The Fine Structure of Synapses in the Ciliary Ganglion of the Chick*

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PLATES 10 TO 16

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ABSTRACT

Ciliary ganglia of chick embryos and newly hatched chicks were examined in the light and electron microscopes. Particular attention was given to the fine structure of calyciform synapses, which are characteristically found in ciliary ganglia of birds. The calyciform endings are characterized by large expansions of the presynaptic axons upon ganglion cells, and the terminal processes extend over a considerable area of the cell surface. Often, indeed they appear to envelop the cell.

In the electron microscope image, the appositional membranes are separated by a space about 300 to 400 A wide; *i.e.*, the synaptic cleft. At irregularly spaced regions, the appositional membranes show areas of increased density. The presynaptic processes contain clusters of synaptic vesicles, localized at these dense regions. Thus the fine structure complex typical of other synapses is evident.

The unique structural features of this synapse are as follows: (*a*) The calyx or presynaptic terminal derives from a single axon, does not arborize, and terminates upon a single ganglion cell. Thus, unlike the classical *bouton terminal*, this represents an anatomical device for firing single cells by single axons. (*b*) The surface area in contiguity, *i.e.*, the area of appositional membranes, is far more extensive than the *bouton terminal*.

The fine structure of this synapse is compared with others, for example, the classical *boutons terminaux* and purely electrical synapses, in an attempt to correlate fine structure with function.

In recent years, numerous studies have been published describing the fine structure of synapses in the nervous system (6, 9, 14, 16, 17). These have demonstrated a remarkable uniformity in architecture at synaptic junctions. For example most synapses are characterized by: (*a*) closely applied limiting membranes of the presynaptic and postsynaptic processes separated by a cleft approximately 200 A wide, with occasional areas of increased density; (*b*) presynaptic terminals which usually contain accumulations of mitochondria and clusters of small vesicles about 200 to 700 A in diameter. Since these descriptions have been derived from observations made in a number of synaptic regions, a generalized picture of the fine structure of the synapse has emerged. There are however, numerous areas in which relatively few neural contacts have been seen as yet, notably the neuropil and the cerebral cortex (17), despite the fact that these areas abound in synapses. Since little is known about the structural variation in synaptic junctions, endings which do not conform to what might be termed the archetype may go unrecognized by the electron microscopist. It has seemed advisable, therefore, to examine synaptic regions which are highly specialized structurally, and in which the nature of the terminations is well

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documented by light microscopy and to compare these endings with the classical *boutons terminaux*.

The group of synapses described as calyces are structurally unique. These are located in several regions of the nervous system; *e.g.*, the nucleus of the trapezoid body, the nucleus tangentialis, and the ciliary ganglion. It is of interest, therefore, to determine whether or not these calyces contain concentrations of vesicles and mitochondria in a form comparable to synapses already described, or whether they exhibit fine structural organizations of another kind.

The following account is a description of calyciform endings in the ciliary ganglion of the chick, in which, it will appear, the fine structural organizations are significantly similar to the *bouton* model. A brief report of this study has appeared elsewhere (7).

Materials and Methods

The tissues utilized in this study consisted of ciliary ganglia obtained from chick embryos (Hamburger-Hamilton series 36 through 45) and newly hatched chicks. For light microscopy, ganglia were fixed and stained by the Bodian silver method (1, 2). For electron microscopy, ganglia were fixed for 1 hour in a 1 per cent osmium tetroxide mixture at 4°C. (5). Dehydration, embedding, and polymerization were carried out by the standard methods. Sections approximately 0.1 to 0.5 micron thick were cut on a Porter-Blum (Servall) microtome and studied in the phase microscope. Contiguous thin sections were examined with an RCA EMU-2E and an EMU-3C electron microscope.

OBSERVATIONS

Light Microscopy

General Appearance.—Since the early descriptions of van Lenhossek (12, 13) and Carpenter (3) little attention has been paid to the cytology of the ciliary ganglion in spite of the fact that it contains a most interesting group of synapses. The ciliary ganglion in the chick lies between the branches of the oculomotor nerve which innervates the ventral oblique and the ventral rectus muscles. It is spindle-shaped and approximately 2 mm. in length. The ganglion receives nerve fibers from two main sources, the ophthalmic division of the trigeminal nerve and the oculomotor nerve. The proximal end of the ganglion contains myelinated nerve fibers of the oculomotor nerve. These fibers enter the ganglion, gradually lose their myelin sheath, and terminate upon ganglion cells (Figs. 1 and 2). The nerve fibers which emerge from the trigeminal nerve are lightly myelinated. They enter the ganglion at its distal end and also terminate upon ganglion cells. The limits of these two regions are not sharply defined and detectable only in favorably oriented transverse sections.

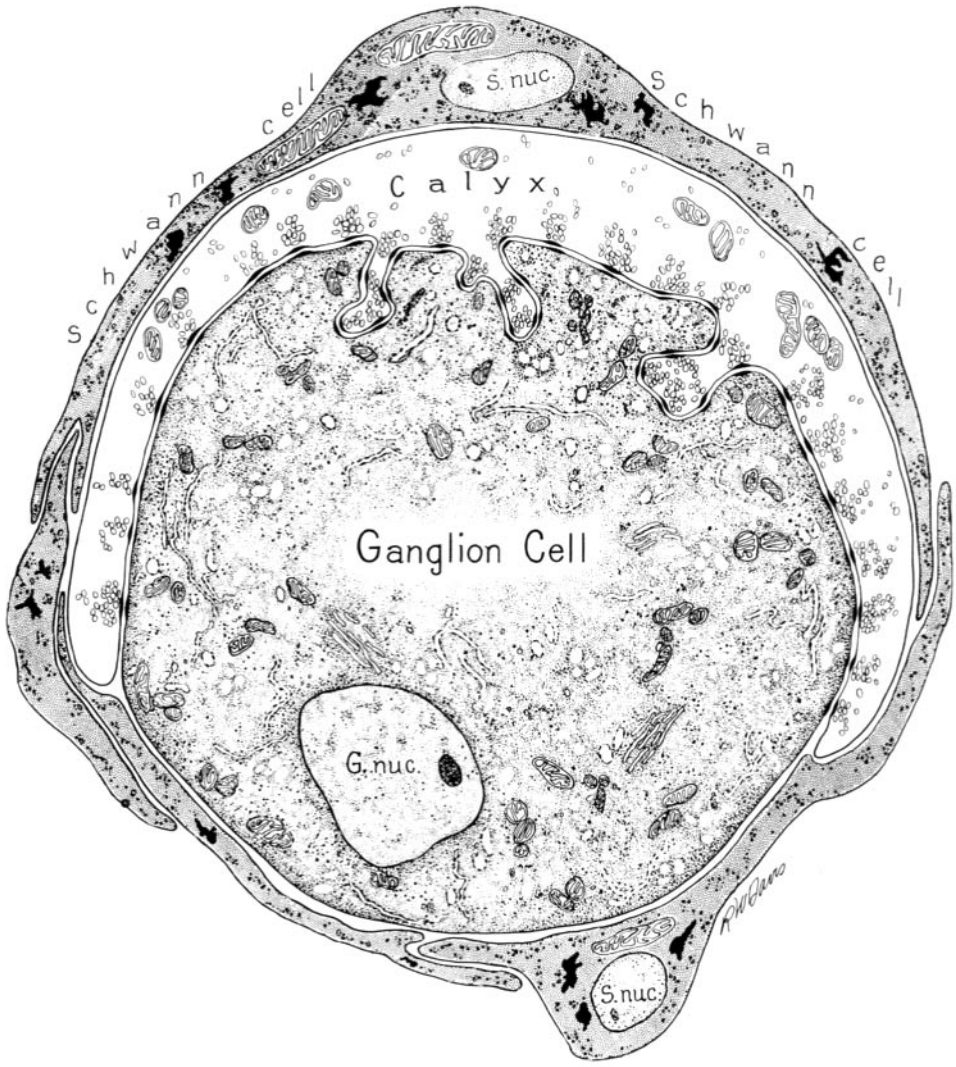
The cells in the ciliary ganglion of the chick are all unipolar. A single process emerges from the cell and becomes a component of the ciliary nerves. The cells are rather closely packed (Figs. 1 and 2) and contain nuclei which are displaced to one end of the cell (Fig. 2). One or more nucleoli may be seen in each nucleus. The individual ganglion cells are enclosed in capsular sheath cells, whose nuclei are very prominent. Interposed between the Schwann cell and the ganglion cell is a process, the neurite, which comprises the calyciform ending (*CE*) characteristic of the ciliary ganglion (Figs. 1 and 2). Scattered myelinated fibers and connective tissue elements occupy the spaces between cells. The calyciform endings are characterized by expansions of the fibers with a variable number of sepal-like processes. In silver impregnated preparations, the calyx appears to envelop the ganglion cell over a considerable distance. However, even with the best silver preparations, the fine structure of the appositional membranes is unresolvable (Fig. 2). In other locations, the ending arborizes into two or three large terminal knobs. The former, however, is the most frequent type (12). Another type of ending is present and can be demonstrated best by silver impregnation methods (Figs. 1 and 2). In this case the neurite divides before reaching the ganglion cell and many small terminal knobs (*BE*) are seen making synaptic contact with cell (Fig. 2 arrow). Although minor variations in these endings have been described using methylene blue staining methods, the synapses observed in this study conform to the above described types.

Electron Microscopy

General Appearance.—The ciliary ganglion cell is easily recognized by its large size and its characteristically dense and compact cytoplasm. Figs. 4 and 7 are views of rather low magnification demonstrating the main morphological structures encountered. In Fig. 4, two large ganglion cells (*G*) are evident and a nucleus (*GN*) appears at the lower left. Surrounding the ganglion cells are light processes (arrows), which comprise the calyciform endings characteristic of the ciliary ganglion. Separating the individual cells is a connective tissue space (*CT*) which contains numerous nerve fibers,

Schwann cells, and collagen. Ensheathing each ganglion cell are processes deriving from Schwann cells. In Fig. 7, small neural processes (*BE*) containing synaptic vesicles are in close apposition to the ganglion cell. This complex in turn is ensheathed by Schwann cells (*S*). These are the basket endings which have been described above. These features are schematically represented in Text-fig. 1.

Nerve Fibers.—The preganglionic nerve fibers which terminate upon the cells in the ganglion are illustrated in Fig. 3. Myelinated axons are scat-



TEXT-FIG. 1. This figure is a highly schematic drawing incorporating the principal fine structure details of calyciform endings in the ciliary ganglion of the chick. The calyx is shown in contiguity with a considerable area of ganglion cell surface. Interdigitations of the presynaptic process with the postsynaptic cell occur frequently in embryonic ganglia and infrequently in newly hatched chicks. However, in all calyces the appositional membranes exhibit localized dense regions. At these locations, clusters of synaptic vesicles are evident in the presynaptic terminal. The synaptic cleft is uniformly 300 to 400 A wide. Occasionally, a dense line is resolved between the appositional membranes in the synaptic cleft.

Schwann cells and their processes invest both the calyx and the ganglion cell (*S. nuc.* identifies the Schwann cell nucleus and *G. nuc.*, the ganglion cell nucleus). Endoplasmic reticulum, cisternae, cytoplasmic granules, and mitochondria are schematically represented and cytological detail is not intended.

tered throughout the connective tissue space (*CT*). Nuclei of Schwann cells (*SN*) and collagen (*C*) fill most of the space between fibers although some large "empty" spaces are evident. The axon labeled (*A*) is a typical fiber sectioned longitudinally. The axoplasm contains very fine neurofilaments, endoplasmic reticulum, and a few mitochondria. Preganglionic axons usually contain more neurofilaments than do the postganglionic nerve fibers; *i.e.*; the ciliary nerves. The myelin sheath and Schwann cell (*S*) complete the structural components of each nerve fiber. A few mitochondria and numerous vesicles are evident in the cytoplasm of the Schwann cells.

Ganglion Cells.—The ganglion cells are characterized chiefly by their large size. The perikaryon of the cell is very dense in appearance (Figs. 4, 5, and 7). The nuclei are not centrally located and usually one or more nucleoli are present. Numerous mitochondria are seen, which are similar in fine structure to mitochondria in other tissues. Perhaps the most striking feature of the perikaryon is the dense concentration of cytoplasmic granules. These granules are usually clumped at the periphery of the cell (Figs. 4 and 5), although occasionally they are near the nucleus. The individual granules are arranged in rosettes in a pattern similar to that described in other neurons (15). There is a paucity of highly ordered endoplasmic reticulum; instead, tubules, vesicles, and cisternae are seen throughout the cell (Figs. 4 and 5). The axon hillock is seen infrequently but easily identified when encountered. In this region, the granular component of the perikaryon terminates abruptly and does not extend into the axon.

Relation of Schwann Cells to Ganglion Cell.—Schwann cell processes form a continuous investment around the ganglion cell, which includes the region of the axon hillock. Occasionally, a single Schwann cell process ensheaths a large surface of the nerve cell. More frequently several processes, perhaps deriving from different Schwann cells, envelop the ganglion cell (Fig. 7). The plasma membrane of the sheath cell is closely apposed to the cell membrane of the neuron. This structural relationship exists in all regions of the ganglion cell, except where the calyciform or basket endings are observed. In these regions, the synaptic endings touch the neuron directly and the Schwann cells lie outside of the synaptic processes (Figs. 4 to 7). The Schwann cell cytoplasm contains the usual organelles and the mitochondria are conspicuous because of their large size. Vesicles, membranes, granules, and lipid inclusions are also present (see Text-fig. 1).

Calyciform Endings.—Fig. 4 is a representative section through two ganglion cells (*G*) demonstrating the calyciform type of synapse. The light process, delineated by the arrows, is the presynaptic calyx terminating upon the cell. Note the extensive ganglion cell surface covered by the calyciform ending. Interdigitations (*E*) of the presynaptic process with the ganglion cell are seen at the region of the middle arrow in Fig. 4. Similar interdigitations were seen in all embryonic ganglia examined. However, in newly hatched chicks they were less frequently encountered. Schwann cell processes are located outside the calyx, thus providing an uninterrupted, continuous outer sheath around the area of synapse. Fig. 5 is a much enlarged portion of the previous figure. The presynaptic process contains mitochondria, a few filaments, and concentrations of minute vesicles which are seen at most synapses. The plasma membrane of the calyx shows several interesting features. First, it is separated from the postsynaptic plasma membrane throughout the area of contiguity by a distance of 300 to 400 A. Secondly, areas of increased density occur in various regions of the membrane (arrows). At these locations, which do not seem to have any particular regularity, the postsynaptic membrane is likewise denser in appearance (Fig. 5). The vesicles in the presynaptic process are also accumulated in these locations in a pattern similar to other synapses. In other locations, increased density is not evident in the apposed membranes. The postsynaptic cell cytoplasm does not show any structural specialization except an occasional accumulation of vesicles. These observations are summarized in Text-fig. 1.

The calyciform endings are located on the ganglion cell surface remote from the axon hillock, and thus these endings are entirely axosomatic. Schwann cell processes do not intervene between the calyx and the ganglion cell except where the presynaptic axon has not yet been delivered to the neuron surface. Fig. 6 is a section cut perpendicular to the long axis of a calyx. The connective tissue space (*CT*) is seen at the lower left. A Schwann cell whose nucleus (*SN*) is located at the upper left, extends its processes (*S*) around the calyx (*CE*) in all regions except the area designated by arrows, which is the zone of synapse. Synaptic interdigitations (*E*) are seen in the upper half of

the figure. Note the increased density of the apposed synaptic membranes and the accumulation of vesicles in this region.

Fig. 7 demonstrates a calyx in the middle of the figure and smaller synaptic processes at the upper and lower left, which comprise the basket endings (*BE*). Ganglion cells containing both types of endings were encountered very infrequently. In Fig. 8, a curious relationship is observed. A nerve fiber (*F*) is seen very close to its ultimate termination. This can be inferred from the densely clustered synaptic vesicles. Schwann cell membranes surround the fiber. In turn, the fiber appears to have penetrated a calyciform ending on the nerve cell (*G*). Synaptic contiguity is evident in the appositional membranes at the regions designated by the arrows. A sheath cell (*S*) encloses the entire complex and the connective tissue space (*CT*) is seen at the upper right. The "gabelformige Endigunden" described by van Lenhossek (12) is illustrated in Fig. 9. In this synapse, the neurite (*NE*) divides near the ganglion cell (*G*) and large terminal knobs (*K*) make synaptic contact with the cell. Other regions of apparent synapse, in the proximal portion of the neurite, are designated by the arrows.

DISCUSSION

Examination of synapses in the ciliary ganglion of the chick has demonstrated fine structural organizations quite similar to the classical *bouton terminal*. The similarities consist of: (*a*) the width of the synaptic cleft, measuring about 300 to 400 A; (*b*) the location and concentration pattern of synaptic vesicles residing in the presynaptic terminal. However, important structural differences have also been described. Synapses studied in other regions such as the medulla oblongata, the spinal cord (14), and the olfactory glomerulus (4) clearly demonstrate numerous presynaptic fibers which divide and end on one or more nerve cells. *Boutons terminaux*, for example, usually consist of collaterals of arborizing axons. In this type of ending, only a small portion of the postsynaptic cell surface is in contiguity with the terminal processes. It is generally agreed by physiologists that many synaptic depolarizations are required to fire some nerve cells (10). In addition, the concepts of facilitation and recruitment require more than one ending on a given cell or population of cells (18). It is of interest, therefore, that in the case of the calyciform ending, only one fiber provides the terminal which is in contiguity with a considerable surface of the postsynaptic appositional membrane. The calyx provides an anatomical device for the firing of single cells by single axons. This suggests, perhaps, facilitation in time rather than in space at such synapses. Rarely, more than one type of ending is seen on the same ganglion cell. Thus the general pattern is the one-to-one-relationship described.

Whether the synaptic interdigitations represent a significant increase in the synaptic area remains to be seen. The evidence suggests the interdigitations are more characteristic of embryonic ganglia than of adult. The exact dimensions of the area of synaptic contiguity are most important to neurophysiologists, since the specific resistance of the fluid in the synaptic cleft and the resistances of the appositional membranes determine the current flow at the synapse. In the classical *bouton*, the terminal is a structure that is very effectively designed for efficient synaptic transmission of either excitatory or inhibitory actions. However, if the area of synaptic contact is considerably increased, the cleft resistance might be so greatly increased, relative to the membrane resistance, that the synaptic mechanism would become inefficient in evoking the flow of postsynaptic currents (10). The calyciform endings present an ideal anatomical structure to test such an hypothesis. The presynaptic fibers and the ganglion cells are sufficiently large to permit the entry of microelectrodes.

Finally, it has been suggested that synaptic vesicles, located at the neuromuscular junction (16) and at other synapses in the presynaptic processes (14) may be involved in the quantal discharge of a chemical transmitter such as acetylcholine. The synapses in the ciliary ganglion seem to fall into this class of synaptic junctions. However, recent examination of strictly electrical synapses, *i.e.*, crayfish lateral giant fiber to motor fiber synapses (11), has demonstrated large clusters of "synaptic vesicles" on the postsynaptic side of the synapses (8). In an electrical synapse "synaptic vesicles" should not be expected. However, they may be related to an entirely different event; *e.g.*, influx or efflux of specific ions. Vesicles, *per se*, do not necessarily mean a transmitter is involved. It would seem wise to approach with caution any interpretation concerning the role of the vesicular component in synaptic junctions, until more evidence is forthcoming.

It is a great pleasure to thank Dr. Rita Levi-Montalcini for suggesting this most interesting group of synapses and for her advice and assistance throughout this investigation. The author is likewise grateful to Professor Viktor Hamburger for the generous use of the facilities of his department during certain phases of this study.

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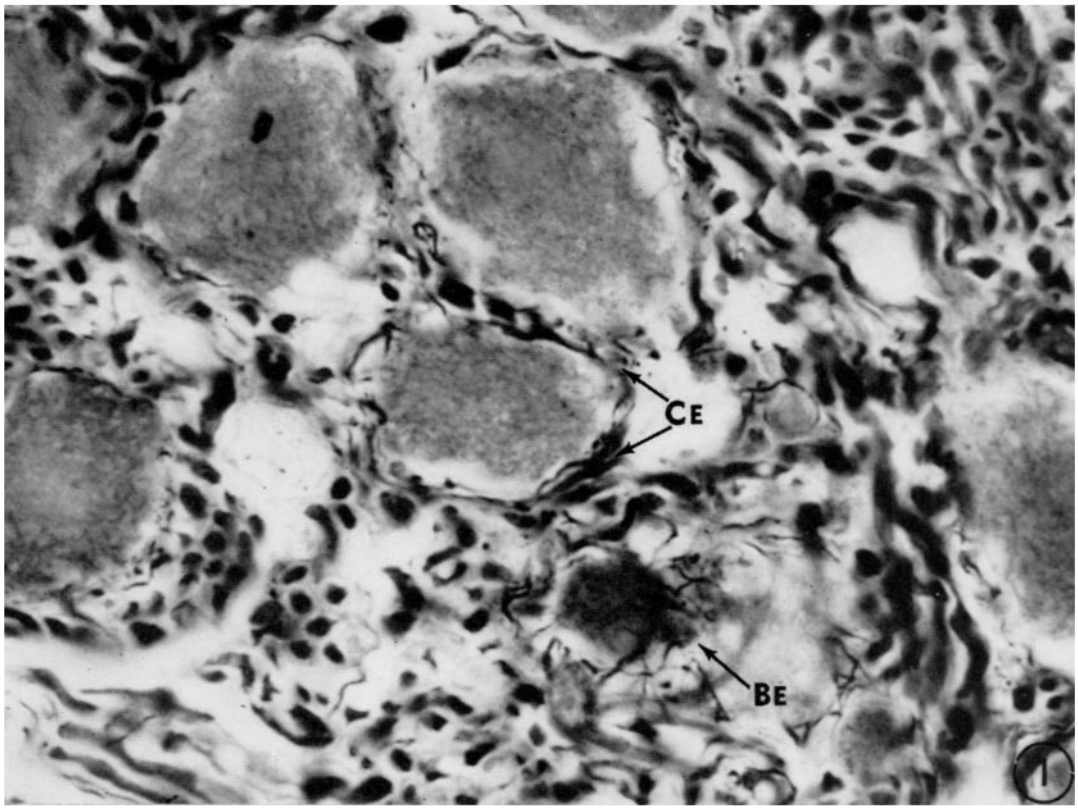
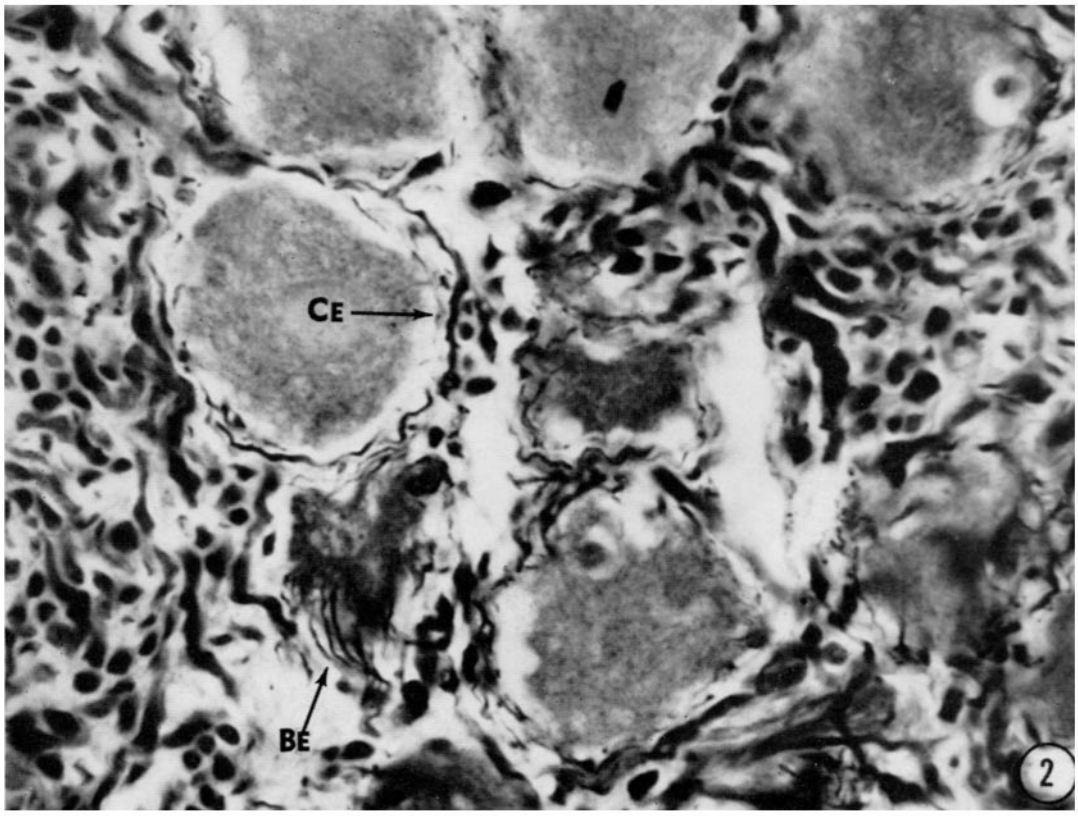
Explanation of Plates

Plate 10

Fig. 1. Section through the ciliary ganglion illustrating the unipolar ganglion cells with their nuclei usually containing a nucleolus. At the region designated by the arrow (*BE*), many small neural processes terminate upon a cell. In the larger cell immediately above, a calyciform ending (*CE*) is evident, which is in contiguity with a large portion of the cell body. Bodian-stained preparation. × 1000.

Fig. 2. Section through a ciliary ganglion stained by the Bodian method. Note at the arrow the basket endings (*BE*) and at the cell immediately above a small calyciform ending (*CE*) which is poorly demonstrated with silver staining, due to shrinkage artifacts. × 1000.

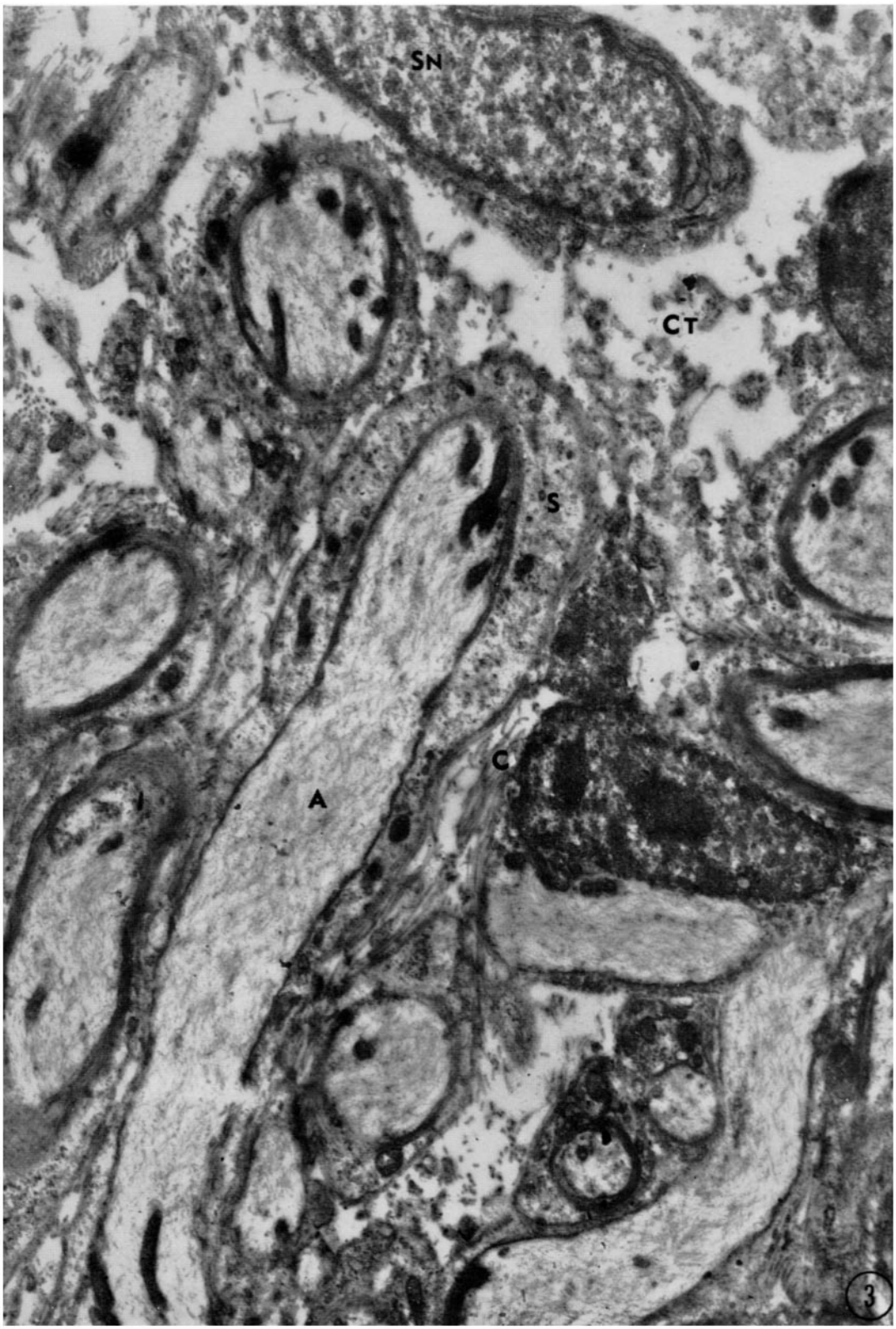




(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 11

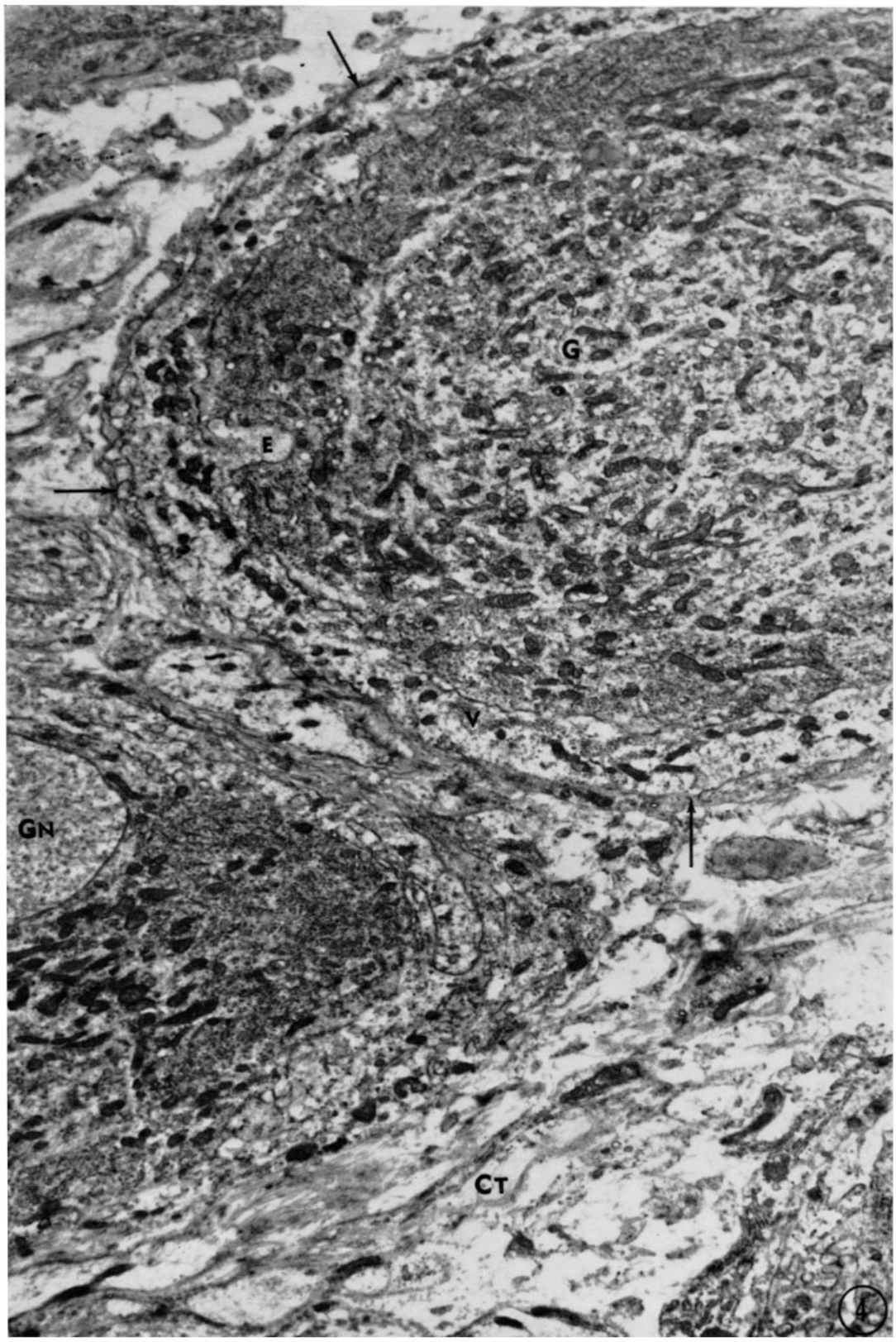
FIG. 3. Electron micrograph of the presynaptic nerve fibers which are lightly myelinated. These nerve fibers are surrounded by a connective tissue space (*CT*) which contains collagen (*C*), Schwann cells (*SN*), and fibroblasts. The fiber labeled (*A*) is a longitudinal section through a typical axon demonstrating the relationship of the Schwann cell (*S*). The axoplasm contains numerous neurofilaments and a few mitochondria. × 6000.



(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 12

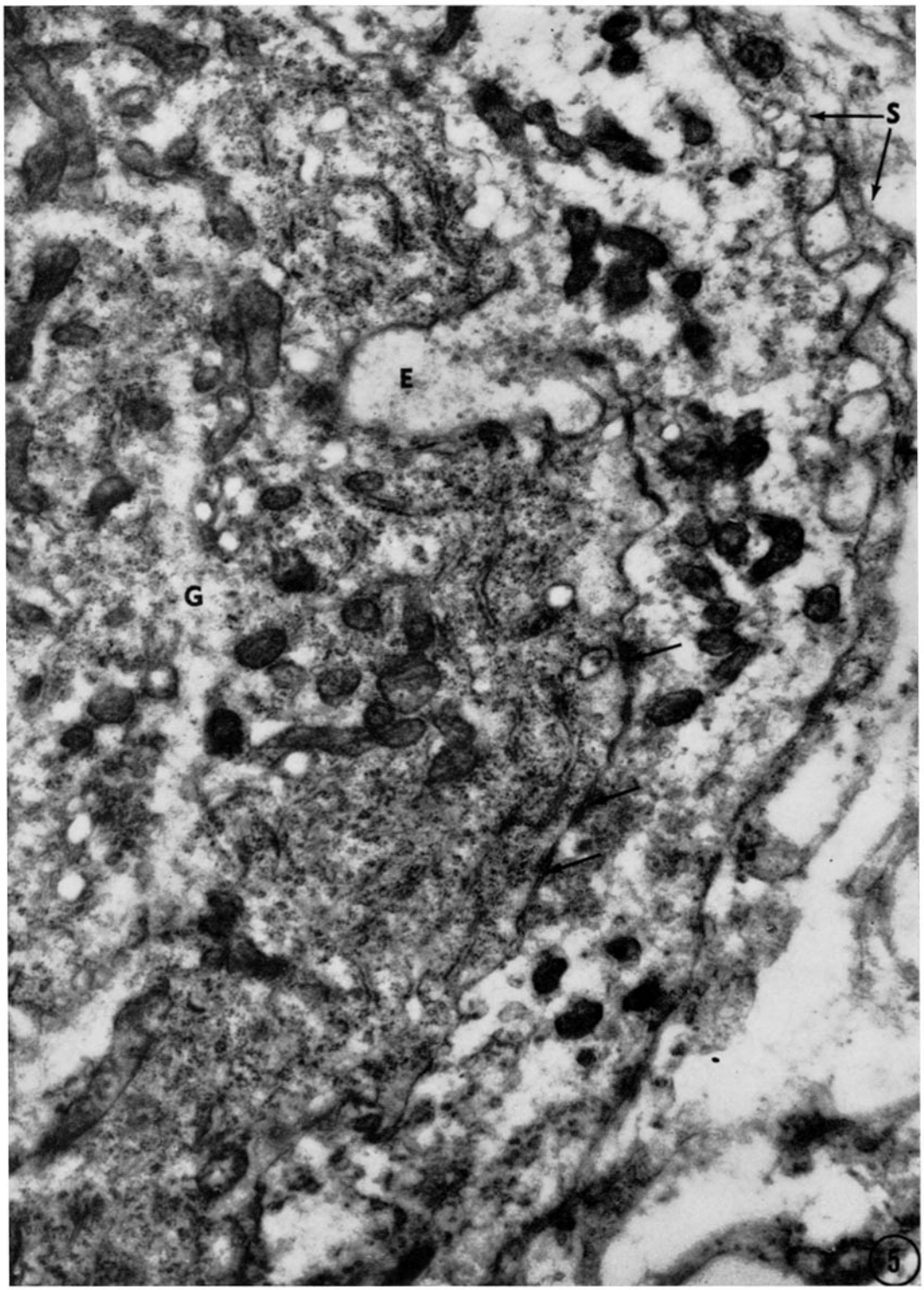
FIG. 4. Low magnification of the ciliary ganglion in which two ganglion cells (*G*) are seen. Connective tissue elements (*CT*) separate the cell at the left, whose nucleus is designated (*GN*), from the other ganglion cells. Note the light process of the neurite which surrounds the cell at the left. This relationship is better demonstrated in the upper cell by the area designated with arrows. These processes comprise the calyciform endings typical of the ciliary ganglia of the chick. The presynaptic process is in contiguity with a large surface area of the postsynaptic cell and contains mitochondria and numerous clusters of minute vesicles (*V*). Interdigitations of the synaptic processes often occur (*E*). × 4000.



(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 13

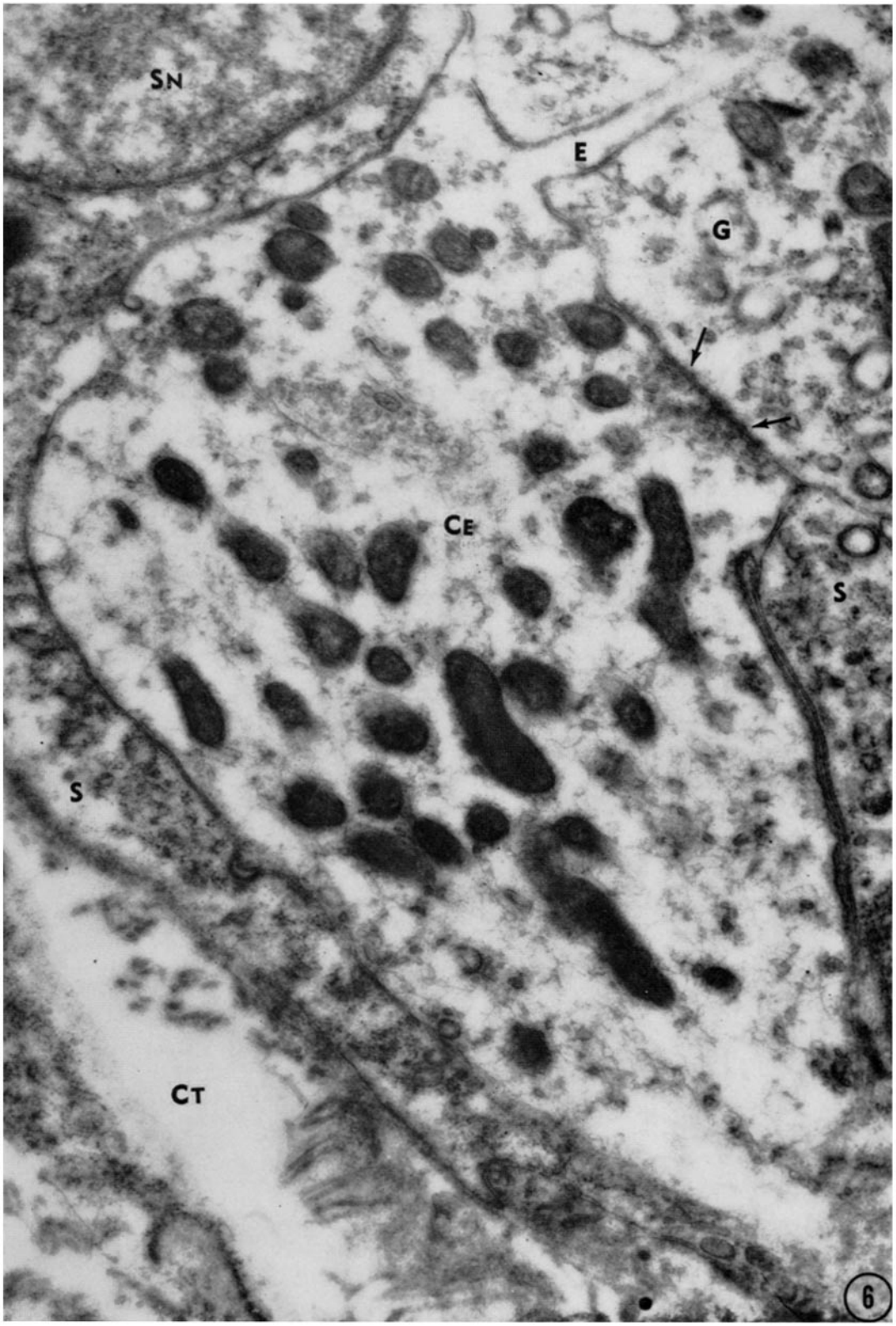
FIG. 5. Higher magnification of a restricted portion of the previous figure. The ganglion cell (*G*) is to the left and the terminal process to the right. The pre- and postsynaptic membranes are separated by a small space about 300 A wide throughout the area of contiguity. The presynaptic process contains mitochondria and minute vesicles, which are concentrated in clusters at regions of increased membrane density (arrows). In the upper half of the figure interdigitation (*E*) of the presynaptic process and the ganglion cell occurs. Such interdigitations occur most frequently in calyciform endings of embryonic ganglia and usually contain clusters of synaptic vesicles. Surrounding the outer edges of the calyx are numerous small Schwann cell processes (*S*). × 24,500.



(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 14

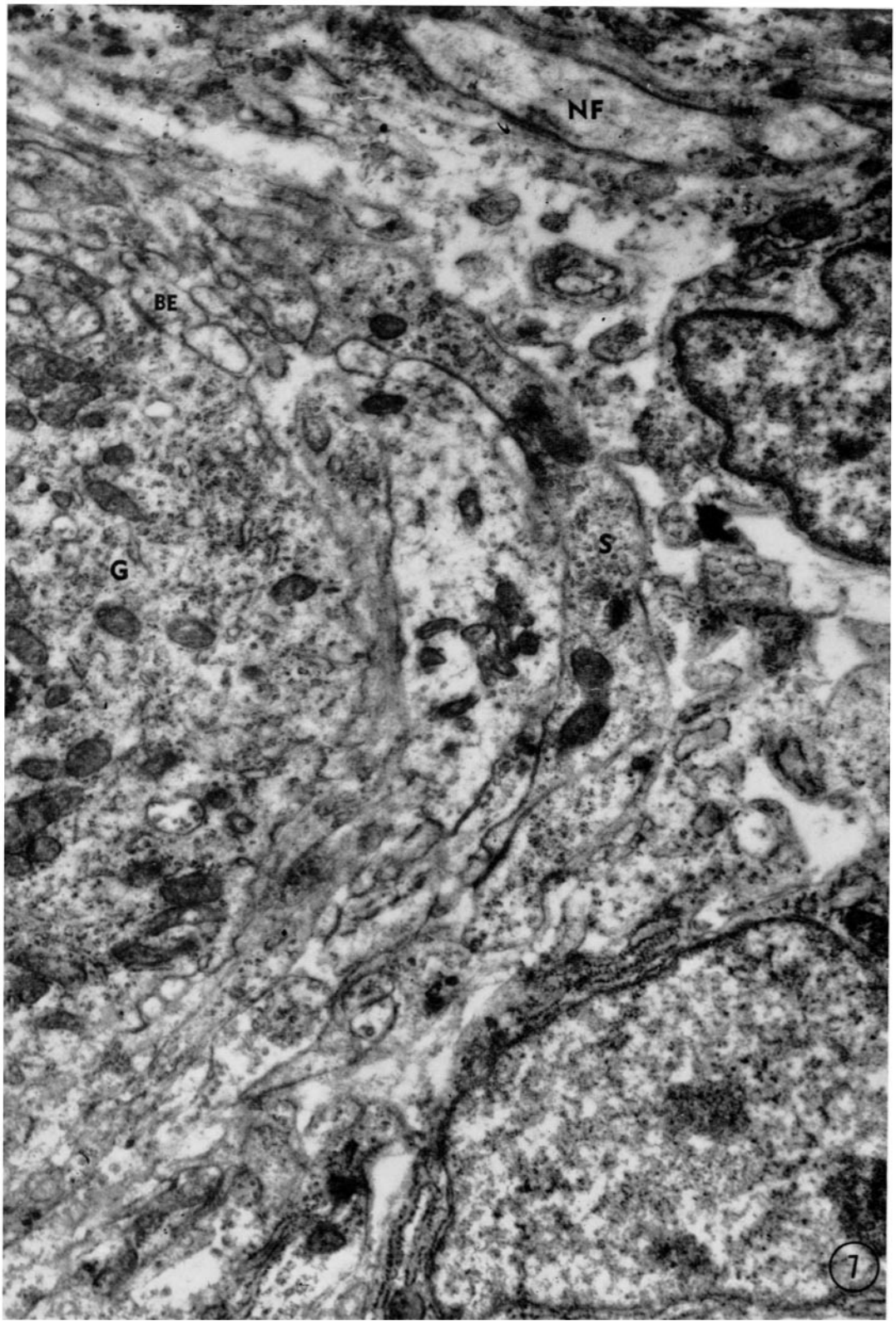
FIG. 6. This is a section through a calyciform ending cut perpendicularly to the longitudinal axis. The calyx (*CE*) is surrounded by a Schwann cell process (*S*) whose nucleus (*SN*) is located at the upper left. The connective tissue space (*CT*) is at the lower left. Synaptic contiguity with the ganglion cell (*G*) is seen at the region designated by the arrows. Note the increased density of the synaptic membranes in this region and the clusters of synaptic vesicles. An interdigitation (*E*) is again evident at the top of the figure. The discontinuity of the membrane near the Schwann cell nucleus (*SN*) is very likely an artifact. × 33,000.



(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 15

FIG. 7. Low magnification of an infrequently observed combination of calyciform endings and basket endings on the same ganglion cell (*G*). The large pale process in the center of the figure is the calyx and the smaller processes containing synaptic vesicles are terminal axons comprising basket endings (*BE*). Nerve fibers (*NF*), Schwann cells (*S*), and collagen occupy the connective tissue spaces. × 6,000.

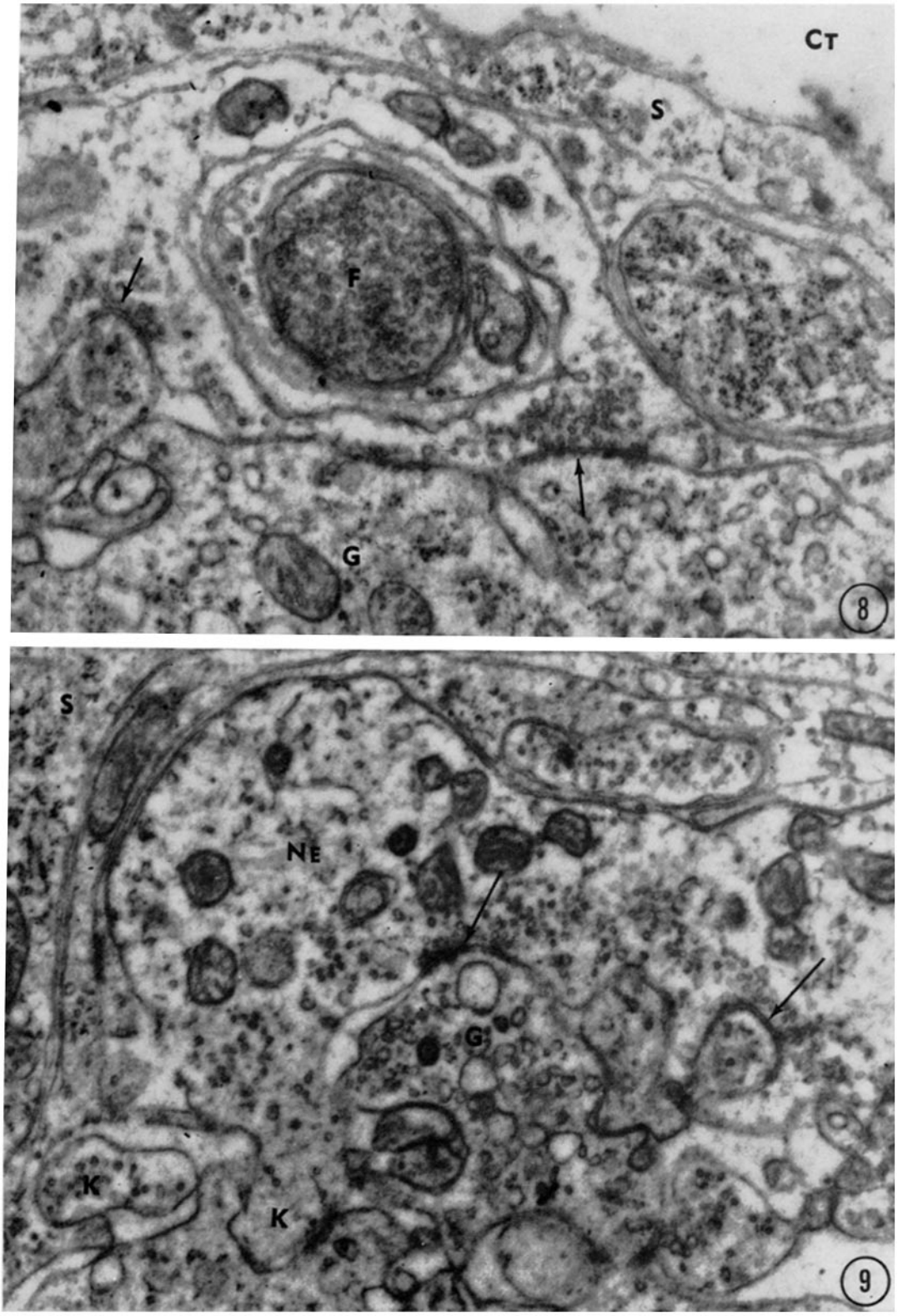


(de Lorenzo: Structure of synapses in ciliary ganglion)

PLATE 16

FIG. 8. Section through the edge of a ganglion cell (*G*) demonstrating a rather unusual relationship. A light process is seen in contiguity with the ganglion cell (*G*), which demonstrates the well documented thickened membranes and synaptic vesicles (arrows) characteristic of a synapse. However, note that another fiber (*F*), which seems to be near its termination (dense clusters of synaptic vesicles), seemingly passes through the synaptic process. It is not possible at this time to resolve whether the membranes surrounding the fiber are entirely of Schwann cell origin (*S*) or whether they are of another kind. × 49,000.

FIG. 9. This figure demonstrates a variation in the calyciform ending and was first described by van Lenhossek (12). In this case, the neurite (*NE*) makes synaptic contiguity with the ganglion cell (*G*) at the points designated by the arrows. At the regions designated (*K*), the distal processes have divided into synaptic knobs which contain some vesicles. The synaptic complex, consisting of thickened apposed membranes and clusters of synaptic vesicles, is evident at several locations in the micrograph. Schwann cell processes (*S*) enclose the ending. × 39,000.



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