SOME FEATURES OF THE SUBMICROSCOPIC MORPHOLOGY OF SYNAPSES IN FROG AND EARTHWORM*

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Plates 8 to 10

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INTRODUCTION

In reviewing the cytological aspects of synaptic function, Bodian (1) stressed the importance of learning more of the ultrastructure concerned, pointing out that since the classical work of Ehrlich (2), Retzius (3), Cajal (4), and others, little except technical refinement has been contributed to the methodology of the study of synapses.

It is evident that the resolving capabilities of the electron microscope can be applied to extend further our knowledge of synaptic morphology by taking advantage of recent improvements in fixation and sectioning techniques.

Using this approach, but basing his studies on sections from which the embedded plastic had been extracted by solvents with consequent distortion of relationships, Robertson (5) reported the distance between presynaptic and postsynaptic axoplasm, as separated by the two axolemmic membranes, to be about 600 A in the giant fibers of the stellate ganglion of the squid and in the abdominal ganglia of the crayfish. Estable *et al.* (6), observing the ventral ganglion of the acoustic nerve of dogs and cats, found the pre- and postsynaptic cytoplasmic masses to be separated by a minimal distance of about 320 A which corresponds approximately to the thickness of a double membrane. Sjöstrand (7), on the basis of very thin sections of well fixed retinal material, found the membrane of the retinal rod cell to be in close contact with that of the bipolar cell at the synaptic areas, with considerable interdigitation of the two cells, processes of the bipolar cell fitting neatly into complex recesses in the rod cell. Minute granules and rodlets were observed in the synaptic cytoplasm.

The preliminary observations mentioned above have thus settled some of the controversies which in the past derived from the limited resolution of the light microscope and the vagaries of silver staining. For example, they appear to

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render invalid the generality of de Castro's (8) postulate of a continuous glial sheath surrounding the presynaptic terminal (see also Robertson (5, 9)), and to support the concept of the individuality of the nerve cell implicit in Cajal's "neuron doctrine" by supplying evidence of continuous cellular membranes separating pre- and postsynaptic elements.

This paper reports a limited and exploratory study of morphological features of synapses encountered in the sympathetic ganglia of frogs and in the neuropile of the earthworm nerve cord. These latter are, according to Bullock (10), axo-axonal synapses, defining axons anatomically rather than on the basis of presumed direction of conduction. Amongst the observations cited here are several which are confirmatory of previous findings based on the light and electron microscopes. In addition, some new details of structure of synaptic membranes are presented. A vesicular or granular component of the synapse is described,[1] and is deemed to be of interest and worthy of further study in view of the special permeability and pharmacological properties attributed to synaptic membranes. However, no general conclusions are warranted on the basis of the observations reported here, since wide variations in synaptic morphology and behavior have been noted in the past, and since the material which has been examined in the present study is very restricted in scope. Moreover, exploration of cytoplasmic detail with the electron microscope is still in its infancy, and a satisfactory classification or understanding of all the cell components encountered is not yet at hand. With the limited experience at our disposal, interpretation of such matters as extent or limits of cell borders and cell membranes, the nature of particulate components, the "granular" or "vesicular" character of bodies encountered, the recognition of many of the profiles revealed in the micrography, and the distinction of artifact from true image, present many hazards and uncertainties which are not likely to be solved definitively without many years of further study and experience on the part of cytologists throughout the world. For these reasons, the interpretations we place upon our micrographs cannot be regarded as more than tentative.

Technique

Leopard frogs (*Rana pipiens*) and bullfrogs (*Rana catesbiana*) were pithed and the abdominal sympathetic nerve chain exposed by careful dissection, leaving the blood supply undisturbed. Earthworms[2] were pinned down in an extended position and the nerve cord exposed

[1] The material in this paper was published in preliminary abstract in *Federation Proceedings*, 1954, **13**, 35, and presented before the American Physiological Society on April 15, 1954. Palade has published simultaneously an abstract (*Anat. Rec.*, 1954, **118**, 335) also describing vesicles in the synaptic end foot. Palade presented his observations before the American Association of Anatomists on April 9, 1954.

[2] The earthworms used in this study were kindly identified for us as immature specimens of *Helodrilus caliginosus* (Savigny) by Professor T. H. Bullock of the University of California, Los Angeles, to whom we are greatly indebted for this taxonomic service, and for his helpful review and criticism of the manuscript.

In both cases the nervous tissue was flooded *in situ* immediately after exposure with Palade's (11) buffered osmic fixative. After a lapse of a few minutes, when fixation was well initiated, the nerve cords were removed, cut, and placed in fresh fixative for 1 to 4 hours. Pieces of fixed nerve cord and ganglia were dehydrated, embedded in methacrylate, and sectioned, using an ultramicrotome modified from the design of Porter and Blum (12), fitted with provisions for mechanical and for thermal advance. Satisfactory thin sections were obtained using both methods of advance. Sections were selected for thinness on the basis of interference reflection colors observed in the sections by means of a Spencer metallurgical microscope. The micrographs were taken with an RCA EMU electron microscope with compensated objective lens, fitted with objective, condenser, and projector apertures. The electron micrographs were obtained at magnifications ranging from 3,600 to 7,000 diameters, with subsequent photographic enlargement up to 65,000.

OBSERVATIONS

In the Frog.—Figs. 1 and 2 illustrate two types of synaptic junctions encountered in the abdominal sympathetic ganglia of the bullfrog. In the upper part of the first figure one can see the cytoplasm of a ganglion cell which is in close relationship to several sections across unmyelinated nerve fibers surrounded by Schwann cells. Two of these fiber sections, F_1 and F_2, are completely separated from the ganglion cell within the plane of the section, but occupy depressions or indentations of the ganglion cell surface. Collagenous fibers surround the Schwann cells of fibers F_1 and F_2.

The area F_3 appears to represent a section of a fiber at a level of synaptic contact with the postsynaptic neuron (*PSN*). The Schwann cell cytoplasm can be seen to contain the vesicular component (*Sch CV*) described elsewhere by De Robertis and Bennett (13). A portion of the Schwann cell and synaptic end foot occupies a depression in the postsynaptic ganglion cell.

The Schwannian covering does not extend over the enlarged end of the presynaptic fiber. The latter terminates in close contact with the surface of the postsynaptic member. The terminal portion of the presynaptic element is characterized by high density and by the presence of numerous scattered granules or vesicles (*SV*), about 100 to 300 A in diameter, which are most dense near the stalk of the end foot, and which scatter out or become less dense in regions close to the postsynaptic neuron. A membrane about 200 A thick, the synaptic membrane (*SM*), can be seen separating pre- and postsynaptic elements. This membrane is not here resolved as a double one, nor is it evident over the entire synaptic interface, being absent or much reduced in density at the central region of the synapse. The synaptic vesicles are largely, if not entirely confined to the presynaptic side of the membrane. However, in the central area where the membrane is defective or reduced in density, a very few vesicles or portions of vesicles can be seen in cytoplasmic areas which cannot be assigned with certainty to either pre- or postsynaptic cells. Here the vesicles show greatly reduced density.

Fig. 2 is identified as representing a section through another synaptic junction. Here again the postsynaptic neuron (*PSN*) occupies the greater part of

the top of the figure. An unmyelinated nerve process about 1.5μ in diameter enters the figure from the lower left and makes contact in a recess in the postsynaptic neuron above the center of the figure. Schwann cell cytoplasm (*Sch C*) can be distinguished sleeving the presynaptic fiber up to the synaptic area. A fibrillar component, the neuroprotofibrils or neurofilaments (*NF*) can be distinguished in the fiber as a series of thread-like densities less than 100 A in diameter, arranged somewhat irregularly, but in general oriented parallel to the longitudinal axis of the fiber, and terminating short of the area containing synaptic vesicles. Rather gross dense bodies designated as mitochondria (*M*) are noted in the presynaptic ending.

Two specially differentiated areas of synaptic contact are recognizable, each characterized by the presence of numerous synaptic vesicles and granules (*SV*). Between these specialized areas is seen a stretch of relatively unspecialized membrane. A synaptic membrane is distinguished also in this figure, presenting an apparent discontinuity at *X*, where pre- and postsynaptic cytoplasm seem to be continuous. The synaptic interface is cut obliquely in the section in most areas, though the vertical segment at the left under the indicating line from the letters "*SM*" appears double under high magnification. Synaptic vesicles are seen in two groups, appearing in each case to be confined entirely to the presynaptic cytoplasm, without the suggestion of encroachment of vesicles into the postsynaptic member, as implied in F_3, Fig. 1. In this figure the diameters of the vesicles range from 200 to 400 A. At high magnification many of the vesicles are seen to be less dense in the center than at the periphery.

In the Earthworm.—In the neuropile of the nerve cord of the earthworm one encounters a confusing tangle of unmyelinated nerve fibers of varying size. In the sections shown in Figs. 3 and 4 profiles of individual nerve fibers can be recognized as distinct units of irregular outline surrounded by a membrane. No Schwann cells are noted, each fiber being in close contact with a neighbor, with the cell membrane or axolemma of each fiber appearing as a distinct line when the interface is cut at an angle sufficiently close to the perpendicular. The fibers interdigitate extensively with each other, so that the sectional profile of some is ramifying and complex.

Within the fibers one can distinguish mitochondria (*M*) and endoplasmic reticulum or ergastoplasm (*ER*) (see Porter (14)). The mitochondria display the characteristic cristae of Palade (15). Neurofilaments are not recognized with certainty in the present material from the earthworm neuropile. As is evident in the section of the large fibers occupying the lower left corners of Figs. 3 and 4, the endoplasmic reticulum and mitochondria appear to be scattered loosely in an almost structureless matrix.

In specialized areas one can recognize a granular and vesicular component (*SV*) similar to the synaptic vesicles described above in the frog sympathetic ganglion. In the worm neuropile the synaptic vesicles likewise vary in diameter

from 200 to 400 A and many display a dense periphery and lighter center. They show interesting special relationships to the nerve fiber membrane, as described below.

Scrutiny of the nerve fiber membranes depicted in Figs. 3 and 4 reveals that the membranes show variations in density and areas of specialization of structure. Some of these variations are well exemplified in the axolemma of the nerve fiber whose profile occupies the lower left corner of Fig. 3. Inspection and tracing of other axonal membranes in Figs. 3 and 4 reveal other examples of similar variability.

Attention is drawn particularly to variations in the axonal membranes associated with clusters of vesicles. Samples of these interesting specialized areas, which are thought to be synaptic, are shown in Figs. 3 and 4 at sites designated by *Y*. Fig. 3A shows a further photographic enlargement of the example designated as *Y* in Fig. 3, whereas Fig. 4 is from a different negative. Focusing attention on Figs. 3 and 3A the neuronal element containing the vesicles is assumed to be presynaptic in nature and is taken to be represented in the section by the cellular mass designated *PRSN*, and bounded by the encompassing cellular axonal membrane of tortuous and irregular geometry. In the insert Fig. 3A, the presynaptic element occupies most of the lower two-thirds of the figure. Mitochondria (*M*), endoplasmic reticulum (*ER*), and unidentified membranous elements, some of which may represent folds or kinks in the axolemma, can be recognized. The postsynaptic neuron (*PSN*) appears to be represented in the section by a much smaller irregular surface about 180 mμ across, bounded all around by a membrane, which is most dense along its lower left surface. The full extent of this postsynaptic neuron is unknown, of course, but the portion here portrayed is taken to be a cross section of a finger-like process invaginating and infolding the presynaptic element, as it seems to be surrounded on all sides by the same cell, the membrane of which, however, is folded against itself at (*FO*) along a line of contact with the postsynaptic member.

Synaptic vesicles (*SV*) are encountered in two places in relation to the synapse in Figs. 3 and 3A; a large mass below it and to the left; a small group of vesicles above it and to the right. Within the large mass of vesicles to the lower left, special attention is drawn to the vesicles immediately in contact with the synaptic membrane itself, designated by *Y*. Some of these vesicles are characterized by high density, and all are elongated in an axis normal to the membrane. Two or three appear to occupy defects or gaps in the presynaptic membrane. Two or three vesicular structures can be detected in the synaptic interspace, in contact with the postsynaptic membrane, which is of great density. A few indefinite, faint, ghost-like, vesicular objects can be seen in the cytoplasm of the postsynaptic member. The second smaller mass of synaptic vesicles above and to the right of the postsynaptic area does not show any remarkable relationships between vesicles and membrane.

Close scrutiny of the presynaptic membrane shows it to be about 90 A thick, to vary in density from place to place, and to be perforated here and there by gaps or stomata in which lie portions of or extensions of synaptic vesicles. The postsynaptic membrane is of about the same thickness, is more uniform in density, and, in general, denser than the presynaptic or than non-synaptic cellular membranes. The intermembranal space is about 120 to 150 A wide, is of much greater density than non-synaptic interneuronal spaces, and contains in it vesicles or portions of vesicles of varying degrees of intactness.

The synapse designated as *Y* in Fig. 4 will now be described. The presynaptic neuron is construed to be represented by the large fiber occupying the center and most of the lower half of the figure, bounded by a membrane which enters the figure just below the middle of the left margin, sweeps upward and to the right, angles downward and to the right at the synaptic area, then curves, tending generally upward, then downward, and to the right, then downward to the left, leaving the figure near the middle of the right hand edge. The circumferential membrane traced above shows variations in density from place to place. The encompassed axonal cytoplasm displays mitochondria (*M*) and strands of endoplasmic reticulum (*ER*). Synaptic vesicles (*SV*) are seen in a cluster on the presynaptic side of the synapse in question. Some of these vesicles are in close relationship to the endoplasmic reticulum; others are contiguous with the synaptic membrane itself. Here again some of them taper or extend towards the membrane and appear to lie in perforations of the presynaptic membrane, so that portions of the vesicles are outlined in the intermembranal space. Vague and ghostly elongated vesicle-like profiles can be distinguished in the postsynaptic cytoplasm immediately adjacent to the membrane. The synaptic membranes themselves are similar in dimension to those shown in Fig. 3, the synaptic membranes measuring about 100 A in thickness respectively, separated by an interspace about 130 A wide. Both pre- and postsynaptic membranes vary in density over the synaptic area, being very dense for about 1,000 A on the left, then presenting a stretch of about the same length where the membranes are much less dense; thence continuing on to the right with further variations in density. In several places the postsynaptic membrane is barely distinguishable, and is appreciably less dense than the corresponding portion of the presynaptic membrane.

Synaptic vesicles can be recognized elsewhere in Figs. 3 and 4, particularly in the right lower quadrant of Fig. 4 and in the right half of Fig. 3. They may be indicative of near terminal axoplasm.

DISCUSSION

Some of these observations on the synaptic area of the frog appear to be confirmatory of those of Bartelmez and Hoerr (16) and of Bodian (17), who described mitochondria and neurofibrils in the presynaptic end foot in the

vertebrate synapse. The distribution of the filamentous component (*NF*) shown in Fig. 2 corresponds to the extent of the neurofibrils described by the above authors, suggesting that aggregates of the submicroscopic filaments here described may have given rise to the appearance of neurofibrils in the fish material studied by the Chicago authors.

In spite of the differences existing between the axosomatic synapse of the ganglion cell of the frog sympathetics, and the axo-axonal complex synapses of the neuropile of the annelids, some common submicroscopic details are apparent. One is that in both cases the two adjacent synaptic membranes are in close contact. However, our present material still does not permit a definite and final formulation of a concept of the nature of the synaptic interface. Presumably the double contours encountered frequently in the earthworm material and here and there in the frog are representative, and can be interpreted as indicating that pre- and postsynaptic neuronal elements each have their own cellular membranes, about 70 to 100 A thick, which approach within 150 to 100 A of each other at areas of synaptic contact. The apparent gaps or deficiencies in membrane density encountered at synapses in the frog and worm cannot be interpreted in a satisfactory manner at present. They may represent artifacts of fixation or tissue preparation, or may represent areas of transient break-down or dissolution of the synaptic membrane, perhaps related to some functional activity. We do not consider these occasional gaps to evidence a syncytial nature of the synapse. Neither frog nor earthworm shows structural material interposed between the synaptic surfaces. In the case of the neuropile, fine interdigitating expansions of neuroglial cells have been seen to separate different synaptic fields but not to extend between axon terminals themselves. In the frog synapse, although the axons leading to the end feet are enclosed within the cytoplasm of the Schwann cells and by a collagenous layer as described by Gasser (18), at the level of the synaptic terminal the presynaptic process becomes entirely naked and makes a direct contact with the postsynaptic membrane.

The vesicular component is likewise common to earthworm and frog material. It consists of oval structures 200 to 400 A in diameter, some of which are granular in nature, others of which present a dense periphery and a less dense center. This latter appearance seems to be most characteristic, and hence these structures have been termed synaptic vesicles. They may correspond to the granules seen in the vicinity of synaptic contacts of the retina by Sjöstrand (7), and in the enlarged terminals of submicroscopic nerve fibers by Fernández-Morán (19). In the frog sympathetic ganglion the synaptic vesicles are unequivocally encountered only in areas deemed to lie within the presynaptic terminal. In the neuropile of the earthworm the complexity of the synaptic field does not permit one to differentiate between pre- and postsynaptic elements on the basis of connections or gross morphological detail. However, the

variations in density encountered in the axo-axonal interfaces in the worm suggest that here and there are areas of specialized contact. Moreover, the distinct polarity evident in the worm where synaptic vesicles are in close relationship to the membrane and are confined to one side provides a picture entirely analogous to the synapses identified in the frog, and permits reasonable assurance in identification of pre- and postsynaptic elements on the basis of this analogy. The interpretation rendered that the synapses pictured in the earthworm represent small finger-like postsynaptic axonal projections indenting a larger presynaptic axonal member is in conformity with some features of the structure of the synapse between second and third order giant fibers in the stellate ganglion of the squid as described by Young (20). Moreover, the interpretation is in several respects similar to the concept of the structure of certain synapses in the crayfish as described by Robertson (5), though in the present worm neuropile no neuroglia or Schwann cells are distinguished, and one axon appears to come into direct contact with another, without intervening Schwann cytoplasm. In the crayfish the extent of invagination of the presynaptic member would appear to be much less striking than in the worm.

The close geometric association of synaptic vesicles with endoplasmic reticulum shown in Fig. 4 suggests that the ergastoplasm may have a role in the synthesis of vesicular contents. The connections of these vesicles with the synaptic membranes designated by *V* in Figs. 3 and 4, the elongation of these vesicles in a direction normal to the membrane, the extension of some of these vesicles into the intermembranal space through stomata, tubule-like extensions or elongations, and the appearance of vesicles or portions of vesicles in the intermembranal space, suggest that vesicles may move to the presynaptic membrane, perforate it, and discharge their contents into the space immediately adjacent to the postsynaptic membrane. This suggestion of movement of vesicles does not imply that movement over a distance of a quarter of a micron or so would necessarily have to occur during the brief period of synaptic delay. The vague ghost-like outlines resembling distorted vesicles in the postsynaptic cytoplasm immediately adjacent to the membrane suggest that some of the vesicular contents may find their way into the postsynaptic cell and there be destroyed rapidly. The validity of these suggestions remains to be established, however, and at this time nothing certain can be said regarding the physiological implications of these findings. Young (20), in describing certain oxyphil granules associated with synaptic surfaces in the squid, rightly cautions against assuming lightly that such bodies might represent any form of chemical synaptic transmitter.

It is of interest in this connection to point out that Feldberg (21) has brought forth and discussed evidence that acetylcholine in the central nervous system of vertebrates is associated with a protein-containing particulate component. Moreover, in the adrenal medulla secretion "droplets" of a size resolvable with the light microscope

have been described in detail by Bennett (22), and have been shown to give chemical reactions characteristic of adrenalin, and to be associated with the secretion of adrenalin into the adrenal veins. More recently Hillarp, Lagerstedt, and Nilson (23) have isolated by centrifugation from the adrenal medulla a granular fraction containing the sympathomimetic catechol amines. These "granules" appear to correspond to the "droplets" described by Bennett (22). Thus there is evidence in the literature that two of the substances pharmacologically active at synapses are associated with particles or viscous droplets as found in the cell. It is not unreasonable to speculate that active compounds of this nature might under some circumstances be associated with particles or granules or droplets or vesicles of submicroscopic size.

Our suggestion that the ergastoplasm may have a role in the synthesis of the vesicular contents is in context with Porter's (14) recognition that the endoplasmic reticulum as seen with the electron microscope in the cytoplasm of cells corresponds to the basophilic "ergastoplasm" of the earlier cytologists, and appears to correspond likewise to the ribonucleic acid–containing cytoplasmic component studied extensively by Caspersson (24), Brachet (25), and others, and which has come to be associated with protein synthesis. Changes which the endoplasmic reticulum of pancreas cells undergo with changes in synthetic activity have recently been studied by Weiss (26).

SUMMARY

Electron micrographs are presented of synaptic regions encountered in sections of frog sympathetic ganglia and earthworm nerve cord neuropile. Pre- and postsynaptic neuronal elements each appear to have a membrane 70 to 100 A thick, separated from each other over the synaptic area by an intermembranal space 100 to 150 A across. A granular or vesicular component, here designated the synaptic vesicles, is encountered on the presynaptic side of the synapse and consists of numerous oval or spherical bodies 200 to 500 A in diameter, with dense circumferences and lighter centers. Synaptic vesicles are encountered in close relationship to the synaptic membrane. In the earthworm neuropile elongated vesicles are found extending through perforations or gaps in the presynaptic membrane, with portions of vesicles appearing in the intermembranal space. Mitochondria are encountered in the vicinity of the synapse, and in the frog, a submicroscopic filamentary component can be seen in the presynaptic member extending up to the region where the vesicles are found, but terminating short of the synapse itself.

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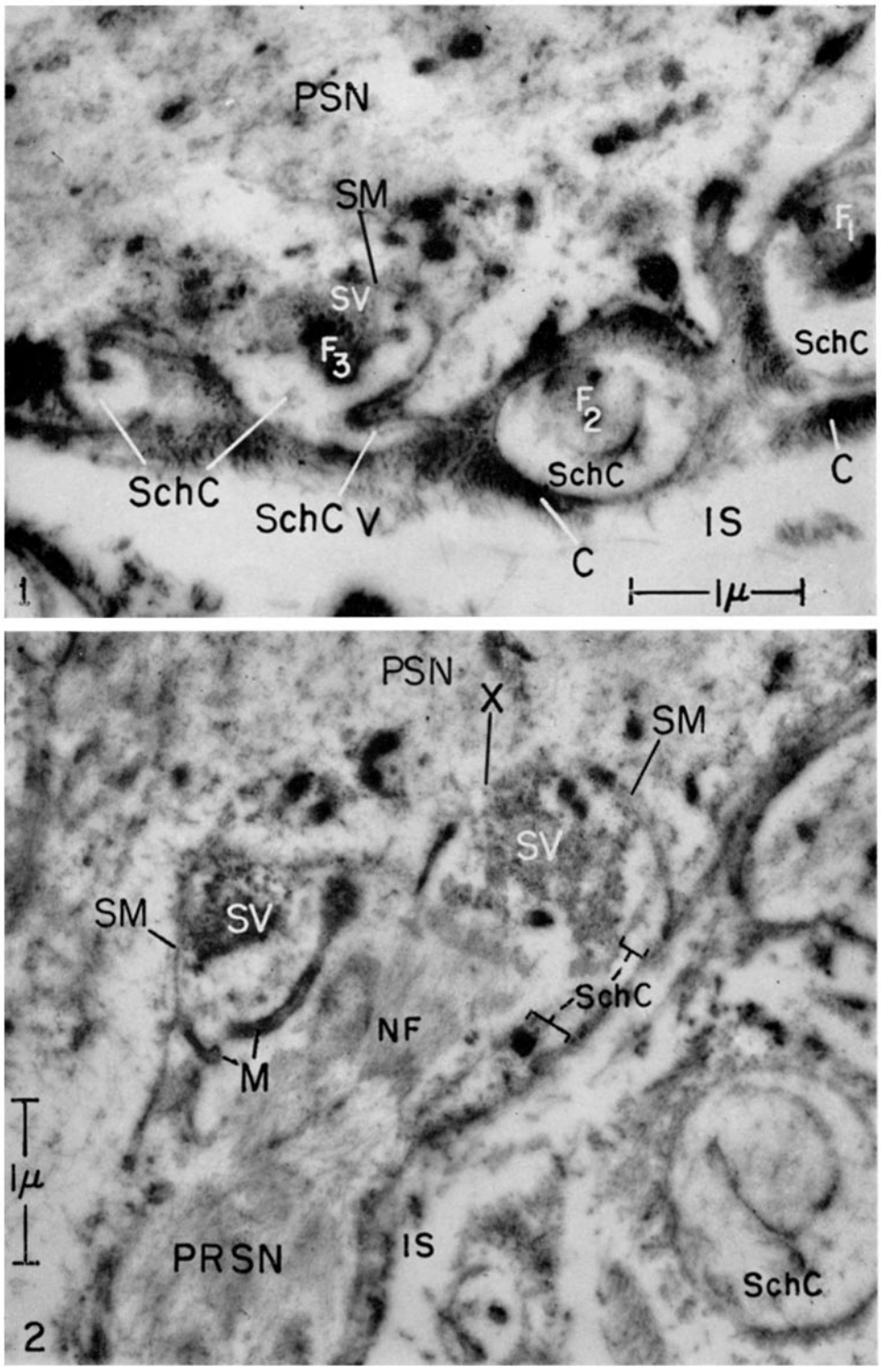
EXPLANATION OF PLATES

C, collagen (of endoneurium).
ER, endoplasmic reticulum, or ergastoplasm.
F, nerve fiber.
FO, fold of axonal membrane.
IS, intercellular space.
M, mitochondria.
NF, neuroprotofibrils, or neurofilaments.
PRSN, presynaptic neuron.
PSN, postsynaptic neuron.
Sch C, Schwann cell.
Sch CV, Schwann cell vesicles.
SM, synaptic membrane.
SV, synaptic vesicles.
X, gap or defect in synaptic membrane.
Y, area of synaptic contact.

PLATE 8

FIG. 1. Electron micrograph of an area in a section from the sympathetic ganglion of the bullfrog. The upper half of the figure is occupied by a portion of a sympathetic ganglion cell (*PSN*). Across the bottom of the figure is a somewhat exaggerated segment of intercellular space, containing scattered fibers of endoneurial collagen. In close relation to the lower border of the ganglion cell one can distinguish three areas (F_1, F_2, F_3) in each of which a nerve fiber with its surrounding Schwann sheath is cut in cross section. To the left is an additional area where part of a Schwann cell is sectioned, with only a small part of the corresponding fiber included. Fibers F_1 and F_2 are completely surrounded by Schwannian sheaths, which are in turn separated from the neuron by collagen fibers. Fiber F_3 is capped rather than surrounded by Schwann cell cytoplasm, in which vesicles can be seen (*Sch CV*). The fiber itself, F_3, appears to fan out into a broad area of contact with the postsynaptic cell. In this expanded terminal are seen numerous granules and vesicles (*SV*). The presynaptic terminal is separated from the ganglion cell by a membrane (*SM*) which cannot be seen to be continuous over the entire area of synapsis. × 24,700.

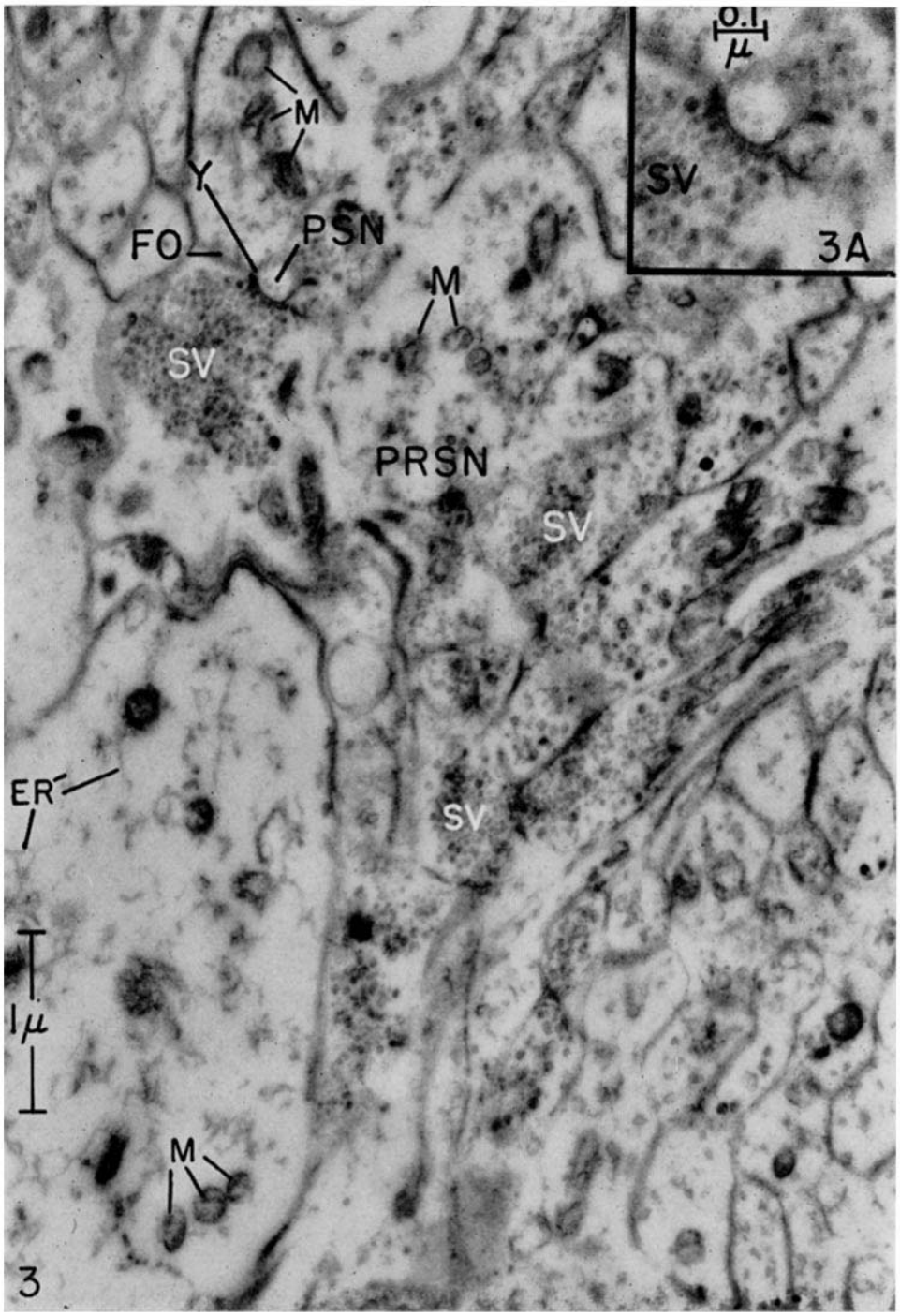
FIG. 2. Electron micrograph of a section taken from the sympathetic ganglion of the bullfrog. The left and upper borders of the figure are occupied by a portion of a sympathetic ganglion cell (*PSN*). An unmyelinated nerve fiber (*PRSN*) enters the field from the lower border and passes upward and to the right, coming into close contact with the postganglionic cell. This nerve fiber is bordered by a Schwannian sleeve (*Sch C*), and contains neurofilaments (*NF*), mitochrondria (*M*), and synaptic vesicles (*SV*). Beyond the limits of the Schwann cell borders, only a membrane (*SM*) separates pre- and postsynaptic elements. Continuity of this membrane is not evident at *X*. × 23,000.



(De Robertis and Bennett: Morphology of synapses in frog and earthworm)

PLATE 9

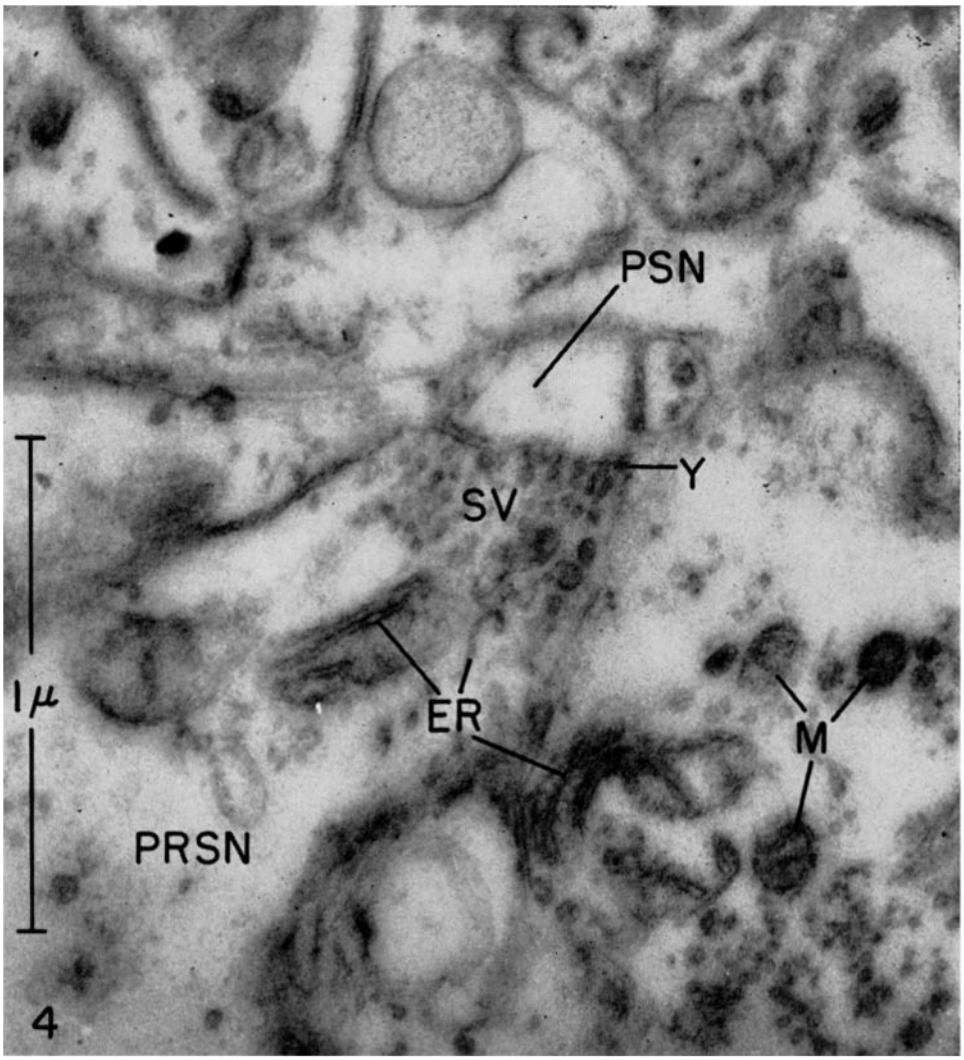
FIG. 3. Electron micrograph of a section of an area of complex axonal entanglement from the neuropile of the earthworm. Numerous profiles of axonal membranes can be distinguished, varying in density from place to place. Mitochondria (*M*) and endoplasmic reticulum (*ER*) are distinguishable in several places. *Y* denotes an area of specialized axonal contact identified as synaptic in nature. Numerous synaptic vesicles (*SV*) are seen in the presynaptic neuron (*PRSN*), whose profile is of irregular outline and which occupies the center portion of the upper half of the figure. The profile of the postsynaptic member (*PSN*) is identified as a section through a finger-like axonal projection indenting the presynaptic axon, producing puckerings or folds (*FO*) in the presynaptic axonal membrane. The insert Fig. 3A, in the upper right corner, shows the synaptic area under higher magnification. Here the pre- and postsynaptic membranes are clearly resolved, synaptic vesicles can be seen aligned along the presynaptic membrane, extending into the synaptic interspace, and coming into direct contact with the postsynaptic membrane. Vague ghost-like annular profiles can be seen in the postsynaptic cytoplasm, suggesting remnants of vesicles. Main figure, × 25,000; insert, × 65,000.



(De Robertis and Bennett: Morphology of synapses in frog and earthworm)

PLATE 10

FIG. 4. Electron micrographs of a portion of the neuropile of the earthworm, showing a confusion of axonal profiles. A section through a large axonal process (*PRSN*) occupies approximately the lower half of the figure. In it mitochondria (*M*) and endoplasmic reticulum or ergastoplasm (*ER*) are apparent. *Y* designates a specialized area, judged to be one of synaptic contact, where the axonal membrane is modified by its relation to numerous vesicles (*SV*). A portion of another axon (*PSN*) identified as postsynaptic, is shown above and close to the synaptic membrane. Synaptic vesicles in this area of the presynaptic member can be seen in close relation to the endoplasmic reticulum. The vesicles close to the synaptic membrane itself are elongated along an axis normal to the membrane. The elongated extensions of some vesicles appear to occupy perforations of the presynaptic membrane, bringing portions of the vesicles into intimate contact with the postsynaptic membrane. Faint ghost-like profiles suggesting remains of or extensions of vesicles can be seen extending into the postsynaptic cytoplasm. × 64,600.



(De Robertis and Bennett: Morphology of synapses in frog and earthworm)