THE EFFECT OF DEPRIVATION OF GLUCOSE ON THE ULTRASTRUCTURE AND FUNCTION OF THE SUPERIOR CERVICAL GANGLION OF THE RAT IN VITRO

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ABSTRACT

The superior cervical sympathetic ganglion of the rat kept in vitro in a bicarbonate-buffered Krebs' solution retains its capacity for synaptic transmission and axonal conduction during more than 36 hr. After glucose withdrawal, synaptic transmission is lost in 2½ hr and this loss is irreversible; on the other hand, axonal conduction can still be measured on the postganglionic nerve for more than 24 hr after glucose deprivation. Electrophysiological measurements as well as electron microscope studies revealed specific changes at the level of the presynaptic terminal processes, while the ganglion cells and the satellite cells remained relatively unaltered. The presynaptic lesion due to lack of glucose can be prevented by keeping the preparation in vitro at 6°C. This strongly suggests that this lesion results from a major disturbance of the metabolism of the presynaptic fibers.

INTRODUCTION

The sympathetic superior cervical ganglion of the rat weighs from 600 to 800 μg, fresh weight. It is thus small enough to be maintained and oxygenated by diffusion in vitro, and large enough to allow metabolic and electrophysiological measurements.

The ganglion can be maintained in bicarbonate-buffered Krebs' solution, equilibrated at pH 7.4 with a mixture of 5% CO_2 and 95% O_2 at 37°C. The functional survival of the ganglion can be estimated by measuring either the uptake of O_2 (31), the utilization of glucose and production of lactate (14, 33), or the synaptic transmission and axonal conduction (15). Records of the tissue polarization (29) or of the slow potentials in normal or curarized ganglions contribute to a better understanding of the transmission mechanisms (16, 17). Finally, the determination of the activities of certain enzymes (14) and their histochemical localization (13) indicates the metabolic status of the tissue at any chosen moment during its survival in vitro. Concurrent morphological study with the light or electron microscope gives information about the state of the structures involved in the conduction and the transmission of the nervous impulse, and about the ultrastructural elements believed to be associated with the cellular metabolism.

The length of the functional survival of the ganglion in vitro assessed by the capacity of the

tissue to transmit a nervous impulse is comparable to that of the ganglion in vivo with its afferent connection severed (32). A pair of ganglions from the same rat presents very similar functional and metabolic parameters; therefore, one of them can be used as a control, while the conditions of survival of the other are modified. On the average, the synaptic transmission can still be measured 36 hr after excision of the ganglion and its incubation in vitro. The conduction may persist much longer especially in the postganglionic nerves, where it has been measured up to 95 hr after the ganglion's excision (15). These observations show the importance of the preservation of the cell body for the maintenance of the functional qualities of the nerve fibers.

In spite of the persistence of function, the tissue undergoes progressive deterioration from the time of excision. The earliest evidence of this progressive change is an increase in the response of the postganglionic nerve after preganglionic stimulation. The nature of the lesion leading to this increase in the postganglionic discharge has been reported elsewhere (17).

When the tissue kept at 37°C is deprived of glucose in vitro, the transmission of an impulse from the preganglionic to the postganglionic nerve declines and then disappears in about 2½ hr. This sequence of events is remarkably constant and the loss of function cannot be reversed by reintroduction of glucose or any other substrate (12, 34, 44).

Contrary to what happens to the electrophysiological activity of the tissue, the oxygen uptake undergoes little change in the absence of glucose: it is decreased only by about 20% 6 hr after glucose withdrawal (31). However, though nervous transmission is not recovered when glucose is reintroduced, the consumption of O_2 does rise again to an almost normal level (34).

Other metabolic changes in the tissue deprived of glucose include production of ammonia concomitant with the loss of the electrophysiological function (10, 34). On the other hand, the activity of a variety of enzymes undergoes only a negligible decrease (9) compared to that recorded under other metabolic conditions (8, 13).

When the glucose-deprived tissue is kept at 6°C, even for periods up to 18 hr, its capacity to conduct and transmit can be recorded again as soon as the solution has been warmed up again to 37°C. Thus, lowering of the temperature protects the tissue against the lesion due to the absence of glucose.

It appeared interesting to investigate whether this irreversible loss of transmission in the absence of glucose could be related to a morphological lesion. Since preliminary observations with the light microscope failed to reveal any structural modifications within the ganglions, it became evident that the changes, if any, had to be revealed by electron microscopy. This lesion would have to affect irreversibly only structures whose integrity is specifically needed for synaptic transmission. The facts that the tissue continues to consume oxygen in the absence of glucose, that its enzymatic machinery remains functional, that it produces ammonia, and, lastly, that it is capable of utilizing glucose again, suggest that the cell bodies of the ganglion cells and satellite cells have not been significantly affected.

In order to evaluate the fine morphological modifications secondary to glucose withdrawal, two preliminary control studies were undertaken. The first one concerned ganglions immediately after their excision (referred to as "normal" ganglions), and the second one concerned ganglions maintained in vitro for periods from 90 min to 40 hr (referred to as "control" ganglions). This work was published elsewhere (22, 24).

The fine structure of the normal sympathetic cervical ganglions of the rat and the cat are similar (20–22). Also, numerous papers published in recent years on the electron microscopic aspects of the autonomic nervous system have shown that the ultrastructure of the neurons, their dendritic and axonic processes, the nerve endings, and the satellite cells is remarkably constant regardless of the vertebrate species ranging from frog to man (50, 40, 55, 28, 47, 8, 49, 51, 37–39, 22, 27, 1–4, 43, 54, 26, 18–21, 25, 6, 41).

The ultrastructure of the neurons in the sympathetic ganglions of the rat resembles that of other neurons. The cell body contains a nucleus, limited by a double membrane with pores, containing one to several nucleoli and finely granular or filamentous material. The perikaryon is composed of neurofilaments, mitochondria, a Nissl substance, Golgi areas, and isolated vesicles; occasionally, there are also multivesicular bodies, granular "Nebenkerne," lipid droplets, and dense inclusions (lysosomes, lipofuscin). The Nissl substance is composed of granular membranes and numerous ribosomes, occurring singly or,

more often, grouped in rosettes. This basophilic substance does not form distinct masses, but it is distributed diffusely throughout the cytoplasm, in a fashion similar to that observed in the sympathetic neurons of the abdominal ganglions of the frog (40). The well developed Golgi complex consists of vesicles of variable sizes and of tubules, aligned closely alongside of one another, forming a discontinuous perinuclear ring.

The neurons, as well as their dendritic and axonic processes, are surrounded by satellite cells which cover them completely with a band of cytoplasm of variable thickness. Only at the levels of the synapses, the plasma membrane of the presynaptic ending is in direct contact with the dendritic or somatic membrane of the neuron. The satellite cells themselves are surrounded by a basement membrane which separates them from the endoneural space.

The dendrites and axons alike are composed of a hyaloplasm containing neurofilaments, vesicles, ribosomes, and occasional mitochondria. According to Elfvin (20, 21), it is possible to differentiate preganglionic fibers from dendrites and axons; the distal portion of the former was said to be more transparent to the electrons than the corresponding portions of the latter two. In our material, investigated by Forssmann (22), it was, indeed, occasionally possible to make this distinction but only because the proximal portions of the axons and dendrites were richer in neurofibrils and mitochondria than the presynaptic fibers. As a general rule, however, this identification was much more difficult to make than in Elfvin's material. Differences in animal species and in techniques may well account for this disparity of views.

The preganglionic nerves can be identified by their synaptic apparatus which is characterized by its microvesicles measuring from 200 to 600 A, by the thickening of the plasma membranes, and by the presence of an electron-opaque material, mostly on the postsynaptic side. These features have already been described by numerous authors in various locations (37, 11, 50, 20, 21, 40). The axodendritic synapses are much more numerous than the axosomatic synapses.

The changes which occur in the superior cervical ganglions maintained in vitro in an oxygenated standard Krebs' solution have been described by Forssmann and Rouiller (24). The changes were slight during the first 20 hr and incubation. In the neurons, after 90 min, the Golgi areas became more numerous, spread out, and occupied the periphery of the cell body. After 7 to 8 hr, the ribosomes broke away from the membranes, and the polysomes dissociated; sometimes, the cisternae of the endoplasmic reticulum became a little dilated. In some cells, the mitochondria were swollen, while in others they were small and more numerous. The nucleus, instead of being rounded, was often of irregular outline.

The satellite cells showed the same changes, but to an even lesser degree. The cell processes, the nerve fibers, the presynaptic terminal processes, and the synapses themselves did not undergo any visible modification. Although the changes in the neurons and satellite cells were slight, they still showed that the incubated ganglions had an early loss of some of their structural integrity. This observation was in keeping with the results of investigations of the functional activity, which showed very early changes (17).

However, marked morphological changes were not observed until after 17 to 22 hr of incubation (24), that is to say, *long after the incubation period used in the present work.*

MATERIAL AND METHODS

Twenty-three male and female Wistar "Foster" rats weighing between 140 and 190 g were used. Both superior cervical sympathetic ganglions were excised under urethane anesthesia (1 g/kg, intraperitoneally). The excised ganglions were rapidly mounted on electrodes, allowing stimulation of the preganglionic nerve and recording of the electrophysiological response on the postganglionic fibers. These electrodes allowed the tissue to remain immersed in the solution during the experimental period (15). Paired ganglions from the same animal were placed in standard Krebs' solution at 37°C for 90 min. Subsequently, both ganglions were transferred: one, serving as control, was placed in fresh standard Krebs' solution whereas the other was placed in a Krebs' solution without glucose. Standard temperature of 37°, pH, and oxygenation were maintained for both series of experiments. The ganglions were kept in vitro under these conditions from 1½ to 30 hr. The height of the recorded action potential served as a control of the functional activity of the tissue. Often the ganglions, after varying periods of incubation in a Krebs' solution, with or without glucose, were mounted in a specially constructed chamber with compartments (Fig. 2) which allowed separate stimulation and recording of axonal conduction or synaptic transmission. In some experiments, macromolecules (polyvinylpyrrolidone) were

added to the solution in order to reproduce not only an osmotic but also an oncotic equilibrium.

Since the synaptic transmission was shown to be irreversibly lost 3 hr after glucose withdrawal (34), the ganglions chosen for electron microscopic investigation were not incubated longer than that. After withdrawal from the incubation medium, the ganglions were cut transversely into 2 or 3 fragments, fixed in a solution of osmium-bichromate tetroxide according to the technique of Forssmann (23), dehydrated with acetone, and embedded in Vestopal (46). The sections were cut with Porter-Blum or L.K.B. microtomes, stained with lead according to Karnovsky (30) or Reynolds (42), and examined with Zeiss EM9 and Siemens Elmiskop I.

RESULTS AND INTERPRETATION

1. *Electrophysiological Localization of the Lesion*

The top of Fig. 1 shows the positions of the stimulating and recording electrodes on the pre- and postganglionic nerves of a ganglion when mounted in a chamber with compartments (Fig. 2). Fig. 1 also shows the action potentials recorded in response to a repetitive stimulation at 6/sec, first in the presence and then in the absence of glucose. It is evident that electrophysiological responses were obtainable for 12 hr after withdrawal of glucose from the preganglionic nerve and for 30 hr from the postganglionic nerve. On the other hand, the synaptic transmission was completely and irreversibly lost 3 hr after placing the ganglion in the glucose-free solution. It is, therefore, at the level of the presynaptic structures that the irreversible lesion must arise. Morphological study with the electron microscope confirmed this conclusion based on the electrophysiological observations.

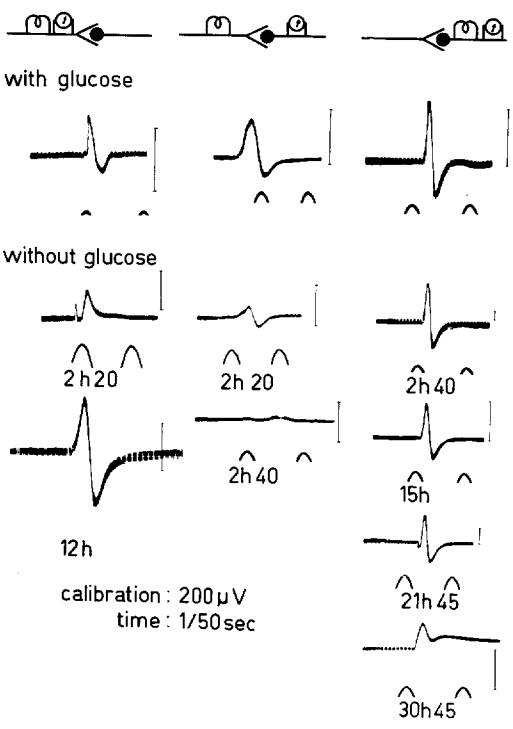


FIGURE 1 Physiological responses of pre- and postganglionic nerves of a ganglion (see text).

2. *The Modification of the Fine Structure of the Ganglions after Deprivation of Glucose for 3 Hr at 37°C*

In comparison with the controls (Figs. 4, 5), the ganglions deprived of glucose presented alterations predominantly in the presynaptic fibers and the terminal processes (Figs. 3, 6, 8, and 10), whereas in the ganglion- and satellite cells the changes were slight and inconstant.

a. The Ganglion Cells

In the perikaryon of the neurons, the cisternae of the endoplasmic reticulum were often enlarged (Fig. 6). The ribosomes were more dispersed: those attached to the membranes were less numerous, and the polysomes were often dissociated. The Golgi areas were enlarged. This was caused by a dilatation of the vesicles and perhaps also to an increase in the number of these vesicles. The mitochondria showed slight changes. Some of them were elongated and narrowed, and others were swollen. There appeared to be the same number of lysosomes, as compared with controls or normal ganglions.

The modifications of the neurons were thus minimal. They were also visible in the control ganglions, but in these the changes in the endoplasmic reticulum appeared later and the vesicles of the Golgi complexes were less dilated (24).

b. The Satellite Cells

The modifications of the satellite cells were comparable to those of the neurons. Frequently, however, the cytoplasmic vesicles were more numerous and larger than in the control ganglions (Figs. 6 and 8, *S*), Such vesicles were especially

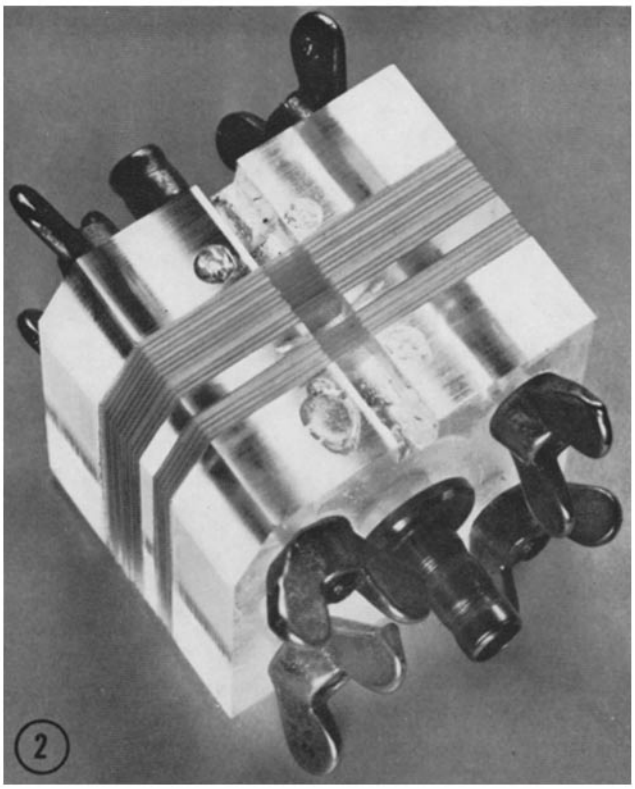


FIGURE 2 Lucite chamber with compartments allowing the recording action potentials using electrodes located on the pre- or postganglionic nerves (*see* Fig. 1). The central tubing allows the block to be warmed by circulating water.

prominent in the satellite cells surrounding the injured cellular processes.

c. The Cell Processes

In the ganglions which lost their function, there were always lesions of the nerve fibers (Figs. 3, 6 to 8), but the frequency and severity of these lesions were variable. In the same tissue section, cell processes with a well preserved structure were adjacent to cell processes with severe lesions (Fig. 8). The latter were usually swollen and the neurofibrils could not be demonstrated. The mitochondria were rare, and their structure modified: some were swollen, others had vesicular cristae, while still others were enclosed in cytolysomes. Frequently, the swollen cell processes contained vesicles which differed from the presynaptic vesicles by virtue of their larger and variable size, wider distribution in the cytoplasm, and denser contents. Cytolysomes and dense amorphous masses of irregular from and unknown nature, typical of myelin degeneration, were also observed in the swollen cell processes. The most extensively deteriorated cell processes were practically reduced to their plasma membrane, either without any electron-opaque contents, or with only a loose network of fine granules of filaments. Synapses and vesicles of presynaptic terminal processes could not be seen, except on one single occasion. None of the lesions described above were observed in control ganglions after an incubation in vitro of comparable duration in a standard Krebs' solution containing glucose (Figs. 9, 10). It was only after 20 hr of incubation that the controls showed lesions such as the mitochondrial injury, formation of cytolysomes, and the appearance of vesicles (24).

3. *Further Identification of Site of Lesion Due to Glucose Deprivation*

Except for the proximal portions of the axons or postsynaptic dendrites, there are few ultrastructural characteristics allowing for the differentiation of presynaptic from postsynaptic fibers or those rare fibers which pass through the

ganglion without synapses (15). In the rat as in the cat, there are no morphologic differences between axons and dendrites. The presynaptic fibers are recognizable only when they can be traced to synapses and synaptic vesicles. Thus, in ganglions deprived of glucose for only 90 min this identification was occasionally possible because the synaptic apparatus was still present.

After 3 hr of incubation in the absence of glucose and the loss of the synaptic apparatus, the exact identification of injured cell processes appeared impossible on morphological grounds alone. Furthermore, even if the proximal portion of a cell process was well preserved this did not rule out an injury more distally located.

However, an additional electrophysiological observation eliminates the doubt left by the electron microscope in the identification of the site of the lesion.

The following experiments were conducted: a sympathetic ganglion of the rat was incubated for 90 min in standard Krebs' solution with physostigmine 5 μg/ml added. Subsequent electric stimulation of the postganglionic nerve for 1 sec (at a frequency of 35/sec) caused the postganglionic nerve to remain spontaneously active for a period of about 50 sec following the initial response (Fig. 12, *1a* and *1b*). This effect is due to the inhibition of cholinesterase by physostigmine, resulting in persistence of the acetylcholine liberated by the presynaptic terminal processes.

If shortly thereafter acetylcholine 1 μg/ml is added to the medium, there is a recordable discharge in the postganglionic nerve in the absence of electric stimulation (Fig. 12, *2a* and *2b*). This indicates direct action of acetylcholine on the postsynaptic membranes. The same responses can be obtained for 4 hr later (Fig. 12, *3a* and *4a*).

If the tissue has been incubated without glucose for 4 hr, the stimulation in the presence of physostigmine gives no response, either immediate or prolonged (Fig. 12, *3b*), because the presynaptic terminal processes are injured; on the other hand, the response to acetylcholine is preserved (Fig. 12, *4b*) indicating that the postsynaptic membranes are still capable of a functional response.

4. *The Ultrastructure of the Ganglions Deprived of Glucose, but Incubated at 6°C*

Ganglions incubated in the absence of glucose at 6°C did not lose their synaptic function irreversibly. Even after 18 hr of incubation without glucose, they were still capable of transmitting a nervous impulse as soon as the temperature of the medium was raised again to 37°C. Low temperature, therefore, protected the tissue deprived of glucose against the presynaptic lesion, presumably by diminishing the rate of metabolism of the tissue.

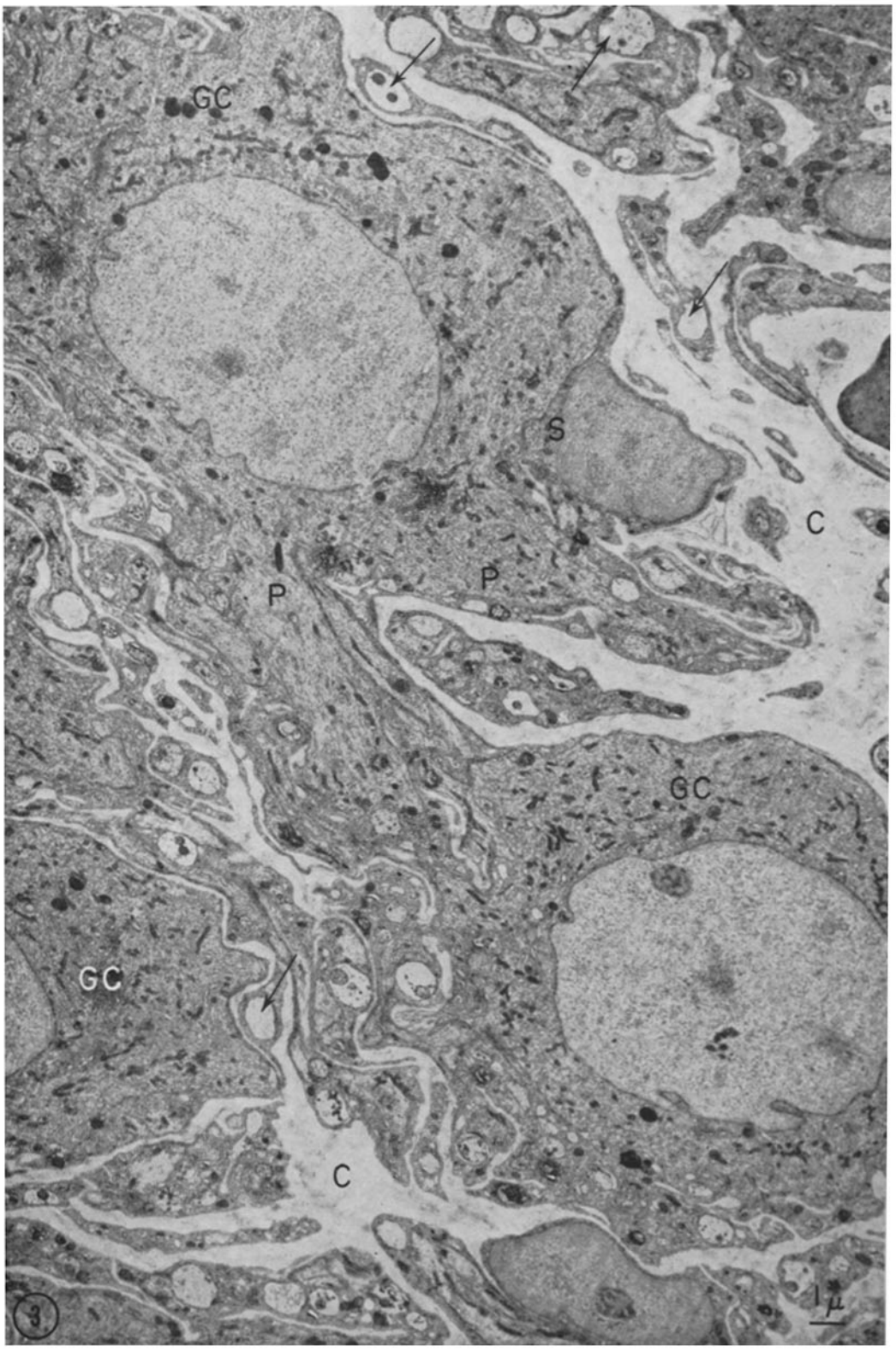
The morphological study of ganglions incubated at 6°C without glucose confirmed the extreme rarity of any lesion (Fig. 13). The presynaptic terminal processes with clearly visible microvesicles and the synaptic junctions were intact (Figs. 13 to 16). The ease with which these normal structures could be identifidd represents the most marked difference between these ganglions and those maintained at 37° with or without glucose.

Abbreviations

C, Connective tissue space	*GC*, Ganglionic cell
E, Nerve ending	*P*, Cell process
er, Endoplasmic reticulum	*r*, Ribosomes
F, Nerve fiber*	*S*, Satellite cell
G, Golgi area	Scale mark, 1 μ.

* *Note:* For the purpose of simplification, the term "nerve fiber" is used for all sections of cell processes, without distinction between axon and dendrite

FIGURE 3 Superior cervical ganglion deprived of glucose for 3 hr. Complete loss of capacity to transmit a nervous impulse. The section is across nerve cells (*GC*), their processes (*P*), and numerous altered nerve fibers, visible as rounded clear profiles (arrows). In ganglions maintained in vitro, the connective tissue space (*C*) is always very transparent to electrons. × 6,000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5. *The Ultrastructure of the Ganglions Incubated in Oxygenated Krebs' Solution with Added Polyvinylpyrrolidone*

Neither from the electrophysiological nor from the morphological point of view were there any differences between the ganglions incubated in a medium with added polyvinylpyrrolidone and those incubated in the normal Krebs' solution, with or without glucose.

DISCUSSION

Superior cervical ganglions of the rat maintained in Krebs' solution in vitro without glucose for approximately 3 hr showed an irreversible loss of their synaptic transmission while axonal conduction in the presynaptic or postsynaptic nerves was maintained. Parallel studies with the electron microscope and electrophysiological techniques revealed a lesion limited to the presynaptic terminal processes. The neurons and the satellite cells, with the exception of occasionally marked vesiculation of the latter, were not significantly altered morphologically. Although distal postsynaptic axonic and dendritic lesions coud not be excluded on morphological grounds alone, they were unlikely because of the good preservation of the proximal segments and of the cell bodies of the neurons. Electrophysiological study confirmed this assumption by demonstrating that the postsynaptic membrane and its receptors were still capable of responding to acetylcholine.

Control ganglions from the same rats incubated in vitro in Krebs' solution containing glucose failed to show any evidence of damage. This strongly suggests that the lesions described were due to lack of glucose and not to other causes, such as bacterial or viral infection, and they can be compared to those recently described in the retina (53).

In some respects, these lesions were also reminiscent of various, previously reported degenerative changes resulting from denervation: such lesions were noted at the level of the "boutons" of the inferior olive 7 to 10 days after experimental injury to the mesencephalon (52); in the preterminal axons or terminal processes in the lateral geniculate ganglion 80 hr or more after bilateral enucleation of the eyes (48); in the presynaptic fibers of the sympathetic ganglions of the frog 2 to 3 days after section of the presynaptic nerve fibers (28); and in the dorsal nerve roots 2 days after laminectomy and section of the dorsal roots (35). In the present experiments, the lesions of the cell processes were evidently not due to denervation, because the controls didn't show them either after an equal or longer time of incubation, up to 40 hr. In the latter ganglions, the presynaptic fibers were remarkably well preserved after a 40-hr incubation in vitro in spite of degenerative changes in the ganglion cells and satellite cells.

One has to consider the possibility that the lesion of the presynaptic processes was secondary to a drop in osmotic pressure due to absence of glucose, but this can be discarded for several reasons. The injury to the fibers was selective and not generalized; also both the neurons and the satellite cells showed no significant changes. The addition of electrolytes to reestablish the osmotic equilibrium or of macromolecules to insure an oncotic equilibrium after the withdrawal of glucose did not prevent the fibers from undergoing the changes described. Thus, the lesion is certainly not of a physicochemical nature.

We feel justified in thinking that the observed tissue damage might be due to the utilization by the tissue of its own stores in the absence of glucose. There are several arguments in favor of this interpretation. The enzymes necessary for such an autocombustion were present. Their activity diminishes only slightly in the absence of glucose and only after a minimum of 6 hr (7, 14, 45, 3). In

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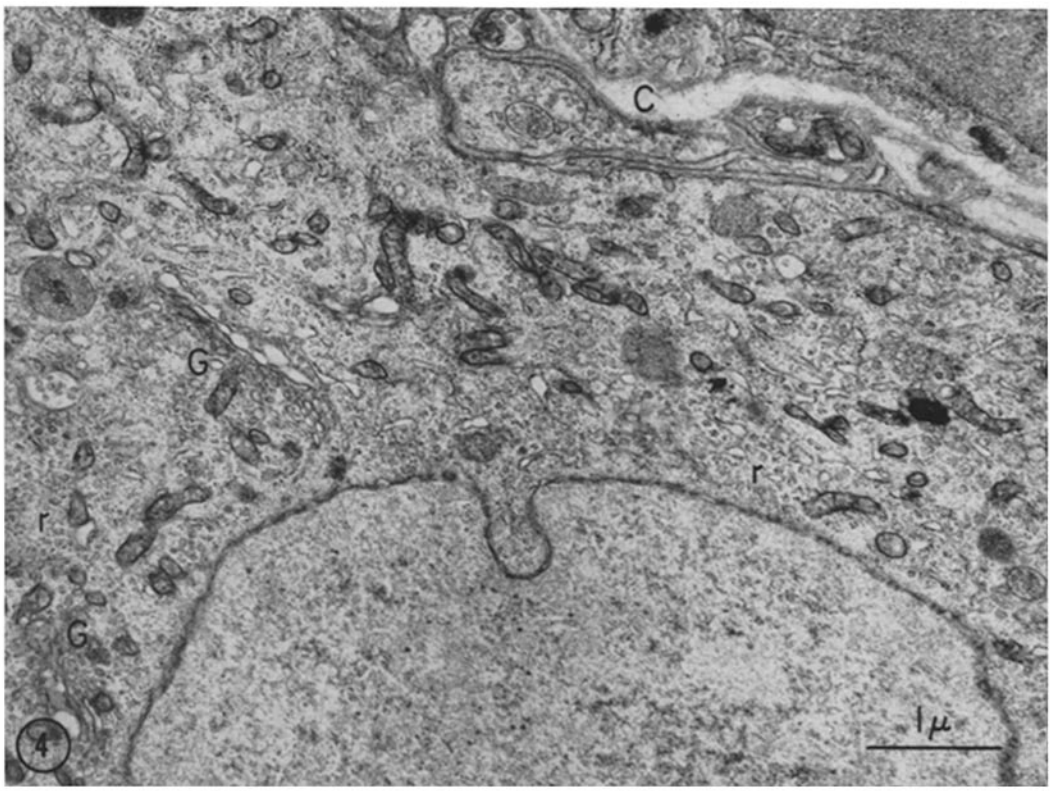
FIGURES 4 and 5 Control superior cervical ganglion, maintained in vitro with glucose.

FIGURE 4 Portion of a nerve cell showing the nucleus (bottom), Golgi areas (*G*), some mitochondria, and some ribosomes. × 17,500.

FIGURE 5 Section through nerve fibers cut longitudinally and obliquely. The fibers, like the ganglionic nerve cells, are always surrounded by a band of cytoplasm, sometimes very thin, which belongs to satellite cells. × 21,500.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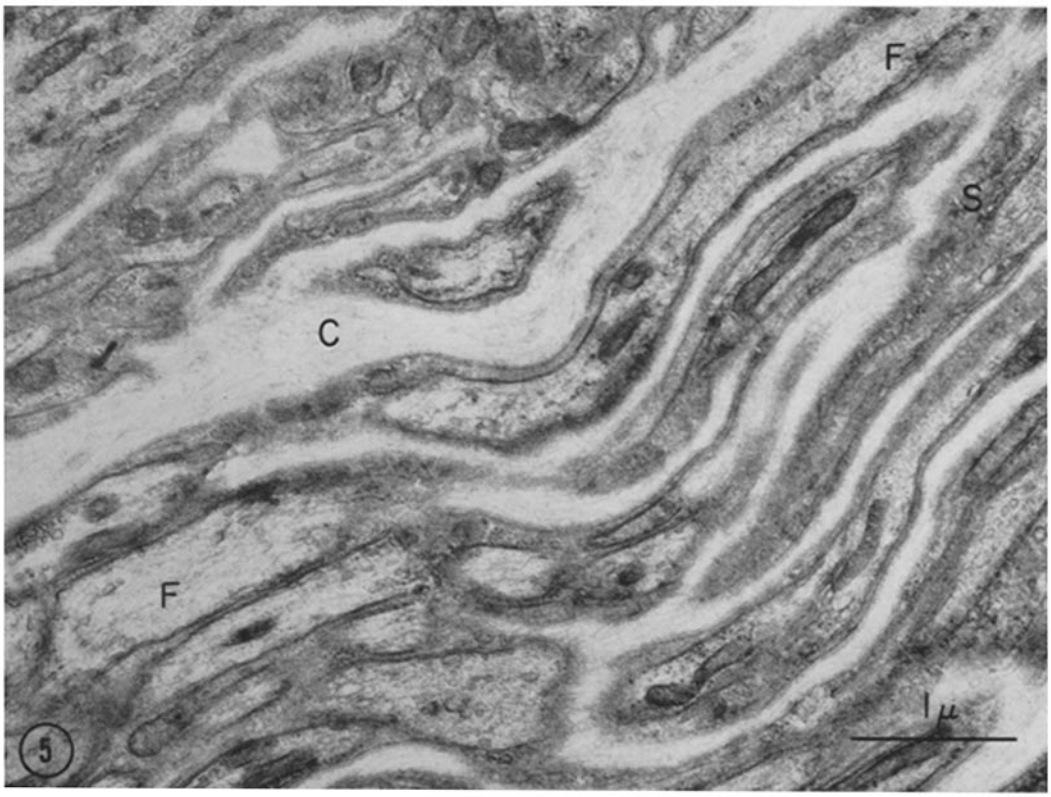
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the experimental setting of the present work, the enzymes necessary for tissue degradation had not yet diffused outside of the tissue and were still active. Other experimental arguments also support the hypothesis of a tissue lesion due to metabolic autoconsumption: consumption of O_2 continues (31); ammonia production is detectable at the moment of irreversible loss of function (34); the tissue can be protected by hypothermic diminution of its rate of metabolism (12); and, lastly, the functional survival of the tissue can be prolonged if the tissue is charged with glycogen before depriving it of glucose (10).

The consumption of an endogenous substrate in the absence of glucose is probably the cause of the lesions of the presynaptic fibers.

One might ask what is the reason for the location of the lesion so specifically limited to the proximal portion of the preganglionic fibers, and affecting neither the neurons nor the postganglionic fibers. Three explanations may account for this:

1. The preganglionic fibers contain less reserve glycogen than the other neighboring structures and hence are more vulnerable to deprivation of glucose.
2. The preganglionic fibers are severed from their cell bodies and this increases their susceptibility to lack of glucose. It must be recalled here that the nerve fibers in continuity with their cell body do survive much longer in vitro (15).
3. Present knowledge suggests that the preganglionic fibers have a more active metabolic rate and greater substrate requirements than the postganglionic fibers (36).

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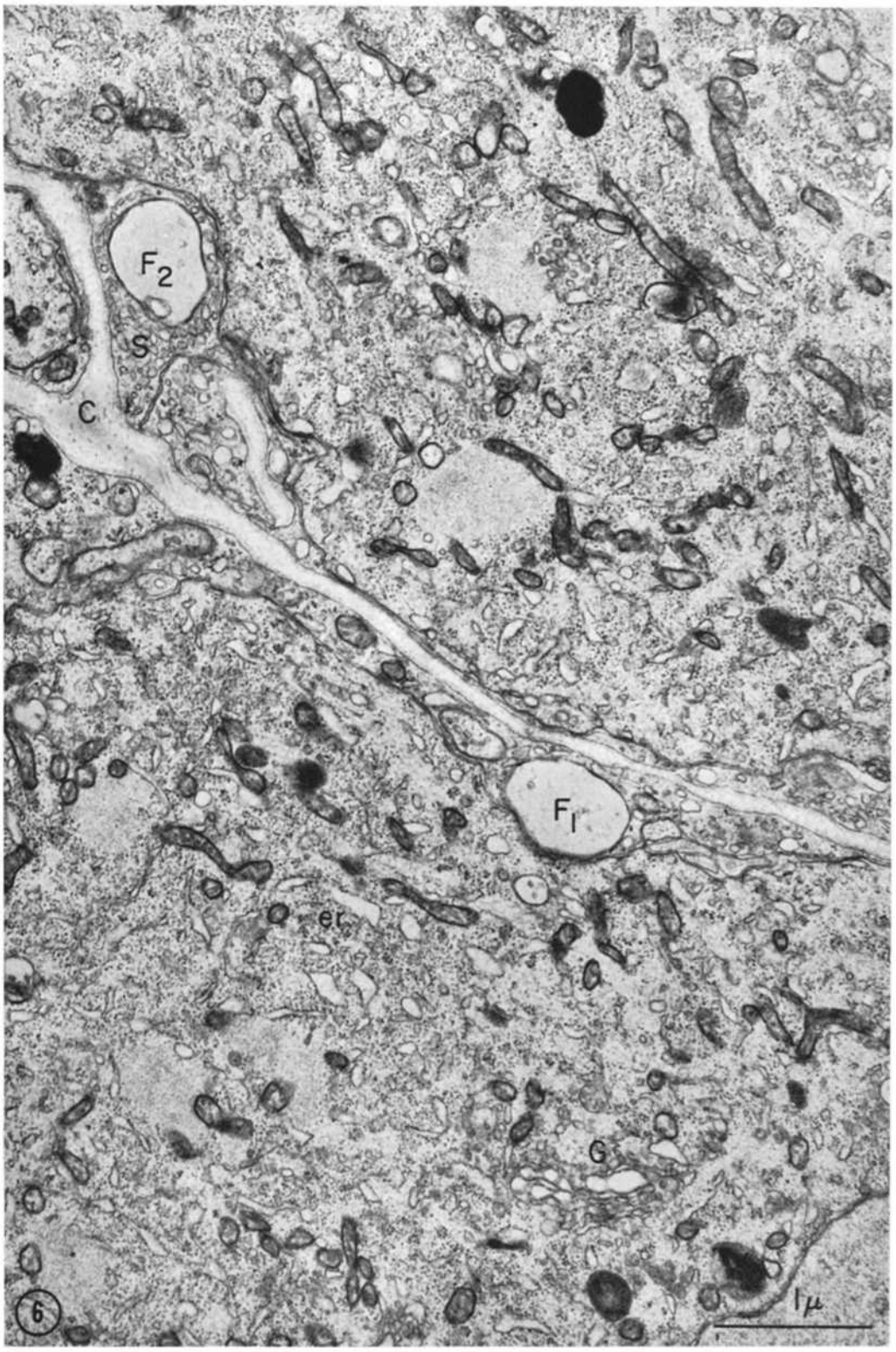
FIGURE 6 Superior cervical ganglion deprived of glucose for 3 hr. Complete loss of ability to transmit a nervous impulse. Section across 2 portions of ganglion cells showing the morphological integrity of the nerve cells whose only visible modification is simply a slight swelling of the cisternae of the endoplasmic reticulum (*er*). F_1 and F_2, 2 transverse sections of injured fibers, of which one (F_2) shows a surrounding satellite cell filled with vesicles (*S*). × 25,000.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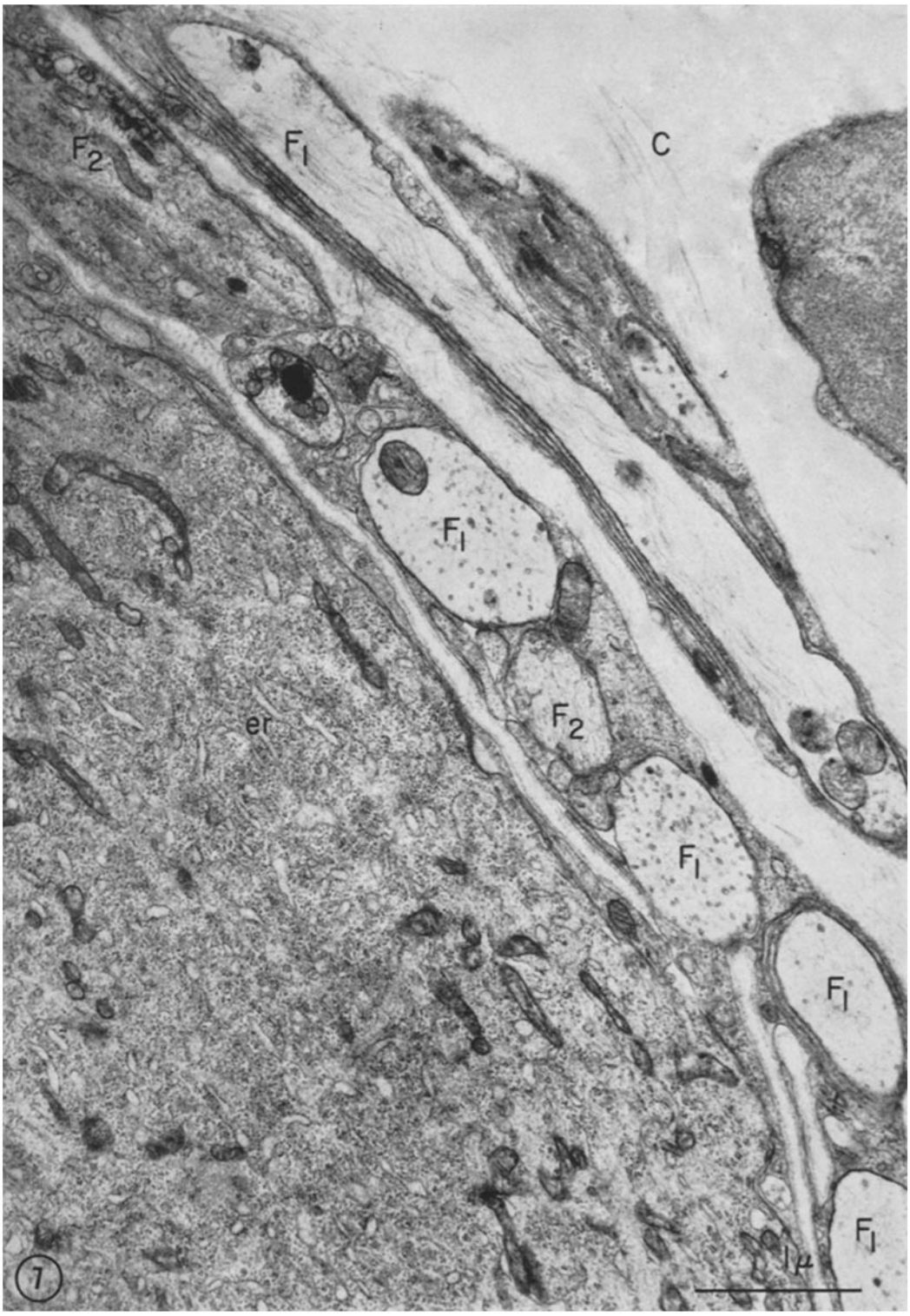

FIGURE 7 Superior cervical ganglion deprived of glucose for 3 hr. Complete loss of ability to transmit a nervous impulse. This electron micrograph shows the contrast between the ultrastructural integrity of the cytoplasm of the ganglionic cell and the considerable alterations of the nerve fibers (F_1). Some of the latter, however, are well preserved (F_2). × 24,000.

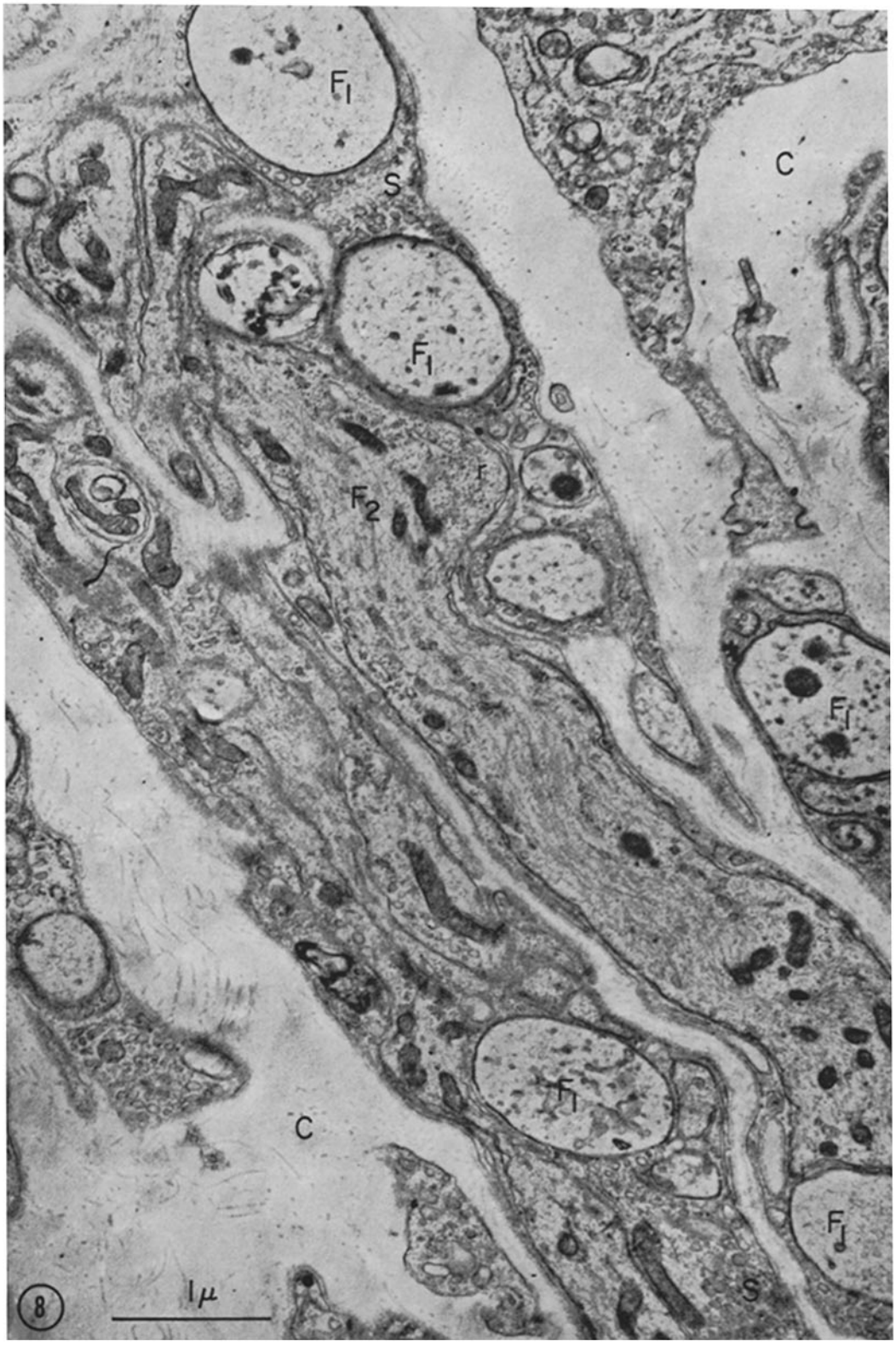

FIGURE 8 Superior cervical ganglion deprived of glucose for 3 hr. Complete loss of ability to transmit a nervous impulse. Note various degrees of injury to the various cell processes. Heavily damaged fibers (F_1) lie immediately alongside a well preserved fiber (F_2). The latter probably corresponds to a post-synaptic process, either axonic or dendritic, because it is rich in neurofilaments and contains some groups of ribosomes (r). × 24,000.

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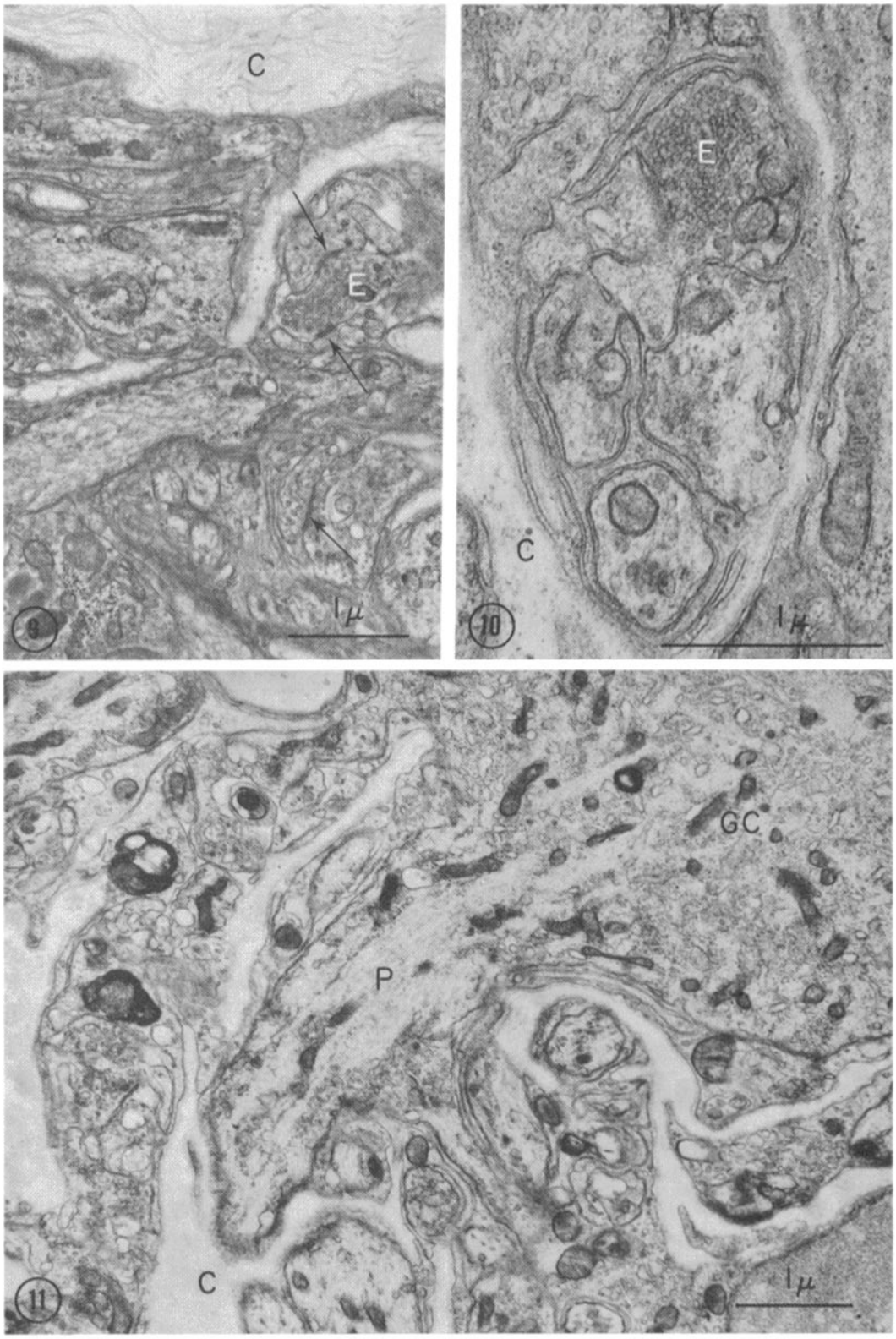
FIGURES 9 and 10 Control superior cervical ganglions, maintained in vitro with glucose. Regions of presynaptic nerve endings. The nerve endings (*E*) are identifiable because of the presence of numerous vesicles and synapses (arrows). Fig. 9, × 17,500. Fig. 10, × 36,000.

FIGURE 11 Superior cervical ganglion deprived of glucose for 3 hr. Complete loss of ability to transmit a nervous impulse. The cell process (*P*) is undamaged. × 18,000.


C
E
1 μ
9
E
C
1 μ
10
GC
P
C
1 μ
11

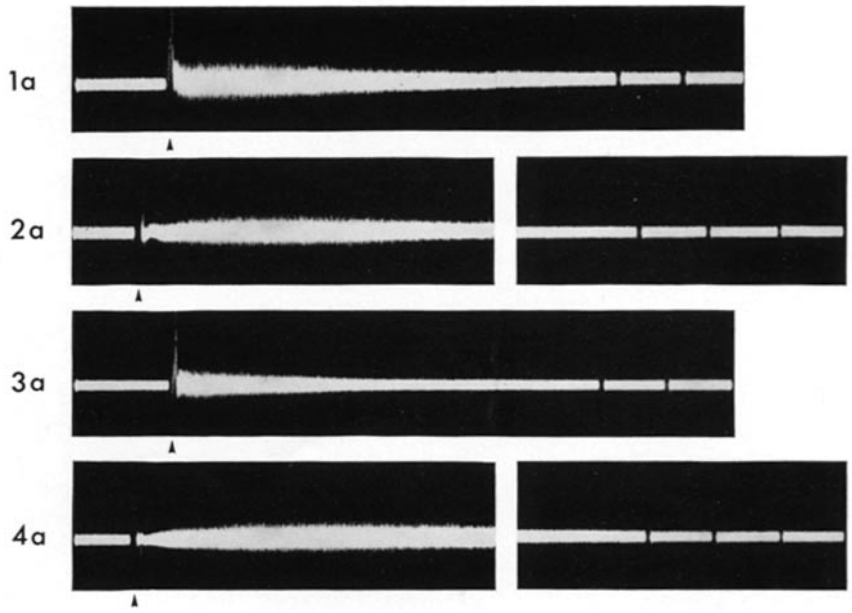


FIGURE 12 *a*

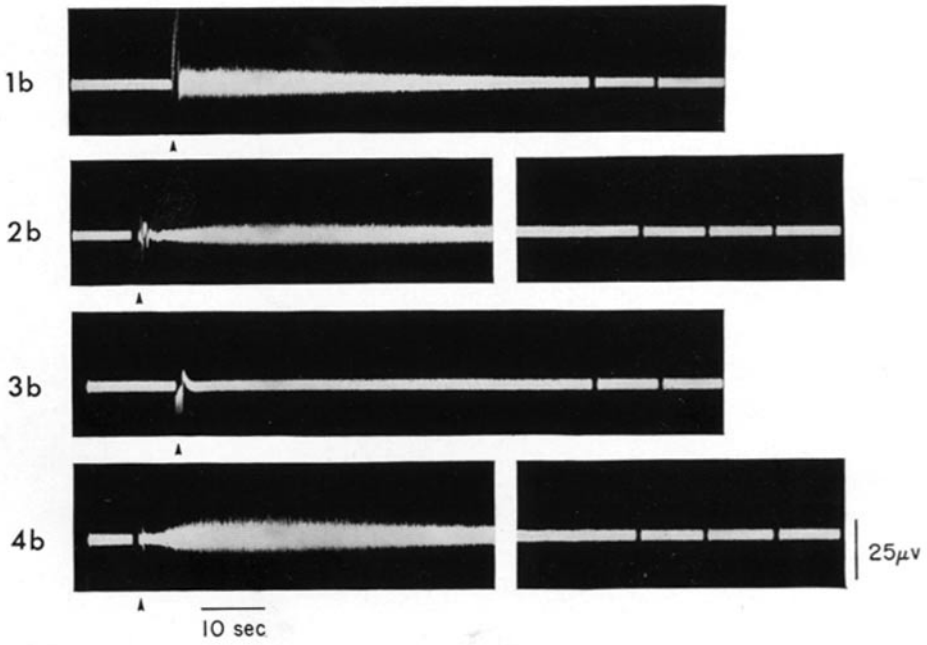


FIGURE 12 *b*

FIGURE 12 Recordings of the electrophysiological activity on the postganglionic nerve of a pair of ganglions (*a* and *b*) excised from the same rat. In *1* and *3*, activity of both ganglions has been elicited by stimulation; in *2* and *4*, acetylcholine 1 μg/ml has been added in the solution (containing physostigmine 5 μg/ml) bathing both ganglions. *1a* and *b* and *2a* and *b* show the activity 90 min after excision, both ganglions having been incubated in a Krebs' solution with glucose. *3a* and *4a* are the responses to the same stimulations after 3 more hr incubation in a standard solution with glucose, and *3b* and *4b* after 3-hr incubation, but in a solution without glucose. See text for explanation of the results.

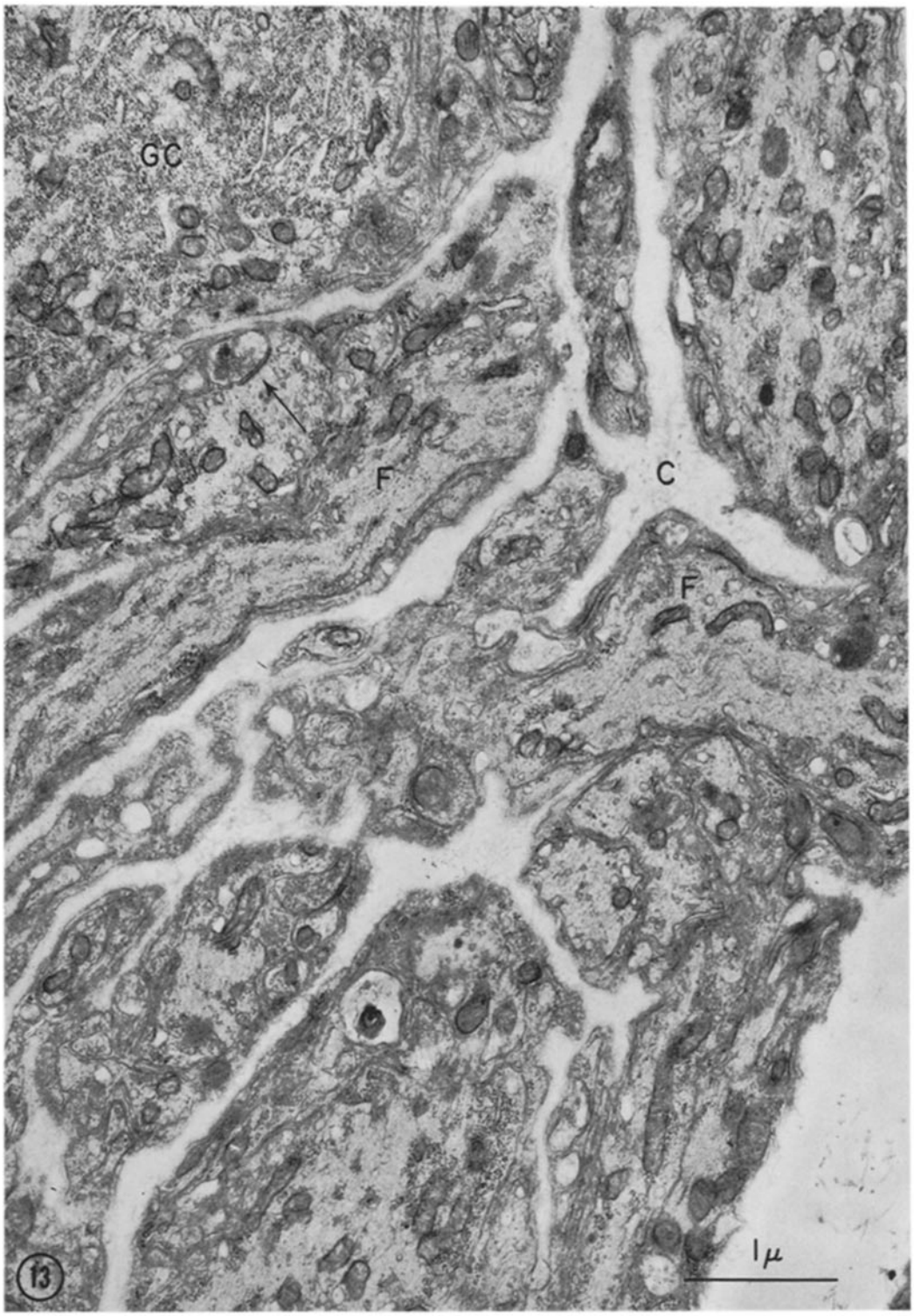
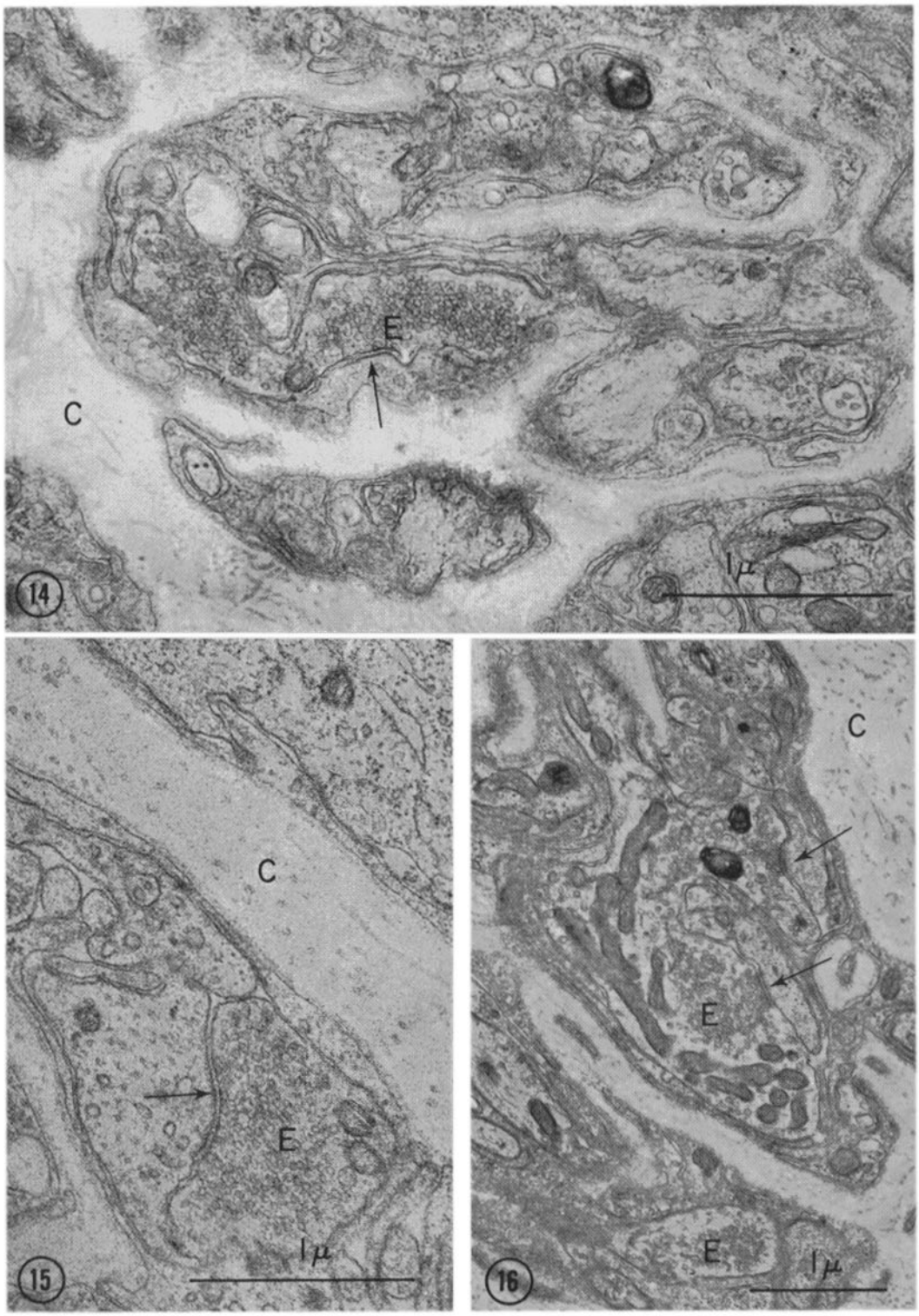


FIGURE 13 Superior cervical ganglion deprived of glucose for 18 hr, but at a temperature of 6°C. The ability to transmit a nervous impulse is intact. The ultrastructure of the nerve fibers is well preserved, and a terminal process with its synapse is visible (arrow). × 23,000.



FIGURES 14 to 16 Superior cervical ganglions deprived of glucose for 18 hr, but at a temperature of 6°C. The nerve endings (*E*) and their synapses (arrows) are easily seen. Fig. 14, × 35,000. Fig. 15, × 34,000. Fig. 16, × 21,000.

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