SOME OBSERVATIONS ON THE
FINE STRUCTURE OF THE
SYMPATHEIC GANGLION OF BULLFROG

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ABSTRACT
Electron microscope observations of abdominal sympathetic ganglia of American bullfrogs, Rana catesbiana, have demonstrated the presence of specific areas of cytoplasm in the superficial zone of the perikaryon which are devoid of granulated endoplasmic reticulum. These areas are occupied almost exclusively by granules 200 to 400 A in diameter which can be stained intensely with lead hydroxide but faintly with uranyl acetate. Each granule shows subgranular internal structure after the lead staining. Granules of similar properties are found in synapses also, and may be glycogen. From the satellite cell there extends a number of leaf- or finger-like cytoplasmic projections around the root portion of the nerve process. Some of these projections directly cover the surface of the nerve process. Many others, however, are separated from the neuron by a fairly wide interspace. Multivesicular bodies of the neuron are occasionally observed in a configuration which suggests that they are being extruded from the root of the nerve process into the interspace. Filaments about 100 A in thickness are found in the satellite cell cytoplasm. They are arranged more or less parallel to each other and are especially well developed around synapses and nerve fibers.

INTRODUCTION
In recent years, a number of electron microscope investigations have provided a great deal more information on the fine structure of the sympathetic ganglion. De Robertis and Bennett (3), observing the abdominal sympathetic ganglia of frogs, found that mitochondria and also a vesicular component 200 to 500 A in diameter, which they designated the “synaptic vesicle,” were constantly present in synaptic endings. Taxi (21, 22) studied the fine structure of synapses in the sympathetic ganglion of mammals and frog. He noted the presence of vesicles containing a dense particle about 500 A in diameter in some synaptic terminations around the frog ganglion cells.

Palay and Palade (14), studying various types of neurons including those of sympathetic origin, reported that Nissl substance consists of masses of granulated endoplasmic reticulum and that an agranular membrane element similar to structures described in other cells as the Golgi apparatus is found scattered throughout the cytoplasm of neurons. They also noted that the cytoplasm between Nissl bodies contains numerous mitochondria, rounded lipid inclusions, and fine filaments. According to Smith (18), besides the ordinary Nissl bodies which correspond to the granulated endoplasmic reticulum, another type of Nissl body comprising compact masses of particles and devoid of endoplasmic reticular elements is observable in the sympathetic neurons of the lizard.

Wyburn (25) described the sympathetic nerve cell as possessing a satellite cell sheath consisting of a narrow ribbon of cytoplasm widening out at
the nucleus. The present author (27) has also reported briefly (Seventy-fifth annual session of the American Association of Anatomists, March, 1962) on the electron microscopy of the sympathetic ganglion of bullfrogs.

This paper will deal with some noteworthy findings on the fine structure of the ganglion cells, satellite cells, and synapses of the abdominal sympathetic ganglion of bullfrogs.

MATERIALS AND METHODS

Ganglia in the abdominal sympathetic trunks from 11 adult bullfrogs, *Rana catesbiana*, of both sexes were used as the material for this study. The animals were kept in the laboratory for 2 or 3 weeks at room temperature, and fed fresh liver several times a week. They were sacrificed in October and November. The ganglia were fixed immediately after removal from the pithed animals in either 3 per cent osmium tetroxide or 1.3 per cent potassium permanganate (9), both buffered at pH 7.4 with *α*-collidine (2) and chilled with ice. The period of fixation was 2 hours in osmium tetroxide or 3 hours in potassium permanganate. After fixation, they were rapidly dehydrated through a series of graded concentrations of ethyl alcohol and embedded either in Epon 812 according to Luft's method (10) or in 8:2 butyl-methyl methacrylate mixture.

Ultrathin sections were cut from the Epon blocks on a Porter-Blum microtome and stained with a half-saturated aqueous solution of uranyl acetate for several hours or with Millonig's lead solution (13) for 15 minutes. RCA EMU 2A and 2C electron microscopes fitted with compensated objectives, 50 μ objective apertures, and special stabilized lens power supplies were employed for observation.

For light microscopy, thick sections (1 ~ 2 μ), some sequential to preceding thin sections, were cut from the Epon blocks and stained with phosphate-buffered (pH 6.8) 0.5 per cent toluidine blue. Thick sections were cut from the methacrylate blocks, and were stained by Hotchkiss' PAS method as well as with toluidine blue after the methacrylate had been removed with xylene. Some methacrylate sections were treated with saliva for 1 or 2 hours at 37°C before PAS staining.

OBSERVATIONS

Subsurface Accumulation of Granules in the Ganglion Cell

The fine structure of the perikarya of sympathetic ganglion cells is similar in principle, with some exceptions, to that previously described for various types of neurons (1, 12, 14, 25).

In the marginal or superficial portion of the perikarya, some peculiar areas of cytoplasm were seen where large numbers of granules ranging from 200 to 400 A in diameter were closely packed (Fig. 1). Such portions of the perikaryon were devoid of granulated endoplasmic reticulum and contained only a very few vesicles, multivesicular bodies, and inclusion bodies. These areas were variegated in shape and size, and also showed considerable variation in their distribution in different cells. In some ganglion cells, for example, so many of these granulated areas were observed that they occupied the majority of the superficial zone of the perikaryon. In the cytoplasm of other ganglion cells, however, such granulated areas were scanty.

The granules in these areas were well preserved by osmium tetroxide fixation; the potassium permanganate fixation of 3 hours produced little density in the granules in question, but appeared to preserve them reasonably well (Fig. 3). They were easily stained by lead hydroxide, but seemed
to be virtually unaffected by the uranyl acetate stain (Fig. 4). After lead hydroxide staining, the granules were observed to contain several tiny dense subgranules (Fig. 2).

It was ascertained from subsequent thick sections for light microscopy that the above-mentioned granulated areas of perikarya correspond to the light portions between the dark Nissl bodies. RNA particles in such abundance would be expected to display a strong basophilia, but the granulated areas in the specimens showed no basophilia whatever with toluidine blue staining. When the PAS reaction was applied, these non-basophilic portions of the cytoplasm became intensely colored. How closely the distribution of the PAS-positive substance corresponds to the light marginal portions of the perikarya is shown in Figs. 5 and 6. These non-basophilic areas did not stain with PAS after treatment with saliva for 2 hours at 37°C, indicating that the positive reaction was probably caused by glycogen.

**The Satellite Cell and Neuronal-Satellite Cellular Relation**

As has already been described in sympathetic (25) and spinal ganglia (15, 26), the perikarya of ganglion cells in the present material were also entirely covered by a satellite cell layer. In some places, the satellite cell cytoplasm was as thin as 100 μ or less, that is, below the limit of resolution of the light microscope. Both in the sections stained with uranyl acetate and in those stained with lead hydroxide, a filament structure was found in the satellite cell cytoplasm. This structure appeared to be especially well developed around incoming nerve fibers and synapses (Figs. 9 and 10), and was still observable even in very thin portions of the cell (Fig. 4). The filaments are about 100 Å in thickness and are arranged more or less parallel to one another. Filaments of the same structure were also found in the cytoplasm of Schwann's cells of unmyelinated fibers running in the ganglion.

Nuclei of the satellite cells are usually located in the angle formed between perikaryon and root of the nerve process of the neuron, as seen in Fig. 7. Satellite cells possess a considerable amount of cytoplasm and abundant cell organelles in the vicinity of their nuclei. In the thin portion of the cell, however, no cell organelles other than vesicles, filaments, and occasionally lipid droplets are seen as a rule.

At the roots of nerve processes, multivesicular bodies of the neurons were sometimes observed bulging the plasma membrane into the spaces between the neurons and the satellite cells. An example of this feature is clearly shown in Figs. 7 and 8. As will be described later, roots of the nerve processes are covered with a thin sheet of satellite cell cytoplasm. The protrusions containing multivesicular bodies, however, have no covering of the satellite cell element, so that the basement membrane lies directly over the protrusions. In addition, a constriction is frequently found between the protrusion and the main portion of the nerve process. It is, therefore, quite likely that these multivesicular bodies are being extruded from the neuron.

**Figure 3**

Potassium permanganate-fixed and lead hydroxide-stained ganglion cell. The ordinary structure of the perikaryon containing organized endoplasmic reticulum (E) and mitochondria (M) is seen in the upper part of the micrograph, and a satellite cell (S) covering the perikaryon is shown near the lower margin. A wide area of the perikaryon where only a small amount of vesicular component and a few multivesicular bodies are distributed corresponds to the granulated area in osmium tetroxide-fixed sections. The granules are not dense with 3 hour potassium permanganate fixation, as is clear in this micrograph. × 16,000.

**Figure 4**

Part of a ganglion cell fixed in osmium tetroxide and stained with uranyl acetate. Granules in the specific superficial area of the perikaryon appear much less dense in this picture than in Fig. 1. Ribosomes around the endoplasmic reticulum (E), on the other hand, are stained deeply. Many thin filaments are seen in the satellite cell (S). M, mitochondrion; N, nucleus of the ganglion cell. × 21,000.
From the satellite cells extend a large number of finger- or leaf-shaped cytoplasmic projections around the root portion of the nerve processes (Fig. 7). Some of these satellite cell projections form a thin layer or sheet to cover directly the surface of the root of the nerve process, except in the synaptic regions and at protrusions containing multivesicular bodies. Many other projections, however, are separated from the neuron by a fairly wide interspace. Some of the projections envelop fine nerve fibers which approach the neuron to form synapses with it. Such ramifying processes of satellite cells are observed around all types of nerve processes with the exception of very tiny ganglionic extensions. Every projection is lined with a basement membrane which often spreads diffusely into the interspace.

### The Synapses

Many synapses are formed around the neuron, especially around the root portion of the nerve process. These synapses contain synaptic vesicles and mitochondria. Besides these, a granular component is also constantly observed in synapses (Figs. 9 and 10). These granules are very similar morphologically to those found in the superficial region of perikarya described above, and are also hardly affected by uranyl acetate stain (Fig. 11). Distribution of the granules in synapses is somewhat irregular. Usually a small number of granules is scattered in a synapse (Fig. 9), but sometimes they are observed in clusters of considerable mass (Fig. 10).

Synaptic vesicles are separated from the neuronal cytoplasm by two layers of plasma membrane, one from the synaptic termination and the other from the neuron. These membranes face each other with a thin intervening space of about 150 A between them. When osmium tetroxide fixation was employed, an increase in density of each of the synaptic membranes was clearly visible, as noted by many authors. In such a dense area, the synaptic membrane appears as though it were conspicuously thickened. However, no thickening of either of the synaptic membranes was detectable after potassium permanganate fixation.

### DISCUSSION

#### Subsurface Accumulation of Granules in the Ganglion Cell

Revel, Napolitano, and Fawcett (16) found that glycogen almost always appears under the electron microscope as roughly circular granules from 150 to 400 A in diameter and that these granules are stained deeply with lead hydroxide using Watson's method. They noticed that in many micrographs the lead occurred in punctate deposits uniformly distributed over the surface of the glycogen granule. Watson (23, 24) and Luft (11) have found that treatment with uranyl acetate, differing from the lead hydroxide staining, contributes little, if any, density increase in glycogen granules. Moreover, it has been pointed out by some authors (9, 16) that glycogen particles are well preserved by permanganate fixation. The present electron microscopic findings, with respect to the granules which accumulate in the subsurface area of the ganglion cell, are in accord with the observations of glycogen granules by the above authors. In addition, the distribution of the granulated area in the ganglion cell corresponds to that of the PAS-positive material digestible by saliva. It is, therefore, quite likely that the granules distributed here are glycogen, or possibly a closely related substance, such as glycoprotein or glycolipid.

My observation that permanganate produced only low density in subsurface granules (presumably glycogen) is in accord with the report of
Revel, Napolitano, and Fawcett (16) on purified glycogen. Luft (9) and Drochmans (4), however, found that permanganate treatment produced a high density of glycogen granules. The reason for this difference may be due to the difference in procedures. In the present study and in that of Revel, Napolitano, and Fawcett, tissues were treated with permanganate solution for 3 and 2 hours, respectively. Luft, however, found that 2 or 3 hours was too short a period to increase the density of glycogen (11), and used 12 hours for treatment. Drochmans applied 5 per cent solution of potassium permanganate for 20 to 30 minutes after sectioning in order to obtain high density in glycogen granules.

The presence of the glycogen in nerve cells has already been observed histochemically in both brains and autonomic ganglia of various mammals. Sulkin and Kuntz (19, 20) have demonstrated considerable amounts of glycogen in feline and canine autonomic ganglion cells by the PAS method combined with the malt diastase digestion test. Shimizu and Kumamoto (17) showed histochemically that some nerve cells in rodent brains contained glycogen. According to their descriptions and micrographs, the distribution of glycogen in these nerve cells is not confined to the superficial portion of the perikaryon; that these areas did not contain any granulated endoplasmic reticulum; that the diameter of granules was 200 to 400 A; and that some granules appeared to have minute subgranular internal structure. However, Smith drew the conclusion, based upon his histochemical data, that these granules are RNA-protein in their chemical nature. He stated that no intraneuronal PAS-positive substance was observable in his material. Thus, the granules in the lizard neuron may differ from those of the present material in their chemical properties, although some resemblance in the electron microscopic structure can be pointed out for both kinds of granules.

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Smith (18) has also reported on the presence of granulated areas in lizard sympathetic neurons. He noted that the distribution of these areas was restricted to the superficial portion of the perikaryon; that these areas did not contain any granulated endoplasmic reticulum; that the diameter of granules was 200 to 400 A; and that some granules appeared to have minute subgranular internal structure. However, Smith drew the conclusion, based upon his histochemical data, that these granules are RNA-protein in their chemical nature. He stated that no intraneuronal PAS-positive substance was observable in his material. Thus, the granules in the lizard neuron may differ from those of the present material in their chemical properties, although some resemblance in the electron microscopic structure can be pointed out for both kinds of granules.

The Satellite Cell and Neuronal-Satellite Cellular Relation

Filaments approximately 100 A in diameter have been described in some types of neuroglia cells (6, 7, 12), as well as in Schwann's cells of unmyelinated fibers in the feline splenic nerve (5). Pannese (15) has observed a network of tiny filaments, less than 100 A in thickness and running in all directions, in satellite cells of sensory ganglia in several kinds of mammals. In his paper he stated that this network is not evident in all kinds of cells; therefore, either it is peculiar to certain cells, or else it depends on artifacts produced in a very delicate and hydrated cytoplasm, insufficiently preserved by the osmium tetroxide fixation. Actually, the network appears in his micrograph...
as a very irregularly arranged and obscure structure.

Filaments in the satellite cells of specimens described here, on the contrary, are commonly present, are of a much more distinct structure, and are arranged more or less parallel to one another. It is conceivable that they are of the same nature as those described for the Schwann's cell by Elfvin (5). No difference in structure between the filaments in the satellite cell and those in the Schwann's cell of the unmyelinated fiber was detected in this study.

Though the general structure of the satellite cell has been much clarified by electron microscope studies, our knowledge of the features of this cell in the vicinity of the root of the nerve process is still far from complete. That the satellite cell extends numerous leaf- or finger-like projections around the root of the nerve process has not, to the knowledge of the author, been previously noted in either an autonomic or a sensory ganglion.

The Synapses

It is highly probable that the granules which were found in synaptic terminations are also glycogen, since their electron microscopic properties are similar to those of the presumptive glycogen particles in the superficial region of the perikaryon. If such is the case, glycogen would be one of the constant components of the synaptic termination in this material. However, attempts to verify the characteristics of these granules histochemically were not successful. Most synapses were too small in size and the granule component of each synapse was usually too small in amount to be examined usefully under the light microscope.

Findings which indicate the process of extrusion of multivesicular bodies from the neuron seem to be significant for the consideration of a possible role of this structure in cell function. However, not enough evidence was obtained in the present study to warrant a discussion as to the significance of this phenomenon.

It is generally held that the contact region between pre- and postsynaptic membranes shows not only localized density increases but also localized thickenings (see Gray (8)). In fact, when osmium tetroxide was used as a fixative, the density increase appeared to be combined with the membrane thickening. It is, however, also possible that false thickening will be caused if the dense substance is close to a membrane of regular thickness. Application of potassium permanganate fixation would be useful to determine whether thickening in a strict sense really exists in the membrane or not, since short permanganate treatment will preserve membranes well, but not other structures. Furthermore, if true thickening does occur, it would be of additional interest to learn which component of the unit membrane of the synaptic membrane is actually thickened.

In fact, no thickening or density increase was detected with the permanganate fixation. Gray (8), who claimed existence of the thickening, also mentioned that the apposed membranes fixed

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**Figure 9**

An electron micrograph of synapses. Besides many synaptic vesicles and mitochondria, small granules are found scattered in the synapses. G, ganglion cell; S, satellite cell containing a well developed filament structure. Osmium tetroxide-fixed, lead hydroxide-stained. X 16,000.

**Figure 10**

Two synapses which contain a considerable amount of granules. Filaments in the satellite cell are also shown in this picture. G, ganglion cell; M, mitochondrion. Osmium tetroxide-fixed, lead hydroxide-stained. X 26,000.

**Figure 11**

A uranyl acetate-stained section through synapses. The granular component of these synapses is not stained at all, in contrast to lead hydroxide-stained sections, while ribosomes in the ganglion cell show high density. Compare with Figs. 9 and 10. The lighter area (A) probably corresponds to a granule-rich area after lead staining. G, ganglion cell. Osmium tetroxide-fixed. X 26,000.
G
S

M
A

G

1 μ

1 μ

1 μ
with permanganate show few, if any, specialized regions of increased density, in contrast to those fixed with osmium tetroxide. The inclination, then, is to deny the occurrence of thickening, in a strict sense, in synaptic membranes.

This study was made while the author was in the Department of Anatomy, University of Washington, Seattle, as a Postdoctoral Fellow of The Rockefeller Foundation, New York.

The studies were supported in part by United States Public Health Service grants 2G-136 and B-401 from the National Institutes of Health, Bethesda. The author wishes to acknowledge the invaluable advice of John H. Luft, M.D.

Received for publication, July 17, 1962.

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