DEVELOPMENTAL CHANGES IN THE STRUCTURE OF THE SYNAPSE ON THE MYELINATED CELL BODIES OF THE CHICKEN CILIARY GANGLION

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ABSTRACT

Electron micrographs, stained Epon-embedded sections, and silver stains of the ciliary ganglion of chickens 19 days prehatched, 4 days, 5 weeks, 6 months, and 1 to 2 years of age were studied. The majority of ganglion cells are large neurons; smaller cells are restricted to a dorsal, distal part of the ganglion. The following description applies to the large neurons. Three to twenty lamellae of loose, semicompact, and compact myelin ensheathe virtually every neuron. All these types of myelin form the sheath of a single neuron. The lamellae greatly increase in number and in compactness during the period between the 19-day embryo and the 4-day-old chick. During the period between the 4-day chick and the adult chicken, the myelin becomes only slightly thicker and denser. The calyx is a large synaptic terminal encircling virtually every neuron in the ganglion up to 5 weeks of age. At 6 months of age, the calyx appears to break up; only about half the number of neurons in the ganglion have this large terminal, while the remaining neurons have numerous relatively small, boutonlike synapses. This rather remarkable transformation in the structure of the calyx is virtually complete in the 1- to 2-year-old chickens. In these older chickens all the cells have boutons, and calyces are no longer present. The entering preterminal fiber, the calyx, the neuron, and the axon hillock can all have myelin lamellae on them. It is possible that this entire complex is effectively insulated by the myelin sheath. No synaptic discs or fusion of the membranes of the preterminal fiber and the postganglionic neuron are seen. The chick ciliary ganglion cells are the only myelinated neurons so far described which receive synapses. The neurons in the small-cell part of the ganglion do not have calyces and are not ensheathed by myelin lamellae at any age.

INTRODUCTION

The presence of a large synapse, called a calyx, on cells of the ciliary ganglion of birds has engendered a great deal of morphological interest. Most recently, de Lorenzo (2) studied this synapse in the electron microscope, and Terzuolo (13), using silver stains, described some of the alterations which this synapse undergoes during aging.

Recent physiological studies (6, 7) have shown that many of the synapses in the ciliary ganglion of 3- to 5-day-old chicks are electrically coupled, in addition to having a chemical transmitter, and that there is an apparent increase in the relative number of electrically coupled synapses in 4-week-old chicks.

During a correlated morphological investigation, some interesting new features of this ganglion have been discovered. Firstly, it was found that the cell bodies are myelinated. Secondly, it was found that staining of Epon-embedded tissues resulted invariably in a staining of the calyx and synapses on the cell bodies. Hence, a detailed study was undertaken in order to determine the structure of the calyx, as revealed in the electron microscope and in the light microscope by silver stains and by staining of Epon-embedded material, and to investigate the changes which this synapse undergoes as the chicken grows. In addition, the structure of the myelin on the cell body and the relation of the myelin sheath to the presynaptic terminal and the synapse on the cell body were investigated.

MATERIAL AND METHODS

Chickens 19 days prehatched, 4 days, 5 weeks, 6 months, and 1 to 2 years of age were used. The ciliary ganglia were fixed in Dalton's fluid and embedded in Epon (5). Thin sections, cut on a Porter-Blum or LKB microtome, were "stained" with lead salts (4, 8) and studied in an RCA EMU 3F or Akashi Tronscope. Sections 1 μ in thickness were stained with a solution consisting of a mixture of equal parts of 1 per cent methylene blue in 1 per cent borax and 1 per cent azure II (9) and studied with a Leitz microscope. For Cajal silver staining, some ganglia were fixed in 15 per cent neutral formol and whole ganglia were stained either by a pyridine-silver method or by an ammoniacal alcohol–silver method. Other ganglia were fixed in 100 ml of 50 per cent alcohol containing 5 grams of chloral hydrate and stained with silver nitrate. After silver staining, the ganglia were dehydrated and embedded in paraffin, and serial sections 10 to 15 μ in thickness were cut. The ammoniacal alcohol–silver method worked best on the ganglia of young chicks, whereas the choloral hydrate method proved most suitable for those of older chickens. The Bodian Protargol method, although successful as a general silver impregnation in chickens of all ages, was nevertheless unsuccessful, in our hands, in revealing calyces in chickens of any age, even in those in which the Cajal stains showed that calyces were present.

RESULTS

The chick ciliary ganglion receives preganglionic fibers from the oculomotor nerve. The postganglionic fibers issue from the ganglion to innervate the intrinsic muscles of the eye. The ganglion has a rather well delineated collection of cells in its dorsal, distal part, which contains cells smaller than the majority of neurons and is called the small-cell part of the ciliary ganglion (Fig. 1). This collection of cells is identified easily, not only by the size of the cells, but also by the dense neuropil occurring between them. All the nerve cells of the ganglion are round and unipolar, the only process coming from them being the axon. The large cells will be considered first. A section on the small cells will be reserved for the end of the paper.

The Large Cells

General Structure

First, the structural features of the cells and their synapses will be described. Then, more specific details of the changes which the ganglion undergoes with age will be considered.

THE PERIKARYON: The cytoplasm of the neurons contains all the organelles typical of nerve cells (Figs. 10 to 12). The mitochondria are nu-

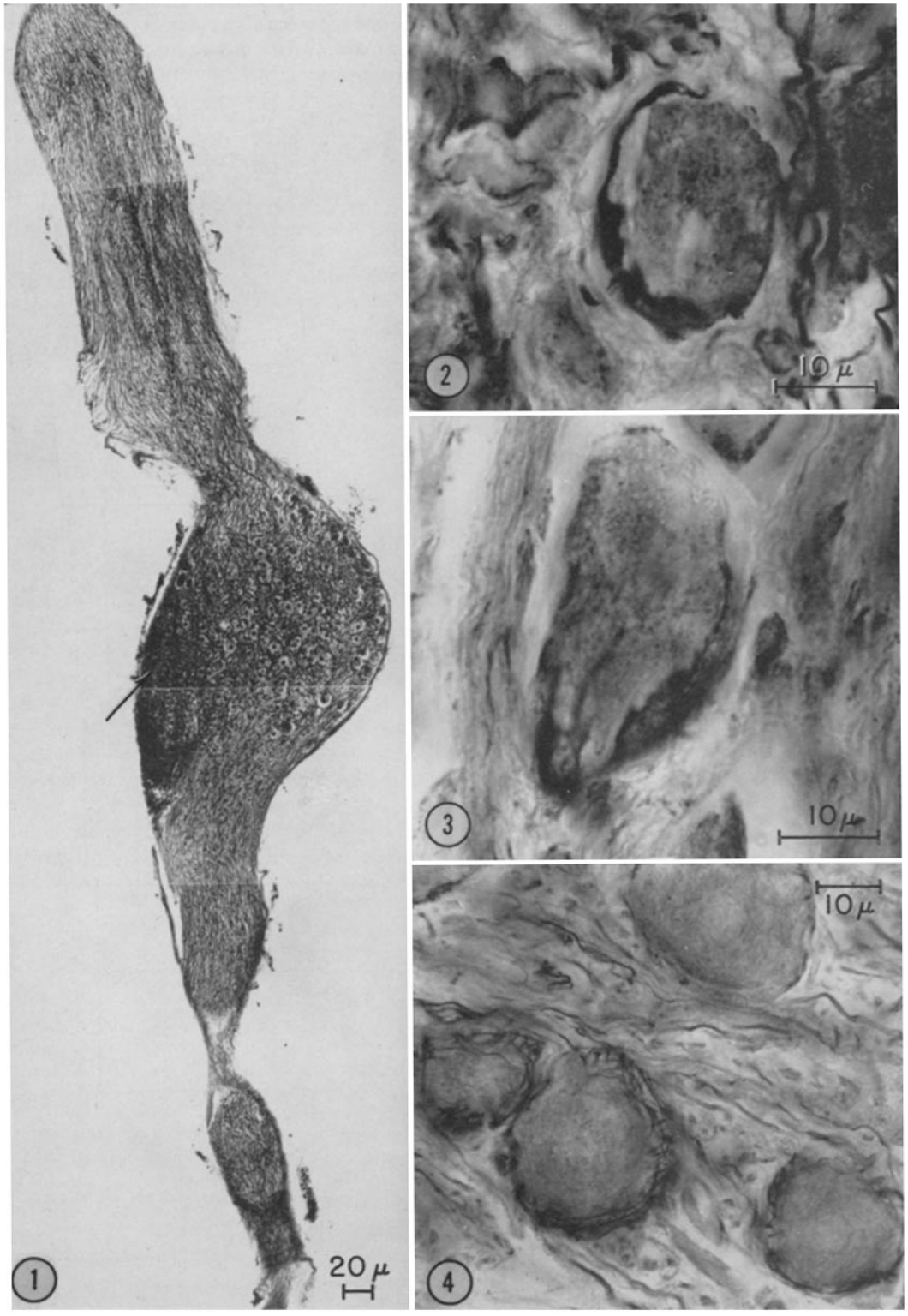
All photographs are of the chick ciliary ganglion.

FIGURE 1 Longitudinal section through the chick ciliary ganglion stained with Protargol. The preganglionic fibers are at the top of the picture, the postganglionic fibers at the bottom. The arrow indicates the small-cell part of the ganglion.

FIGURE 2 Four-day-old chick. A calyx encircles the postsynaptic cell. Cajal silver method. × 1500.

FIGURE 3 Five-week-old chick. A calyx is present on the neuron. Cajal silver method. × 1500.

FIGURE 4 One- to two-year-old chicken. Calyx is no longer present. A dense network of fibers encloses the neuron. Cajal silver method, × 1000.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merous, are scattered throughout the cell, and contain internal folds. Most of the cells have many rounded clusters of dense granules scattered throughout the cytoplasm. These granules have the appearance of ribonucleoprotein particles seen elsewhere, and compose the Nissl substance of the nerve cell. A few cisternae of the endoplasmic reticulum and vesicles (probably cisternae in cross-section) are seen in the cells. The clusters of granules are not, in general, located upon the cisternae and hence the chick ciliary neurons do not have oriented ergastoplasm or Nissl bodies, but rather a more scattered Nissl substance.

THE SYNAPSES ON THE CELLS: The description of the synapses on the nerve cells in this section will be mostly concerned with a description of the calyx. The term "calyx" will be used to denote axo-somatic endings which are very large and which cover extensive areas of the postsynaptic cell, at times almost completely enclosing it. The difference between this type of ending and the more common, sharply circumscribed bouton is readily apparent.

Cajal silver stains show the calyx as a large, brown-stained structure clasping or encircling the more lightly stained yellow postsynaptic cell (Figs. 2 and 3). Stained Epon sections reveal the calyx as an intensely blue structure surrounding the more lightly blue-stained cell (Figs. 5 and 6). Electron micrographs of the calyx show that it is a terminal axon containing many vesicles, about 200 A in diameter, and mitochondria (Fig. 10). At times, the vesicles are very numerous and crowded into the nerve terminal, which appears dense as a consequence. This terminal is very extensive and in some sections can be seen to surround completely the postsynaptic cell. Densities occur on the pre- and postsynaptic membranes and the synaptic cleft space between them. In some sections, they occur frequently along long stretches of synapse and postsynaptic cell. In other sections, the membrane thickenings appear quite at random or not at all. Also in some sections, the synaptic vesicles appear to be highly concentrated behind the membrane thickening and to pile up in a very small area adjacent to the dense area of the membrane of the presynaptic terminal. In still other sections, membrane thickenings can be seen without any particular concentrations of vesicles on them; or, contrariwise, accumulations of vesicles can occur in areas without membrane thickenings.

The extensive line of contact between the calyciform terminal and the cell body frequently is not so smooth as in Fig. 10. At times, a small process of neuronal cytoplasm pushes into the calyx or the latter sends out a short process invaginating the nerve cell. Sometimes these prolongations of the calyx can be rather long. Most frequently, these relatively long processes of the calyx pushing into cell body do not have synaptic vesicles within them, but are rather devoid of organelles.

Some striking and characteristic features are seen in the cytoplasm of the postsynaptic cell immediately adjacent to the calyx or synapse. In all cells, it can be seen that the cytoplasm around the periphery of the cell is not so dense as in the interior (Figs. 10 and 11). This feature is especially marked in the postsynaptic cytoplasm near a calyx or terminal, and it can usually be seen that the postsynaptic cell has a "shell" of less dense cytoplasm in the region of the synapse (Figs. 10 and 11). The decrease of density is due to a decrease in the occurrence of organelles, especially of the clusters of ribonucleoprotein particles, which are dis-

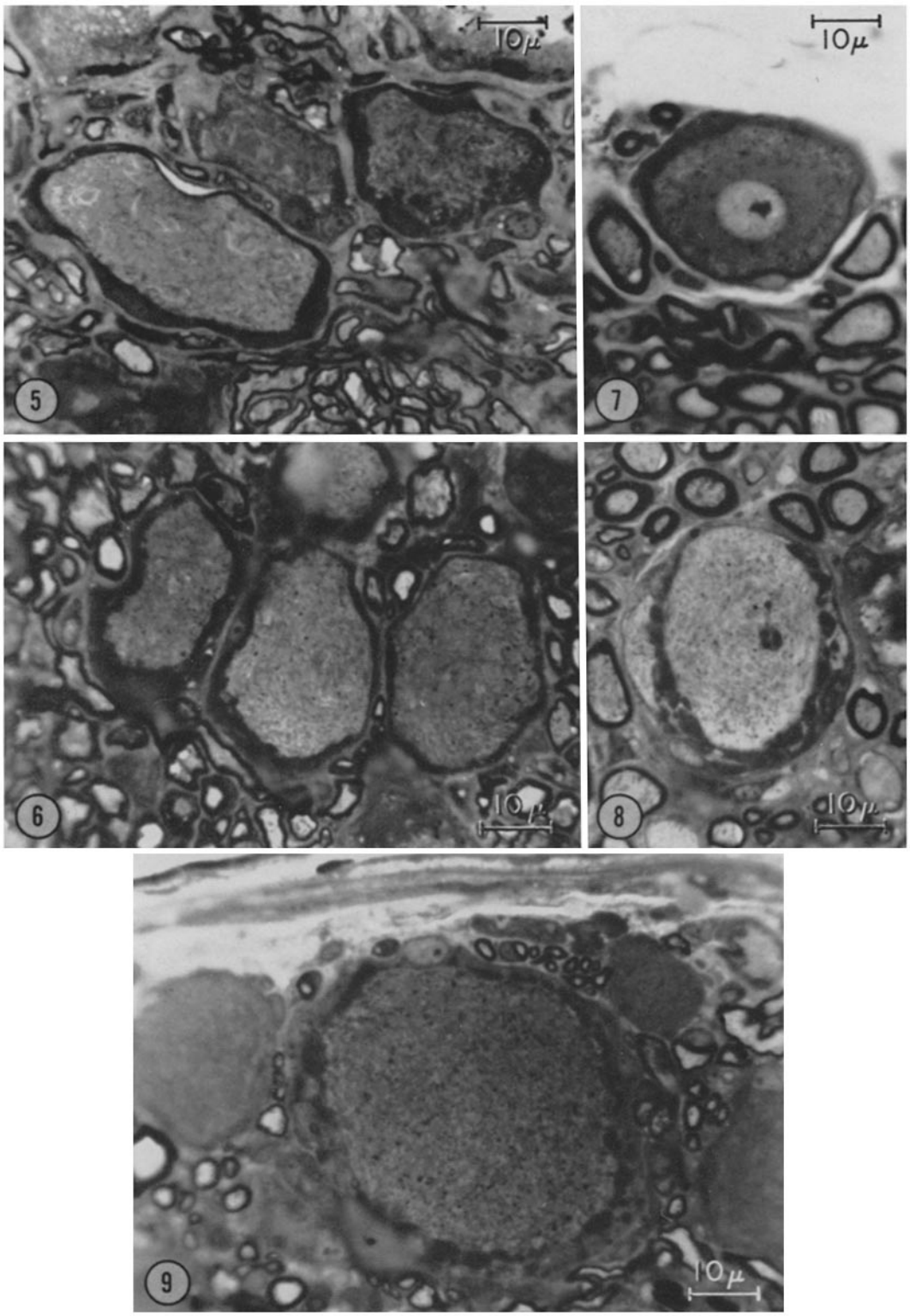
FIGURE 5 Four-day-old chick. Calyces are present around neurons. Stained Epon section. × 1000.

FIGURE 6 Five-week-old chick. Calyces are present around neurons. Stained Epon section. × 1000.

FIGURE 7 Six-month-old chick. A neuron with a calyx. Stained Epon section. × 1000.

FIGURE 8 Six-month-old chick. Calyx not present. Neuron is enclosed by a series of stained droplets. Stained Epon section. × 1000.

FIGURE 9 One- to two-year-old chicken. Calyx not present. Neuron is surrounded by a collection of stained droplets. Stained Epon section. × 1000.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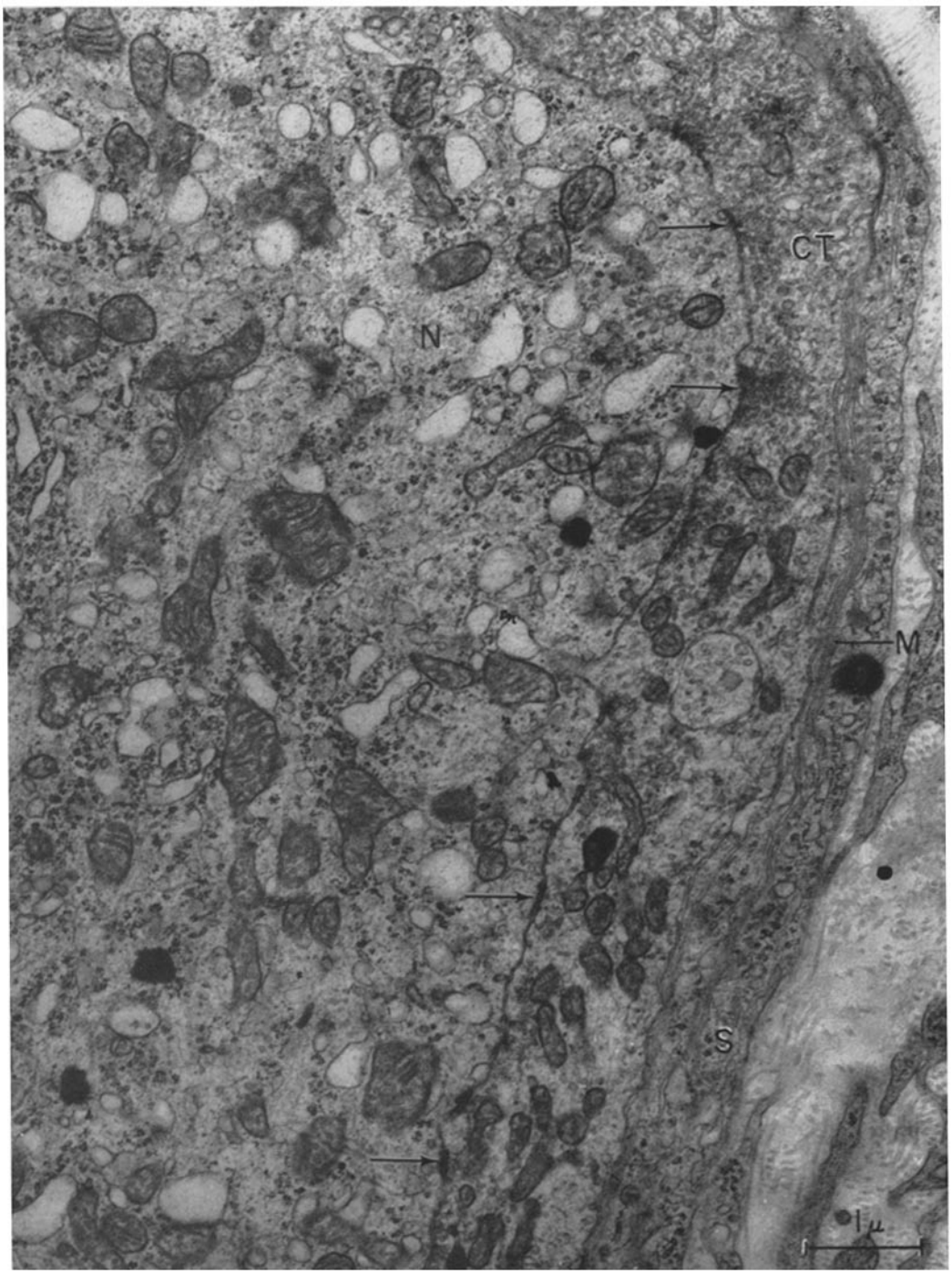


FIGURE 10 Electron micrograph of a neuron from a 19-day chick embryo. *N* indicates the neuronal cytoplasm covered for a long distance by the extensive calyciform terminal (*CT*), which has synaptic vesicles and mitochondria and is covered in its turn by some lamellae of semicompact and loose myelin (*M*) of the Schwann cell (*S*). Ribosomes are present in the nerve cell body and in the Schwann cell. A scattered series of membrane thickenings (arrows) occurs between the nerve cell and the calyx. × 16,000.

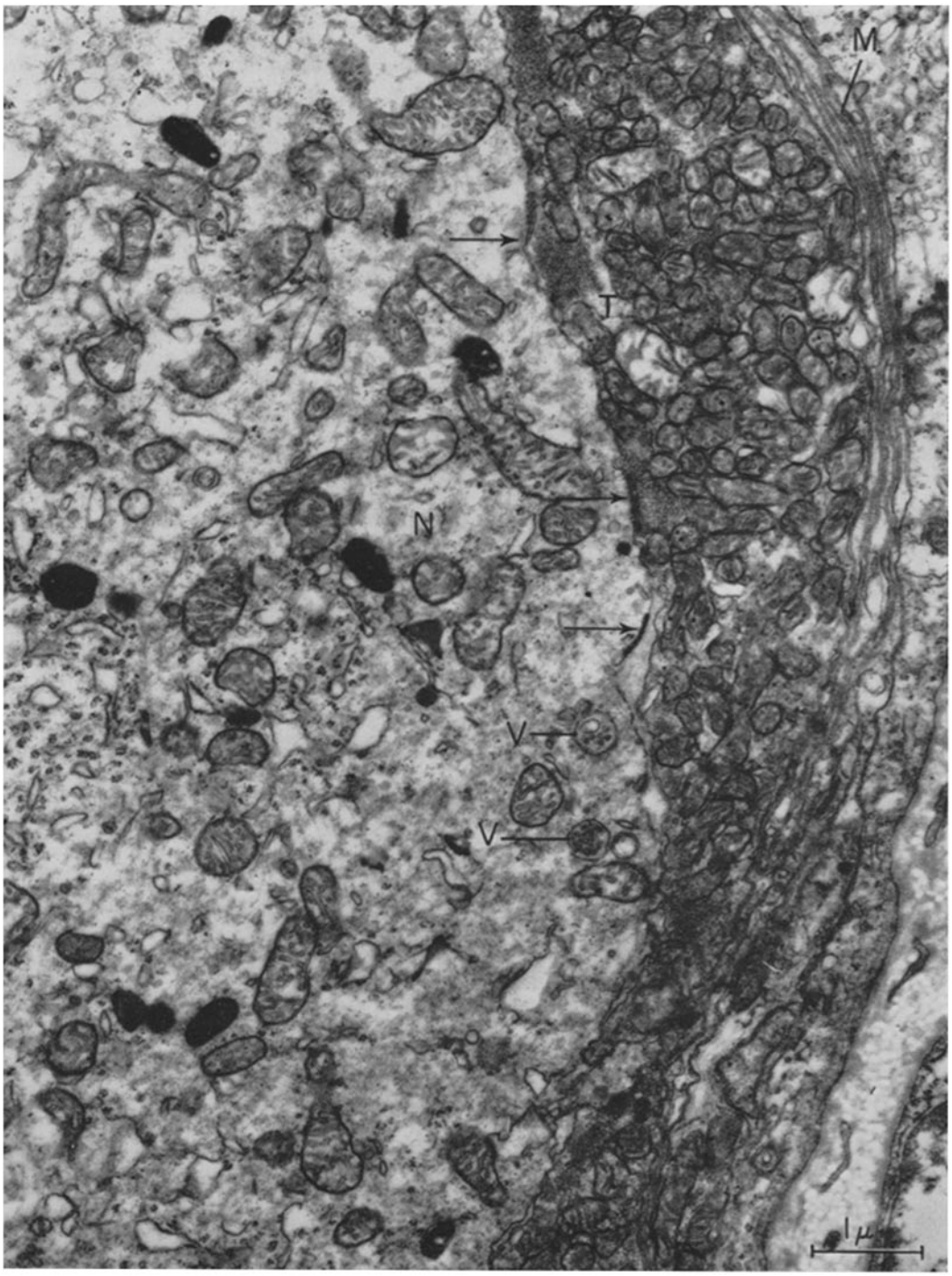


FIGURE 11 Electron micrograph of a neuron from a 1- to 2-year-old chicken. The large terminal (*T*) has synaptic vesicles and many mitochondria. Membrane thickenings (arrows) are seen between the terminal and the nerve cell (*N*). Multivesicular bodies (*V*), ribosomes, and mitochondria are present in the neuronal cytoplasm. The lowest arrow points to a dense subsynaptic rod. The terminal is covered by some lamellae of loose and semicompact myelin (*M*). × 16,000.

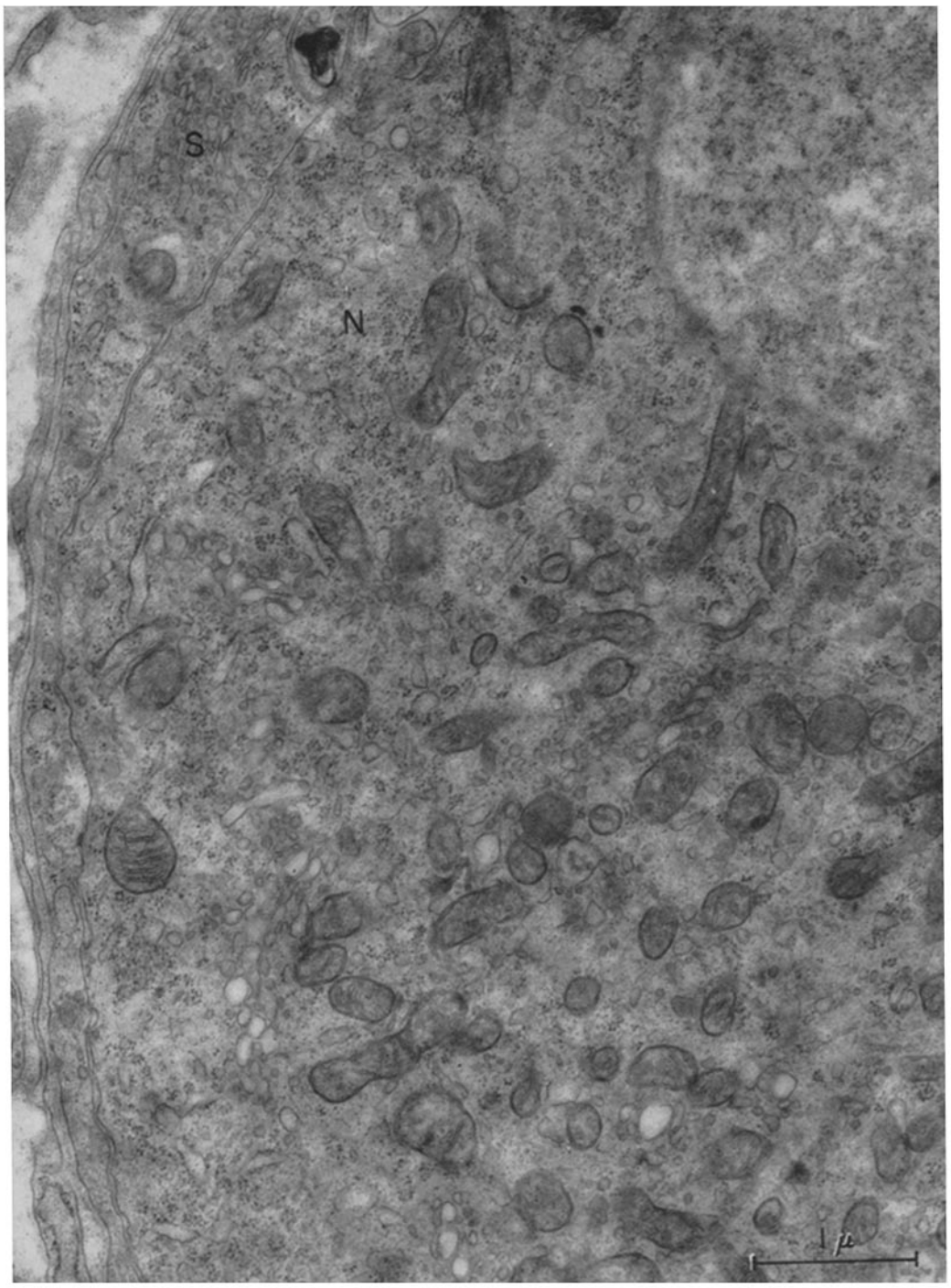


FIGURE 12 Electron micrograph of a neuron from a 19-day chick embryo. The neuron (*N*) is covered by Schwann cell (*S*) cytoplasm. Myelin lamellae are not present. × 24,000.

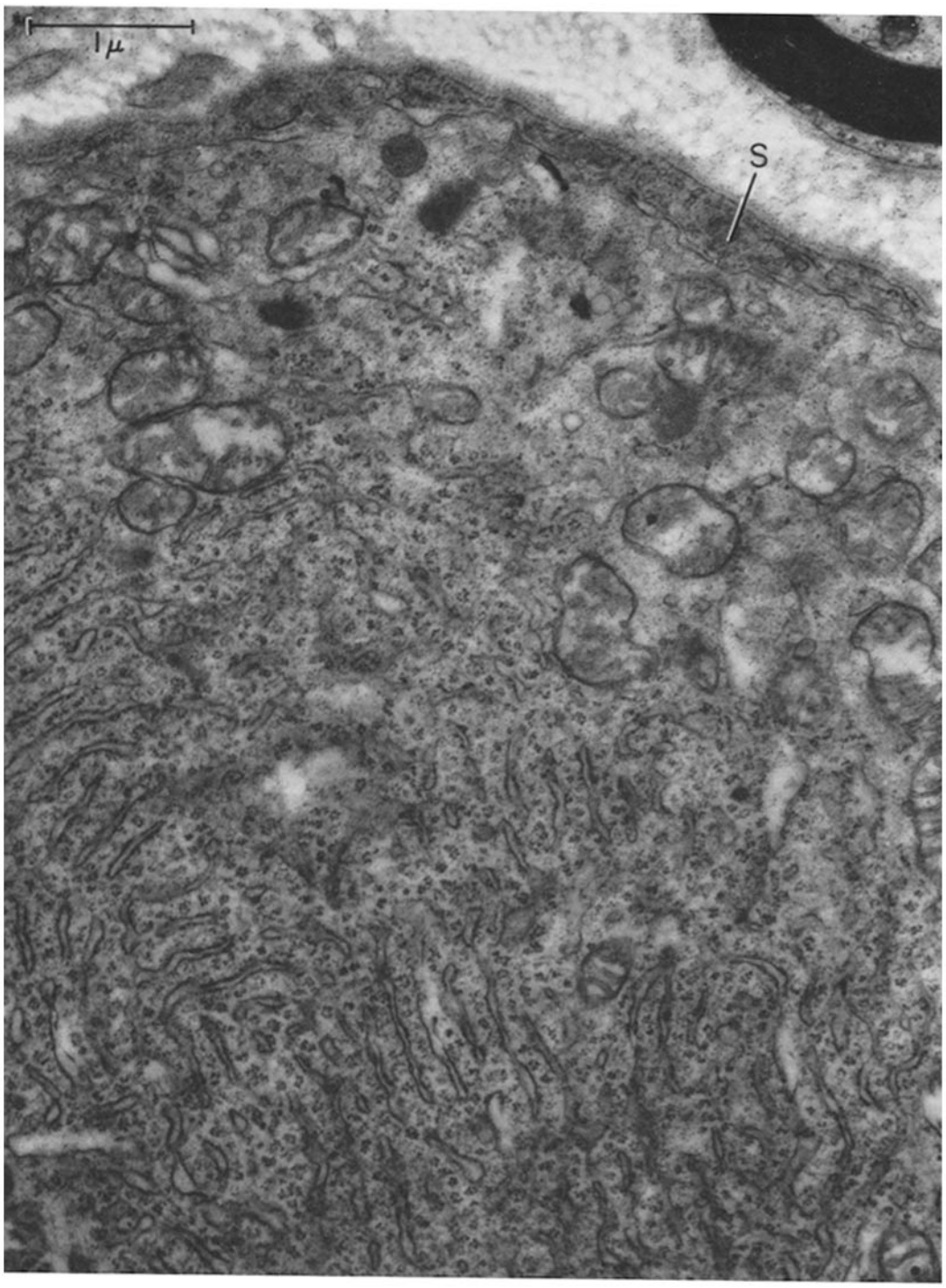


FIGURE 13 Electron micrograph of a neuron from the small-cell part of the ganglion of a 6-month-old chicken. The neuronal cytoplasm is characterized by a great number of double membranes and a marked accumulation of ribosomes. Compare with the cytoplasm of the other type of neuron as seen in Figs. 10 and 11. A thin layer of Schwann cell (*S*) cytoplasm covers the neuron. No calyx is present, nor are myelin lamellae seen. × 24,000.

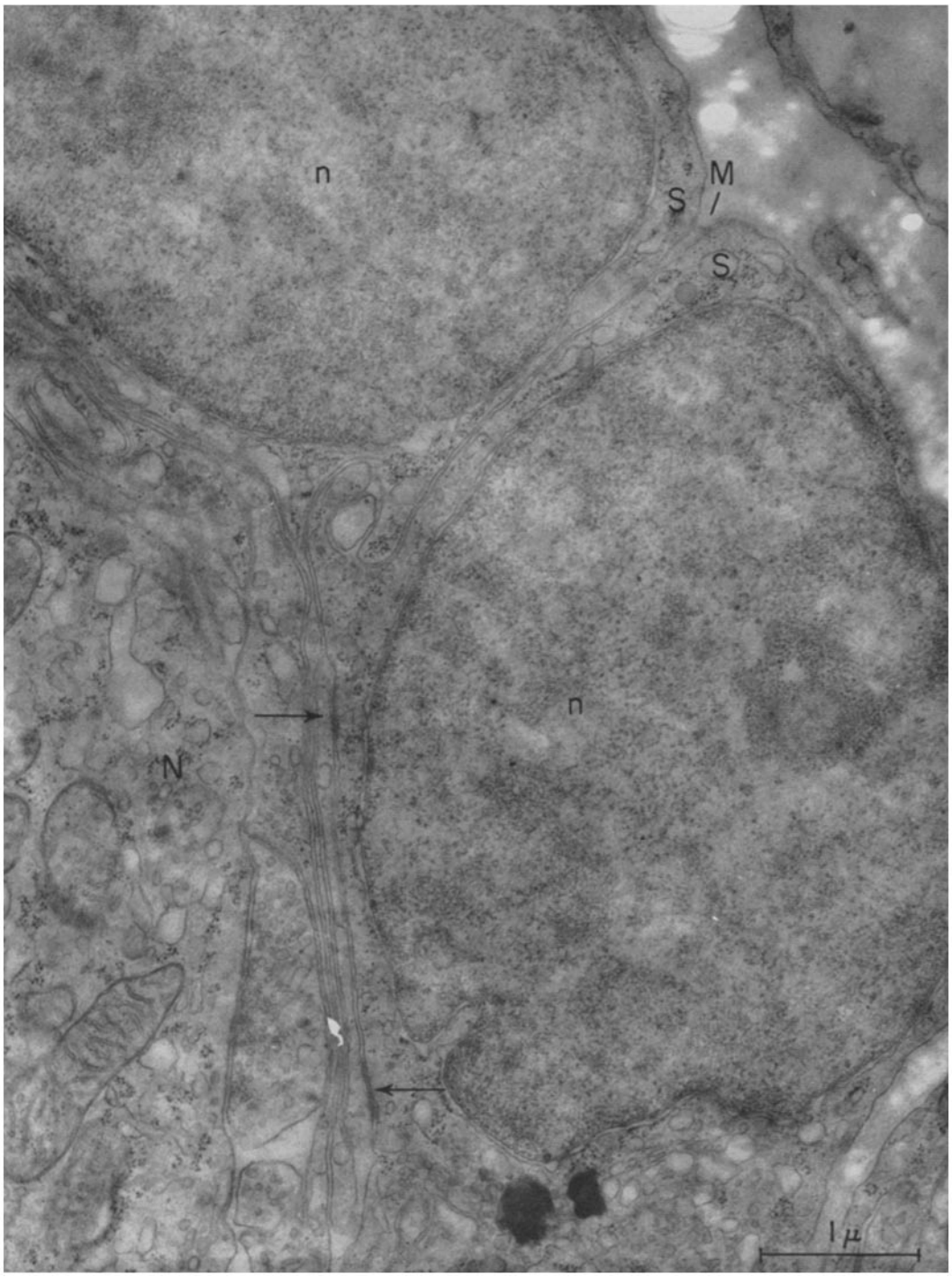


FIGURE 14 Electron micrograph of the ganglion of a 19-day embryo. At least two Schwann cells (*S*), whose nuclei (*n*) are present, contribute to the myelin lamellae on the nerve cell (*N*). *M* indicates the external mesaxon of the myelin sheath. Membrane densities between adjacent myelin lamellae are shown at the arrows. × 24,000.

tinctly deficient in number in the area of a nerve cell under the calyx. This is a constant feature of postsynaptic cells.

Another structural feature of postsynaptic cytoplasm is the occurrence, near the calyx or synapse, of multivesicular bodies (Figs. 11 and 15). In the chick, these organelles are not necessarily very near or adjacent to the synapse or calyx, but whenever they are noticed, they are always in the peripheral portions of the perikaryon in the vicinity of the synaptic terminal, and they are usually not found in the interior of the nerve cell.

Another structure is seen near the synapse or calyx. This structure is found in several instances on the postsynaptic cell just under the synaptic terminal (Figs. 11 and 21). This postsynaptic structure is a dense rod or disc about 250 A thick and 2 μ long. A dense stratum parallel to and separate from the postsynaptic membrane has been described in the sympathetic ganglion of some frogs (1), but in the chick, this rod is apposed to and, indeed, appears to be part of the postsynaptic membrane. It is not a constant feature of every section of every calyx. Whether it was missed in some sections or whether it was absent in most cells is not yet known.

The calyx is the only synapse that has thus far been seen on the perikaryon. As far as we could determine, no boutonlike terminations shared a cell with a calyx. However, other apparently different synapses can occur on the axon hillock.

The axon issuing from the cell can be identified easily. It reveals a sharp reduction in number of clusters of the dark ribonucleoprotein particles and also a distinct difference in the number and shape of mitochondria. These organelles are less numerous and more elongate in the axon and its hillock than in the perikaryon. The synapses on the axon hillock are many. They appear as round structures packed with synaptic vesicles and lined up along the axon hillock and the immediate distal part of the axon (Fig. 16). Thickenings frequently occur on the pre- and postsynaptic membranes of the terminal fiber and axon hillock. These last-described synapses apparently correspond to the basketlike terminations described by others on the chick ciliary ganglion cells.

THE SCHWANN CELL AND MYELIN SHEATH: The neurons are ensheathed by Schwann cells and their processes. The outermost layer of the sheath surrounding a nerve cell is cytoplasmic. Two or more Schwann cells, or the nuclei of these cells, can be seen surrounding a nerve cell and hence contribute to the composition of the neuronal sheath (Fig. 14). These cells send elongated processes to wrap around the neuron. This situation is entirely analogous to that of Schwann cells and peripheral nerve axons, where the former cells send processes to wrap around the nerve fiber. Two or more layers of Schwann cell wrappings separated from each other by a small and regular distance can be considered a myelin sheath. Hence, the neurons of the chick ciliary ganglion can be considered to be myelinated, since they are ensheathed by two or more orderly wrappings of Schwann cell cytoplasm. The lamellae in all the types of myelin to be described present an orderly arrangement. Two overlapping processes of Schwann cell on a neuron are not considered a myelin sheath; to be called myelin in this study, the lamellae must extend for considerable distances around the cell body and be separated from each other by a regular periodicity.

The outermost covering of the neuron is usually a relatively thick layer of Schwann cell cytoplasm (Fig. 14). The nucleus of the Schwann cell is located here. The cytoplasm of the Schwann cell (Figs. 10 and 14) contains cisternae of the endoplasmic reticulum, dark clusters of ribonucleoprotein particles, some of which are located on the cisternae, and vesicles and mitochondria. In some sections, the concentration of clusters of ribonucleoprotein particles is quite dense and the granules are quite numerous. Wrappings of the Schwann cell around the neuron form the myelin sheath.

The limiting membranes of two adjacent Schwann cells or the juxtaposed processes of a single cell can be seen entering into the formation of the myelin sheath of the neuron (Fig. 14). This is equivalent to the outer mesaxon seen on myelinated nerve fibers. Several mesaxons can be found on a single neuron (Fig. 15). Also several Schwann cell nuclei can be seen around each neuron (Fig. 14). It thus appears that the myelin sheath of these nerve cells, unlike that of vertebrate peripheral nerve internodes, is formed by several Schwann cells rather than just one.

The relations of the Schwann cell and neuron in the chick are similar to those described by Rosenbluth (11) in the rat. The "loose" myelin is composed of processes of Schwann cells and hence consists of Schwann cell cytoplasm surrounded by its limiting membranes (Figs. 11, 15, 17, and 18).

These lamellae vary in thickness. Membrane thickenings can sometimes be seen between adjacent lamellae (Fig. 14). A more regular periodicity of the lamellae is seen in "semicompact" myelin (Figs. 17 and 18). Here, cytoplasm is absent, and the lamellae consist of cell membranes separated by rather regular distances of about 300 A. Most frequently, this type of myelin is the thinnest and consists of relatively few lamellae. The last type of myelin, the "compact" (Figs. 15, 17, and 18), is similar to that seen on the nerve fiber and consists of closely packed cell membranes without any intervening Schwann cell cytoplasm; the center-to-center distance of the lamellae is about 150 A. This type of myelin has in it the fused Schwann cell membranes which form major dense lines, and appears very dark in the electron microscope.

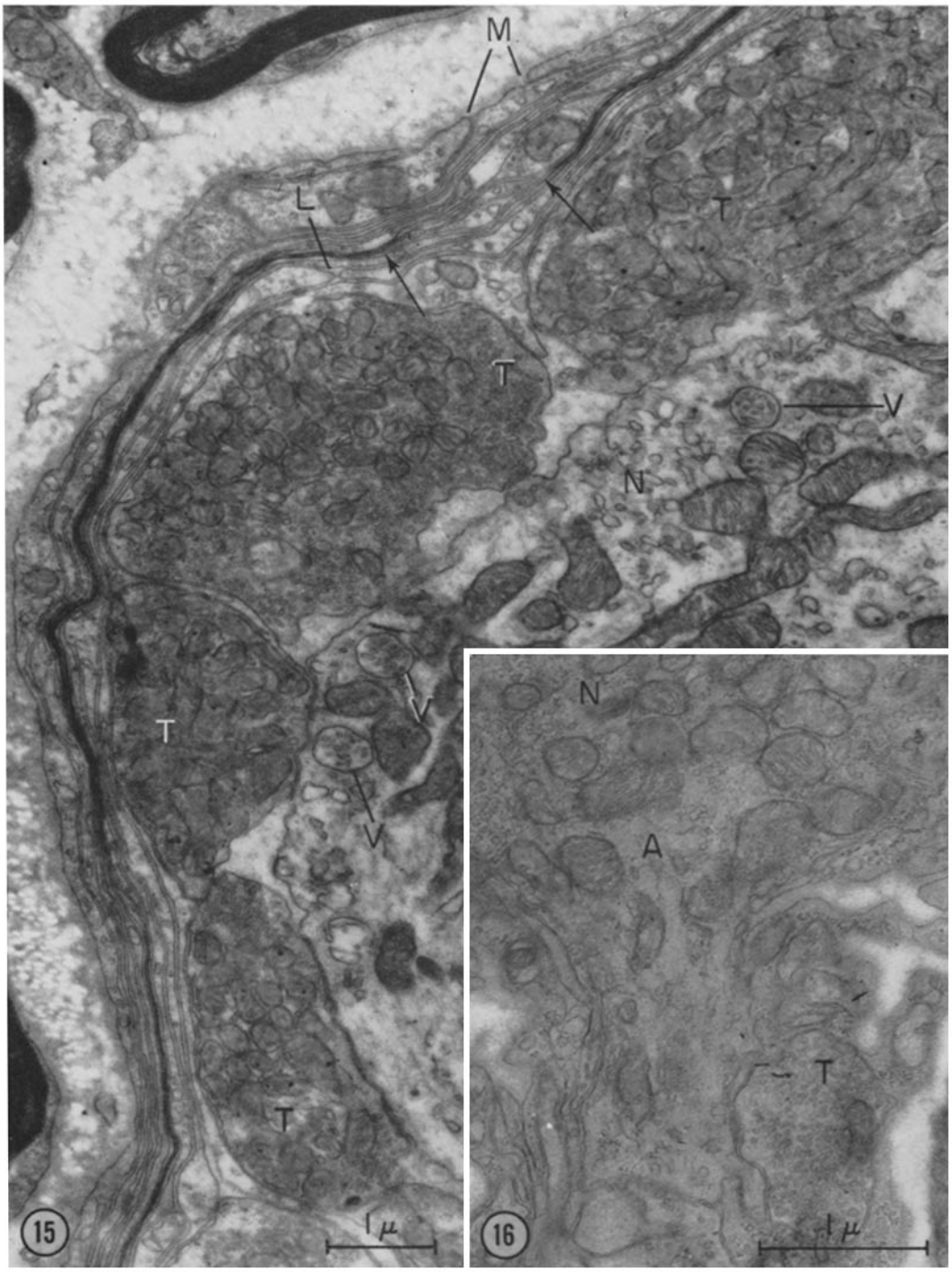
The myelin sheath on an individual neuron is very variable in appearance. Almost all the variations detailed by Rosenbluth (11) in the rat can be found in the chicken. Firstly, it is not necessarily a rule, as one might perhaps think, that a correlation exists between number of lamellae and type of myelin. A patch of loose myelin can contain a smaller or larger number of lamellae than compact myelin. Also, the type of myelin on a neuron usually varies from place to place along the cell body, and virtually always two or more types of myelin are found on the same nerve cell (Figs. 15 and 18). Also, as will be seen below, there is not necessarily a close correlation with age, for compact and loose myelin can be seen to surround a neuron in both a 4-day-old chick and a 1- to 2-year-old. It is possible to see lamellae in compact myelin abruptly separate from each other to form a short stretch of loose myelin and then join together again to become compact myelin (Fig. 15). Thus, the myelin varies considerably in type as it ensheathes the neuron, and the lamellae of one type of myelin freely join in the formation of another type as they pass around the nerve cell body. There are many discontinuities in individual lamellae of the myelin sheath, and the neuron is surrounded by a very variable number of lamellae. At times, lamellae end abruptly in a blind loop of cytoplasm on the neuron (Fig. 15). Sometimes several lamellae end at or about the same level on the nerve cell. However, when a perikaryon is said to be myelinated, it is almost always covered over its entire surface by multiple lamellae (although highly variable in number).

Myelin figures consisting of whorls of compact lamellae can be seen in the Schwann cell layer (Fig. 22). Connections can be seen between the lamellae of these myelin figures and the lamellae of the myelin ensheathing the neuron. These figures might be due to disorders of development.

RELATION OF CALYX TO MYELIN SHEATH: As the afferent fiber approaches the postsynaptic cell and divides into its calyciform terminal, some myelin sheath lamellae run up onto the preterminal fiber for a short distance (Fig. 19). These lamellae gradually end on the afferent fiber. Thus, the preterminal fiber has a few myelin lamellae on it. The calyx or terminal itself is covered on one side by the myelin lamellae. The exiting axon is similarly covered by a few myelin lamellae which end on it as it leaves the nerve cell (Fig. 20). The myelin sheath opens, as it were, to permit entry of the afferent fiber and exit of the axonal process, and as these fibers pass through the myelin sheath they carry with them a few lamellae, which causes the entire complex of preterminal fiber, calyciform terminal, perikaryon, and axon hillock area to be ensheathed by myelin lamellae. The inherent irregularity in the disposition of the myelin lamellae described above causes variation in this scheme, and the number of lamellae and the

FIGURE 15 Electron micrograph of a neuron in ganglion of a 1- to 2-year-old chicken. The terminals (*T*) are seen as a series of boutons on the nerve cell (*N*). Multivesicular bodies (*V*) are present in the neuron. Myelin lamellae, highly variable in disposition, cover the terminals and the neuron. The dark compact myelin can become loose myelin and then become compact again (arrows). Lamellae can also be seen ending abruptly along the surface of the neuron (*L*). Two outer mesaxons (*M*) are seen. × 16,000.

FIGURE 16 Electron micrograph through an axon hillock in ganglion of a 4-day-old chick. The nerve cell (*N*) with ribosomes is at the top. The ribosomes stop rather abruptly as the axon (*A*) issues from the cell. A bouton terminal (*T*) with synaptic vesicles ends on the axon hillock. A membrane density occurs between the terminal and the axon. × 24,000.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thickness of the myelin sheath on each of these structures are highly variable, although ensheathement of the perikaryon and its related synapse and axon is frequently seen.

Developmental Changes

THE CALYX: The calyx undergoes remarkable changes during development. In stained Epon sections, the calyces are revealed consistently. They are present in the 19-day embryo, the 4-day-old chick (Fig. 5), the 5-week-old chick (Fig. 6), and the 6-month-old chick (Fig. 7). The calyx appears as a blue structure encircling the neuron. In the 19-day chick embryo and the 4-day and 5-week chick, virtually every nerve cell is encircled over a large area of its circumference by a blue structure. However, in the 6-month-old chicken, this appearance of a calyx on almost every cell changes. Many cells still have calyces on them, but in many instances the blue terminal, instead of encircling the nerve cell for a long distance, now appears as a series of small dots on the neuron (Fig. 8). The calyx thus appears to begin to break up in the 6-month-old chicken. In chickens 1 to 2 years of age, this breaking up of the calyx appears essentially complete, and large structures encircling the neuron for long distances are not seen. Rather, the terminals on the neuron appear as blue dots in almost every instance (Fig. 9). Hence, it is apparent, from the study of stained Epon-embedded material, that the calyx, a large terminal encircling the nerve cell in chicks up to 5 weeks of age, breaks up and appears as a series of boutons on many cells of 6-month-old chickens and on almost every cell in chickens 1 to 2 years of age.

Silver stains also show in a striking manner the developmental changes in the calyx. No calyx is seen in chickens 1 to 2 years of age (Fig. 4). In the place formerly occupied by the calyciform terminal in younger chicks (Figs. 2 and 3), bundles of thin axons now form a dense neuropil around the perikarya. The thin nerve fibers follow a variable course and enmesh the cell bodies; enlarged terminals on the neuron are not revealed.

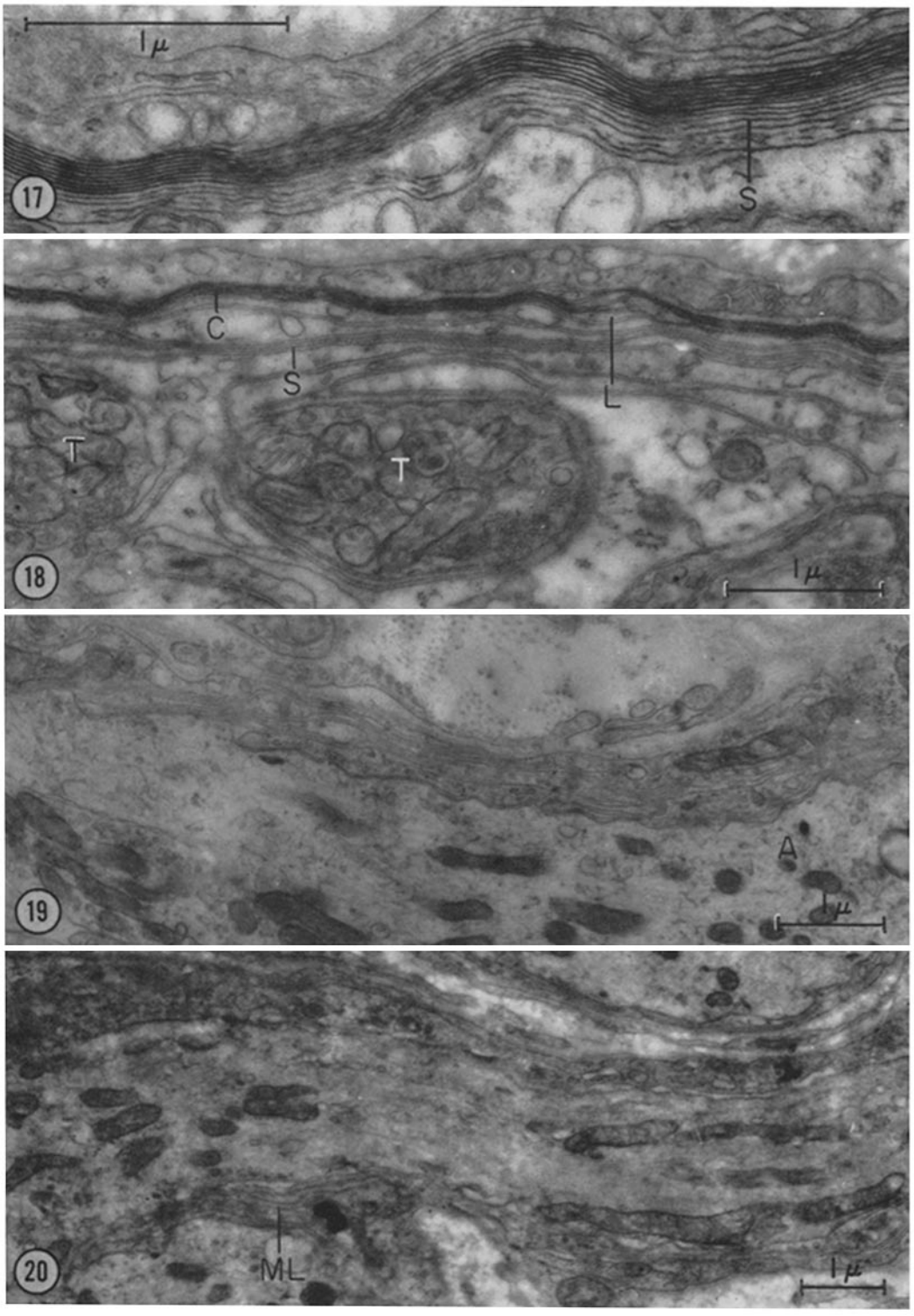
This rather remarkable alteration in structure of the synapse with development is seen also in electron micrographs. As shown thus far, the calyx covers an extensive area of the nerve cell (Fig. 10). In almost any section of ciliary ganglion of chicks up to 5 weeks of age, long stretches of the membrane of the calyciform terminal can be seen apposed to the membrane of the postsynaptic nerve cell and separated from it by a distance of about 200 A. In the 6-month-old chick, many cells are similarly covered by calyx, but a series of boutons can also be seen on many cells (Fig. 23). The terminal is no longer a continuous element, but rather consists of several individual endings on the surface of the neuron. There are now many endings rather than one long one, and they are separated by a portion of Schwann cell cytoplasm. In some cases, the endings are lined up on the surface of the neuron, but they are not continuous and are separated by only a thin tongue of Schwann cell cytoplasm. In chickens 1 to 2 years of age, this multiple terminal disposition of the endings is seen on all neurons (Figs. 15, 18, and 21). Long stretches of apposition of the membranes of the terminal and postsynaptic cell are no longer found, but rather the endings consist of a series of boutons on the nerve cell. Membrane thickenings of the terminal and postsynaptic membranes are found. The boutons have in them the

FIGURE 17 Electron micrograph of the myelin lamellae on a nerve cell of a 1- to 2-year-old chicken. Semicompact (*S*) and dark compact myelin lamellae are seen. × 40,000.

FIGURE 18 Electron micrograph of the myelin lamellae and terminal boutons (*T*) of a ganglion cell of a 1- to 2-year-old chicken. Loose (*L*), semicompact (*S*), and compact (*C*) myelin are seen. × 24,000.

FIGURE 19 Electron micrograph of myelin lamellae on the preterminal part of the axon (*A*) as it enters into formation of the calyx on a ganglion cell in a 1- to 2-year-old chicken. The myelin lamellae are continuous with those covering the nerve cell body. × 16,000.

FIGURE 20 Electron micrograph of myelin lamellae (*ML*) on the axon hillock as it issues from a ganglion cell in a 1- to 2-year-old chicken. The myelin lamellae are continuous with those covering the nerve cell body. × 12,400.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same organelles seen in the calyx: vesicles and mitochondria.

Lamellae of the myelin sheath can be seen passing over the series of boutons (Figs. 15 and 18). Hence, the boutons have the same relation to the myelin sheath as the calyx, and the terminals are covered on one side by the myelin sheath. It is difficult to find preterminal portions of the parent axons of the boutons to see whether they are ensheathed by Schwann cell lamellae as they pass through the myelin sheath. Failure to locate them is probably due to the breaking up of the large parent axon of the calyx, as development proceeds, into thinner fibers of variable course, as described above in silver stains.

THE MYELIN SHEATH: The number of myelin lamellae can be up to about 20. There is no extensive correlation between the stage of development and the type of myelin. A 19-day chick embryo and chicks 4 days, 5 weeks, 6 months, and 1 to 2 years of age were studied. Even in adult chickens, loose, semicompact, and compact types of myelin can be found sharing in the formation of the myelin sheath on a single neuron. Perhaps, as a general impression, compact myelin is of more frequent occurrence in adult chickens 1 to 2 years of age than in 4-day chicks, but the variability in the structure of the myelin described above precludes the drawing of a definite conclusion. Only one neuron was seen ensheathed completely by compact myelin, and that was in an adult chicken. The number of lamellae, in general, appears to increase with age; the neuron of the adult chicken 1 to 2 years of age appears to be surrounded by a thicker sheath (with more compact myelin) than, for instance, the neuron of the 4-day chick. However, the difference in neuron myelinization between an adult chicken of 1 to 2 years and a 4-day chick is not striking. In the former many parts of the neurons are surrounded by relatively few myelin lamellae, while in the 4-day chick there are places on nerve cells covered by many myelin lamellae.

In all the posthatched chicks studied (from 4 days onward), every nerve cell body has myelin lamellae somewhere along its circumference. The situation in the 19-day chick embryo (Fig. 12) is different from that in all the other chickens studied. Here, many cells do not have anything that can be called myelin; the neurons are surrounded by one or two thin layers of Schwann cell, the arrangement being like that of satellite cells around ganglion cells in almost all other locations. Loose myelin is seen on a few neurons, but it is composed usually of only a few lamellae (Fig. 10). Semicompact myelin can also be found (Fig. 10), but compact myelin is especially rare and has been seen on only one nerve cell. The differences in thickness and compactness of the myelin sheath between a 19-day embryo and a 4-day chick are far greater than those between a 4-day chick and a 1- to 2-year-old chicken. Perhaps hatching gives a strong stimulus to myelin sheath formation.

The Small Cells

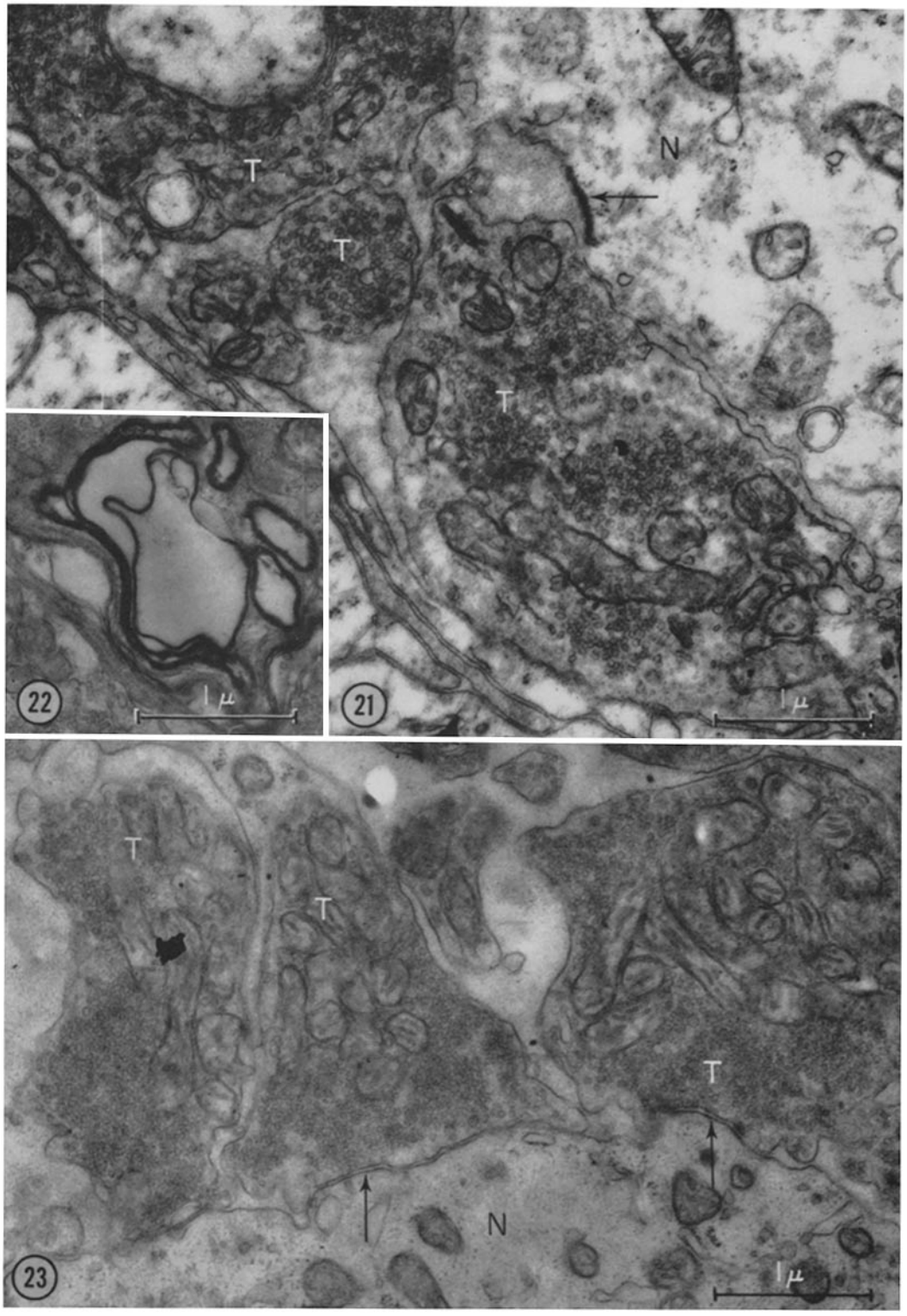
In contrast to the large neurons, the other cells, which are believed to be in the small-cell part of the ganglion, do not have the usual arrangement of Nissl substance. The disposition of Nissl substance is quite different (Fig. 13). These small cells have many more double membranes upon which are many clusters of ribonucleoprotein granules, and they also have many more granules and clusters of granules. Hence, the small cells appear denser in the electron microscope and have more organized basophilic components than the larger cells.

The neurons in the small-cell portion of the

FIGURE 21 Terminal boutons (*T*) with synaptic vesicles on a ganglion cell (*N*) in a 1- to 2-year-old chicken. Compare these boutons with the extensive calyciform terminal of young chicks shown in Fig. 10. A subsynaptic rod is seen at the arrow. × 24,000.

FIGURE 22 Electron micrograph of a whorl of myelin lamellae (myelin figure) in the Schwann cell cytoplasm in ganglion of a 4-day-old chick. × 24,000.

FIGURE 23 Electron micrograph of terminal boutons (*T*) with synaptic vesicles on a nerve cell (*N*) in ganglion of a 6-month-old chick. Many other cells in this chick had an extensive calyciform terminal on them, rather than a series of boutons. Membrane thickenings are seen at the arrows. × 24,000.


T
T
N
T
22
1 μ
21
1 μ
T
T
T
N
23
1 μ

ganglion do not have calyciform terminals upon them (Fig. 13), in chickens of all ages studied. Their synapses are relatively small bouton type endings. It is interesting that this difference in form of synaptic termination occurs in this ganglion, since all the fibers ending in both the large-cell and the small-cell parts are said to come from the third nerve. Terzuolo (13) has ruled out fifth nerve terminations in this ganglion.

The cells of the small-cell portion of the chick ciliary ganglion differ radically in their investment of Schwann cell from the large cells described above. There is no myelin sheath on these small cells (Fig. 13), at any age studied. The cell is covered with a very thin layer of Schwann cell cytoplasm, usually only single.

DISCUSSION

The nerve cells of the chick ciliary ganglion are unique in that they are the only myelinated neurons thus far observed that have synapses. The myelinated cells of the cochlear and vestibular ganglia (11, 12) are sensory bipolar neurons devoid of synaptic terminations.

Physiological studies (6, 7) have shown that about 60 per cent of the chick ciliary ganglion cells in 4-day-old chicks are electrically coupled and that the incidence of electrical transmission increases in 4-week-old chicks. It was thought by the present investigator that the coupling potentials obtained were due to saltatory conduction and that the parent preterminal afferent fiber, the calyx, the postsynaptic nerve cell, and the exiting axon were effectively insulated by the myelin lamellae and hence acted as an internode, with the impulse jumping from preterminal afferent fiber to exiting axonal process. The irregular disposition of the myelin lamellae could allow for ineffective insulation of some neurons and hence would account for the 40 per cent of the neurons (in 4-day chicks) which do not exhibit electrical transmission or saltatory conduction. The increase in thickness or change in type of myelin, as development proceeds, could allow a more effective insulation of some neurons and could account for the finding that the incidence of electrical transmission is larger in 4-week-old chicks than in 4-day chicks.

The suggestion that saltatory conduction takes place in the chick ciliary ganglion does not imply that electrical coupling cannot exist at the site of calyx and nerve cell. Indeed, electrical coupling between calyx or bouton and nerve cell is probably necessary for saltatory conduction to occur.

The current flow during saltatory conduction would be expected to occur through calyx and neuron, as well as outside the myelin sheath, to complete the local electrical circuit. Such a local electrical circuit occurs in the internodal portion of the axon during saltatory conduction in a peripheral nerve fiber, where the action potential at each node excites the next node by current flowing forward in the axis cylinder and back in the fluid outside the myelin sheath (3). Some sort of electrical coupling of calyx or bouton and postsnyaptic neuron might perhaps be expected to occur during saltatory transmission of the nerve impulse through the chick ciliary ganglion, so that the action potential at the last node of the preterminal fiber can excite the next node of the exiting axon hillock by current flowing forward in the calyx and neuron and back in the fluid outside the myelin sheath. Hence, the electrical coupling of calyx and neuron described by Martin and Pilar (6) might well be a component of the mechanism of saltatory conduction of the nervous impulse in the chick ciliary ganglion.

If only the calyx is necessary for electrical coupling to occur (and myelin lamellae are not involved significantly), the occurrence of electrical transmission in only 60 per cent of the cells of the 4-day chick ganglion (and not in all the cells) cannot depend merely on the presence or the absence of a calyx, since in both stained Epon-embedded material and electron micrographs it appears that virtually every large neuron has a calyx. Perhaps some calyces are ineffective in producing electrical coupling because of size; measurement of the size of calyces has not been performed in the present study. Physiological investigations in adult chickens 1 to 2 years of age, where calyces are absent and the myelin is relatively well organized, might provide more information about the physiological role of the calyx. A series of closely aligned boutons (such as occurs in adult chickens) as well as a calyx might serve to effect electrical coupling through the chicken ciliary ganglion and allow for the occurrence of saltatory conduction. Similarly, physiological studies in chick embryos, where myelin lamellae are virtually absent and calyces are already present, should yield information about the role of the myelin lamellae in effecting transmission through the ciliary ganglion.

It should be mentioned that no type of fusion of

pre- and postsynaptic membranes is seen in the chick ciliary ganglion, at least with the techniques employed in the present study. Such fused membranes, called synaptic discs, have been correlated in some instances with the occurrence of electrical synaptic transmission (10). If such discs occur in the chick ciliary ganglion (and perhaps have been missed in the present study), they are of such low frequency that it would appear unlikely that they could affect significantly the physiological properties of the synapse.

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