J. Physiol. (1971), **216**, *pp.* 87–98
With 8 plates and 2 text-figures
Printed in Great Britain

SYNAPTIC TRANSMISSION IN THE SUPERIOR CERVICAL GANGLION OF THE CAT AFTER REINNERVATION BY VAGUS FIBRES

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(*Received 2 November 1970*)

SUMMARY

1. A vagus-sympathetic anastomosis was performed in the cat by connecting end to end the cranial trunk of the vagus to the cranial end of the cervical sympathetic trunk, both severed under the ganglia.

2. Forty to sixty days after the anastomosis, the ocular signs of sympathetic paralysis (such as myosis and prolapse of the nictitating membrane) which had developed shortly after the operation, had completely disappeared, thus suggesting the recovery of synaptic transmission in the ganglion. In case of plain preganglionic denervation after the same period the ocular signs of cervical sympathetic paralysis were still present.

3. Contraction of the nictitating membrane could be induced by electrical stimulation of both the vagus preanastomotic and the sympathetic post-anastomotic–preganglionic trunks. Ganglionic blocking agents induced the blockade of the 'new' ganglionic synaptic function, while nicotine and pilocarpine provoked a marked contraction of the nictitating membrane.

4. Electron microscopy showed that the preganglionic regeneration of vagus fibers resulted in the formation of new synapses, mainly of axo-dendritic type, identical to normal ganglionic synapses. Moreover, after cutting the preanastomotic trunk of the vagus, these new ganglionic presynaptic profiles degenerated, thus proving their vagal origin.

5. During restoration of the synaptic contacts readjustment of dendritic tips occurred.

INTRODUCTION

It is known that shortly after severing nerve fibres, in the portion below the cut synaptic transmission as well as conductivity fail while synapses and axons undergo degeneration (Hunt & Nelson, 1965; Hamori, Lang & Simon, 1968; Sotelo, 1968). In the peripheral nervous system severed fibres

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are usually capable of an active regeneration which may eventually result in partial or almost complete functional recovery (Butson, 1950; Gibson, 1940; Guth, 1956*b*).

It has been shown experimentally that reinnervation and functional recovery can be obtained by regeneration of not only fibres deriving from the same nerve, but also from fibres originating in different nerves (Guth, 1956*a*; Hillarp, 1946; De Castro, 1951). A vago-sympathetic reinnervation, obtained by anastomosing the cranial trunk of the cervical sympathetic nerve, has been studied morphologically by De Castro (1934, 1951) and Hillarp (1946). They observed that the reinnervation coincides with the reappearance of the pericellular apparatus on the somatic surface of the ganglionic cells. No synaptic connexions could be directly demonstrated, the identification of these structures being beyond the possibilities of light microscopy. In a more recent physiological investigation Guth reported that after a similar vagus-sympathetic anastomosis some functions, such as the ear temperature regulation and the pupillary function at feeding, were restored, while ocular signs of sympathetic paralysis remained (Guth, 1956*b*).

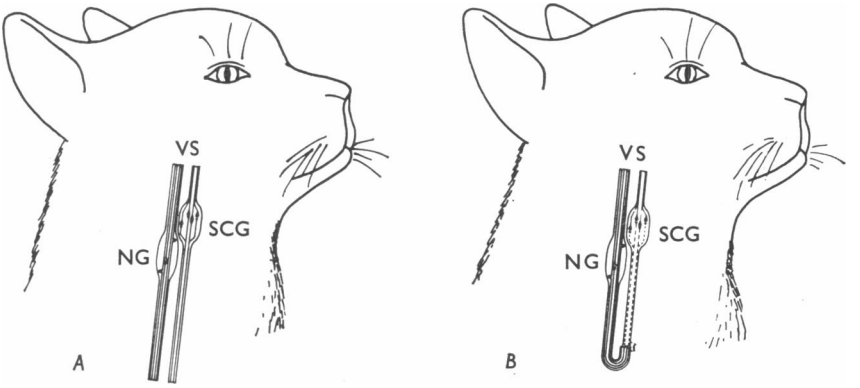
The mechanisms involved in the reinnervation which have obviously a great neurophysiological and neuropharmacological interest, are very little explored and explained, and particularly the morphological basis of the phenomenon has not been clarified. We decided to reinvestigate the problem by means of electron microscopic techniques, with the aim of studying the morphological events related to functional recovery, and correlating them with the results of a physiopharmacological study.

METHODS

Surgical procedure

In fourteen adult cats of either sex weighing 2500 g (average) a vagus sympathetic anastomosis was performed under Nembutal anaesthesia (35 mg/kg I.P.) and in aseptical conditions. Approx. 3 cm of the vagus sympathetic trunk were bilaterally exposed and the two nerves were carefully separated from each other. The complete separation of the trunks was checked by observing the pupillary response after separate electrical stimulation of the two nerves. On the right side ~ 2 cm of both nerves were removed ~ 2 cm caudally with respect to the ganglia. Caudal trunks, folded downward and spaced from each other, were fixed between the skin and the sternomastoid muscle in order to prevent a caudal regeneration. An end to end anastomosis of the cranial trunks of the vagus and sympathetic nerve was performed by means of a single stitch (Ethicon 6–0) passing only through the perineurium, as illustrated in Text-fig. 1. On the left side about 2 cm of the sympathetic trunk were removed and the caudal trunk, previously tied, was lifted and fixed on top of the muscle plane according to the procedure used on the right side. After the operation cats were kept within single cages. In order to check the pupillary diameter and the degree of relaxation of the nictitating membrane of both eyes they were periodically photographed under uniform and constant illumination.

Groups of two animals were sacrificed 24, 36 and 72 h after surgery. A group of six animals was submitted to physio-pharmacological investigation, as described below, 50–60 days after surgery and then sacrificed. In two additional cats the preanastomotic trunk of the vagus was excised caudally to the nodose ganglion 60 days after the first operation. These animals were sacrificed 36 hr after the second operation. All cats were sacrificed by intra-arterial perfusion with fixative solutions as described in detail below.



Text-fig. 1. Scheme of the vagus-sympathetic anastomosis (*B*). Both nerves were severed below the ganglia and the cranial trunk of the vagus was anastomosed end to end to the cranial trunk of the cervical sympathetic nerve. The scheme of the normal anatomical relationship is shown in *A*. V = vagus nerve; S = cervical sympathetic nerve; NG = nodose ganglion; SCG = superior cervical ganglion.

Stimulation and recording procedures. Superior cervical ganglion–nictitating membrane preparation

In chloralose anaesthesized cats (70 mg/kg I.V.) the trachea was cannulated and the right vagus sympathetic anastomosis carefully isolated from surrounding tissues. The preanastomotic trunk of the vagus and the post-anastomotic–preganglionic trunk of the sympathetic nerve were carefully exposed and prepared for electrical stimulation. In order to prevent the central propagation of stimuli, the vagus was tied below the nodose ganglion. Nervous trunks were then covered with warm (about 37° C) liquid paraffin. For recording purpose the tip of the nictitating membrane was connected to a frontal isotonic lever by means of a thin silk thread passing through a pulley. Weight on the nictitating membrane was of approx. 6 g and contractions were amplified about 12 times. Contractions were recorded by means of either a chymograph or a polygraph (Battaglia-Rangoni, Bologna, Italy). In the latter case the nictitating membrane was connected to an isometric transducer (Battaglia Rangoni TR-B/100).

Rectangular pulses, 0·3 msec in duration, from an electronic stimulator, were applied through platinum electrodes for 20 sec every 3–4 min. In each experiment the voltage of pulses was slightly greater than the minimum required for maximal retraction of the membrane. Stimuli of graded intensity were obtained by varying the frequency of the pulses, from 0·05 to 30 shocks/sec.

During the experiments the femoral arterial pressure was recorded. Drugs were injected intravenously through the previously cannulated femoral vein.

Tissue preparation for electron microscopy

All animals were sacrificed by intra-aortic perfusion (Ceccarelli & Pensa, 1968) with a solution of 2·5 % glutaraldehyde and 2 % formaldehyde in 0·12 M phosphate buffer, pH 7·4 (Karnovsky, 1965). Osmolarity of the fixative was 1330 ± 10 m-osmole. The fixative perfusion was preceded by a rapid washing with approximately 1 l. Ringer lactate solution at 18 ± 2° C containing heparin (1000 u./l.) and procaine hydrochloride (0·2 %); the total amount of the fixative solution perfused was about double the animals' weight. Both superior cervical ganglia were cut in small pieces and post-fixed in 1 % osmium tetroxide in 0·12 M phosphate buffer pH 7·4 for approx. 2 hr, at 4° C. Specimens were embedded in Epon 812. Sections obtained with a diamond knife in LKB Ultratome 2 and stained with uranyl acetate and lead citrate (Venable & Coggeshal, 1965) were examined with a Philips EM 300 electron microscope.

RESULTS

Structure of the normal ganglion

The fine structure of the cat superior cervical ganglion is well known (Elfvin, 1963*a*, *b*) and does not require a further detailed description. We shall briefly report on the relationship between presynaptic profiles and post-synaptic areas.

In the cat most of the synapses are known to be of axodendritic type, axosomatic synapses being very few (Elfvin, 1963*b*; Hamori *et al.* 1968). Dendritic processes, unmyelinated preterminal fibres and synaptic profiles are wrapped by cytoplasmic expansions of Schwann cells. Only at the level of the synaptic contact pre- and post-synaptic membranes appear very close, without interposition of cytoplasmic processes of Schwann cells (Pl. 1, fig. 1).

Synaptic profiles containing vesicles, 300–500 Å in diameter, intermingled with dense core vesicles, 800–1200 Å in diameter, consist of enlargements of unmyelinated preganglionic axons (Pl. 1, fig. 2; Pl. 2, fig. 3). These are arranged in series along the axon alternated with thinner portions containing only neurotubules and filaments (Elfvin, 1963*b*; Hamori *et al.* 1968).

In a very few dendrites of ganglionic cells we have observed large dilatations of varicosities loaded with mitochondria closely packed together and containing numerous small dense particles, probably glycogen. In agreement with the previous observations by Sotelo & Palay (1968) in the lateral vestibular nucleus of the rat, we could establish that, at least in some cases, such structures are terminal varicosities of dendrites.

Ultrastructure of the ganglion 24–72 hr after anastomosis (degenerative phase)

In agreement with previous findings (Hamori *et al.* 1968) we observed that the early and most striking events taking place after preganglionic deconnexion of the superior cervical ganglion are the rapid degeneration of synaptic terminal profiles and the activation of Schwann cells. Twenty-four hours after the cervical sympathetic trunk excision most of presynaptic enlargements show definite alterations and contain several dense multivesicular or myelin-like bodies, probably derived from the aggregation and fusion of synaptic vesicles (Pl. 2, fig. 3, 4). At this stage mitochondria appear often well preserved. Later the whole presynaptic terminal is transformed into a large cytolysome engulfed by the cytoplasm of the Schwann cells (Pl. 2, fig. 5). These cytolysomes are generally highly osmiophilic and sometimes contain residual organelles still recognizable. Some normal presynaptic unmyelinated preterminal axons may also be seen at this time point.

The degenerative process develops so quickly that 72 hr after axotomy no normal presynaptic profiles can be observed any more. The very few terminals still recognizable are in an advanced degenerative stage. Schwann cells exhibit within their cytoplasm dense or clear cytolysomes which sometimes contain remnants of presynaptic organelles, like mitochondria. Myelinated axons in various stages of degeneration appear contained within the cytoplasm of Schwann cells.

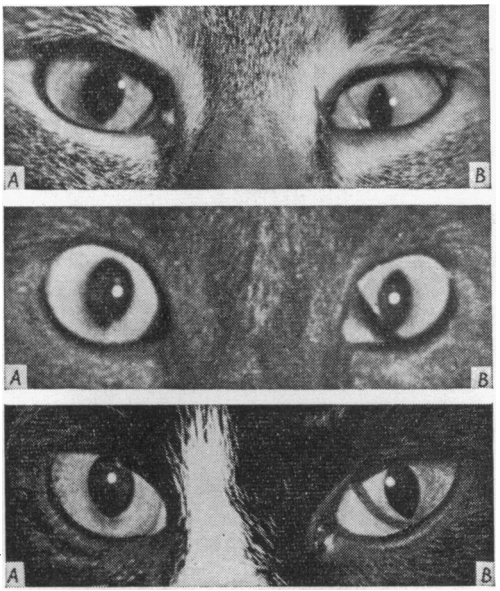
A much larger number of degenerating myelinated axons are evident at later stages of the process, which are not described in detail in this study.

Physiopharmacological and ultrastructural observations 50–60 days after anastomosis

Seven out of eight animals displayed a clear anisocoria 50 days after the intervention. The diameter of right pupil (reinnervated side) was normal and this functional recovery was accompanied by a complete reduction of the previously prolapsed right nictitating membrane. By contrast, on the left side (sympathectomy) all the ocular signs of the cervical sympathetic paralysis, such as myosis and prolapse of the nictitating membrane, were still evident (Text-fig. 2).

Recording of contractions of the nictitating membrane elicited by electrical stimulation of the preanostomotic trunk of the vagus, as well as the post-anastomotic–preganglionic trunk of the sympathetic nerve, was made in five animals showing clear signs of ocular functional recovery. Stimulation always resulted in a marked contraction of the membrane (Pl. 3, fig. 6). Moreover, the stimulation at various frequencies of both

nervous trunks revealed a slight degree of supersensitivity: contraction of the membrane could be obtained even at a frequency of 0·1 stimuli/sec for the vagus preanastomotic trunk and of 0·05 stimuli/sec for the sympathetic post-anastomotic trunk, as shown in Pl. 3, fig. 6. These responses elicited by electrical stimulation were almost completely abolished by the ganglionic blocking agent hexamethonium, at doses larger than 2·5 mg/kg (Pl. 3, fig. 7).



Text-fig. 2. Pupils and nictitating membranes of cats 50 days after surgery. The recovery of the pupillary tone and the reduction of the prolapse of the nictitating membrane is clearly evident on the right side of the animals (*A*) where the vagus–sympathetic anastomoses were performed. On the side of the plain sympathectomy (*B*) the ocular signs of cervical sympathetic paralysis are still present.

The response of the nictitating membrane to stimulation of the preanastomotic trunk of the vagus strongly suggests that functional connexion has been restored by reinnervation of the superior cervical ganglion by regenerated fibres originating in the central trunk of the vagus.

Since it is known that nicotinic and muscarinic receptors, located at the post-synaptic area, are involved in ganglionic synaptic transmission (Takeshige, Pappone, De Groat & Volle, 1963; Trendelenburg, 1967), the sensitivity of reinnervated ganglia to nicotine as well as to muscarinic agents, such as pilocarpine, was investigated. Nicotine at doses between 200 and 400 μg/kg, I.V., evoked a clear contraction of the nictitating membrane

(Pl. 3, fig. 7). As shown in Pl. 3, fig. 8 also pilocarpine at doses as low as 50 μg/kg induced contraction of the nictitating membrane; such an effect was abolished by atropine at a dose (500 μg/kg) which was ineffective on the response to the electrical stimulation of the preanastomotic trunk of the vagus.

The morphological study revealed that the structure of the superior cervical ganglion of the reinnervated side was comparable with the structure of the normal ganglion. Synapses were almost exclusively of the axo-dendritic type (Pl. 4, fig. 9; Pl. 5, fig. 11; Pl. 6, fig. 13). Presynaptic profiles were very numerous and usually slightly smaller than in the normal ganglion; they appeared surrounded by expansions of the cytoplasm of Schwann cells often containing large clusters of dense particles (probably glycogen) (Pl. 4, fig. 10). At this stage of regeneration large amounts of glycogen are usually present also in numerous nerve endings and neurones, whose fine structure is practically normal (Pl. 5, fig. 12). The large dendritic varicosities filled by mitochondria and dense particles were encountered much more frequently than in the normal ganglion (Pl. 4, fig. 9; Pl. 6, fig. 13).

On the left side (plain sympathectomy) a complete lack of both myelinated axons and synaptic profiles was observed (Pl. 7, fig. 14). In neurons polysomes and ER membranes were reduced in small masses dispersed throughout the cell (Pl. 7, fig. 15). Numerous bundles of microtubules and neurofilaments were arranged around these masses and scattered in the cytoplasm.

Ultrastructure of reinnervated ganglion in degenerative state 36 hr after preanastomotic vagotomy. In order to check whether the functional and morphological recovery observed in the superior cervical ganglion after vagus sympathetic anastomosis was dependent only on reinnervation of the ganglion by fibres originating in the vagus, a preanastomotic vagotomy was performed in two cats showing clear pupillary signs of functional recovery. Animals were sacrificed 36 hr after the second intervention.

Almost all regenerated unmyelinated synaptic profiles degenerated into cytolysomes contained in the cytoplasm of the Schwann cells. Only a few residual preterminal profiles in an advanced involutive stage could be seen, containing the osmiophilic aggregations of vesicles analogous with those previously described in the ganglia 24–72 hr from ganglionic sympathicectomy (Pl. 8, fig. 16). Large processes with recognizable mitochondria and containing many dense and myelin-like bodies were seen. They might be tentatively identified as degenerating tips of dendrites (Pl. 8, fig. 17).

DISCUSSION

Previous studies demonstrated that a reinnervation of the denervated superior cervical ganglion by vagus fibres can be established (Hillarp, 1946; De Castro, 1934; Guth, 1956*a*, *b*). However, neither actual synaptic connection was demonstrated in the ganglion nor adequate physiological and pharmacological studies were performed.

The results obtained in this study clearly demonstrate that the regenerating fibres establish new synaptic connexions. The contraction of the nictitating membrane evoked by stimulation of the preanastomotic trunk of the vagus as well as the rapid degeneration of the new synaptic profiles after section of the vagus between the nodose ganglion and the anastomosis led us to conclude that reinnervation of the ganglion has been brought about by vagal fibres and to exclude the possibility of reinnervation by fibres originating in the caudal ending of the sympathetic trunk.

It has been reported that cholinergic fibres can reinnervate directly the nictitating membrane (Vera, Vial & Luco, 1957; Vera & Luco, 1967). In our experimental conditions, however, there is clear evidence that efferent vagal fibres do not regenerate straight through the ganglion to reach the nictitating membrane, since the response to electrical stimulation was unaffected by high doses of atropine whereas it was abolished by hexamethonium.

Both the pupillary and the nictitating membrane tonus were restored by regenerated fibres originating in the vagus. It seems likely that such a puzzling result could also imply the establishing of complex central adaptations.

In contrast with the results by Guth (1956*b*) who reported the persistence of signs of ocular paralysis 8 weeks after the same vagus sympathetic anastomosis, we have observed a complete recovery. We have not a definite explanation to this discrepancy; however, it could simply depend on different degrees of success in anastomosis.

The new synapses are mainly of the axo-dendritic type. Thus, their site as well as their ultrastructural features reproduce the synaptic pattern present in the normal superior cervical ganglion. Moreover, our physiological observations and pharmacological results suggest that new synaptic and post-synaptic areas involved in transmission have properties similar to those displayed by normal synapses.

Our data show that regenerated preganglionic axons establish connexions almost exclusively with the dendritic processes of ganglionic neurones.

It seems therefore that, on the surface membrane of ganglionic neurones, areas exist which are specialized for establishing connexions with axon terminals. Such areas are mainly localized in the membrane of dendritic

expansions. In this respect, the frequent occurring in the reinnervated ganglion of dendritic expansions loaded with closely packed mitochondria and glycogen seems also of importance, since we were able to identify them as terminal dendritic expansions. Sotelo & Palay (1968) who first described such structures in another area of the C.N.S. put forward the hypothesis that they may be growing or expanding tips of growing or regenerating nerve cells. It seems therefore that these structures might represent a dynamic stage of dendrites, involved in the process of regenerating synaptic connexions. The absence of such dendritic varicosities in the cronically denervated ganglion indirectly supports this hypothesis.

Such an interpretation may be strengthened by the finding that, in reinnervating ganglion the section of the preanastomotic trunk of the vagus not only brings about a rapid degeneration of presynaptic terminals but also results in the appearance of structures such as that shown in Pl. 8, fig. 17 which are difficult to identify but are reminiscent of the terminal varicosities of dendrites, in an advanced stage of degeneration. If confirmed by future investigations this would be a definite example of transneuronal degeneration.

The skilful technical assistance of Messrs Franco Crippa, Numa Jezzi and Paolo Tinelli is gratefully acknowledged.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Superior cervical ganglion of a normal cat. The Figure shows an unmyelinated axon profile containing numerous synaptic vesicles (V), large dense core vesicles (LDV), glycogen granules (G), and mitochondria. The synaptic contact (arrows) occurs in a zone of a dendrite rich in microtubules (mt.) × 41,500.

Fig. 2. Superior cervical ganglion of a normal cat. The field is occupied by a large preterminal axon profile filled up with synaptic vesicles (V). No synaptic contact is visible in this section. × 45,000.

PLATE 2

Fig. 3. Superior cervical ganglion of a cat 24 hr after vagus–sympathetic anastomosis. A presynaptic profile is shown containing, besides synaptic vesicles (V) and large core vesicles (LDV), two dense osmiophilic bodies (B) which are generally considered an early sign of peripheral synaptic degeneration × 41,000.

Fig. 4. Superior cervical ganglion of a cat 36 hr after vagus–sympathetic anastomosis. Numerous myelin-like bodies (MB) are contained in an enlargement of a presynaptic axon profile in which normal mitochondria, a few synaptic vesicles (arrows) are still recognizable. × 33,000.

Fig. 5. Superior cervical ganglion of a cat 36 hr after vagus–sympathetic anastomosis. The cytoplasm of a Schwann cell (Sc) is engorged with a large cytolysome containing amorphous dense material and dense membrane-bound structures (arrows). × 38,500.

PLATE 3

Fig. 6. Cat 50 days after vagus–sympathetic anastomosis. Upper record: blood pressure (B.P.). Lower record: nictitating membrane (Nict.M.). Contraction of the membrane was elicited every 4 min by graduated electrical stimulation of either

the sympathetic post-anastomotic (Post-A.S.T.) or the vagus preanastomotic (Pre-A.V.T.) trunks. At Ad. the contraction of the nictitating membrane was elicited by an I.V. injection of epinephrine.

Fig. 7. Cat 50 days after vagus–sympathetic anastomosis. From top to bottom: respiration (R), heart rate (HR), blood pressure (B.P.) and contractions of the nictitating membrane (Nict.M.). Contraction of the nictitating membrane was elicited every 3 min by graduated electrical stimulation of the vagus preanastomotic trunk. Contraction of the nictitating membrane was also elicited by I.V. injection of adequate doses of nicotine (Nic). Responses to both electrical stimulation and nicotine were blocked by a previous I.V. injection of hexamethonium bitartrate (Hex).

Fig. 8. Cat 50 days after vagus–sympathetic anastomosis. Upper record: blood pressure (B.P.); lower record: nictitating membranes (Nict. M.). Contraction of the nictitating membrane was elicited by either electrical stimulation (repeated every 4 min) of the vagus preanastomotic trunk or by I.V. injection of pilocarpine (P). The latter response was abolished by a previous I.V. injection of atropine (Atr.)

PLATE 4

Fig. 9. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis. A dendritic expansion (D) filled with many mitochondria and glycogen particles is surrounded by two presynaptic profiles, containing the usual types of vesicles. Synaptic contacts with the dendrite expansion are indicated by arrows. A third presynaptic profile is evident in the lower left corner. × 25,000.

Fig. 10. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis. The micrograph shows a presynaptic enlargement of normal appearance, enwrapped by the cytoplasm of a Schwann cell (Sc) particularly rich in glycogen granules (G). C: collagen. × 24,500.

PLATE 5

Fig. 11. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis. A large preterminal axon is shown. Numerous synaptic vesicles of the usual types are scattered throughout the presynaptic area. × 35,000.

Fig. 12. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis. A synaptic enlargement of a regenerated axon particularly rich in glycogen granules (G) is shown in the centre of the picture. On the bottom a small portion of the cytoplasm of a neurone (N) is visible; its fine structure appears normal. × 34,500.

PLATE 6

Fig. 13. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis. A large dendritic expansion (D) filled with mitochondria is present: such finding is particularly frequent in reinnervated ganglia. On the bottom of the Figure a fibroblast (F) and numerous collagen fibrils (C) are shown. × 27,000.

PLATE 7

Fig. 14. Superior cervical ganglion of a cat 50 days after cervical sympathectomy. Neither myelinated fibres nor unmyelinated presynaptic axon profiles and synapses are visible in this low magnification picture. × 9500.

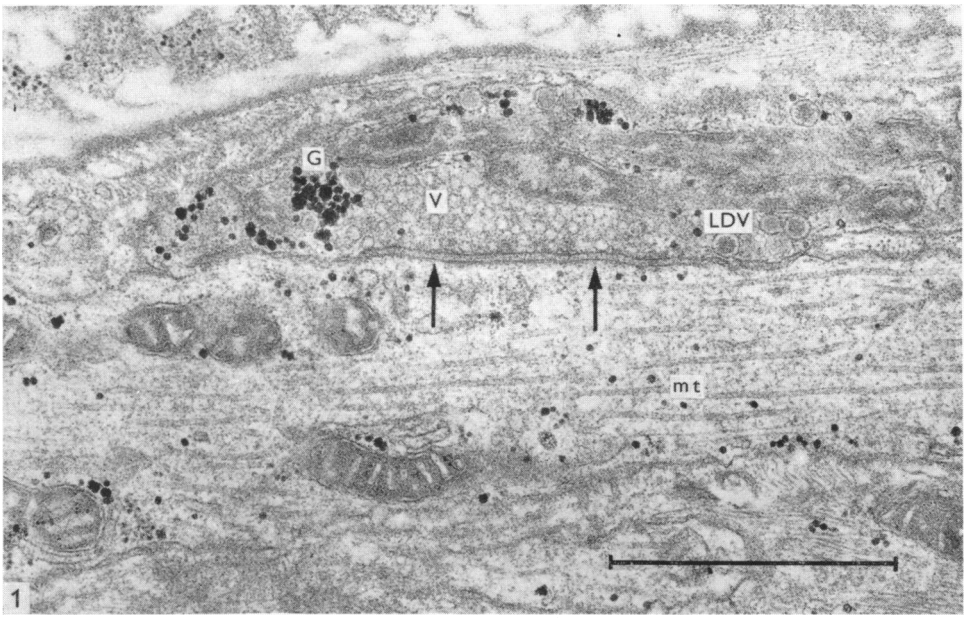
Fig. 15. Superior cervical ganglion of a cat 50 days after cervical sympathectomy. In the cytoplasm of a ganglionic neurone only a few elements of the rough surfaced endoplasmic reticulum are evident. They are arranged in clusters (Nissl bodies, Nb)

together with many free polysomes, surrounded by bundles of microtubules (mt) and neurofilaments (nf). At the bottom right the nucleus, with nuclear pores (P), is evident. × 13,000.

PLATE 8

Fig. 16. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis and 36 hr after preanastomotic vagotomy. A regenerated presynaptic profile exhibiting clear signs of degeneration is evident. Numerous electron-dense bodies (B) are present containing amorphous material and structures reminiscent of large dense core vesicles. A large dendritic expansion full of mitochondria and glycogen particles is evident at the bottom right. × 30,000.

Fig. 17. Superior cervical ganglion of a cat 50 days after vagus–sympathetic anastomosis and 36 hr after preanastomotic vagotomy. A large process, that can be tentatively identified as a degenerating tip of a dendrite, is shown in the micrograph. It contains mitochondria and many dense (B) and myelin-like (arrows) bodies. × 35,000.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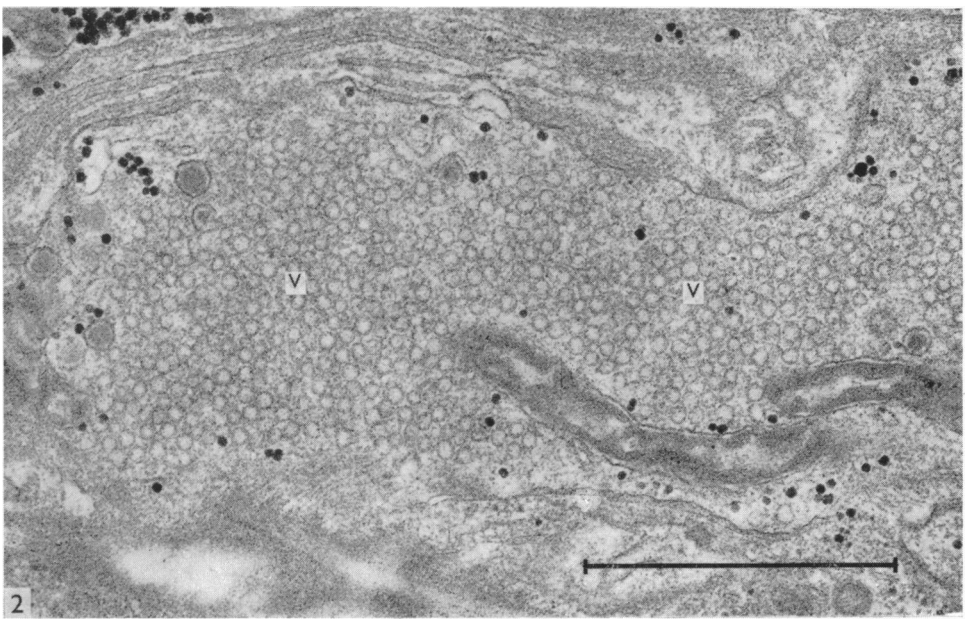
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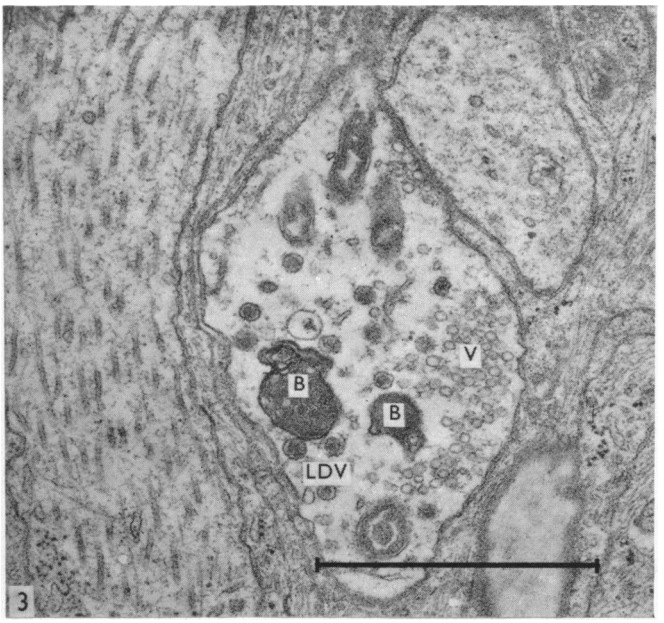
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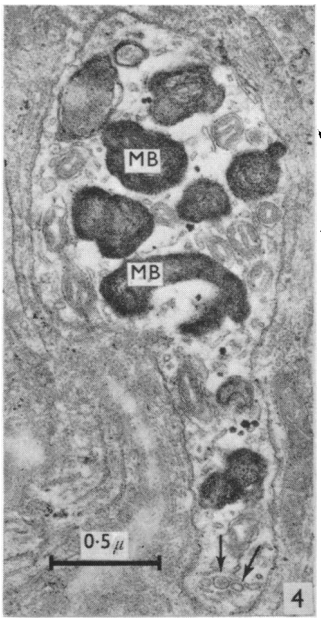
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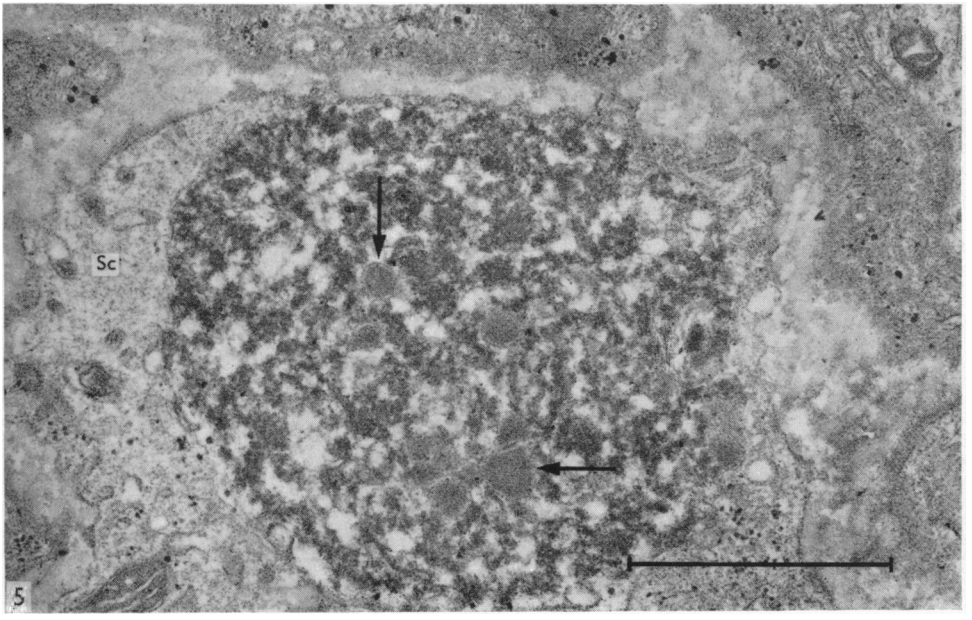
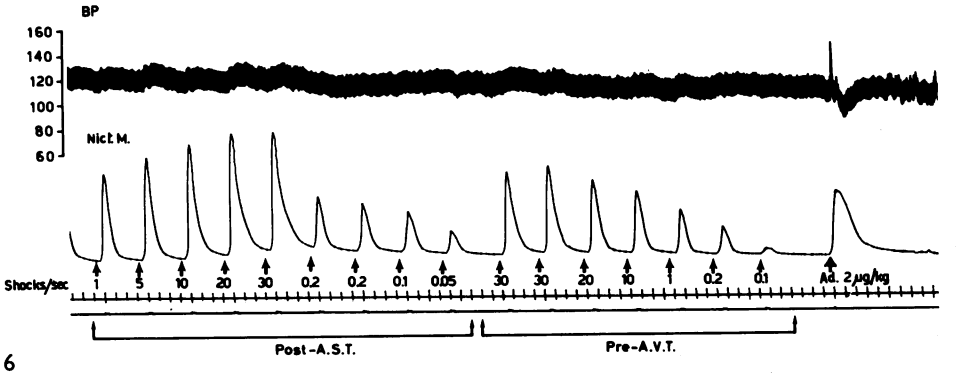
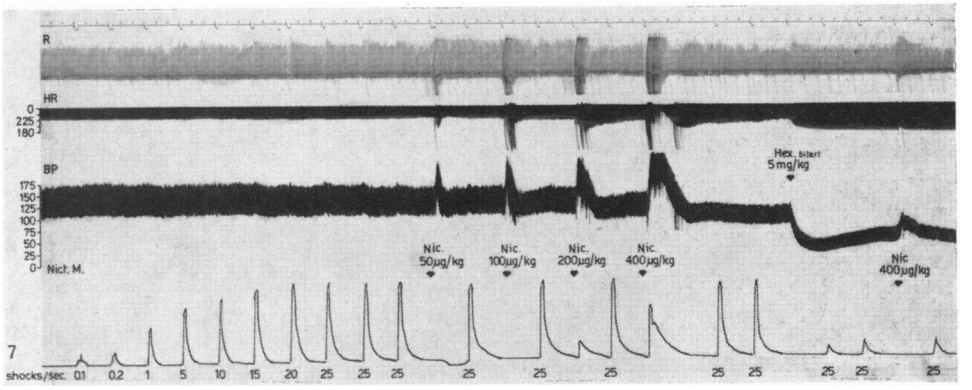
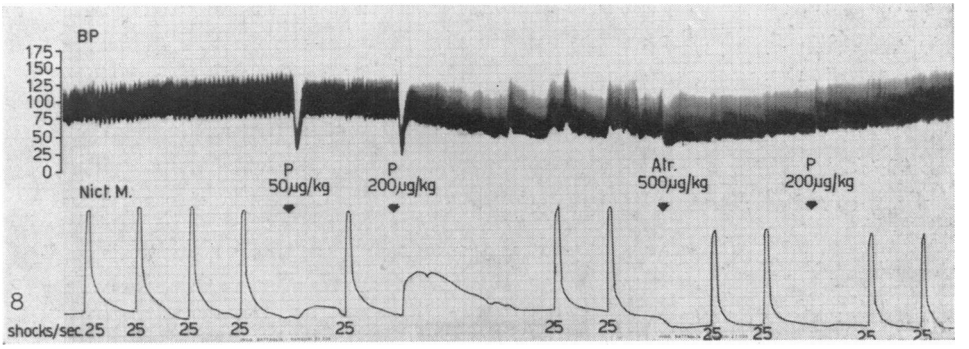

Sc
5


BP
160
140
120
100
80
60
Nict M.
Shocks/sec
1
5
10
20
30
0.2
0.2
0.1
0.05
30
30
20
10
1
0.2
0.1
Ad. 2 µg/kg
Post-A.S.T.
Pre-A.V.T.

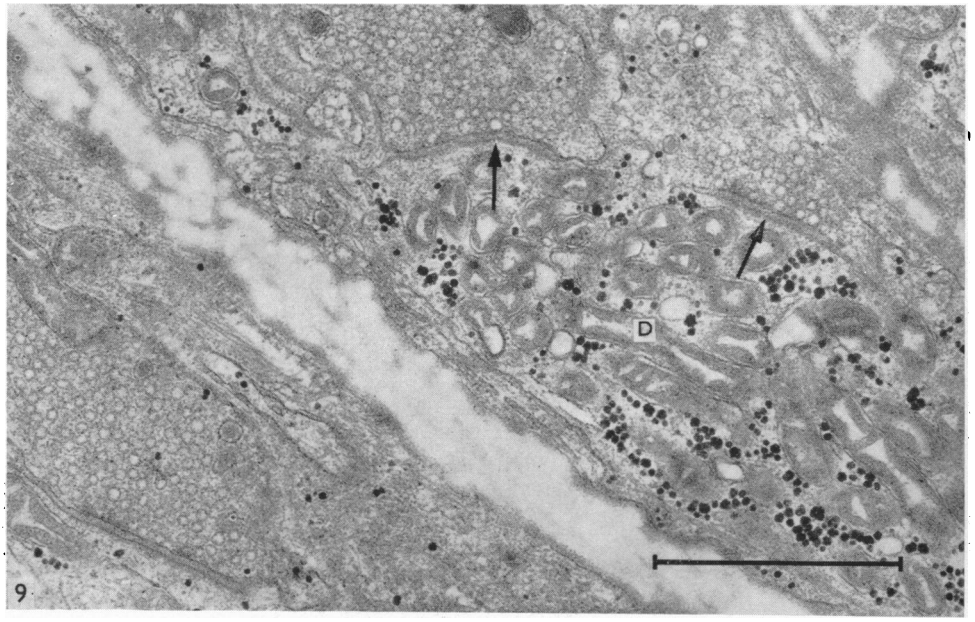
6

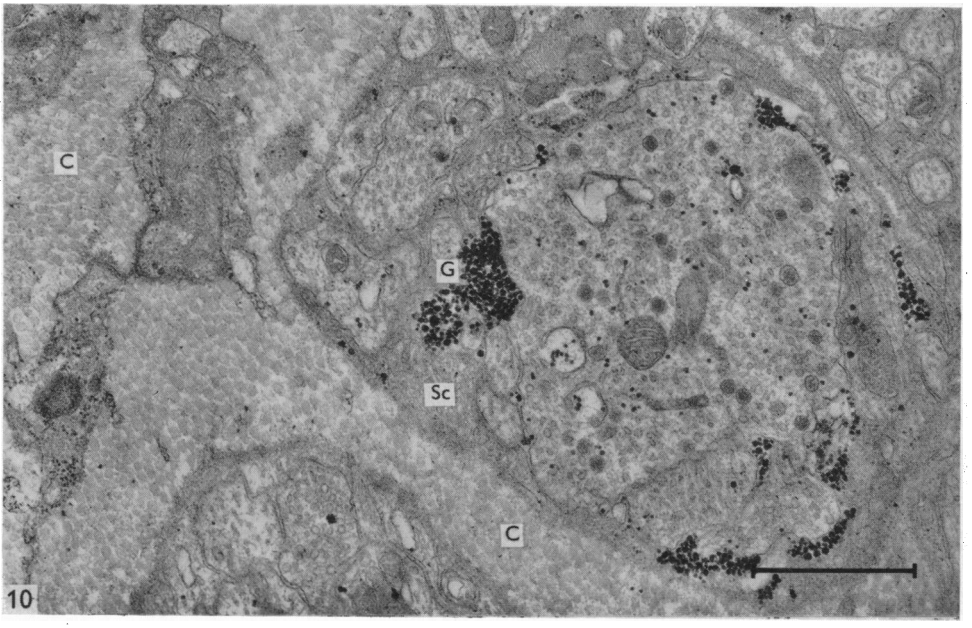

R
HR
0
225
180
BP
175
150
125
100
75
50
25
0
Nict. M.
Nic. 50µg/kg
Nic. 100µg/kg
Nic. 200µg/kg
Nic. 400µg/kg
Hex. bitart 5 mg/kg
Nic 400µg/kg
shocks/sec. 0.1 0.2 1 5 10 15 20 25 25 25 25 25 25 25 25 25 25

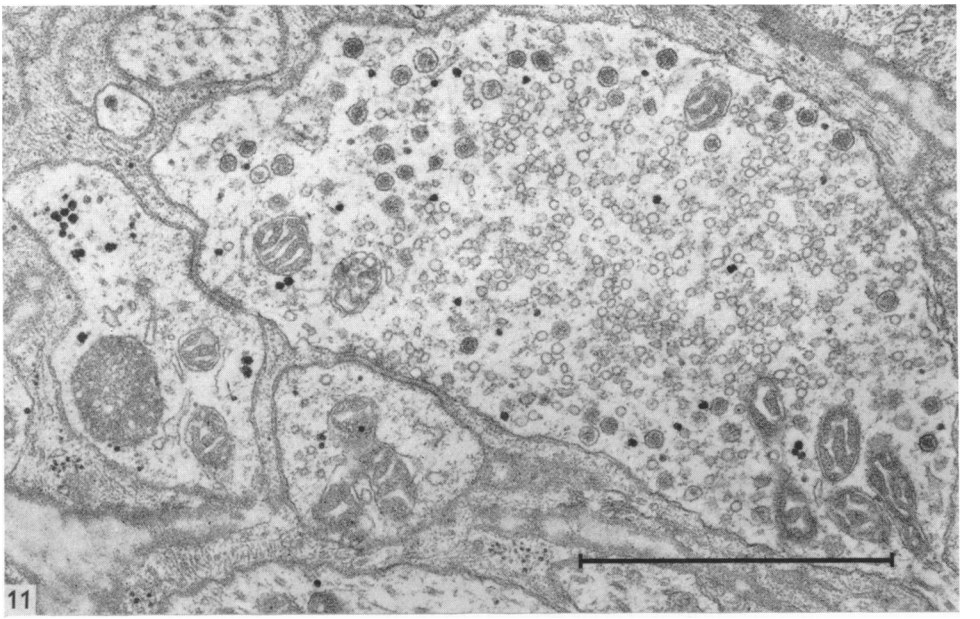
7


BP
175
150
125
100
75
50
25
0
Nict. M.
P 50µg/kg
P 200µg/kg
Atr. 500µg/kg
P 200µg/kg
shocks/sec. 25 25 25 25 25 25 25 25 25 25 25

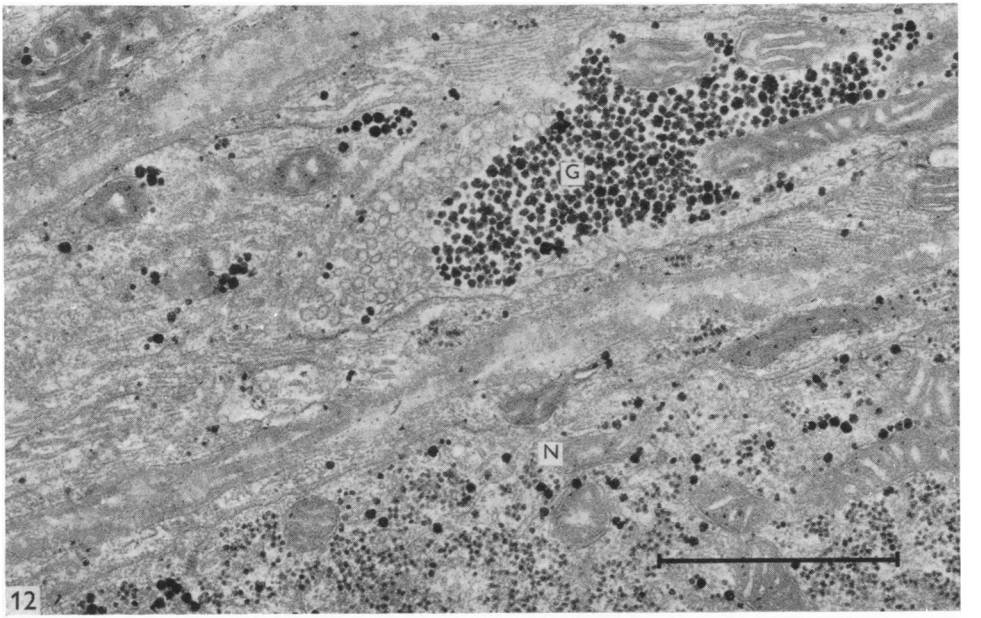
8


D
9

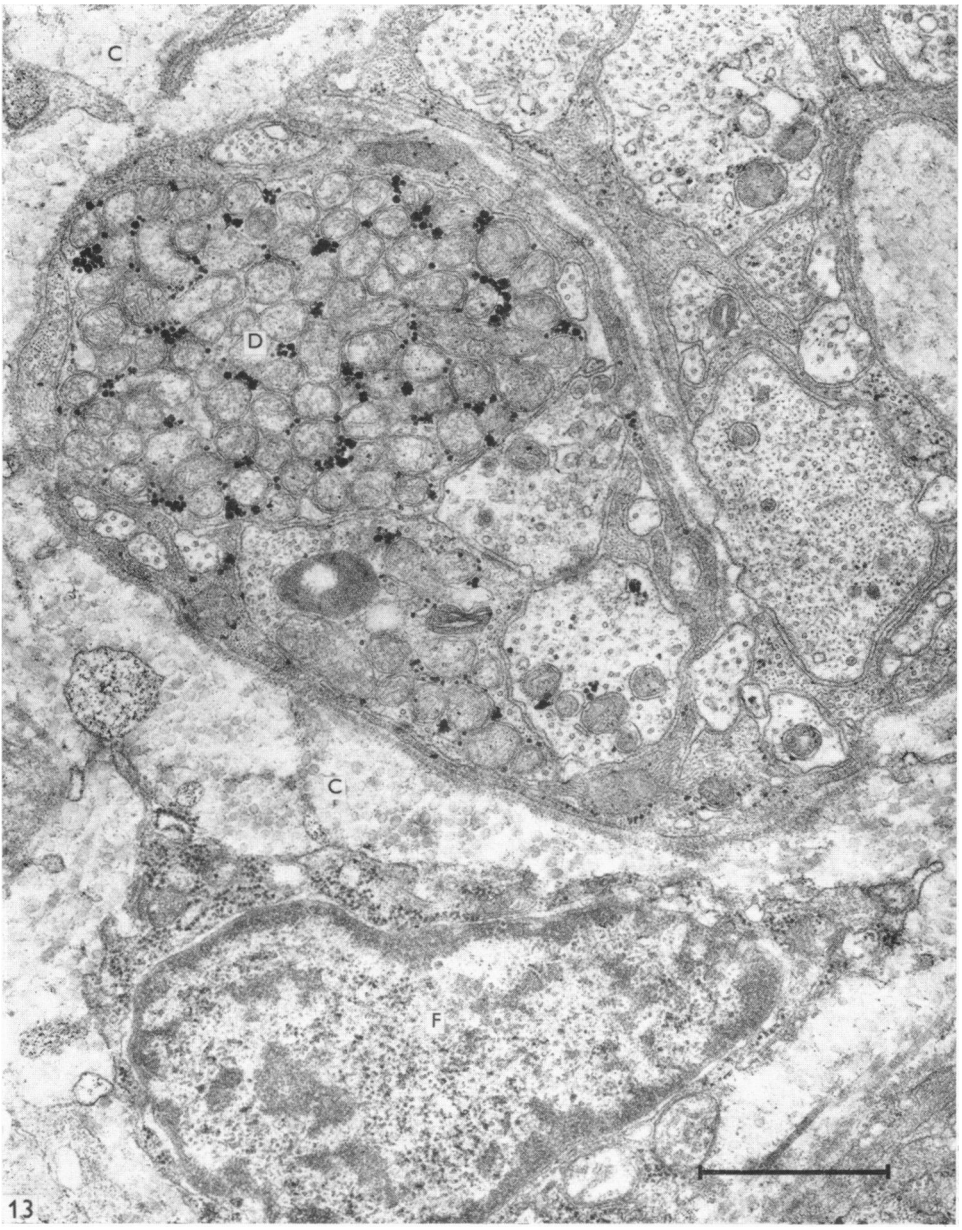

C
G
Sc
C
10

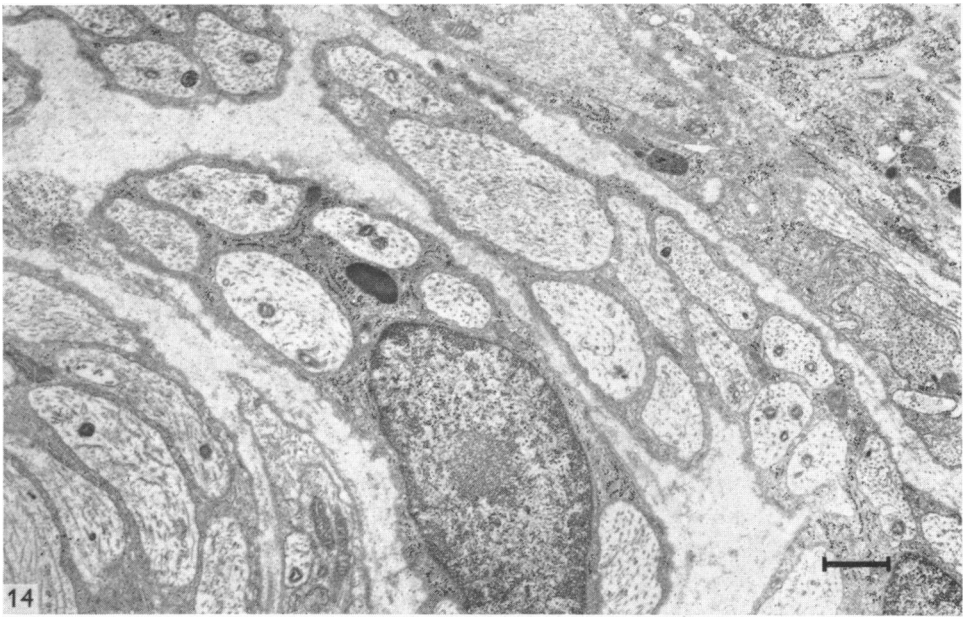


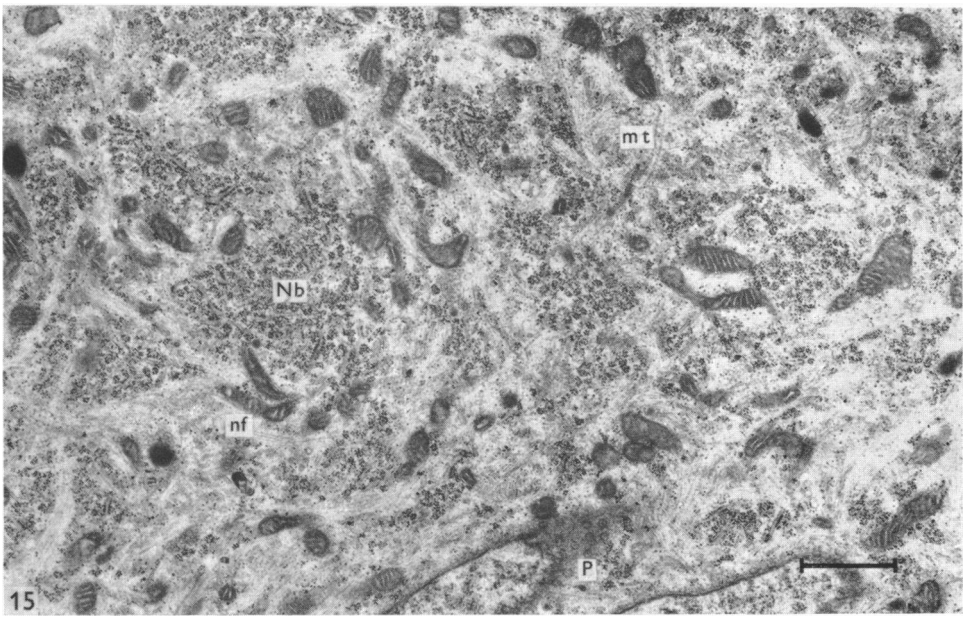
11

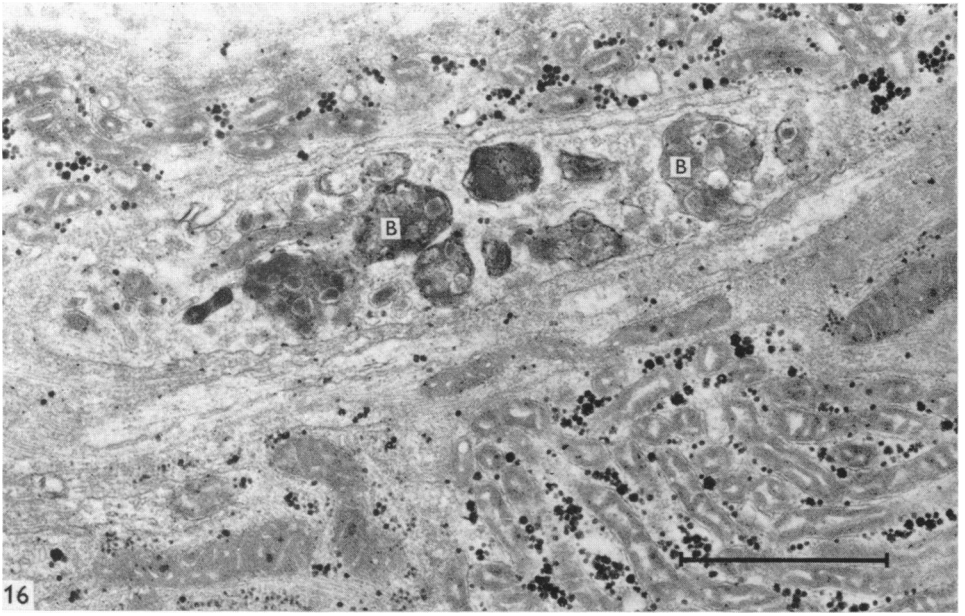


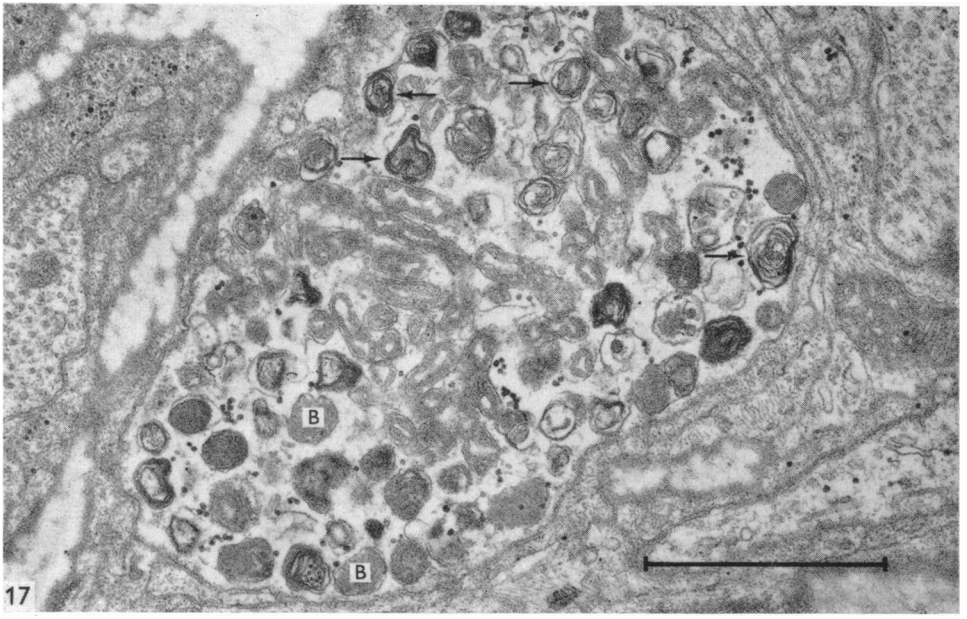
12


C
D
C
F
13


14


mt
Nb
nf
P
15


B
B
16


B
B
17