micrograph of a transverse section through a small arteriole. Portions of a smooth muscle cell (*sm*) are evident which encircle portions of several endothelial cells, one of which is sectioned through its nucleus (*n*). Several sectioned nerve processes (*np*) can be seen amongst the collagen fibrils of the adventitia. Notice the vesiculated nerve processes (*vnp*) containing synaptic vesicles and mitochondria, applied to the surface of the smooth muscle cell. Dense material, presumably basement membrane, is present between vesiculated nerve processes and the muscle cell. This tissue was taken from the wall of the pylorus of a rat. × 13,000.

be present in the interior of the process. The contents of the synaptic vesicles in all the processes shown is of a higher density than the material surrounding the vesicles. On rare occasions, vesicles of relatively large diameter, which contain dense granules, have been observed within nerve processes of unstained sections of the muscularis externa of the gastrointestinal tract (Fig. 11) but not in sections of the wall of the urinary bladder. These granulated vesicles compare favorably with those shown by Hager and Tafuri (23) in the colon of guinea pigs, by Taxi (51) in the sympathetic ganglia of frogs, and by Richardson (42) in the vas deferens of rats.

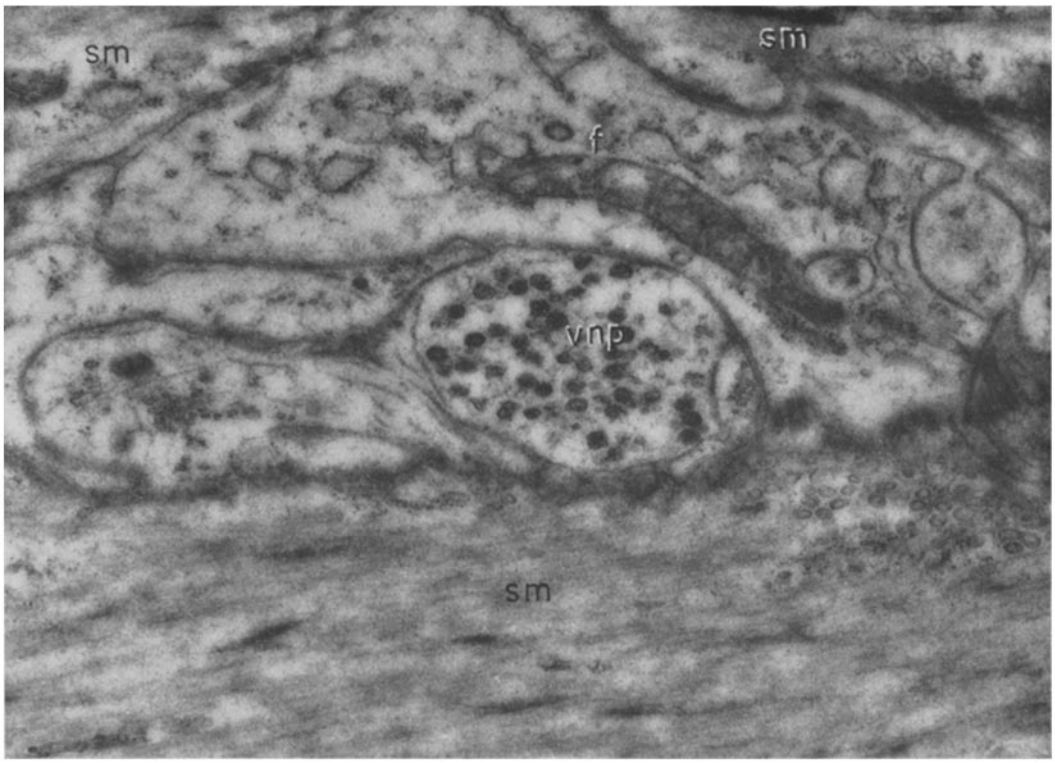

FIGURE 11

Electron micrograph of an oblique section through portions of three smooth muscle cells (*sm*) and the contents of the intercellular space. A nerve process containing granulated vesicles (*vnp*) is apparent within the intercellular space together with portions of other nerve processes and a process of a cell thought to be a fibroblast (*f*). The fibroblastic process is in close relationship with the plasma membranes of two of the smooth muscle cells and with the plasma membrane of the vesiculated nerve process. This tissue was taken from the inner circular layer of the muscularis externa of the descending colon of a rat. × 33,000.

Mitochondria, which are considered to be normal constituents of nerve endings, are present in all vesiculated nerve processes shown except for the processes in Figs. 5 and 6. Figs. 8 to 10 show sectioned profiles of mitochondria in greater numbers than do other figures. The mitochondria average 0.15 micron in diameter. The processes in the wall of the urinary bladder seem to be more heavily endowed with mitochondria than those seen elsewhere. Occasionally, mitochondria with longitudinally oriented cristae can be seen within the processes, as demonstrated in Figs. 2 and 8. These have been described in axonal endings within the central nervous system (34).

Neurofilaments are often seen within nerve processes as they make their way between smooth muscle cells (Figs. 1 and 2). They are, however, for the most part, absent within the vesiculated nerve processes. They are especially apparent within the nerve processes in Fig. 2. In this same figure myofilaments within the smooth muscle cell are also prominently demonstrated.

Schwann cells, which are normal constituents of nerve bundles, have been studied in the myenteric plexus and within the intercellular spaces of smooth muscle tissue. The cytoplasm of these cells contains mitochondria, granular endoplasmic reticulum, Golgi complex, and assorted vesicles in the vicinity of their nuclei. However, that portion of the cytoplasm which surrounds the enclosed nerve processes is devoid of organelles except for occasional vesicles. The cytoplasm of these Schwann cell extensions in most instances is less dense than the enclosed nerve process.

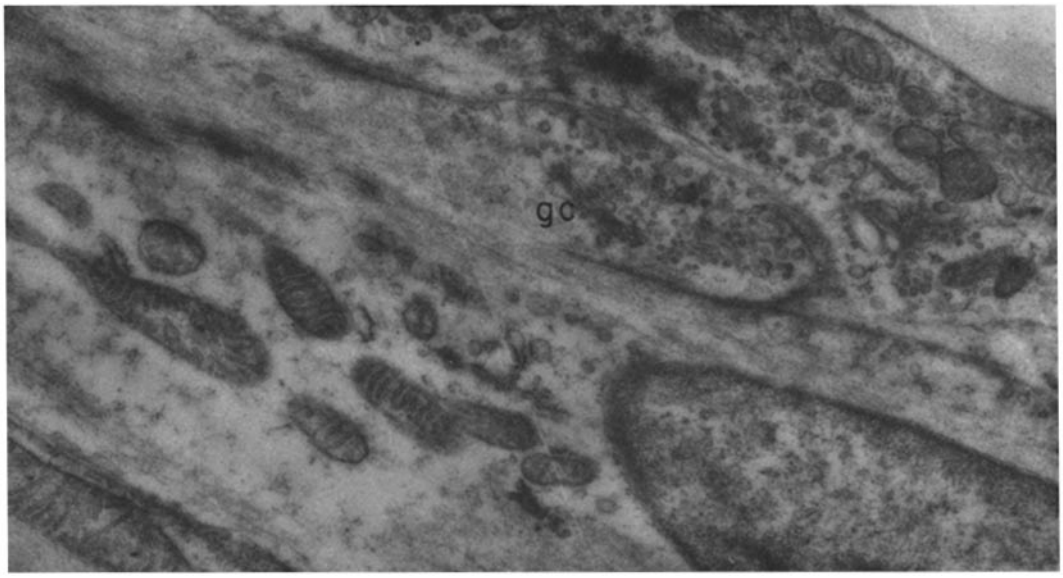

FIGURE 12

Electron micrograph of a longitudinal section through portions of two smooth muscle cells, one of which is just visible in the lower left corner of the field. A protrusion of the muscle cell, capped by what is thought to be a macrophage, is apparent in the upper third of the field. Notice the Golgi complex (*gc*) and its associated vesicles within the protrusion. Vesicles of a larger diameter are located in the periphery of the protrusion. Sarcoplasm, containing mitochondria, RNP particles, and vesicles, can be seen at the polar region of the muscle cell nucleus. This tissue was taken from the inner circular layer of the muscularis externa of the descending colon of a rat. × 28,500.

However, occasionally, as seen in Fig. 9, the enveloping portion of the Schwann cell may be more dense than its enclosed nerve process. It is assumed that most vesiculated nerve processes are ensheathed by Schwann cells except at the point of close apposition with smooth muscle cells. It is possible, however, that some naked nerve processes occur.

Frequently, protrusions of the surface of smooth muscle cells occur. In some instances, they may possess characteristics which resemble those of nerve processes. Such a resemblance can be seen in Fig. 12. Here the protrusion is filled with vesicles, some with an average diameter of 300 A and some 600 A. The smaller vesicles which supposedly are part of the Golgi complex present in the protrusion are very similar to the synaptic vesicles of vesiculated nerve processes, whereas the larger vesicles are characteristic of the ones normally seen beneath the plasma membranes of smooth muscle cells. In vesiculated nerve processes, the smaller vesicles usually occur in the periphery; the large vesicles, if present, usually occur deep within the process (Figs. 2, 5, and 6). The situation in Fig. 12, however, is just the opposite. If this protrusion were sectioned in a plane perpendicular to that shown, it would appear as an individual entity and would be difficult to distinguish from a vesiculated nerve process, except for the position of the two sizes of vesicles. In Fig. 13, many protrusions (*p*) are emanating from smooth muscle cells in both the left and right portions of the field. This sort of picture has been seen frequently in smooth muscle tissue from the wall of the gastrointestinal tract and from the wall of the urinary bladder. Due to the absence of myofilaments and the diminished density of their contents, the protrusions may be mistaken for nerve processes. Even the profiles which show no continuity with smooth muscle cells are considered to be protrusions of the surface of smooth muscle cells not in this plane of section. Some of the protrusions do contain large vesicles and mitochondria. Another frequent occurrence with regard to protrusions is the example demonstrated in Fig. 14. Here a protrusion

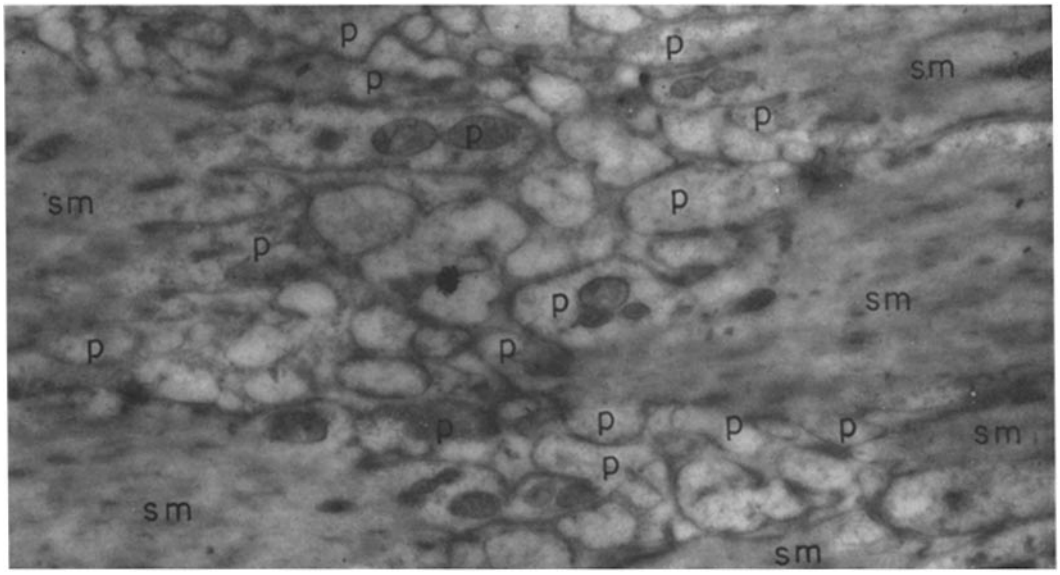

FIGURE 13

Electron micrograph of a longitudinal section through portions of smooth muscle cells (*sm*). The center of the field contains many protrusions (*p*) from muscle cells extending from the left and right portions of the field. The protrusions appearing as individual entities (unmarked), supposedly are from portions of muscle cells which are not in this plane of section. Notice the mitochondria and vesicles in some of the protrusions. No sectioned nerve processes are thought to be present. This tissue was taken from the outer longitudinal layer of the muscularis externa of the descending colon of a rat. × 18,500.

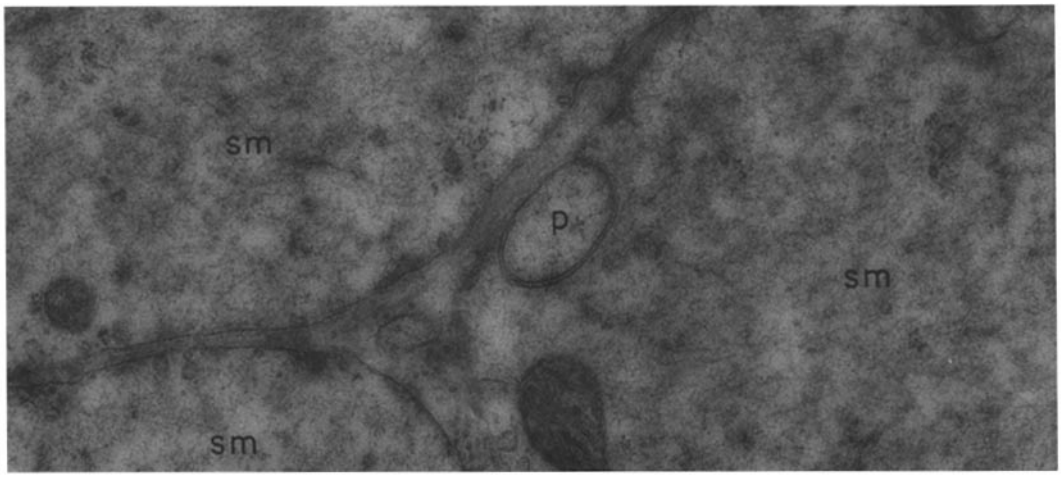

FIGURE 14

Electron micrograph of a transverse section through portions of three smooth muscle cells (*sm*). Notice the protrusion (*p*) of a smooth muscle cell, not in this plane of section, embedded in a depression of one of the muscle cells. Notice the absence of vesicles in this protrusion. Many thread-like myofilaments are apparent in the sarcoplasm of the muscle cells. This tissue was taken from the outer longitudinal layer of the muscularis externa of the descending colon of a rat. × 42,500.

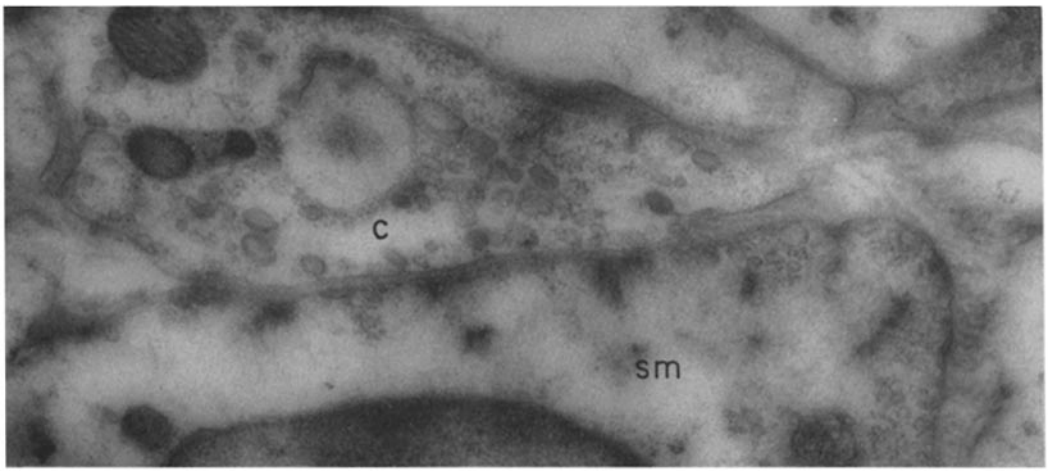

FIGURE 15

Electron micrograph of a transverse section through a portion of a smooth muscle cell (*sm*) and what is thought to be a process of an interstitial cell of Cajal (*c*). Notice the relatively large vesicles and vacuole in the process. A close relationship exists between the interstitial cell process and the muscle cell. This tissue was taken from the outer longitudinal layer of the muscularis externa of the descending colon of a rat. × 40,000.

from a smooth muscle cell not in the plane of section has embedded itself within a deep depression of the surface of another smooth muscle cell. In other instances, the protrusions may fit within shallow depressions. The protrusion shown lacks vesicles but does contain remnants of myofilaments.

In addition to the existing relationships of smooth muscle cells with vesiculated nerve processes and other smooth muscle cells, the muscle cells also show a close relationship with connective tissue cells (Figs. 11 and 12), Schwann cells, capillaries, and cells which are tentatively identified as interstitial cells of Cajal. The tentative identification of the interstitial cell process in Fig. 15 is based on previous descriptions of interstitial cells (40, 50). This process contains a large vacuole and numerous large vesicles which are characteristic of interstitial cells. The space between this process and the smooth muscle cell is approximately 150 A.

DISCUSSION

The observations reported in this study clearly demonstrate that vesiculated nerve processes occur in smooth muscle tissue and that they are frequently in close apposition to the surface of the muscle cells. Even though the material used for this study consisted of rat tissue only, it appears from other evidence that vesiculated nerve processes also occur in the smooth muscle tissue of a wide variety of animals. Indications of such have been found in the electron microscopic studies of tissues from mice (6, 52), rabbits (40), guinea pigs (20), and man (57). Taxi (52), however, is of the opinion that the nerve-muscle relationships which he observed do not constitute discrete neuromuscular junctions. The reasons given for this belief are concerned with the variable diameters of the vesicles within the nerve fibers, the variability of the space width between nerve fibers and the smooth muscle cells, the lack of areas of increased density of the apposed membranes, and the supposed lack of nerve fibers within the outer longitudinal muscle layer of the intestine. Gansler (20), in her report of an electron microscopic study of the innervation of smooth muscle of the guinea pig colon, the rat uterus, and the frog stomach, also denies the occurrence of synaptic contacts between nerve fibers and smooth muscle cells. Gansler and Taxi are of the opinion that it is possible for the diffusion of a chemical mediator to take place from nerve to muscle over a considerable distance. This concept was suggested by Rosenblueth *et al.* (44) and later reiterated by Fawcett and Selby (18) in their report of the nerve-muscle

relationship within the turtle atrial wall. However, some evidence for the occurrence of discrete neuromuscular junctions in smooth muscle tissue has previously been advanced. Caesar *et al.* (6) consider the nerve-muscle relationships which they observed as "synapses between the autonomic nerves and the smooth muscle cells." Richardson (40), in his study of the smooth muscle innervation of the rabbit intestine, reported the presence of only two possible nerve endings making contact with the surface of smooth muscle cells. However, he recently presented more convincing evidence for the occurrence of discrete nerve endings on smooth muscle cells within the wall of the vas deferens of the rat (42).

Although the present findings add somewhat to our knowledge of the end formations of the autonomic nervous system, the organization of these end formations and the nature of the actual neuromuscular junctions in smooth muscle tissue are, for the most part, incompletely understood. No junctional folds such as are found in the motor end-plate of skeletal muscle are present and the supposed synaptic cleft is not always of a constant width. In the neuromuscular junctions of tonus skeletal muscle (33, 38), the striated muscle of blood vessels (29), and the muscle of invertebrates (16, 36, 48), it is found that junctional folds are lacking and that the width of the synaptic cleft is variable. These reports would indicate that these features are not necessarily essential for all neuromuscular junctions. Also, it may not be assumed at the present time that all nerve processes which contain the so called synaptic vesicles and mitochondria and which are but a few hundred angstrom units from the surface of the innervated structure are efferent in nature. Many reports (7, 11, 14, 17, 46, 47) indicate that afferent nerve processes also show similar characteristics. For this reason, great care must be exercised when vesiculated nerve processes are identified as afferent or efferent. Some or all of the vesiculated nerve processes observed in this study may be efferent or they may be afferent, and, if efferent, they may be sympathetic or parasympathetic. Richardson (42) has reported that nerve processes containing granulated vesicles are numerous within the wall of the rat vas deferens. The vas deferens appears to be primarily supplied by sympathetic nerve fibers (5). The infrequent occurrence of nerve processes containing granulated vesicles within the smooth muscle tissue of the gut wall of the rat may indicate that it has a meager sympathetic innervation. These unknowns, however, cannot be resolved merely on the basis of the morphological evidence presented here. Work is now in progress in an attempt to determine the true identity of all vesiculated nerve processes within the muscularis externa of the rat gastrointestinal tract.

As previously indicated (40, 52, 53), protrusions of smooth muscle cells and the processes of other cells such as Schwann cells, connective tissue cells, or interstitial cells may be mistaken for nerve fibers or even vesiculated nerve processes when vesicles are present within them. This is especially true when these protrusions or processes occur as individual entities within the intercellular spaces of smooth muscle tissue, or when they lie within a depression of the surface of smooth muscle cells. A confusion of this kind appears to be present in the material demonstrated by Yamamoto (57).

In some cases, nerve processes contain only a few synaptic vesicles as shown in Figs. 2 and 7. It may be possible that these processes are merely a short distance from an actual synaptic contact or that these processes are in a depleted state (14). Similar examples of this kind of relationship can be found in numerous reports of neuroeffector junctions (2, 3, 6, 12, 14, 37, 45, 47, 48, 52).

The electrophysiological experiments of Burnstock and Holman (5) on the vas deferens of the guinea pig indicate that neuromuscular junctions are present in smooth muscle tissue. They summarize their conclusions as follows: "Our results have shown that the mechanism of transmission of excitation from sympathetic nerve to smooth muscle is essentially similar to that of transmission at other neuro-effector junctions; stimulation of the effector nerve producing depolarization of the post-junctional membrane. The results also show that the sympathetic nerve endings must be distributed widely amongst the smooth-muscle cells of the vas deferens. Further, that there is a marked degree of convergence of each of the postganglionic axons on each individual or small group of muscle cells." Their technique was similar to the one used by Del Castillo and Katz (10) to study the skeletal neuromuscular junction.

It was determined by Richardson (41), in a combined light and electron microscopic study of the intestinal wall of rabbits, that the quality of

fixation and staining for light microscopic preparations is dependent upon many factors such as the composition and pH of the fixing and staining solutions used. He found it possible, by varying these factors, to produce results which are contradictory. Because of this source of contradiction and others, many conflicting reports are present in the literature dealing with the morphology and neuromuscular relationships of the peripheral autonomic nervous system.

The results of the present study and those of other electron microscopic investigations (6, 20, 40, 41, 50, 52) support the concept that the autonomic nervous system is composed of individual neurons and their definitive processes. No evidence has been found to uphold the view (28, 31) that the interstitial cells of Cajal act as intermediators between the nerve fibers of the autonomic neurons and the smooth muscle cells, even though processes of interstitial cells are sometimes closely applied to the surface of smooth muscle cells (Fig. 15). Furthermore, the concept (4, 30) of a syncytium of neurofibrils or small nerve fibers within a Schwann plasmodium is also untenable in view of the present observations. It appears, then, that the nerve bundles within smooth muscle tissue which emanate from the myenteric plexus are similar to the bundles of unmyelinated nerve fibers found in other portions of the nervous system. These individual nerve fibers, ensheathed by Schwann cells, eventually make contact with the surface of smooth muscle cells to form neuromuscular junctions.

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