J. Physiol. (1975), **245**, *pp.* 91–135
With 7 plates and 14 text-figures
Printed in Great Britain

DETACHMENT OF STRUCTURALLY INTACT NERVE ENDINGS FROM CHROMATOLYTIC NEURONES OF RAT SUPERIOR CERVICAL GANGLION DURING THE DEPRESSION OF SYNAPTIC TRANSMISSION INDUCED BY POST-GANGLIONIC AXOTOMY

BY MARGARET R. MATTHEWS AND VICTORIA H. NELSON*

From the Department of Human Anatomy and University Laboratory of Physiology, Oxford

(*Received 8 April 1974*)

SUMMARY

1. Electrophysiological studies showed that injury of post-ganglionic nerve fibres leads to severe and prolonged depression of synaptic transmission through the rat superior cervical ganglion, beginning within 24 h. This is in line with the results of previous studies in other species and upon other neurones.

2. Electron microscopy after post-ganglionic axotomy revealed nerve endings of presynaptic type with all the specialized membrane-related features of a synaptic zone, but which were not apposed to any post-synaptic nervous element. These unusual profiles were interpreted as detached presynaptic nerve endings. In normal and control ganglia, such profiles formed at most 0·5 % of all vesicle-containing profiles of presynaptic type; in ganglia with all major post-ganglionic branches cut the proportion rose to approximately 7 %, between 3 and 7 d post-operatively. Over this period, the mean incidence of chromatolytic neurones was 74·6 %.

3. Concomitantly, the incidence of synapses within the ganglion fell by about 75 %, reaching its lowest levels between 3 and 7 d post-operatively. There was strikingly little evidence of persistence of post-synaptic membrane specializations ('membrane thickenings') following detachment of synapses.

4. At longer survival intervals the incidence of synapses gradually increased, and that of detached nerve endings gradually decreased; recovery was well advanced by 42 d.

5. The fall in the incidence of synapses was closely paralleled by a fall in the incidence of desmosome-like attachments in the ganglion; the

* M.R.C. Scholar.

incidence of such attachments was found to be correlated to a significant degree with that of synapses.

6. It is concluded that most or all of the synapses upon sympathetic neurones become physically dissociated during the chromatolytic reaction of these neurones to axotomy. The failure to persist of ultrastructurally specialized post-synaptic sites, and the loss of desmosomes (particularly marked for those involving purely post-ganglionic nervous elements) suggest that the post-ganglionic neurone is losing all its specializations for attachment.

7. Some evidence suggests that the satellite cells may effect the final separation between pre- and post-synaptic structures.

INTRODUCTION

The response of a nerve cell to injury of its axon, the 'retrograde' or 'axon' reaction, involves complex changes in its electrophysiological and biochemical properties, and in its morphology. In neurones whose axons enter the peripheral nervous system the reaction may take the form of chromatolysis. Various aspects of this reaction have recently been reviewed by Lieberman (1971). One of the accompaniments of chromatolysis in lower motor neurones is a reduced responsiveness to synaptic activation. This was described for the ventral horn motor neurone of the mammalian spinal cord by Campbell in 1944, and has been pursued further in a number of more recent studies (e.g. Campbell, Mark & Gasteiger, 1949; Downman, Eccles & McIntyre, 1953; Eccles, Libet & Young, 1958; McIntyre, Bradley & Brock, 1959; Kuno & Llinás, 1970 *a*, *b*). Recording with intracellular micro-electrodes in the cat has indicated that there are abnormalities in the monosynaptically evoked excitatory post-synaptic potentials of the injured spinal motor neurone (Eccles *et al.* 1958; Kuno & Llinás, 1970*a*, *b*), although the quantal release of transmitter by the exciting synaptic terminals appears to be within normal limits (Kuno & Llinás, 1970*a*). This phenomenon is not confined to the somatic motor system. Brown & Pascoe (1954), working with cats and rabbits, found that axotomized sympathetic post-ganglionic neurones similarly show reduced responsiveness to synaptic activation, induced by preganglionic stimulation. Comparable findings have been obtained for the cat by Acheson & Remolina (1955) and for the frog by Hunt & Riker (1966), who recorded intracellularly and found alteration of synaptic potentials. Brown & Pascoe (1954) showed that the diminished response to preganglionic stimulation occurred despite a normal level of release of acetylcholine from the preganglionic endings. They found that the post-ganglionic neurones showed a reduced sensitivity to intra-arterially injected acetylcholine. It therefore

appeared likely that the abnormality was confined to the axotomized neurones and did not extend to the nerve endings which formed synapses upon them.

Exploring for degeneration of axon terminals ending upon spinal motor neurones during chromatolysis, Barr (1940) found by light microscopy that there were at most only minor changes in the number, size and appearance of end-bulbs on the injured neurones. More recent electron microscope work, however, has revealed a reduction in the number of synaptic contacts upon the cell bodies and (proximal) dendrites of motor neurones undergoing chromatolysis (Blinzinger & Kreutzberg, 1968; Hamberger, Hansson & Sjöstrand, 1970; Torvik & Skjörten, 1971*b*; Price, 1972; Kerns & Hinsman, 1973; Sumner & Sutherland, 1973), although there was no evidence in these studies to suggest degeneration of nerve endings. These findings would indicate that, in addition to any alteration in the sensitivity to transmitter of the surface membrane of the injured neurone, there may be a separation of the presynaptic nerve terminals from their post-synaptic sites on the neurone. Apart from that of Sumner & Sutherland (1973) the electron microscope studies were not quantitative, or were semi-quantitative, and some of the evidence was relatively indirect, so that the exact nature and extent of such a process remain to be defined.

The present experiments were undertaken with the object of exploring for synaptic changes during the period of reduced responsiveness of chromatolytic neurones to synaptic activation. The population of neurones chosen for study was the superior cervical ganglion of the rat, where recent work (Matthews & Raisman, 1972; Matthews, 1973) has defined the pattern and time course of certain of the morphological changes which follow section of post-ganglionic axons close to the ganglion. In the sympathetic ganglion, the synaptic situation is rather simpler than in most parts of the central nervous system: the great majority of the intraganglionic synapses are formed between the preganglionic nerve endings and the post-ganglionic neurones, and other synaptic possibilities are of limited number (Grillo, 1966; Tamarind & Quilliam, 1971; Matthews, 1974). A series of electrophysiological experiments has been performed to establish the severity and time of onset of the synaptic depression following post-ganglionic axotomy. Quantitative electron microscopy of ganglia has shown that this depression is accompanied by a reduced incidence of synapses together with an increased incidence of profiles of an unusual type, tentatively interpreted as detached presynaptic endings. It is concluded that separation occurs between the presynaptic ending and the post-synaptic neurone during chromatolysis of the latter, and it is suggested that this may be of advantage in facilitating regeneration. The

mechanism of detachment appears to be via the loss by the injured neurone of its specializations for attachment, together with the insinuation of tongues of satellite cell cytoplasm between the pre- and post-synaptic elements of the synapse. A preliminary account of part of this work has already been given (Matthews & Nelson, 1973).

METHODS

Young adult Wistar rats (1·5–3 months old, 150–200 g) of both sexes were used.

Recovery experiments

Two series of animals were prepared, one primarily for subsequent electrophysiological recording and the other for electron microscopy. Under anaesthesia with intraperitoneal chloral hydrate an injury was made to the external carotid nerve (ECN; one of the principal post-ganglionic branches of the superior cervical ganglion). The type of lesion varied according to the series of experiment.

First series. In thirty-four rats which were to be used for electrophysiological experiments, a major division of the left ECN was crushed or cut as it lay along the external carotid artery, as far cranially as possible beyond the first ventral branch of the external carotid artery (superior thyroid artery). In all cases the nerve was ligated with 10/0 monofilament nylon at the site of crushing, or just proximal to the point of cutting, for ease of subsequent identification. Care was taken not to expose the superior cervical ganglion, nor to injure other nerves or vessels. The right ganglion was left undisturbed, to be used as a control.

Second series. In rats which were to be used for light microscopy and ultrastructural study as many divisions as possible of the ECN (at a corresponding level) and the internal carotid nerve were crushed or cut, with or without nylon ligatures, either on the left side (ten rats) or on both sides (seven rats). Other branches of the ganglion (spinal and recurrent cardiac branches) were left undisturbed.

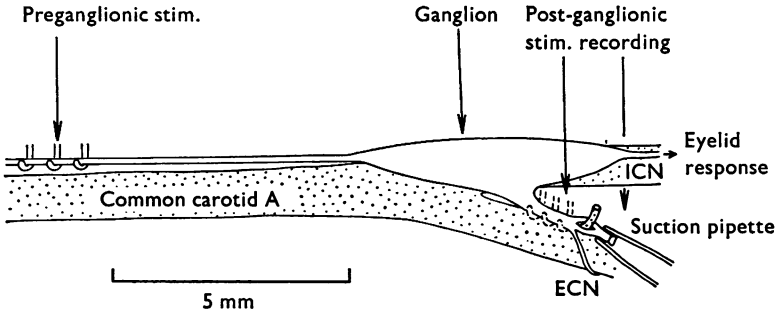
In all recovery experiments the wound was closed with interrupted sutures and penicillin was given intramuscularly. After survival intervals ranging from 6 h to 10 weeks the ganglia of the first (electrophysiological) series were prepared for *in situ* preganglionic stimulation and post-ganglionic recording. The ganglia of the second series were removed at a second (terminal) operation, cut into three blocks and fixed by immersion for 1–2 h in 1% osmium tetroxide buffered with 0·1 M phosphate. Contralateral control ganglia, and ganglia from normal rats, were removed and fixed in the same manner. After a brief wash in distilled water the tissue blocks were dehydrated in graded ethanols and embedded in TAAB embedding resin. Sections 1–2 μm thick were taken for light microscopy, and selected regions were trimmed and cut as thin sections for electron microscopy. These were stained with uranyl acetate and lead citrate for viewing in a Philips E.M. 200 or Siemens Elmiskop 1 electron microscope.

Electrophysiological experiments

A simplified diagram of the preparation is shown in Text-fig. 1. In normal and operated rats, under anaesthesia with intraperitoneal chloral hydrate, the preganglionic cervical sympathetic trunk was exposed and freed from neighbouring structures where it lies alongside the common carotid artery at a level caudal to the omohyoid muscle, about 6 mm proximal to the superior cervical ganglion. The nerve was placed in continuity on tripolar platinum electrodes (cathode central) for

stimulation in air by square wave pulses of variable intensity and duration (Text-fig. 1). Care was taken to avoid drying of the nerve and injury to its blood supply.

The injured branch of the external carotid nerve, or a corresponding branch of normal and control ganglia, was identified and gently freed from surrounding tissues as far proximally as the superior thyroid artery, round which it usually branches and re-unites. The ligature was left in place. Normal or control nerves were ligatured and cut as far cranially as possible, as at the initial operation in the experimental series. About 0·5–1·0 mm of the prepared external carotid nerve



Text-fig. 1. Schematic diagram of the rat superior cervical ganglion to show its relationships with the carotid arteries and the arrangements for stimulation and recording; drawn approximately to scale indicated. ICN, internal carotid nerve; ECN, external carotid nerve; as shown, the latter commonly branches and rejoins round the origin of the superior thyroid artery from the external carotid artery (not labelled separately). The smaller post-ganglionic branches (e.g. spinal and recurrent cardiac) are omitted for simplicity (cf. Dunant, 1967).

branch was drawn by gentle suction into a close-fitting saline-filled glass pipette for the recording of transganglionic compound action potentials in response to preganglionic stimulation (Text-fig. 1). A second electrode of silver wire lay immediately alongside the tip of the pipette. The responses after amplification were displayed and photographed on a storage oscilloscope. Areas under compound action potentials were measured using a Quantimet image analysing computer on tracings made from enlarged projections of the 35 mm film records.

Post-ganglionic transmission in the external carotid nerve branch was tested by transferring the stimulating electrode to the trunk of the external carotid nerve, between the ganglion and the superior thyroid artery, which provided a convenient earthing point. Recordings were made as before, by the same suction pipette, which was disturbed as little as possible during the transfer of the electrodes.

Transganglionic responses via the intact internal carotid nerve were monitored during preganglionic stimulation by observing the resulting widening of the palpebral fissure, using a small mirror placed beneath the animal's head. In some experiments the mechanical response of the upper eyelid was recorded by attaching the upper tarsal plate by thread to a strain gauge myograph, the amplified output of which was displayed on the cathode ray oscilloscope.

At the end of each experiment the ganglia were removed and fixed for subsequent microscopy and the animal was killed. Fixation was in most cases by 1 % glutaraldehyde in 0·1 M phosphate buffer, 18–48 h, followed by post-osmication and

processing as above; in some cases the ganglia were fixed directly in phosphate-buffered osmium tetroxide, as described earlier.

Light microscopy

Semithin (1–2 μm) sections of all ganglia, stained with a mixture of methylene blue and Azur II, were examined in order to evaluate the state of the nervous tissue

The incidence of chromatolysis of neurones was assessed in the semithin sections of the ganglia of the second series. In each ganglion, successive microscope fields were scanned using a × 40 objective until 100 or more neurones had been encountered which showed the nucleus in the plane of section. Each of these neurones was classified as chromatolytic or non-chromatolytic. Chromatolysis may be defined as reduction or dispersal and peripheral displacement of the Nissl material. A neurone was judged to be chromatolytic if it lacked any clumps of Nissl material in the deeper part of the cytoplasm (Pl. 2*b*, *c*; cf. Matthews & Raisman 1972). In addition, at the longer survival intervals the non-chromatolytic neurones were subdivisible into those which appeared completely normal and those which, while possessing clumps of Nissl material in the deeper part of the cytoplasm, had various persisting abnormalities which indicated that they were recovering from chromatolysis. These abnormalities included sparseness of Nissl material, extreme displacement and distortion of the nucleus, and aggregation of darkly-staining dense bodies in the deeper part of the cytoplasm, as described by Matthews & Raisman (1972).

In sections adjacent to the thin sections used for quantitative electron microscopy, the mean incidence of neuronal nuclear profiles was assessed by counting in three overlapping × 40 light microscope fields, placed over areas of the specimen more or less uniformly filled with neurones, i.e. free from large bundles of intraganglionic axons. These were the regions in which quantitative sampling was made with the electron microscope. The area of the × 40 field was approximately 162,800 μm^2, and was about twice the area sampled at the ultrastructural level.

Quantitative electron microscopy

The thin sections (silver interference colour) for electron microscopy were collected on squared copper grids of 200 mesh, the square apertures having a side of 96 μm and thus an area of close to 9000 μm^2. In a few cases, 400 mesh grids with squares of approximately one quarter this area were used. A map was drawn at low magnification of each ultrathin section as it lay on the grid in the electron microscope, and about 10 squares (or 40 at 400 mesh) were selected at random for quantitative survey from among those which satisfied the following criteria; that they should be free from major defects such as stain deposits, holes and scores, and that they should be covered by areas of the section occupied by more or less uniformly distributed neurones, and free from large bundles of intraganglionic axons or major blood vessels. Such areas are included in Pl. 2, *a* to *c*. The square traced on Pl. 2*a* indicates the approximate dimensions of the 200-mesh grid square.

Each of the selected squares was then scanned at high magnification for the counting and examination of various features, including synapses, presynaptic nerve endings, appositions and attachments between nervous profiles and the state of the neurones and of the Schwann and satellite cells. (In the sympathetic ganglion no obvious ultrastructural distinction has yet been established between the Schwann cells supporting the many unmyelinated nerve fibres and the satellite cells which invest the neurones and their dendrites, and the functions of the two appear to overlap; but Chamley, Mark & Burnstock (1972) have found it possible to make a distinction between their behaviour in tissue culture. The two terms have therefore

been used as accurately as possible in this article but may nevertheless be interchangeable.) Three observers took part in the scanning, and criteria were carefully established, were made as objective as possible and were repeatedly checked to ensure comparability. Any doubtful features were photographed for later discussion and evaluation.

In some of the counts, lengths of synaptic thickenings and of desmosome-like attachment plaques were recorded at a standard magnification in terms of the diameter (5 mm) of a small central circle engraved on the viewing screen of the electron microscope.

RESULTS

Physiological investigations

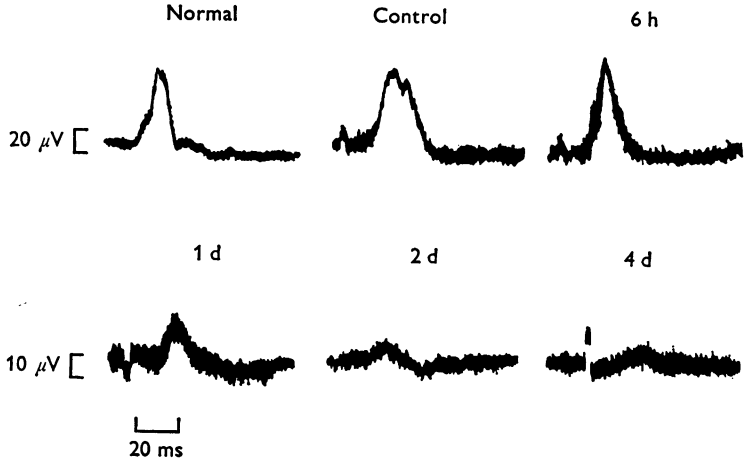
Transganglionic action potentials of normal and control ganglia

The transganglionic compound action potentials obtained from normal and from control external carotid nerve branches in response to single preganglionic stimuli showed a general resemblance in form. At low stimulus intensities the compound action potential regularly had two more or less well separated peaks, the earlier of which had slightly the lower threshold. These corresponded with the B and C peaks of the response described for this ganglion by Dunant (1967). Only for five out of sixteen ganglia was a distinct separation into two peaks maintained on maximal stimulation. In the remaining cases one or other peak predominated as the transganglionic action potential increased in size with increase of stimulus intensity (e.g. Text-fig. 2). Local application of Flaxedil to the ganglion abolished the transganglionic potential, confirming that it was synaptically mediated. The sizes of the maximal transganglionic potentials determined for normal and control ganglia are shown in Text-fig. 3. The wide variation in size of these potentials is probably due partly to variation in size and in fibre composition of the post-ganglionic nerve bundles available and partly to small differences in the application of the recording electrode. There could also have been varying degrees of double or triple firing of the post-ganglionic neurones, as was demonstrated in the rabbit superior cervical ganglion by Erulkar & Woodward (1968) and in the rat and guinea-pig by Perri, Sacchi & Casella (1970).

Previous repetitive stimulation with trains of maximal stimuli at 5, 10 or 20 Hz for periods of 5, 10 or 20 sec regularly (nine out of ten ganglia) gave enhancement of the transganglionic compound action potential (increase in height and/or duration), when this was tested by single shocks with the same maximal stimulus within a few seconds after the repetitive stimulation. The effect varied from animal to animal (range after 20 sec stimulation 7 % to 220 %; mean at 5 Hz 64·3 % (eight experiments), at 20 Hz 45·3 % (five experiments)). As these data imply, the enhancement was sometimes considerably less at 20 Hz than at 5 Hz in the same

ganglion. The effect had usually disappeared on re-testing at 30 sec after the end of the train of stimuli.

The preparation gave results which were stable and repeatable over several hours. Observations were continued for up to 6 h in some of the experiments, and the results remained consistent throughout. It was concluded that the acute effects of severing the nerve and recording by suction pipette, and the exposure and stimulation of the preganglionic trunk, did not impair the functioning of the various nerve fibres, or transmission at the ganglionic synapses, under these experimental conditions.

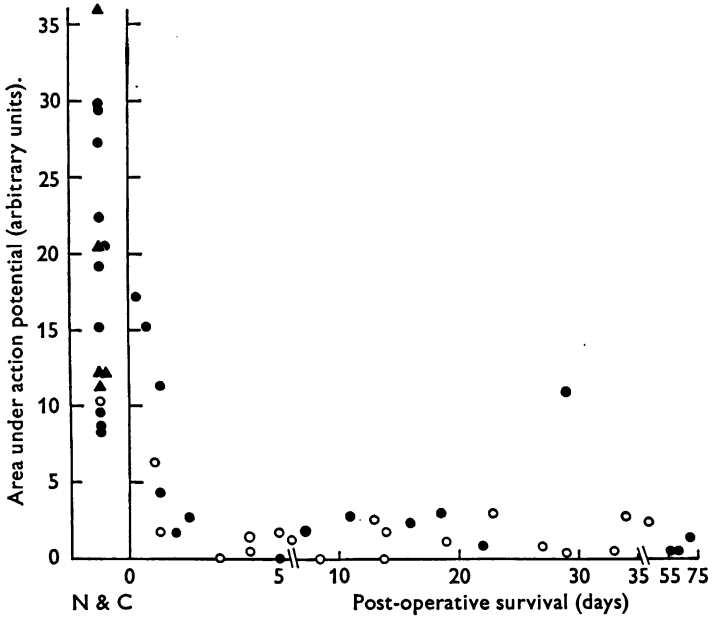


Text-fig. 2. Compound action potentials, recorded by suction pipette during stimulation of the preganglionic trunk by single maximal shocks, obtained from a normal external carotid nerve branch, from a 29-day control branch and from injured external carotid nerve branches at various post-operative intervals; ten to twenty traces superimposed. Records retouched. Positivity upwards.

Survival experiments: effects of injury of post-ganglionic axons

Cut or crush with permanent ligature. As was to be expected from the acute experiments, the transganglionic compound action potential recorded from the injured external carotid nerve branch showed no departure from the normal within the first few hours after operation (Text-fig. 2, upper row of records). In the course of the second 12 h, however, it declined sharply, reaching very low levels between 3 and 7 days post-operatively (Text-fig. 2, lower row of records; Text-fig. 3). The difference is highly significant statistically. At longer survival intervals the transganglionic action potential tended to increase again slightly. The mean of the ten values obtained between 36 h and 10 d (1·08, S.E. 0·29, in the arbitrary units used in Text-fig. 3) is lower than the mean of the seven

values obtained between 10 d and 20 d (1·93, S.E. 0·40, same units). Comparison of these sets of values by the Mann–Whitney U-test (one-tailed) gives the result $0{\cdot}025 < P < 0{\cdot}05$. This suggests that from about 10 d post-operatively there is some minor degree of recovery from the initial severe depression of the transganglionic response. This degree of recovery was not consistently maintained, however, and there was no evidence for further substantial recovery at intervals ranging up to 73 d (Text-fig. 3).



Text-fig. 3. Graph showing in arbitrary units the area under the compound action potential elicited by maximal preganglionic stimulation with single shocks from normal and control external carotid nerve branches (left-hand column), and from nerves at various intervals after experimental injury. ▲, Normal; ●, nerve division with ligature; ○, nerve crush with ligature; same symbols used for controls as for experimental ganglia. Within 1–2 d after injury the areas have fallen below the range of the normal and control values. There is little sign of subsequent recovery, except in one experiment at 29 d after injury. This could represent true recovery, or could have resulted from a possible post-operative attachment of an intact nerve branch to the injured one.

The wave form of the transganglionic response was however altered so that it showed a slower component. This was likely to be due to the presence of increased numbers of small axons in the post-ganglionic trunk at this stage (Pl. 1*a*, b), a factor complicating the interpretation of the size of the potential (cf. Hopkins & Lambert, 1972).

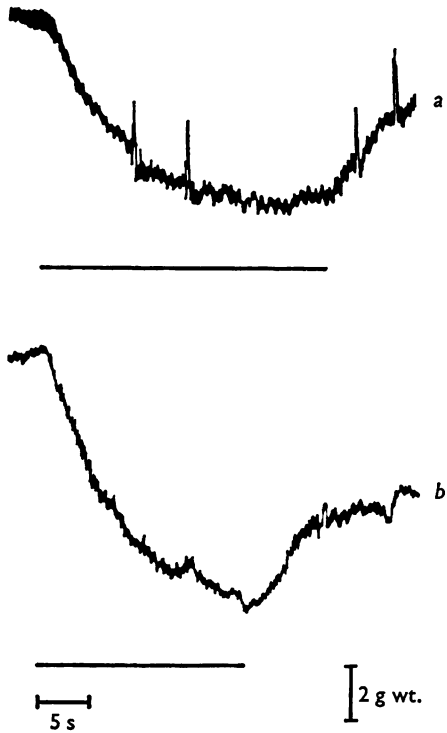
Repetitive preganglionic stimulation as described above could still induce enhancement of transganglionic responses up to 36 h post-operatively, and a variable enhancement was obtained again in twelve out of thirteen ganglia from the 7th to the 44th post-operative day (mean increase in six ganglia stimulated at 5 Hz for 20 sec, 28·8 %, range 8–50 %; in four ganglia stimulated at 20 Hz for 20 sec, 124 %, range 67–210 %; not measurable for all ganglia). In two ganglia at 5 d after operation, in which the transganglionic response was very small or absent, no enhancement could be obtained.

Simple crush. In one experiment the external carotid nerve was injured by crush alone, and 7 d later the transganglionic compound action potential was within the lowest part of the normal and control range (8·86 in the units of Text-fig. 3), although 30 % of the neurones in the middle region of the ganglion showed chromatolysis.

Control of strength of preganglionic stimulation: response of internal carotid nerve. It was desirable to confirm, in experiments in which there was no detectable transganglionic response in the injured external carotid nerve branch, that the preganglionic nerve fibres were responding to the stimulus, and that conditions within the ganglion were potentially favourable for synaptic transmission. For this purpose the internal carotid nerve was left intact in the experimental animals. (There is appreciable overlap within the ganglion of the territories occupied by neurones whose axons leave by the internal and external carotid nerves; e.g. Matthews & Raisman, 1972, and cf. Jacobowitz & Woodward, 1968.) The preganglionic stimuli were adjusted to elicit a maximal transganglionic response in the external carotid nerve, and were in the same range as those which proved maximal for the normal and control ganglia (2·2–3·0 V). If no response was obtained from the external carotid nerve, then stimuli rather stronger were used. In all cases, whether the external carotid nerve had been injured or not, these stimuli were sufficient, when delivered repetitively, to produce brisk eyelid opening via the intact internal carotid nerve innervating the smooth muscle component of levator palpebrae superioris. In some experiments the mechanical response of the upper eyelid was recorded (six experimental, four control sides) and the responses were found to lie within the same range, without significant differences between the two groups (Mann–Whitney U-test). Pairs of experimental and control records (four pairs) were not greatly disparate, indeed, the experimental could exceed the control value (e.g. Text-fig. 4). It appeared therefore that the effects of injury to the post-ganglionic fibres were confined to the territory of the injured external carotid nerve branch, and did not involve transmission via the internal carotid pathway. This is in agreement with an earlier observation of Brown & Pascoe (1954). It

indicates, moreover, that there had not been any significant impairment of the blood supply of the ganglion.

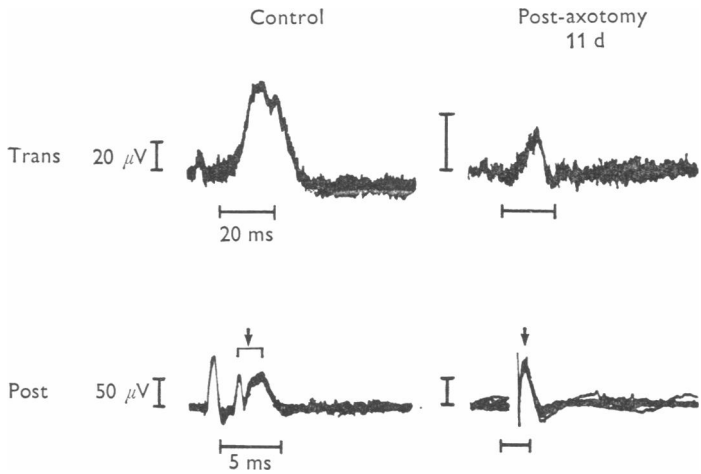
Conduction in the post-ganglionic trunk. The post-operative depression of the transganglionic action potential could have resulted from failure of synaptic transmission in the ganglion, or from altered conduction in the post-ganglionic axons close to the point of injury and subsequent recording. After survival intervals greater than a few days, the tip of the injured



Text-fig. 4. Eyelid responses of control (upper) and operated (lower) sides of a rat 3 days after injury to external carotid nerve. Tension records obtained by isometric myograph attached to the upper eyelid in each case. Records retouched. Horizontal lines below records indicate duration of stimulation of the preganglionic sympathetic trunk at 10 Hz, by stimuli maximal for the external carotid response. The transganglionic response of the external carotid nerve on the operated side was so small as to be unrecordable, and on the control side was within the normal range.

nerve was enlarged by sprouting and attached to surrounding structures by a mixture of regenerating axons and scar tissue, from which it had to be dissected. Moreover, the bundle now contained large numbers of tiny newly formed nerve fibres of whose electrical properties we were uncertain

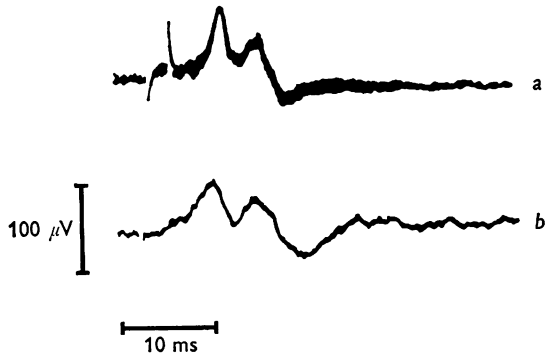
(Pl. 1*a*, *b*; cf. Hopkins & Lambert, 1972). Conduction in the external carotid axons was tested for fourteen normal and control ganglia and sixteen experimental ganglia, after the transganglionic response had been assessed. The external carotid nerve was activated orthodromically between the ganglion and the superior thyroid artery, as described in the Methods section, and recording was made as previously from the suction electrode on the cut end of the nerve. As the conduction distance between the arterial side-branch and the recording pipette was not more than 1 mm at most, it was not possible in more than a few experiments completely



Text-fig. 5. Transganglionic and post-ganglionic compound action potentials elicited on maximal stimulation by single shocks from external carotid nerve branches, for 29 d control and 11 d post-axotomy. Although the transganglionic response at 11 d after axotomy is much smaller, the post-ganglionic responses of the two nerves (arrowed) are seen to be more nearly comparable, despite incomplete separation from the stimulus artifact in the 11 d experiment. Records retouched; about twenty traces superimposed.

to separate the compound action potential in the nerve from the stimulation artifact. Nevertheless it was established that compound action potentials of moderate size could be conducted along the post-ganglionic trunk, at least for several days after injury, even though transganglionic transmission was severely depressed or abolished (Text-fig. 5). A slow later component of the post-ganglionic response was seen in some experiments from 36 h onward. This would probably correspond with the slow wave seen by Hopkins & Lambert (1972) for regenerating preganglionic unmyelinated axons and attributed by them to axons of small diameter (cf. Dyck & Hopkins, 1972).

In two further control experiments the preganglionic nerve trunks of both sides were cut or crushed, with ligature, and three days later responses were recorded by suction electrode from the injured proximal ends during orthodromic activation via tripolar electrodes 3–5 mm proximally; this provides a more favourable site for recording than when the lesion is post-ganglionic. Compound action potentials were obtained which corresponded closely with the responses of the acutely divided preganglionic trunk (Text-fig. 6). In the rat the preganglionic cervical sympathetic axons do not differ greatly from the post-ganglionic axons, both groups being largely unmyelinated and of comparable size. It is probable that the proximal parts of the post-ganglionic axons were similarly capable of conducting action potentials after the injury.

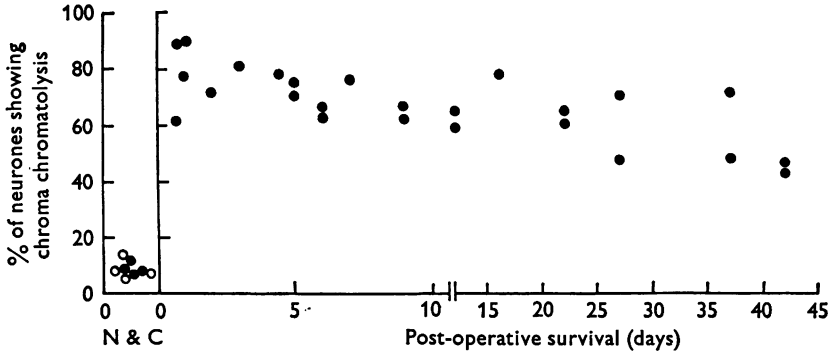


Text-fig. 6. Compound action potentials recorded by suction pipette from the preganglionic cervical sympathetic trunk in response to orthodromic stimulation with single shocks adjusted to give a maximal response. *a*, Normal preganglionic trunk, acutely ligated and divided; *b*, stump of preganglionic trunk ligated and divided 3 d previously; about 0·5–1·0 mm of stump drawn into pipette. About ten traces superimposed; records retouched.

It is likely, therefore, that there was indeed depression of intraganglionic synaptic transmission, confined selectively to the injured neurones. These experiments indicate that the depression was most severe from about 36 h to 10 d post-operatively, and that there was only a partial recovery within the limits of the experimental period. At the longer survival intervals, however, the size of the summated post-ganglionic response may have been limited by the electrical properties of the altered population of post-ganglionic nerve fibres, and any such limitation would also govern the transganglionic responses elicitable by this method.

Light microscopy

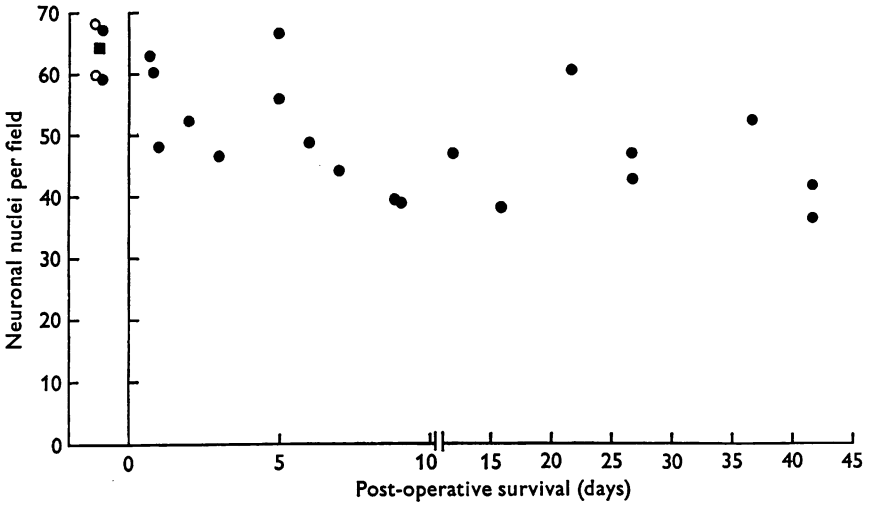
In the ganglia used for the electrophysiological experiments it was confirmed that there was regularly a proportion of neurones undergoing retrograde chromatolysis from about 1 d after the injury to the external carotid nerve branch. These neurones were distributed within a restricted region in the caudal two thirds of the ganglion, where they were interspersed with many normal neurones. The neurones in the cranial one third of the ganglion were almost all normal in appearance.



Text-fig. 7. Incidence of neurones showing chromatolyiss, on the criteria explained in the text, plotted as percentages encountered in samples of approximately 100 neurones each, in normal (○) and control ganglia (●, left-hand column) and at various intervals after injury of all external and internal carotid nerve branches (●, main body of graph).

In the series of ganglia prepared for electron microscopy by lesions of all the external and internal carotid nerve branches, a high proportion of neurones throughout the ganglion became chromatolytic (Pl. 2, *a* to *c*). In each of these ganglia, the incidence of strictly chromatolytic neurones was determined as described in the Methods section. The results of this survey are shown in Text-fig. 7. The proportion of chromatolytic neurones rose rapidly over the first day after injury to a level of 80 % or more, and remained close to this figure until about 5 days post-operatively (mean incidence of chromatolytic neurones, 3 d to 7 d, 74·6 %). The proportion decreased gradually with time, showing more variation at the longer survival intervals, but by 42 d was still in the region of 40–45 %. After the first few days post-operatively, the 'non-chromatolytic' neurones included an increasing proportion of neurones which had begun to re-form Nissl clumps but did not yet have normal quantities of Nissl material. They were generally smaller than the normal neurones and still showed cytological abnormalities such as displacement and distortion of the

nucleus, aggregations of cytoplasmic dense bodies and scanty Nissl material, by which they could be distinguished from completely normal neurones (cf. Matthews & Raisman, 1972). The proportion of neurones indistinguishable from the normal was low and showed rather little change throughout the earlier part of the series, rising slightly later (means 1–7 d, 4·3 %; 9–22 d, 5·3 %; 27–42 d, 10·7 %); complete recovery from chromatolysis was therefore slow. Only a very few neurones, at intermediate survival intervals (9–22 d approx.), showed severe shrinkage and atrophy suggestive of imminent cell death.



Text-fig. 8. Mean numbers of neuronal nuclei observed per × 40 microscope field in normal and control ganglia and at various intervals after injury of all external and internal carotid nerve branches. Further description in text. Left-hand column: ○, normal; ●, 13½ h and 4½ d control; ■, 27 d control.

The apparent packing density of neurones in the experimental ganglia, as determined by counting nuclear profiles in the semithin sections, fell by about one-fifth in the first 24 h (Text-fig. 8). This initial fall may be accounted for largely by shrinkage of the neurones or of their nuclei (Pl. 2*a*, *b*). By 9 d post-operatively the incidence of neuronal nuclei in the tissue sections had settled to a level about one-third below the normal, and remained so thereafter in most ganglia (Text-fig. 8). The later fall is only partly attributable to further shrinkage and distortion of nuclei and to loss of neurones: in addition, the increased intervals between the neurones were largely occupied at these longer survival intervals by an increased volume of Schwann cell profiles supporting many small, newly formed unmyelinated axons (Pl. 2*c*). This is a characteristic and striking

feature of the regenerative axon reaction when the lesion is close to the ganglion (M. R. Matthews & G. Raisman, in preparation).

From these observations it appears that of the 80% or more of neurones which became chromatolytic about half showed signs of incipient recovery and relatively few died during the period of the experiments. Appreciable changes occurred, however, in the volume fraction occupied within the ganglion by the cell bodies of the chromatolytic neurones, which not only shrank but became further dispersed by the interposition of bundles of small new nerve fibres with associated Schwann cells.

Electron microscopy

The features of neuronal chromatolysis and the axonal and Schwann reactions observable with the light microscope in the experimental ganglia were confirmed, and it was established that the ganglia otherwise showed no abnormalities of tissue reaction, such as might be attributable to direct injury or anoxia.

Intraganglionic synapses

In the normal ganglion the vast majority of the synapses are of the type preganglionic axon to post-ganglionic neurone illustrated in Pl. 3.

The preganglionic axons end as terminal boutons or as trails of varicosities which form *en passant* as well as terminal synapses. The preganglionic nerve ending contains small clear-centred 'synaptic' vesicles, of about 20–40 nm diameter, with usually some larger dense-cored vesicles of 60–80 nm diameter and a varying number of mitochondria. Other organelles, including dense bodies, are occasionally present. The small clear-centred vesicles are clustered towards the specialized synaptic region, and are associated there with triangular tufts of dense material projecting from the membrane, and the large dense-cored vesicles are typically situated at or near the periphery of the vesicle cluster; but sometimes, as in Pl. 3*a*, a large dense-cored vesicle is found at the synaptic membrane in a synapse of this type. (This feature was seen in 12·7 % of 284 synapses, in two normal ganglia.) In the cytoplasm of the post-synaptic element there is a more or less thick and well defined layer of dense material apposed to the post-synaptic membrane in the specialized synaptic region (the post-synaptic thickening). Irregular clear vesicles or a further 'sub-synaptic band' (Taxi, 1961) are sometimes associated with the deep aspect of this.

The present study has concerned itself with all the synapses involving principal post-ganglionic neurones as the post-synaptic elements. A preliminary survey was made of 1060 synapses in four normal ganglia. A few of the synapses received by the post-ganglionic neurones lie upon their

cell bodies (four were found in the above survey), but most are situated on their dendrites; these neurones are multipolar and may have much-branched dendrites (e.g. de Castro, 1932). The dendritic synapses are received either upon the dendritic shafts (which are characterized by longitudinally oriented microtubules and neurofilaments, together with ribosomes and/or granular endoplasmic reticulum; Pl. 3*a*) or upon spine-like projections of various forms (which lack microtubules and neurofilaments, and have typically a finely filamentous cytoplasmic matrix; Pl. 3*b*). A ratio of spine to shaft synapses of 2·4:1 was found for 891 synapses for which this distinction could be made. A certain number of short spine-like processes project from the cell bodies of the post-ganglionic neurones, and these also may receive synapses (Pl. 3*c*; Elfvin, 1971*a*). In the present survey twenty-two synapses of this type were encountered, an incidence of approximately 2 %.

A small proportion of the preganglionic axons terminate upon the small granule-containing cells or small intensely fluorescent (SIF) cells of the ganglion (e.g. Matthews, 1971; Matthews & Ostberg, 1973), and these cells in turn can form synapses upon the post-ganglionic neurones (Matthews & Nash, 1970). In the entire study of normal and experimental ganglia, clusters of small granule-containing cells were only seldom chanced upon and were not included in the areas sampled; their characteristic efferent synapses accounted for only two of the 2799 synapses observed. In addition, there are possible synaptic interconnexions between the principal post-ganglionic neurones, which would have been included in the survey. No unequivocal examples of these were found, and indeed such synapses might not be readily differentiable by the standard fixation procedures used, except in the most fortunate circumstances, e.g. Matthews (1974).

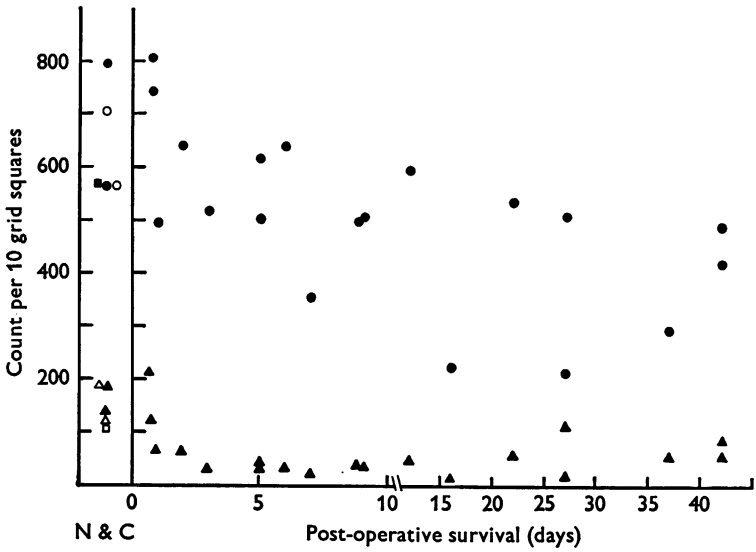
Incidence of synapses after injury of post-ganglionic axons

A quantitative survey was next made in ganglia of the series prepared for electron microscopy by cutting, or by crushing and tying, all the major post-ganglionic branches. This was done so as to produce chromatolysis in as many neurones as possible, in order to maximize the incidence of any synaptic changes.

Synapses and other features were counted and analysed in two normal, three control and nineteen experimental ganglia. Most of the counts were made over the area of section contained within 10 squares of a 200-mesh electron microscope grid, i.e. over approximately 90,000 μm^2. In a few cases up to 15 squares were counted, where incidences of various features were low, and in four experimental and two control ganglia fewer squares were counted: 8 and 9 at 42 d and in the control ganglia, and 5 in each of two ganglia at 9 d.

Regions of the ganglion containing evenly distributed neurones were selected for the counts, in preference to regions containing major nerve bundles (where the incidence of synapses is low). It was also decided to

relate the counts to some internal feature of the tissue, in order to overcome sampling problems and effects of any volume changes in the ganglion, and to provide a basis for comparison between ganglia. Since a neurone is a large and infrequent feature in the scale of the electron microscope, and since the chromatolytic neurones were undergoing such major changes in size and shape, it was decided to avoid relying directly on them. The feature which was chosen instead as the reference point was the vesicle-containing region of the preganglionic nerve ending. The observations of Barr (1940), of Brown & Pascoe (1954) and of Kuno & Llinás (1971*a*) would suggest that these endings do not immediately undergo gross



Text-fig. 9. Incidences of vesicle-containing profiles (●) and of synapses (▲). Circles and filled square, numbers of vesicle-containing profiles of presynaptic type (including those forming synapses) encountered per 10 grid squares of side 96 μm; figures scaled to correspond in cases where area sampled was greater or less than this (see text). ○, Normal; ●, experimental and short-term control; ■, 27 d control. Triangles and open squares, numbers of synapses encountered in the same area. △, Normal; ▲, experimental and short-term control; □, 27 d control. In this and in succeeding Figures, the short-term control values were from experiments with 13½ h and 4½ d survival.

alteration as a consequence of post-ganglionic axotomy, and it was anticipated that they should therefore provide a suitable reference feature for synapses. Profiles of the terminal and near-terminal regions of the preganglionic axons contain the characteristic grouping of vesicles, mitochondria etc. described above for the synapse (Pl. 3). Such

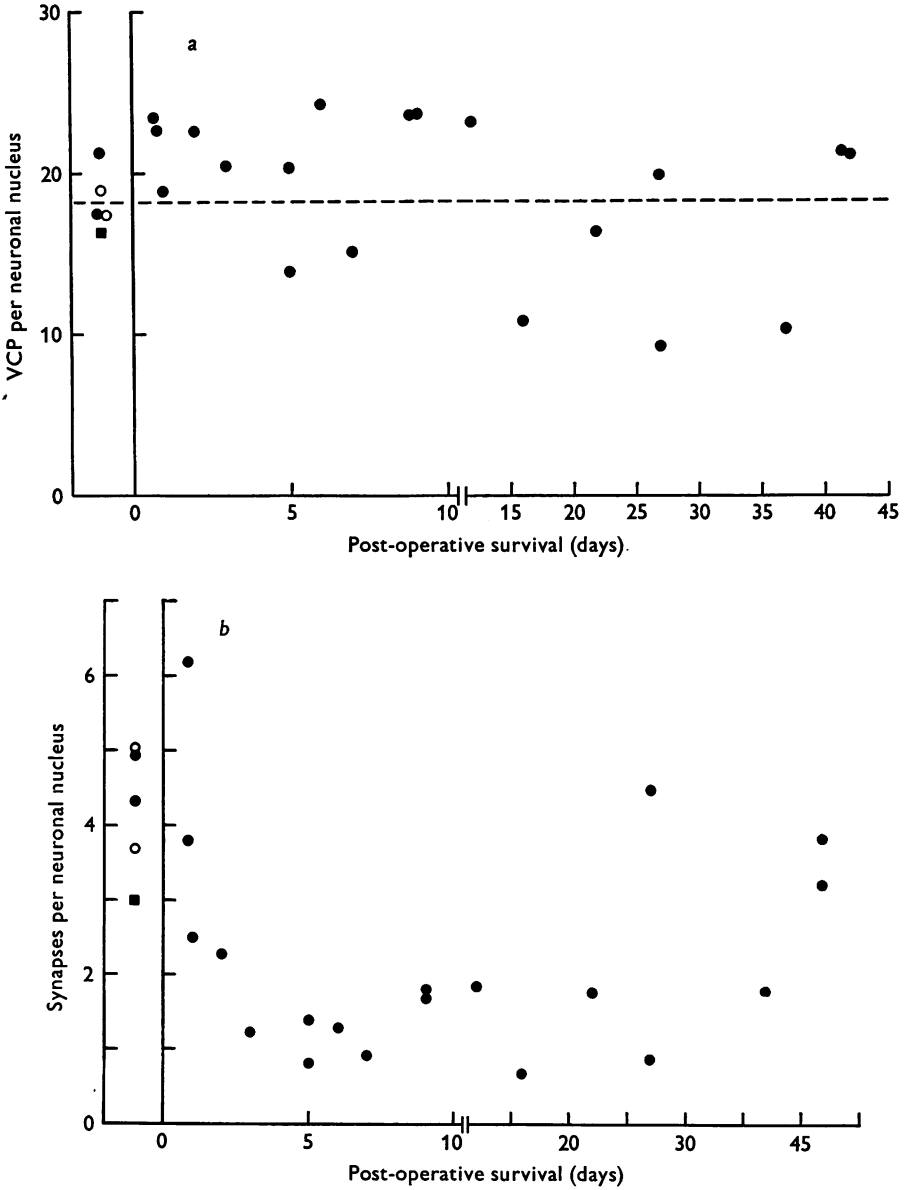
vesicle-containing profiles (VCP) are taken to represent potential synaptic regions, although for reasons of geometry they may not show a synapse in the plane of section.

In normal and control ganglia a count over ten grid squares yielded between 560 and 800 VCP, and between 100 and 180 synapses (Text-fig. 9). From 1 d post-operatively to the end of the first week the counts of VCP were rather smaller, but did not fall below about 500 in the area sampled. The counts of synapses however fell sharply, to approximately thirty in the same area (Text-fig. 9). At greater survival intervals the count of VCP became much more variable, probably owing to sampling problems, but showed a general tendency to fall; the count of synapses by contrast tended to rise again. When these counts are plotted as incidences per neuronal nuclear profile (data from Text-fig. 8), then it is seen that in relation to the neurone population the VCP in many ganglia remain at or a little above the normal and control levels post-operatively, falling later in some ganglia (Text-fig. 10*a*), whereas the synapses fall steeply from a figure of about 4 per nuclear profile to a low level over the first few days, and show indications of a later recovery (Text-fig. 10*b*).

In order to obtain stricter evaluation of samples, and to facilitate comparisons between ganglia, the VCP seen forming synapses in each ganglion were expressed as a percentage of the total VCP counted for the same ganglion. The result is shown in Text-fig. 11*a*. In the normal and control ganglia between 18 and 25 % of the VCP show synapses in the plane of section. After post-ganglionic axotomy this proportion falls rapidly and is lowest between about 3 d and 7 d, when it is reduced to roughly one quarter of the normal value. About one quarter of the neurones are non-chromatolytic over this same period (cf. Text-fig. 7). A gradual recovery follows, and appears to be almost complete by 42 d. The persisting synapses which were found at the shorter survival intervals were within the normal range in respect of their ultrastructural appearance (Pl. 3); at longer survival intervals some had very poorly defined post-synaptic thickenings, and the vesicle population of the presynaptic profile was sometimes unusually low.

In one ganglion at 27 d after post-ganglionic nerve section the indices of synaptic recovery were high (Text-figs. 10*b*, 11*a*), but only 47 % of the neurones were still chromatolytic and about 16 % looked completely normal; it is likely therefore that the initial lesion was rather less extensive than in other experiments.

Detached presynaptic profiles. Of particular interest was the discovery of a new class of profiles in these experimental ganglia. Preganglionic-type nerve endings were found which were completely normal in appearance, showing a typical presynaptic clustering of vesicles toward a specialized



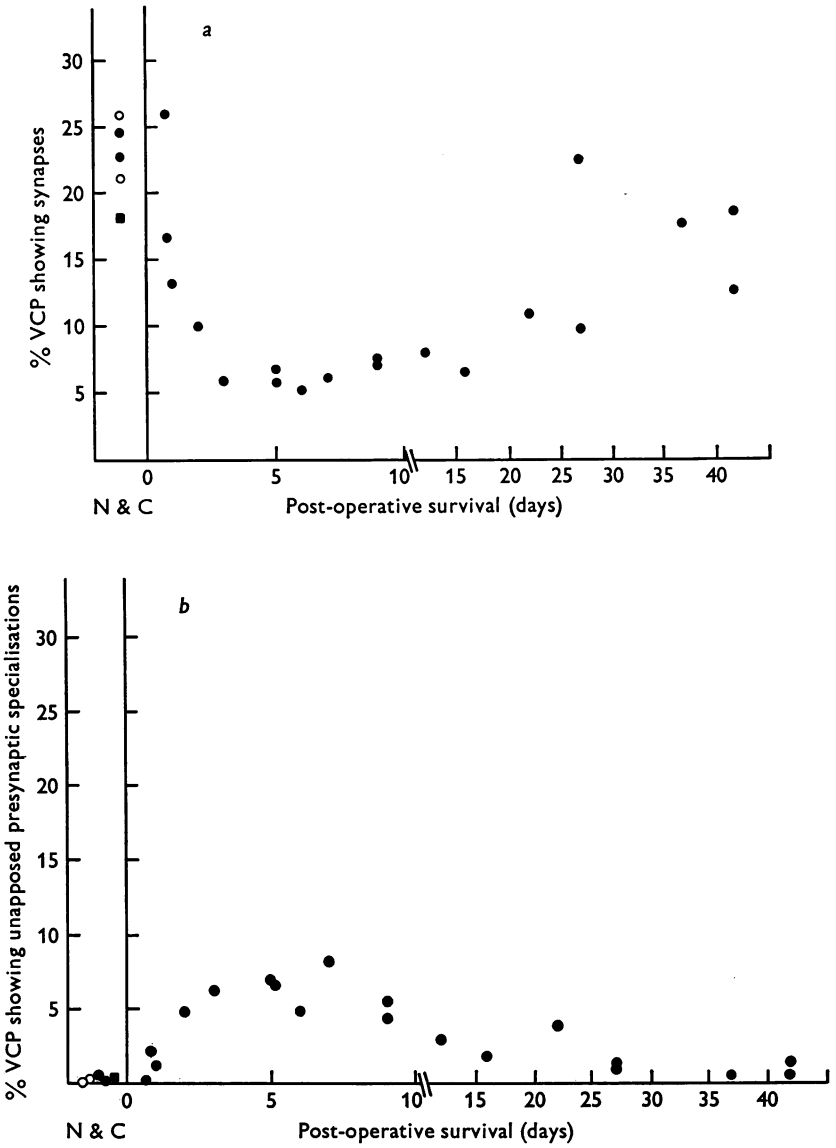
Text-fig. 10. *a*, Numbers of vesicle-containing profiles (VCP) per neuronal nucleus; *b*, numbers of synapses per neuronal nucleus; values derived for each sample by dividing total counts of VCP and synapses by mean incidence of neuronal nuclei in the same area as the sample (data represented in Text-figs. 10 and 11). ○, Normal; ●, experimental and short-term control; ■, 27 d control. Dashed line in *a* indicates mean of normal and control values.

area of the surface membrane, but this was not apposed to any post-synaptic nerve element, and instead the profile was entirely enveloped in Schwann or satellite cell cytoplasm (Pl. 4; Pl. 5, fig. 1). We have interpreted these unusual profiles as presynaptic endings which have become detached from their normal post-synaptic sites, but without undergoing any other obvious change, and which have retained their presynaptic specializations. Such profiles did not show any unusual signs of degeneration. The retention of specialized presynaptic features could even include the presence of dense intra-cleft material in the interval between the specialized presynaptic zone and the adjacent Schwann cell, although the latter showed no evidence of any corresponding membrane specialization (Pl. 4*b*, *c*, *d*). Sometimes the specialized region was inwardly bowed and slightly wrinkled, a feature taken by Streit *et al.* (1973) to be suggestive of recent release of transmitter by exocytosis.

Unapposed presynaptic specializations were found in ganglia which had been used for the electrophysiological experiments and which showed severe depression of the transganglionic response. They have also been found in a ganglion 5 d after simple crushing of the post-ganglionic branches.

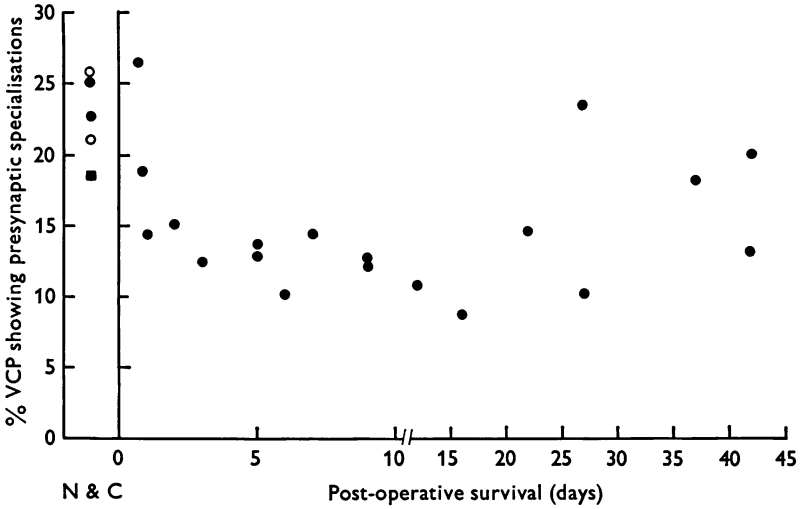
The incidence of VCP showing unapposed presynaptic specializations in the series of ganglia prepared for electron microscopy was recorded during the survey of synapses, and was expressed like that of synapses as a percentage of the total number of vesicle-containing profiles. The results are shown in Text-fig. 11*b*. In the normal and control ganglia only five unapposed presynaptic sites were found in 2579 vesicle-containing profiles. The maximum incidence was 0·5 % in a control ganglion $18\frac{1}{2}$ h after contralateral axotomy. In axotomized ganglia the incidence rose to a maximum of the order of 7 % (mean 3–7 d, 6·94 %; 2690 VCP in five ganglia). The highest levels were found between 3 d and 7 d post-operatively, when the incidence of synapses was lowest. At this stage there were slightly more unapposed presynaptic specializations than there were persisting synapses. As the incidence of synapses increased again, at longer survival times, the incidence of VCP showing unapposed specializations decreased (Text-fig. 11).

Elimination of a possible source of confusion. It might be objected that there is one type of profile in the ganglion which could be erroneously identified as a detached presynaptic profile. In normal ganglia the dendrites and cell bodies of the post-ganglionic neurones contain occasional clumps of small vesicles, which are sometimes clustered toward the surface membrane and associated there with dense projections from the membrane (as illustrated for recently denervated ganglia by Taxi, Gautron & L'Hermite, 1969). In most cases the additional presence of ribosomes



Text-fig. 11. *a*, Percentages of vesicle-containing profiles (VCP) seen forming synapses in the plane of section. ○, Normal; ●, experimental and short-term control; ■, 27 d control. Further description in text. *b*, Percentages of VCP showing typical presynaptic specializations (localized clustering of synaptic vesicles in association with inward projections of dense material at the surface membrane) which are not apposed to any post-synaptic nervous profiles in the plane of section. ○, ●, ■ As in *a*.

within the profiles permits their exclusion as possible detached preganglionic endings. These could however conceivably account for some of the very few 'detached presynaptic profiles' which may be found in normal and control ganglia. They can hardly have been of importance as a source of error post-operatively, however, as it was confirmed in the course of the present experiments that the incidence of clumps of small vesicles in the post-ganglionic dendrites and cell bodies falls to a low level at an early stage after axotomy (Table 1; cf. Matthews & Raisman, 1972). It did not begin to show recovery until survival intervals of 27–42 d.



Text-fig. 12. Percentages of all vesicle-containing profiles which showed presynaptic specializations, either apposed (forming synapses) or unapposed, in normal (○), experimental (●) and control ganglia (●, ■ as before). The relative incidence of presynaptic specializations is reduced post-operatively.

Total presynaptic specializations: are there changes in the nerve endings after detachment? If all the detached presynaptic elements of synapses were to persist unaltered, then there should be no change in the total proportion of VCP bearing presynaptic specializations revealed in a section. In fact this appears not to be true, as the graph of all presynaptic specializations plotted as a percentage of VCP (Text-fig. 12) shows an abrupt fall over the first 2–3 d to approximately two-thirds of the normal, with a further slight decline to rather less than half the normal level during the next 10 d or so. There is a later increase which reflects the improved proportion of synapses. The incidence of presynaptic specializations is still tending to fall at a stage when the recovery in the proportion of

synapses has begun (Text-fig. 11*a*). It should be noted that this graph inevitably reflects aberrations in the counts of both synapses and unapposed specializations, from which it is compiled, and therefore is more subject to fluctuation than either alone. There seems to be no doubt, however, that the proportion of vesicle-containing profiles with detectable presynaptic specializations in the plane of section falls during the chromatolysis of the post-ganglionic neurones, and that much of this decrease coincides with the early fall in the proportion of synapses.

This result could arise in a variety of ways. An obvious possibility is that some VCP may lose their presynaptic specializations during or after detachment. Other factors could however contribute to produce this apparent effect. In the normal synapse, the presynaptic specialization may sometimes appear discontinuous or may be less extensive than the post-synaptic thickening, and may therefore be less easily detected after detachment. The detached VCP might swell or form sprouts, or the synaptic specialization might decrease in extent, in either case coming to occupy a smaller proportion of the surface of the profile. The characteristic vesicles might become redistributed (or might increase in number) so as to extend further into the preterminal regions of the nerve endings, giving rise to an increase in the number of VCP without specializations per section. Changes in shape of the preganglionic endings might produce a similar result.

Not all these possibilities are readily testable, but some have been explored. Those tending to increase the number of profiles of VCP would also cause a decrease in the proportion of VCP found forming synapses. They are however unlikely to have influenced the results to a serious extent. As Text-fig. 10*a* indicates, there appears to have been a small increase in the incidence of VCP relative to that of neurones in the tissue sections during the first 12 d post-operatively, but this could have been due to shrinkage of neurones and is in any case insufficient to account for either the fall in the incidence of synapses or the shortfall in the proportion of presynaptic specializations. At some of the longer survival intervals the samples contained relatively few VCP, and this may represent an actual decline of VCP; it is unlikely to have been due entirely to problems in sampling.

The shape, size and vesicle content of the VCP are very variable at all stages, including the normal, and have not been analysed quantitatively. They do not seem to show dramatic differences in the early post-operative period, though the impression was obtained that many profiles of VCP tend to be smaller at intermediate survival intervals, and some VCP at longer intervals show very scanty vesicles. These observations suggest that the detached nerve endings do not show immediate major changes

but may undergo a slow atrophy in the longer term. Two further pieces of evidence would support this. A sustained increase was found in the incidence of cytoplasmic dense bodies in profiles of nerve endings (VCP) from the end of the first day post-operatively (Table 1), and this could indicate a heightened autophagic activity, suggesting an altered turnover of cytoplasmic constituents, consistent with atrophy. At the same time, there was little direct evidence for loss of VCP. Degenerating fragments enclosed in Schwann and satellite cells were recorded during the counts,

TABLE 1. Incidences of certain features shown by neurones, satellite or Schwann cells and presynaptic nerve endings, in normal and control ganglia and at various intervals post-operatively. Comparable regions of the ganglia were surveyed in all cases. These are given either per grid square of side 96 μm (for neurones and satellite cells) or per 100 profiles observed (for nerve endings). Figures in parentheses indicate, for satellite cells, the number of squares in which a feature was surveyed, where this differs from the figure given in the first column; for nerve ending profiles, the number of profiles surveyed

	Neurones:	Satellite cells		Nerve ending profiles (presynaptic VCP)	
	No. of grid squares analysed	clumps of small vesicles (per square)	Stellate lipid droplets (per square)	Degenerating profiles in satellites (per square)	% showing dense bodies	% apposed to other nervous profiles
Normal	16	12·13	1·38 (32)	4·06	1·8 (1195)	16·25 (726)
Control	9	13·89	3·07 (27)	3·15 (13)	1·72 (464)	13·28 (128)
17½ h	5	8·4	29·46 (11)	14·4	1·79 (390)	12·31 (390)
18½–24 h	5	6·6	32·85 (20)	2·8	4·31 (371)	13·21 (371)
2 d	7	0·86	11·0 (6)	5·43	4·40 (455)	14·91 (322)
5–6 d	11	1·18	1·74 (31)	1·91	3·22 (666)	9·61 (666)
9 d	10	1·5	0·9	2·5	2·99 (501)	21·7 (727)
12–22 d	22	1·46	3·34 (32)	1·82	4·72 (974)	15·37 (1204)
27–42 d	20	9·3	3·91 (33)	2·75	7·07 (863)	11·85 (1148)

and were not found to be unusually numerous, except in one ganglion at 17½ h post-operatively (Table 1). Profiles identifiable as degenerating VCP were extremely few, among those of which the origin could be decided: one is illustrated in Pl. 5*b*. Degenerating nerve profiles in the ganglion are rapidly digested by the Schwann (or satellite) cells, which accumulate irregularly stellate droplets of lipid-like material in this process (Pl. 5*b*, inset; Pl. 7*a*; Matthews, 1973). This appeared in large quantities only during the first 1 to 2 d following axotomy (Table 1). At this stage there was also positive evidence of penetration of satellite (Schwann) cell processes into neurones, with pinching-off or sequestration of fragments of neuronal cytoplasm which apparently became digested within the satellite cells (Pl. 5*c*, *d*; Matthews & Raisman, 1972). The reduced incidence of VCP in relation to neurones in some of the longer-term experiments, if

it was not due to errors in sampling, may thus have been the result of a slow atrophy or resorption of some of the detached profiles, rather than an acute degeneration and digestion.

Lengths of detached presynaptic specializations. Measurement of approximate lengths of synapses and of unapposed presynaptic specializations in the course of some of the counts showed that unapposed presynaptic specializations tended to be of the same length as the shorter synapses, so that their *mean* length was shorter than that of either shaft or spine synapses as measured in control ganglia and at short post-operative intervals (mean length of 121 unapposed presynaptic specializations, 0·132 μm; of 168 spine synapses, 0·208 μm; of 164 shaft synapses, 0·249 μm; synapses measured in normal and control ganglia, and values within 4 % of these obtained in first 24 h post-operatively). This may reflect the tendency, observable in the normal, for the presynaptic vesicle clustering to be less extensive than the post-synaptic zone of dense material applied to the membrane, but it could also be due to a reduction in extent occurring as a result of separation. It would have the effect that presynaptic specializations would appear in roughly two-thirds as many sections after detachment as before (cf. Text-fig. 12). It seems unlikely, therefore, that any great number of these specialized presynaptic regions was lost immediately after detachment, or in the course of detachment; and many may have remained available for subsequent re-formation of synapses.

Relative changes in shaft and spine synapses. There was some indication that synaptic sites upon dendritic shafts and upon spines might differ in the timing of their response to the post-ganglionic axotomy. Both types of synapses decreased in incidence post-operatively, neither type being lost preferentially, but shaft synapses showed a more immediate fall and a slightly earlier onset of recovery.

As indicated above, sections through the specialized membrane regions of shaft synapses in normal and control ganglia tended to include longer profiles than those of spine synapses: the means differ by about 20 %. Post-operatively, similar ratios of lengths were observed at all survival intervals. The mean lengths of surviving synapses showed little change (less than 7 %) and no consistent alteration until the longer survival intervals (27–42 d), when both types of synapses showed an increased preponderance of the longer sectional profiles (mean length of 119 spine synapses, 0·233 μm, and of 100 shaft synapses, 0·29 μm, i.e. +11·2 % and +11·6 % respectively). In these longer-term experiments the populations of synapses almost certainly include a proportion which have been re-formed, or newly formed, during recovery of the ganglionic neurones from chromatolysis; the observed changes in mean size of synapses would not

seem sufficiently great to suggest that the recovery in incidence of synaptic profiles could be due entirely to hypertrophy of surviving synapses.

The observations on synapses and VCP indicate that after post-ganglionic axotomy there is a detachment of many or all of the synapses upon the injured neurones without immediate gross alteration in the majority of the presynaptic nerve endings. Many unanswered questions remain, among them, how is the separation effected? Some evidence points to the injured post-ganglionic neurones as the primary agent.

Post-synaptic sites on post-ganglionic neurones

Extremely few unequivocal vacated post-synaptic membrane densities were found in the course of the counts (only seventeen in nineteen experimental ganglia, in which there were found over 350 unapposed presynaptic sites, and 920 fewer synapses than would have been expected in normal ganglia in relation to the observed number of VCP). This is in striking contrast with the effect of cutting the preganglionic axons, when the resulting degeneration of the preganglionic nerve endings leaves the post-synaptic sites clearly identifiable by apparently unaltered post-synaptic dense material (frog: Taxi, 1964; Sotelo, 1968; rat: personal observations).

The seventeen vacant post-synaptic sites observed in the present experimental material were distributed among eight ganglia at between 2 and 12 d survival; for comparison, one such site was found in each of the two normal and in two of the three control ganglia, where they presumably reflect an incidental turnover of synapses. The dense material of the post-synaptic site is thought to be in some way concerned with adhesion of the synapsing elements. It was concluded that there had been loss or alteration of this material in the experimental ganglia post-operatively, and that this might have been a factor in the separation of the synapses.

Attachment plaques or 'desmosomes'

Other specializations for attachment and/or other interactions also exist between nerve profiles in the ganglion, in the form of desmosome-like attachment plaques (Pl. 6). These are not identical with true epithelial desmosomes, but like them are symmetrical regions of attachment between profiles. They will be termed desmosomes for convenience. They are generally thought to have a purely mechanical function. These also were enumerated in the quantitative survey. Most of the intraganglionic desmosomes are placed entirely between nervous profiles. Their mean length is close to that of the shaft synapses (mean length of 129 neuronal desmosomes in normal and control ganglia, 0·241 μm). Occasional desmosomes are found between Schwann or satellite cells and nervous

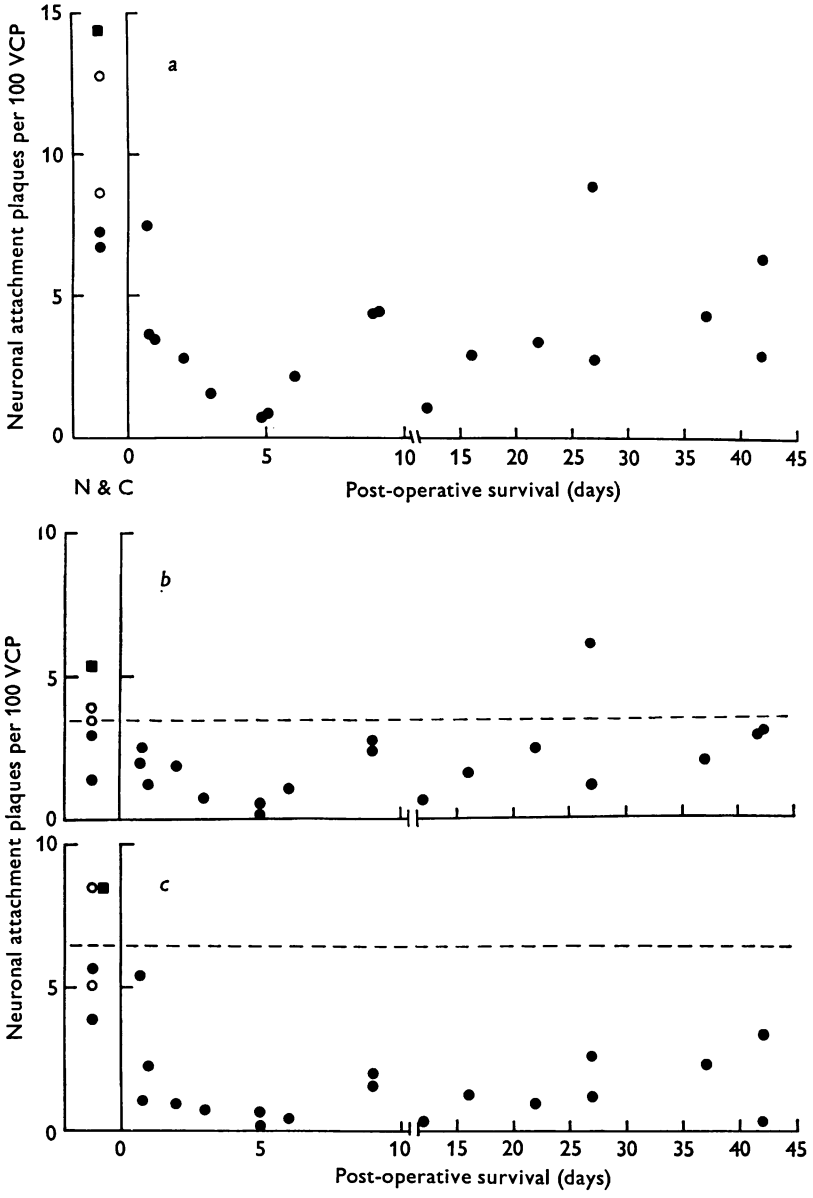
elements; the incidence of the latter was 5·7 % of 368 desmosomes involving nervous profiles in normal and control ganglia.

Of the desmosomes between nervous profiles alone, in normal and control ganglia, many are placed between purely post-synaptic elements, e.g. between adjacent dendrites, or between a dendrite and the cell body of a neurone (Pl. 6*c*). About two-thirds in all are between nerve profiles which are not presynaptic VCP (but could include non-terminal regions of preganglionic axons). The remaining one third involve vesicle-containing profiles of presynaptic type, and are mostly placed between presynaptic and post-synaptic profiles, where they would seem to supplement the attachment at the synapse (Pl. 6*a*, *b*). Desmosomes between nervous profiles of all types were found to become fewer after axotomy (Text-fig. 13*a*). The mean length of those surviving was less than in unoperated ganglia and tended to be rather less than that of the surviving spine synapses; the difference persisted at the longer survival intervals (mean length of 71 desmosomes, 27–42 d, 0·208 μm, i.e. 14·3 % less than in unoperated ganglia). The incidence of desmosomes between nervous and satellite profiles (not included in Text-fig. 13) was less affected, and these thus became relatively though not absolutely more frequent post-operatively (9·25 % of 357 desmosomes in experimental ganglia). Among the desmosomes between nervous profiles, it was those desmosomes involving non-VCP elements (largely post-ganglionic) which showed the greater change in incidence (Text-fig. 13*b*, *c*). It seemed likely that the post-ganglionic neurone was losing all its specializations for attachment, both synaptic and non-synaptic, and that the remaining component of attachment provided by the preganglionic nerve endings was insufficient to maintain apposition at the synapses.

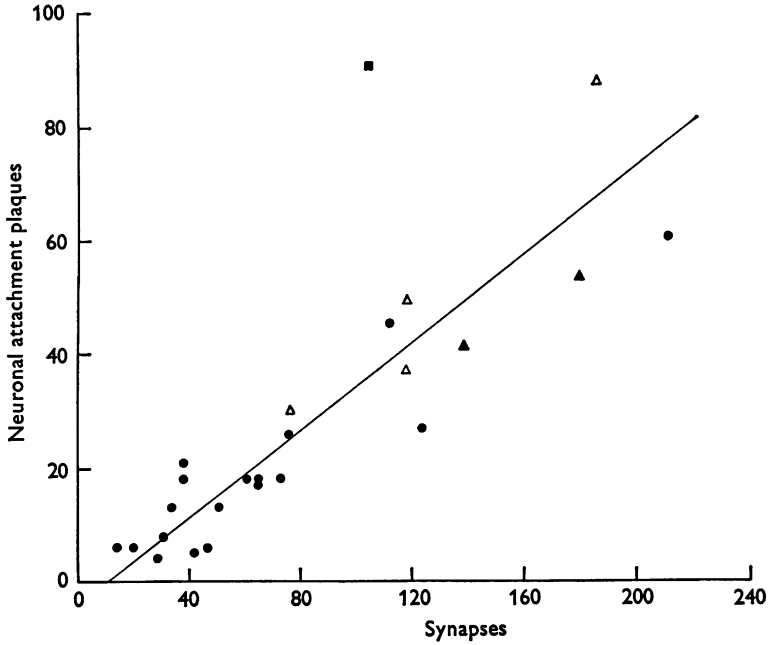
Correlation between desmosomes and synapses. The degree of resemblance between the post-operative incidence of neuronal desmosomes (between nerve profiles) and that of synapses prompted the experiment of plotting the total counts of these desmosomes against the total counts of synapses, adjusted in each case to a mean per ten grid squares (Text-fig. 14). This gave evidence of a highly significant correlation ($P < 0{\cdot}001$, Spearman rank correlation test), which is observable also for the normal and control ganglia. It would seem that an important function of the desmosome-like attachment plaques between nerve profiles in the ganglion may be the mechanical stabilization of the intraganglionic synapses, either directly or indirectly.

Final agent of separation of synapses

It seems probable that, following the loosening of the hold of the post-ganglionic neurone, the final separation may be effected by the satellite



Text-fig. 13. Desmosome-like attachments between neuronal profiles, plotted as incidences per 100 vesicle-containing profiles (VCP) for purposes of comparison between samples. *a*, Incidence of all neuronal desmosomes; *b* and *c*, respectively, incidences of desmosomes involving VCP and of those not involving VCP (i.e. probably mostly post-ganglionic to post-ganglionic). ○, Normal; ●, experimental and short-term control; ■, 27 d control. Further description in text.



Text-fig. 14. Correlation between desmosomes and synaspes. Numbers of desmosome-like attachments between nervous profiles (ordinate) have been plotted against numbers of synapses (abscissa) for all ganglia sampled, the figures having been adjusted to correspond to an area of 10 grid squares for those ganglia in which more or fewer squares were surveyed (cf. text). △, Normal; ●, experimental; ▲, short-term control; ■, 27 d control. Continuous line = regression line calculated for all points except the 27 d control: compensatory readjustments in this ganglion post-operatively, e.g. in response to an increased barrage of preganglionic impulses, may have led to the unusually high incidence of desmosomes here. The slope of the regression line is described by the equation: (no. of desmosomes) = $-4{\cdot}344 + 0{\cdot}388$ (no. of synapses). If ■ is excluded, the following coefficients of correlation (r) are obtained: for all remaining points, $r = 0{\cdot}912$; for experimental points alone, $r = 0{\cdot}922$; for normals and short-term controls (7 points) $r = 0{\cdot}804$.

or Schwann cell. Occasional evidence has been obtained that this may be so (Pl. 7). It appears that probing tongues of satellite cytoplasm may become insinuated between the synapsing elements, at a stage when the post-synaptic attachments have already disappeared but while the pre-synaptic dense material associated with the attachments (both synaptic and desmosome-like) is still recognizable; Pl. 7 shows examples of each type.

Re-attachment: possible reformation of synapses. A mechanism seems to exist whereby the detached presynaptic profile might become reattached to a suitable post-synaptic structure. Simple appositions of VCP to other

nerve profiles, without synapse or desmosome, were seen at all stages in these ganglia. Their incidence was recorded in the areas of ganglion sampled (Table 1). They showed initially rather consistent levels, followed by a dip towards the end of the first week post-operatively, when low values were observed in three of five ganglia sampled between 3 and 6 d, and a sharp rise shortly afterwards, seen in three of four ganglia sampled at 9 d and 12 d. The decrease occurs at a time when synapses and desmosomes are at their least numerous, and the rise coincides with the early stages of recovery (and with quite a high incidence of desmosomes in the 9 d ganglia, as shown in Text-fig. 13). Variable incidences were seen at longer survival intervals. It may be that there is a constant shifting of simple contact relationships between nervous elements within the ganglion, which could be brought about by movement and rearrangement of the Schwann cell processes, and possibly supplemented post-operatively by growth, e.g. by sprouting from detached VCP (although we have no direct evidence for this). Random appositions thus formed might be consolidated into synapses when the circumstances again become favourable.

DISCUSSION

These results indicate that there is a rapid and severe decline of transganglionic synaptic transmission to neurones of the superior cervical ganglion in the rat after their axons have been cut, and that this may be correlated with detachment of preganglionic, presynaptic nerve endings and loss of ultrastructurally identifiable post-synaptic sites.

The electrophysiological observations are in line with previous findings, notably those of Brown & Pascoe (1954), Acheson & Remolina (1955) and Hunt & Riker (1966), all of whom worked with sympathetic ganglia. Further to this, the present experiments establish the time of onset of the decline in transmission in the situation explored. Depression of transmission occurred early (within the first day post-operatively), but this was paralleled by an early onset of chromatolysis, as seen by light microscopy, in neurones having an appropriate distribution within the ganglion, and both are consistent with the close proximity of the lesion to the ganglion (within 2–3 mm). Matthews & Raisman (1972), after lesions slightly closer to the ganglion, detected the onset of chromatolysis in many neurones by 6 h after operation. From the data of Watson (1968), Cragg (1970) has concluded that the 'signal' for chromatolysis travels up the hypoglossal nerve in the rat from a point of injury at 4–5 mm per day; and Torvik & Heding (1969) found that actinomycin D could block the chromatolytic reaction in mouse facial motoneurones only if given within the first 9 h after they had cut the nerve at the stylomastoid foramen.

The changes which have been found in the present experiments are attributed to the axonal injury rather than to any interference with blood supply to the ganglion, because (1) not all the post-ganglionic axons were cut, and chromatolytic and normal neurones were correspondingly intermixed and juxtaposed with an appropriate distribution in the tissue; (2) the ganglion tissue did not show ultrastructurally anoxic changes such as have been described by Rouiller *et al.* (1971); (3) the response of the upper eyelid, mediated via the intact internal carotid nerve, was consistently normal post-operatively, although the preganglionic fibres concerned traverse the region of the ganglion which contains the injured neurones, and some of the neurones whose axons leave by the internal carotid nerve are also situated among these neurones (Matthews & Raisman, 1972; cf. Jacobowitz & Woodward, 1968).

Capacity of post-ganglionic axons to conduct. Brown & Pascoe (1954) and Acheson & Remolina (1955) established that the proximal parts of the post-ganglionic axons were still capable of conducting nerve impulses at a time after axotomy when the transganglionic transmission was greatly reduced, demonstrating that the defect was apparently selectively intraganglionic; transmission to uninjured post-ganglionic nerves was unaffected (Brown & Pascoe, 1954). The same general conclusions have been established in the present experiments (Text-figs. 4, 5). To the extent that sprouting of new small axons occurs along the length of the injured post-ganglionic axon bundle, by-passing some of its axons, a new population of more slowly-conducting fibres is added, along which the propagation of action potentials is rather less secure (Hopkins & Lambert, 1972) but there is no reason to suppose that propagation along the many larger surviving axons (e.g. Pl. 1) should have failed to occur. Matthews (1973) found that only about 0·2 mm of the post-ganglionic axons died and was resorbed in the early stages proximal to a ligature made with the same fine monofilament nylon as was used in the present experiments. Moreover, the experiment of placing an exactly comparable ligature upon the preganglionic sympathetic trunk, where a greater distance was available between the stimulating electrodes and the recording pipette, confirmed that these axons after 3 days were still able to conduct action potentials orthodromically up to a recording pipette placed similarly to that upon the post-ganglionic axons. It is likely that with a greater distance between stimulating and recording electrodes it would regularly be possible to demonstrate that the post-ganglionic axons behave in the same manner.

Duration of depression of transganglionic transmission. The period when transganglionic transmission to the injured neurones might fail appeared to be fully established by 3–6 days post-operatively. There was no definite evidence for any substantial recovery of transmission, but it is not certain

to what extent the presence of many small, immature axons in the injured post-ganglionic nerve bundle may have influenced the experimental results in the longer term, by giving smaller, slower action potentials. The circumvention of a cut or tight permanent ligature requires sprouting; and the maturation of axonal sprouts depends upon their re-establishing functional connexions (Weiss, Edds & Cavanaugh, 1945; Aitken, Sharman and Young, 1947; Hopkins & Lambert, 1972). The chances of the post-ganglionic axons finding their way back to their original sites within 70 d after a lesion involving much fibrosis might not be high (e.g. Tuckett, 1895; Langley, 1897; Butson, 1950). On the other hand, it is possible that alternative sites of termination, perhaps closer to the ganglion, may have been colonized (Langley, 1897), and that this may have induced the restoration of transganglionic transmission to some of the injured neurones. Acheson & Remolina (1955) found for cat inferior mesenteric ganglion that the trans- and post-ganglionic potentials became equal again (though about half the original) between 80 and 120 d post-operatively. Some of the injured neurones indeed fail to survive (cf. Acheson & Remolina, 1955; Torvik & Skjörten, 1971*a*); occasional profiles of dying neurones were seen in ganglia from 9 d onward. Lieberman (1971) comments on the rarity with which such profiles are actually encountered, although an appreciable number of chromatolytic neurones may die eventually. What appeared to be good cytological and ultrastructural recovery of neurones was, however, seen in many instances. It is concluded that in the absence of intracellular recording it is not possible to draw firm conclusions about the state of transganglionic synaptic transmission at the longer survival intervals.

Ultrastructural changes in intraganglionic synapses. The electron microscope findings are relatively clear-cut, particularly in the early stages. The rapid fall in the proportion of intraganglionic synapses, both in relation to the presynaptic type of vesicle-containing profile and to the number of neuronal nuclear profiles, closely followed the decline in transganglionic transmission and the development of chromatolysis. Synapses upon dendritic shafts of the chromatolytic neurones decreased in incidence sooner than those upon dendritic spines, and began to recover first. Synapses upon the cell bodies are too seldom encountered to be analysed in this way. It has earlier been shown that chromatolytic lower motor neurones in the central nervous system may bear fewer synapses on their cell bodies and basal dendrites than the normal, and may become enveloped instead by sheets of glial cytoplasm, identified as belonging to microglial cells (Blinzinger & Kreutzberg, 1968; Hamberger *et al.* 1970; Torvik & Skjörten, 1971*b*; Price, 1972; Kerns & Hinsman, 1973). Sumner & Sutherland (1973) have observed shrinkage of dendrites and a reduced

incidence of dendritic synapses in the case of rat hypoglossal motoneurones, but the electrophysiological observations of Kuno & Llinás (1970*a*, *b*) for axotomized cat spinal motoneurones would suggest that the remoter dendritic synapses may continue to function normally. They demonstrated a deficiency of synaptic input to the cell bodies of the chromatolytic motoneurones, which accords well with the ultrastructural findings for central motoneurones in general. Their experiments could not indicate what had happened to the nerve endings on the cell bodies, and the earlier ultrastructural evidence on this was negative: no degenerating synaptic endings were found in the vicinity of the chromatolytic motoneurones (Blinzinger & Kreutzberg, 1968; Hamberger *et al.* 1970; Torvik & Skjörten, 1971*b*). This was in harmony with the light microscopic observations of Barr (1940) and Schadewald (1940). Barnard (1938), however, reported 'progressive degenerative changes' in end feet upon axotomized motoneurones; and Sumner & Sutherland (1973) in their recent ultrastructural study have observed a long-lasting reduction and later recovery in mean widths of 'boutons', and confirmed that degenerating boutons were infrequent.

In the present study the discovery of vacant but normal-looking presynaptic terminals provides direct and positive evidence of separation of synapses from the post-synaptic site, without any major ultrastructural changes in the presynaptic element. Indeed, on occasion a synapse has been seen apparently in process of detachment, and the presynaptic profile does not appear abnormal in any significant respect (Pl. 7*b*). Thus, it appears that the synaptic attachment is in some way loosened, and the synapsing profiles become physically separated by a satellite cell process. The results strongly suggest that the primary change occurs in the post-ganglionic element of the synapse, i.e. in the chromatolytic neurone itself. Some of the specialized presynaptic regions of the ganglionic nerve endings would appear to be lost during or after detachment, but for various reasons already explored this loss may be more apparent than real. Brown & Pascoe (1954) found that there was a normal release of acetylcholine from a sympathetic ganglion 3 weeks after post-ganglionic axotomy had virtually abolished transganglionic transmission, and concluded that the preganglionic axons were still functioning normally. The specialized presynaptic sites of the detached nerve endings may persist for some time, possibly later serving in the reconstitution of synapses when receptive post-synaptic sites are again available, since the incidence of vacant presynaptic regions progressively falls at a stage when the incidence of synapses is beginning to recover.

The separated presynaptic nerve endings do not seem to degenerate and disappear in any large numbers, for at least the first week after

separation. They do, however, show a tendency to have a higher than normal incidence of cytoplasmic dense bodies, especially in the longer term (cf. Horoupian, Ghetti & Wisniewski, 1973). This and other evidence suggests a slow atrophy, shrinkage or resorption of those presynaptic endings which have not been afforded a chance to re-establish a synaptic connexion. This could be the result of withdrawal of an inductive influence derived from the post-ganglionic neurone: Black, Hendry & Iversen (1972) have shown that the presence of the post-ganglionic neurone is necessary for the normal maturation of the preganglionic nerve endings during development, and this may be so also for maintenance of the endings in the mature state.

Maintenance of adhesion at the synapse. The finding of detached presynaptic profiles with intact presynaptic specializations implies that these specialized regions *per se* do not provide an attachment adequate to hold them on to the post-synaptic element, and that the latter must play an important part in maintaining the synaptic contact. In the sympathetic ganglion the converse is also true: rather rapid separation occurs if the presynaptic element is damaged, in contrast with the situation at central nervous synapses (Quilliam & Tamarind, 1967; Raisman & Matthews, 1972). Further evidence that the primary cause for separation at the synapse comes from the injured post-ganglionic neurone in the present situation is provided by the almost complete failure to persist of any recognizable unoccupied post-synaptic membrane densities. After preganglionic denervation these are readily found in quite high incidence in the sympathetic ganglion (Taxi, 1964; Sotelo, 1968): there may be 10 or more of these per 200-mesh grid square (M. R. Matthews, unpublished); and they are found to persist also in other denervated sites in the nervous system (e.g. Westrum, 1966; Westrum & Black, 1971; Raisman & Field, 1973). The membrane-associated dense material of the post-synaptic site, and in particular that component which forms the external membrane coat, is thought to include attachment among its functions; and its loss suggests that the injured neurone ceases to produce or to maintain in its normal state the material requisite for attachment at its synaptic surface. The abrupt fall in the incidence of neuronal attachment plaques or desmosomes in the ganglion, coincidentally with the reduction of synapses, supports this possibility. The decline affects particularly those desmosomes involving profiles of post-ganglionic type, rather than the uninjured presynaptic vesicle-containing profiles. It appears that the chromatolytic post-ganglionic neurone is losing all its specializations for attachment. The close correlation between the incidence of synapses and that of desmosomes in both normal and operated ganglia suggests that the desmosome-like attachments may be an important adjunct in the

mechanical stabilization of synapses; indeed, they probably function also to maintain the steric stability of the ganglion as a whole, and their loss here might well be a prerequisite of the type of retraction of dendrites described by Sumner & Watson (1971) for axotomized hypoglossal neurones. Changes in proteins at the cell surface, or possibly in ionic binding, might be the initial cause of separation of both synapses and desmosomes: Borysenko & Revel (1973) have recently shown that 'labile' classes of desmosomes may be separated by treatment with EDTA, and more 'stable' types by trypsin or desoxycholate; and Pfenninger (1971) has found that synapses, though resistant to EDTA, may be separated by solutions of high ionic strength or by proteolytic enzymes. The reappearance and coupling up of neuronal desmosomes might be an important preliminary stage in the restoration of synaptic contacts; possibly the increased incidence of neuronal attachment plaques seen in the two 9-day experiments in the present series marks a transient phenomenon of this nature.

Satellite cells as agents of separation. Evidence such as that given in Pl. 7 suggests that probing extensions of the satellite cell may effect the final separation of the pre- and post-synaptic structures. Detached presynaptic profiles are often wrapped by one or more narrow lamellae of satellite cytoplasm (e.g. Pl. 4), which envelop the specialized presynaptic region, and these may be newly formed extensions of the satellite cells. There are various signs of vigorous activity of the satellite cells in these axotomized ganglia; not only do they form intrusions into the neurones but they also appear to pinch off and sequestrate, possibly to digest, bits of neuronal cytoplasm (Pl. 5*c*, *d*; Matthews & Raisman, 1972). It is known from tissue culture that the cytoplasm of ganglionic neuronal satellite cells in recent explants may make frequent active movements (Pomerat *et al.* 1966; Chamley *et al.* 1972). There is evidently a very closely balanced relationship between the neurones and their satellite cells; and the increased activity of the satellite cells might indeed be triggered by material released as the synapses and desmosomes begin to separate. A response of this nature has been envisaged by Watson (1972) for the glial cells of the central nervous system, some of which similarly show increased activity following axotomy of adjacent neurones. Multiplication of (micro-) glial cells (Kreutzberg, 1966; Sjöstrand 1966) and the formation of intrusions into neuronal cell bodies (Kirkpatrick 1968), in addition to the elaboration of processes which envelop the cell bodies, are typical of early stages of the chromatolytic reaction. Watson (1972) has shown an early metabolic response of astroglial cells in the hypoglossal nucleus following section of the hypoglossal nerve, and reports that this response coincides with the synaptic changes.

Re-formation of synapses; are these functional? Some later re-formation of synapses did apparently occur in the present experiments; the incidence of synapses per neuronal nuclear profile increased, and began by 42 d to approach the normal value, although almost half the neurones still showed chromatolysis and most of the others were in various stages of recovery from chromatolysis. There were by now more synapses than would have been expected for the small population of neurones which looked completely normal, and a proportion of these synapses is likely, therefore, to have been newly formed. It is not known whether these synapses represent a hyper-innervation of uninjured neurones, or a restoration of synaptic input to neurones recovering from chromatolysis, although on numerical grounds the latter seems more likely. Nor is it known whether all these synapses were functional: possibly those which restored contact with injured neurones were still too few to ensure security of transmission in response to single preganglionic stimuli, especially if the post-synaptic responses of the ganglionic neurones were still abnormal. Brimble, Wallis & Woodward (1972) found that there was a large subliminal fringe in the rabbit superior cervical ganglion, and the same may be true of the axotomized rat ganglion. There would seem to be no strong reason, however, for expecting such synapses as were formed to be non-functional in the long term: apart from a temporary phase during development of synapses, evidence for non-functional junctions of apparently normal ultrastructure has so far been derived from situations in which there is established an overlap between physiologically appropriate and inappropriate sources of innervation (motor end-plates upon fish muscle fibres: Marotte & Mark, 1970; Mark, Marotte & Mart, 1972). In the case of the neurone, separation of synapses during chromatolysis in the manner indicated by the present experiments makes it rather less likely that appositions with all the ultrastructural features of a synapse can exist stably in a completely non-functional state. It appears, however, that effects of any persisting synapses may be modified during chromatolysis. The membrane properties and excitability of the chromatolytic neurone show alterations, so that it may not fire action potentials, although some synaptic inputs may still demonstrably have access to it (Eccles *et al.* 1958; Kuno & Llinás 1970*a*, *b*). Brown & Pascoe (1954) showed that the responsiveness to intra-arterially injected acetylcholine of the sympathetic neurone is reduced during chromatolysis. If the neurone were able to stop responding altogether to activation from structurally intact synapses, then there would seem to be no advantage to be gained from physical separation at the synapse.

In addition to activation of the post-synaptic membrane, however, the functions of a synapse include inductive influences, which are mediated

probably by local uptake into the neurone of material released from the presynaptic endings. In the sympathetic neurone, these influences include the induction of increased activity of enzymes concerned in transmitter synthesis, both in the mature neurone (Molinoff & Axelrod, 1971) and during development (Black, Hendry & Iversen, 1971). This inductive influence is proportional to the amount of preganglionic impulse traffic (Thoenen, 1970), and since this may be expected to increase reflexly after post-ganglionic axotomy then it would follow that the pressure upon the post-ganglionic neurone to synthesize transmitter would be enhanced at a time when it has no longer any extraganglionic physiological outlet for transmitter, and has instead an urgent requirement to rebuild its axon. By withdrawal from the presynaptic endings the neurone may escape from their inductive influence, and from the expenditure of energy for the generation and conduction of impulses, and be better able to reorientate its productive capacity in the direction of axon regeneration. The output of transmitter into the axons of the injured neurones is indeed demonstrably reduced (e.g. Boyle & Gillespie, 1970), and material related to axon regeneration appears instead in the stumps of the damaged axons, which begin to sprout freely within a short time after the injury (Matthews, 1973).

In conclusion, these experiments indicate that an axotomized sympathetic neurone releases its hold upon most or all of its synaptic inputs and becomes physically separated from them during the period when it is reorganizing itself so as to regenerate its axon and re-establish functional connexions with the periphery. It is closely supported in this process by the neuronal satellite cells. The presynaptic elements initially are apparently unaltered in structure, but may show a consequential atrophy; they seem to remain capable of restoring synaptic contact as the neurones recover from chromatolysis.

This work was supported by a grant from the Medical Research Council. The authors wish to thank Mr C. P. Case for excellent technical assistance, including assistance with the quantitative electron microscopy, and for the electron micrographs of Pl. 6, and Mrs J. Brazier and Miss M. Langford for skilled photographic work.

REFERENCES

ACHESON, G. & REMOLINA, J. (1955). The temporal course of the effects of post-ganglionic axotomy on the inferior mesenteric ganglion of the cat. *J. Physiol.* **127**, 603–616.

AITKEN, J. T., SHARMAN, M. & YOUNG, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connexions. *J. Anat.* **81**, 1–22.

BARNARD, RUTH (1938). Experimental changes in end feet. *Anat. Rec.* **70**, suppl. 3, 6.

BARR, M. L. (1940). Axon reaction in motoneurons and its effect on the end bulbs of Held-Auerbach. *Anat. Rec.* **77**, 367–374.

BLACK, I. B., HENDRY, I. A. & IVERSEN, L. L. (1971). Trans-synaptic regulation of growth and development of adrenergic neurones in a mouse sympathetic ganglion. *Brain Res.* **34**, 229–240.

BLACK, I. B., HENDRY, I. A. & IVERSEN, L. L. (1972). The role of post-synaptic neurones in the biochemical maturation of presynaptic cholinergic nerve terminals in a mouse sympathetic ganglion. *J. Physiol.* **221**, 149–159.

BLINZINGER, K. & KREUTZBERG, G. (1968). Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z. Zellforsch. mikrosk. Anat.* **85**, 145–157.

BORYSENKO, J. Z. & REVEL, J. P. (1973). Experimental manipulation of desmosome structure. *Am. J. Anat.* **137**, 403–422.

BOYLE, F. C. & GILLESPIE, J. S. (1970). Accumulation and loss of noradrenaline central to a constriction on adrenergic nerves. *Eur. J. Pharmacol.* **12**, 77–84.

BRIMBLE, M. J., WALLIS, D. I. & WOODWARD, B. (1972). Facilitation and inhibition of cell groups within the superior cervical ganglion of the rabbit. *J. Physiol.* **226**, 629–652.

BROWN, G. L. & PASCOE, J. E. (1954). The effect of degenerative section of ganglionic axons on transmission through the ganglion. *J. Physiol.* **123**, 565–573.

BUTSON, A. R. C. (1950). Regeneration of the cervical sympathetic. *Br. J. Surg.* **38**, 223–239.

CAMPBELL, B. (1944) The effects of retrograde degeneration upon reflex activity of ventral horn neurons. *Anat. Rec.* **88**, 25–37.

CAMPBELL, B., MARK, V. H. & GASTEIGER, E. L. (1949). Alteration of neuron excitability by retrograde degeneration. *Am. J. Physiol.* **158**, 457–464.

CASTRO, F. DE (1932). Sympathetic ganglia, normal and pathological. In *Cytology and Cellular Pathology of the Nervous System*, vol. I, ed. PENFIELD, W., pp. 317–379. New York: Hoeber.

CHAMLEY, J. H., MARK, G. E. & BURNSTOCK, G. (1972). Sympathetic ganglia in culture. II. Accessory cells. *Z. Zellforsch. mikrosk. Anat.* **135**, 315–327.

CRAGG, B. G. (1970). What is the signal for chromatolysis? *Brain Res.* **23**, 1–21.

DOWNMAN, C. B. B., ECCLES, J. C. & MCINTYRE, A. K. (1953). Functional changes in chromatolysed motoneurones. *J. comp. Neurol.* **98**, 9–36.

DUNANT, Y. (1967). Organisation topographique et fonctionelle du ganglion cervical supérieur chez le rat. *J. Physiol., Paris*, **59**, 17–38.

DYCK, P. J. & HOPKINS, A. P. (1972). Electron microscopic observations on degeneration and regeneration of unmyelinated fibres. *Brain*, **95**, 223–234.

ECCLES, J. C., LIBET, B. & YOUNG, R. R. (1958). The behaviour of chromatolysed motoneurones studied by intracellular recording. *J. Physiol.* **143**, 11–40.

ELFVIN, L.-G. (1971*a*). Ultrastructural studies on the synaptology of the inferior mesenteric ganglion of the cat. I. Observations on the cell surface of the post-ganglionic perikarya. *J. Ultrastr. Res.* **37**, 411–425.

ELFVIN, L.-G. (1971*b*). Ultrastructural studies on the synaptology of the inferior mesenteric ganglion of the cat. III. The structure and distribution of the axo-dendritic and dendrodendritic contacts. *J. Ultrastruct. Res.* **37**, 432–448.

ERULKAR, S. D. & WOODWARD, J. K. (1968). Intracellular recording from mammalian superior cervical ganglion in situ. *J. Physiol.* **199**, 189–203.

GRILLO, M. A. (1966). Electron microscopy of sympathetic tissues. *Pharmac. Rev.* **18**, 387–399.

HAMBERGER, A., HANSSON, H.-A. & SJÖSTRAND, J. (1970). Surface structure of isolated neurons. Detachment of nerve terminals during axon regeneration. *J. cell Biol.* **47**, 319–331.

HOPKINS, A. P. & LAMBERT, E. H. (1972). Conduction in regenerating unmyelinated fibres. *Brain* **95**, 213–222.

HOROUPIAN, D. S., GHETTI, B. & WIŚNIEWSKI, H. M. (1973). Retrograde transneuronal degeneration of optic fibers and their terminals in lateral geniculate nucleus of rhesus monkey. *Brain Res.* **49**, 257–275.

HUNT, C. C. & RIKER, W. K. (1966). Properties of frog sympathetic neurons in normal ganglia and after axon section. *J. Neurophysiol.* **29**, 1096–1114.

JACOBOWITZ, D. & WOODWARD, J. K. (1968). Adrenergic neurons in the cat superior cervical ganglion and cervical sympathetic nerve trunk. *J. Pharmac. exp. Ther.* **162**, 213–226.

KERNS, J. M. & HINSMAN, E. (1973). Neuroglial response to sciatic neurectomy. II. Electron microscopy. *J. comp. Neurol.* **151**, 255–280.

KIRKPATRICK, J. B. (1968). Chromatolysis in the hypoglossal nucleus of the rat: an electron microscopic analysis. *J. comp. Neurol.* **132**, 189–212.

KREUTZBERG, G. W. (1966). Autoradiographische Untersuchung über die Beteiligung von Gliazellen an der axonalen Reaktion im Facialiskern der Ratte. *Acta neuropath.* **7**, 149–161.

KUNO, M. & LLINÁS, R. (1970*a*). Enhancement of synaptic transmission by dendritic potentials in chromatolysed motoneurones of the cat. *J. Physiol.* **210**, 807–821.

KUNO, M. & LLINÁS, R. (1970*b*). Alterations of synaptic action in chromatolysed motoneurones of the cat. *J. Physiol.* **210**, 823–838.

LANGLEY, J. N. (1897). On the regeneration of pre-ganglionic and of post-ganglionic visceral nerve fibres. *J. Physiol.* **22**, 215–230.

LIEBERMAN, A. R. (1971). The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int. Rev. Neurobiol.* **14**, 49–124.

MCINTYRE, A. K., BRADLEY, K. & BROCK, L. G. (1959). Responses of motoneurones undergoing chromatolysis. *J. gen. Physiol.* **42**, 931–958.

MARK, R. F., MAROTTE, L. R. & MART, P. E. (1972). The mechanism of selective reinnervation of fish eye muscles. IV. Identification of repressed synapses. *Brain Res.* **46**, 149–157.

MAROTTE, L. R. & MARK, R. F. (1970). The mechanism of selective reinnervation of fish eye muscles. II. Evidence from electron microscopy of nerve endings. *Brain Res.* **19**, 53–69.

MATTHEWS, M. R. (1971). Evidence from degeneration experiments for the preganglionic origin of afferent fibres to the small granule-containing cells of the rat superior cervical ganglion. *J. Physiol.* **218**, 95–96*P*.

MATTHEWS, M. R. (1973). An ultrastructural study of axonal changes following constriction of postganglionic branches of the superior cervical ganglion in the rat. *Phil. Trans. R. Soc.* B **264**, 479–508.

MATTHEWS, M. R. (1974). Ultrastructure of ganglionic junctions. In *The Peripheral Nervous System*, ed. HUBBARD, J. I. New York: Plenum Publishing Corp.

MATTHEWS, M. R. & NASH, J. R. G. (1970). An efferent synapse from a small granule-containing cell to a principal neurone in the superior cervical ganglion. *J. Physiol.* **210**, 11–14*P*.

MATTHEWS, M. R. & NELSON, V. (1973). Ultrastructural changes at the synapse associated with depression of synaptic transmission in rat superior cervical ganglion after injury of post-ganglionic axons. *J. Physiol.* **234**, 36–37*P*.

MATTHEWS, M. R. & OSTBERG, A. (1973). Effects of preganglionic nerve section upon the afferent innervation of the small granule-containing cells in the rat superior cervical ganglion. *Acta physiol. pol.* **24**, 215–223.

MATTHEWS. M. R. & RAISMAN, G. (1972). A light and electron microscopic study of the cellular response to axonal injury in the superior cervical ganglion of the rat. *Proc. R. Soc.* B **181**, 43–79.

MOLINOFF, P. B. & AXELROD, J. (1971). Biochemistry of catecholamines. *A. Rev. Biochem.* **40**, 465–500.

PERRI, J., SACCHI, O. & CASELLA, C. (1970). Electrical properties and synaptic connections of the sympathetic neurons in the rat and guinea-pig superior cervical ganglion. *Pflügers Arch.* **314**, 40–54.

PFENNINGER, K. H. (1971). The cytochemistry of synaptic densities. II. Proteinaceous components and mechanisms of synaptic connectivity. *J. Ultrastruct. Res.* **35**, 451–475.

POMERAT, C. M., HENDELMAN, W. J., RAIBORN, C. W. & MASSEY, J. F. (1967). Dynamic activities of nervous tissue *in vitro*. In *The Neuron*, ed. HYDÉN, H., pp. 119–178. Amsterdam: Elsevier.

PRICE, D. L. (1972). The response of amphibian glial cells to axonal transection. *J. Neuropath, exp. Neurol.* **31**, 267–277.

QUILLIAM, J. P. & TAMARIND, D. L. (1967). Ultrastructural changes in the superior cervical ganglion of the rat following preganglionic denervation. *J. Physiol.* **189**, 13–15*P*.

RAISMAN, G. & FIELD, P. M. (1973). A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res.* **50**, 241–264.

RAISMAN, G. & MATTHEWS, M. R. (1972). Degeneration and regeneration of synapses. In *The Structure and Function of Nervous Tissue*, vol. IV, ed. BOURNE, G. H., pp. 61–104. New York: Academic Press.

ROUILLER, Ch., NICOLESCU, P., ORCI, L. & RUFENER, C. (1971). The effect of anoxia on the ultrastructure of the superior cervical ganglion of the rat *in vitro*. *Virchows Arch.* (Abt. B, Zellpath.) **7**, 269–292.

SCHADEWALD, M. (1940). Number and size of the boutons about the cells of the trochlear and abducens nuclei in the cat after unilateral section of the corresponding nerves. *Anat. Rec.* **76**, 48–49.

SJÖSTRAND, J. (1966). Studies on glial cells in the hypoglossal nucleus of the rabbit during nerve regeneration and morphological changes in glial cells during nerve regeneration. *Acta physiol scand.* **67**, suppl. 270, 1–43.

SOTELO, C. (1968). Permanence of postsynaptic specializations in the frog sympathetic ganglion cells after denervation. *Expl Brain Res.* **6**, 294–305.

STREIT, P., AKERT, K., SANDRI, C., LIVINGSTON, R. B. & MOOR, H. (1973). Dynamic ultrastructure of presynaptic membranes at nerve terminals in the spinal cord of rats. Anaesthetized and unanaesthetized preparations compared. *Brain Res.* **48**, 11–26.

SUMNER, B. E. H. & SUTHERLAND, F. I. (1973). Quantitative electron microscopy on the injured hypoglossal nucleus in the rat. *J. Neurocytol.* **2**, 315–328.

SUMNER, B. E. H. & WATSON, W. E. (1971). Retraction and expansion of the dendritic tree of motor neurones of adult rats induced *in vitro*. *Nature, Lond.* **233**, 273–275.

TAMARIND, D. L. & QUILLIAM, J. P. (1971). Synaptic organization and other ultrastructural features of the superior cervical ganglion of the rat, kitten and rabbit. *Micron* **2**, 204–234.

TAXI, J. (1961). Etude de l'ultrastructure des zones synaptiques dans les ganglions sympathiques de la grenouille. *C. r. hebd. Séanc. Acad. Sci., Paris* **252**, 174–176.

TAXI, J. (1964). Etude de certaines synapses interneuronales du système nerveux autonome. *Acta neuroveg.* **26**, 360–372.

TAXI, J., GAUTRON, J. & L'HERMITE, P. (1969). Données ultrastructurales sur une éventuelle modulation adrénergique de l'activité du ganglion cervical supérieur du rat. *C. r. hebd. Séanc. Acad. Sci., Paris* D **269**, 1281–1284.

THOENEN, H. (1970). Induction of tyrosine hydroxylase in peripheral and central adrenergic neurones by cold-exposure of rats. *Nature, Lond.* **288**, 861–862.

TORVIK, A., & HEDING, A. (1969). Effect of actinomycin D on retrograde nerve cell reaction. Further observations. *Acta neuropath.* **14**, 62–71.

TORVIK, A. & SKJÖRTEN, F. (1971*a*). Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. I. Changes in the nerve cell cytoplasm. *Acta neuropath.* **17**, 248–264.

TORVIK, A. & SKJÖRTEN, F. (1971*b*). Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. II. Changes in the glial cells. *Acta neuropath.* **17**, 265–282.

TUCKETT, I. L. (1895). On the structure and degeneration of non-medullated nerve fibres. *J. Physiol.* **19**, 267–311.

WATSON, W. E. (1968). Observations on the nucleolar and total cell body nucleic acid of injured nerve cells. *J. Physiol.* **196**, 655–676.

WATSON, W. E. (1972). Some quantitative observations upon the responses of neuroglial cells which follow axotomy of adjacent neurones. *J. Physiol.* **225**, 415–435.

WESTRUM, L. E. (1966). Electron microscopy of degeneration in the prepyriform cortex. *J. Anat.* **100**, 683–685.

WESTRUM, L. E. & BLACK, R. G. (1971). Fine structural aspects of the synaptic organization of the spinal trigeminal nucleus (pars interpolaris) of the cat. *Brain Res.* **25**, 265–288.

WEISS, P., EDDS, M. V. & CAVANAUGH, M. (1945). The effect of terminal connections on the caliber of nerve fibers. *Anat. Rec.* **92**, 215–233.

EXPLANATION OF PLATES

PLATE 1

Electron micrographs showing post-ganglionic axons of the external carotid nerve just before its exit from the superior cervical ganglion. Scale bars indicate 1 μm.

a, Normal ganglion; each nerve fibre is individually wrapped by its Schwann cell and the range of fibre diameters is roughly twofold. *b*, Ganglion 9 d after division of external carotid nerve about 3 mm from the ganglion. The nerve fibres shown in *b* include some of normal diameter and many small, newly formed fibres, which often share the same invaginations into the Schwann cells and are seldom individually wrapped; the range of fibre diameters is roughly eightfold, and the fibre diameter histogram would be distinctly bimodal. Apart from their variation in size the ultrastructural appearance of these nerve fibres is within normal limits.

N, nucleus of Schwann cell; c, cytoplasm of Schwann cells. At × a Schwann cell shows dense floccular material within a cistern of the granular endoplasmic reticulum; such material increases while the Schwann cells are accommodating newly formed nerve fibres.

PLATE 2

Light micrographs of 1–2 μm sections of resin-embedded material, stained with methylene blue and Azur II. Scale bars indicate 50 μm.

a, Normal ganglion; the neurones have spherical and approximately central nuclei, and their cytoplasm contains many large flakes of deeply staining Nissl material, especially in its deeper regions. The intercellular spaces are relatively open. *b*, Ganglion 2 d after crushing and ligation of external and internal carotid nerve branches. All the neuronal nuclei shown are now eccentric and some are distorted; the Nissl material is displaced away to the cell periphery from the deeper cytoplasm,

which begins to show many cytoplasmic dense bodies. *c*, Ganglion 6 d after ligation and division of post-ganglionic branches. The neuronal nuclei show extreme displacement and many are flattened or indented. Numerous large, deeply staining cytoplasmic dense bodies lie in the deeper part of the cytoplasm in many neurones. Some of the intervals between the neurones are occupied by enlarged bundles of intraganglionic nerve fibres (f).

Plate 3

Examples of types of synapses found upon post-ganglionic neurones in rat superior cervical ganglion. All are from experimental ganglia, but are within the range of the normal in appearance. These presynaptic profiles all show the characteristics of preganglionic nerve endings. Scale bars represent 0·3 μm.

a, Synapse upon dendritic shaft. Among the small synaptic vesicles which are crowded toward the specialized region of membrane in the presynaptic element is a single larger dense-cored vesicle (arrow); other dense-cored vesicles lie further away, at the periphery of the mass of small vesicles. A well defined post-synaptic thickening or layer of dense material is present in the cytoplasm immediately beneath the post-synaptic membrane, and further dense material is present in the synaptic cleft. m = mitochondria. All these features are typical of preganglionic synapses upon the post-ganglionic neurones.

b, Synapse upon the base of a short spine-like process from a dendrite. Arrow indicates a coated pit in the post-synaptic element, encroaching on the margin of the synaptic zone; these are not infrequently seen.

c, Synapse upon a very short spine-like projection from the cell body of a post-ganglionic neurone. Such synapses are relatively infrequent (see text). Arrows indicate coated vesicles in the base of the projection; numerous vesicles and two multivesicular bodies lie in the adjacent neuronal cytoplasm. Primary fixation in this ganglion was by aldehydes; all other micrographs show material fixed by primary fixation in buffered osmium tetroxide.

(*a*, 17½ h after division of post-ganglionic axons; *b* and *c* respectively, 2 d and 15 d after crush and ligation of post-ganglionic axons.)

Plate 4

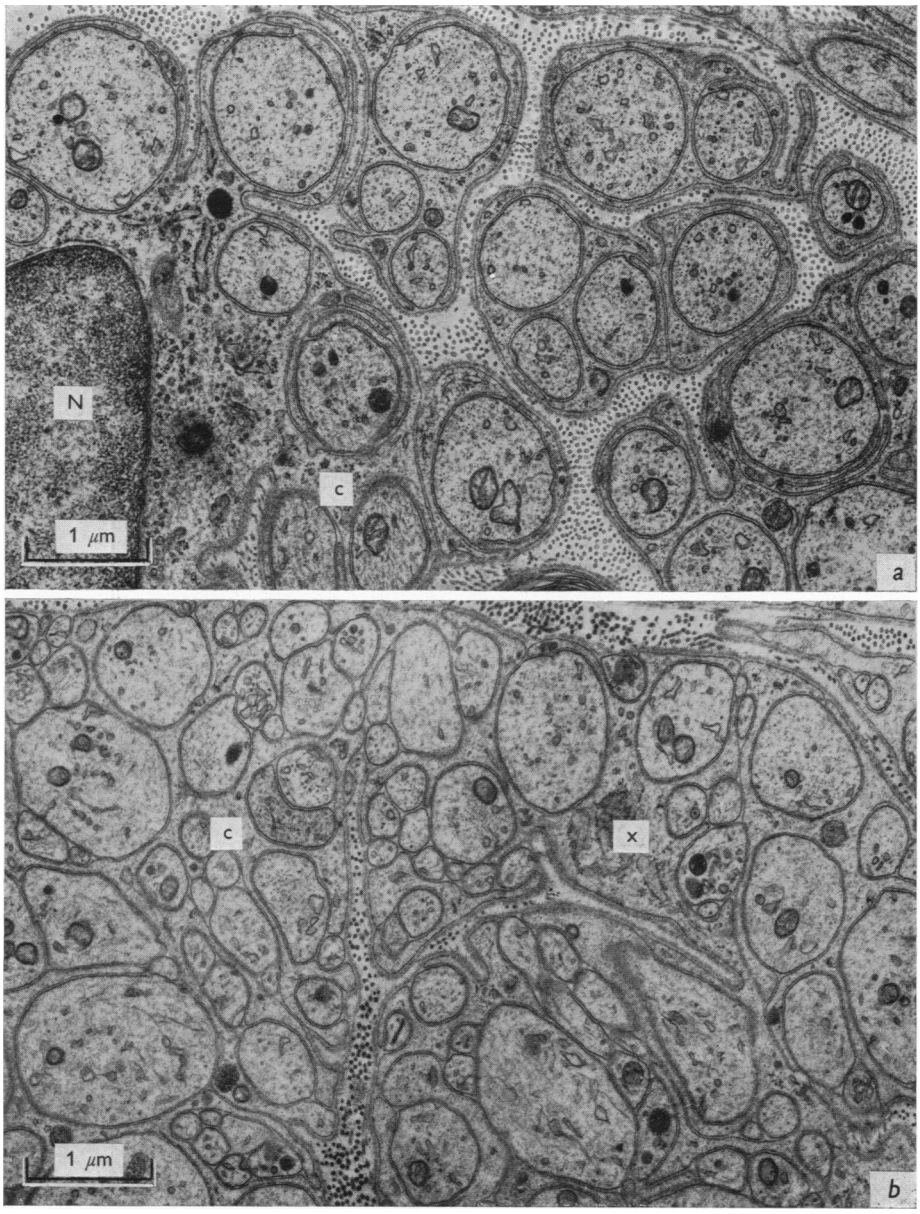
Detached presynaptic profiles found in ganglia after injury of post-ganglionic axons; *a*, *b*, at 2 d; *c*, at 9 d; *d*, at 5 d post-operatively. Scale bars indicate 0·5 μm in *a* and *d*, 0·3 μm in *b* and *c*. The presynaptic profiles show well defined presynaptic specializations, with clustering of vesicles associated with dense material at the surface membrane (with separate dense projections in *c*; cf. Pl. 3 *c*). The profile shown in b has a large dense-cored vesicle in the vesicle cluster at the membrane (arrow). Each profile is enveloped in satellite cell cytoplasm (s), and the post-synaptic nervous element of the synapse is lacking; dense material is, however, distinctly present in the cleft between the presynaptic profile and the satellite cell membrane in the region of the membrane specializations in *b* to *d*. There is no definite evidence of any reciprocating membrane specialization in the underlying satellite cell cytoplasm. Satellite cells are nonetheless capable of forming specialized surface attachments: the upper part of *c* shows a short attachment between the two layers of the mesaxon, just before it opens out to enclose the presynaptic profile. Both *c* and *d* enable positive identification of the satellite cell cytoplasm as being the layer which enwraps the nervous elements and separates them across a basement membrane from the connective tissue spaces of the ganglion; in addition, *a* and *c* show that the specialized presynaptic region may be faced by one or more very narrow lamellae of satellite cytoplasm, which could be newly formed.

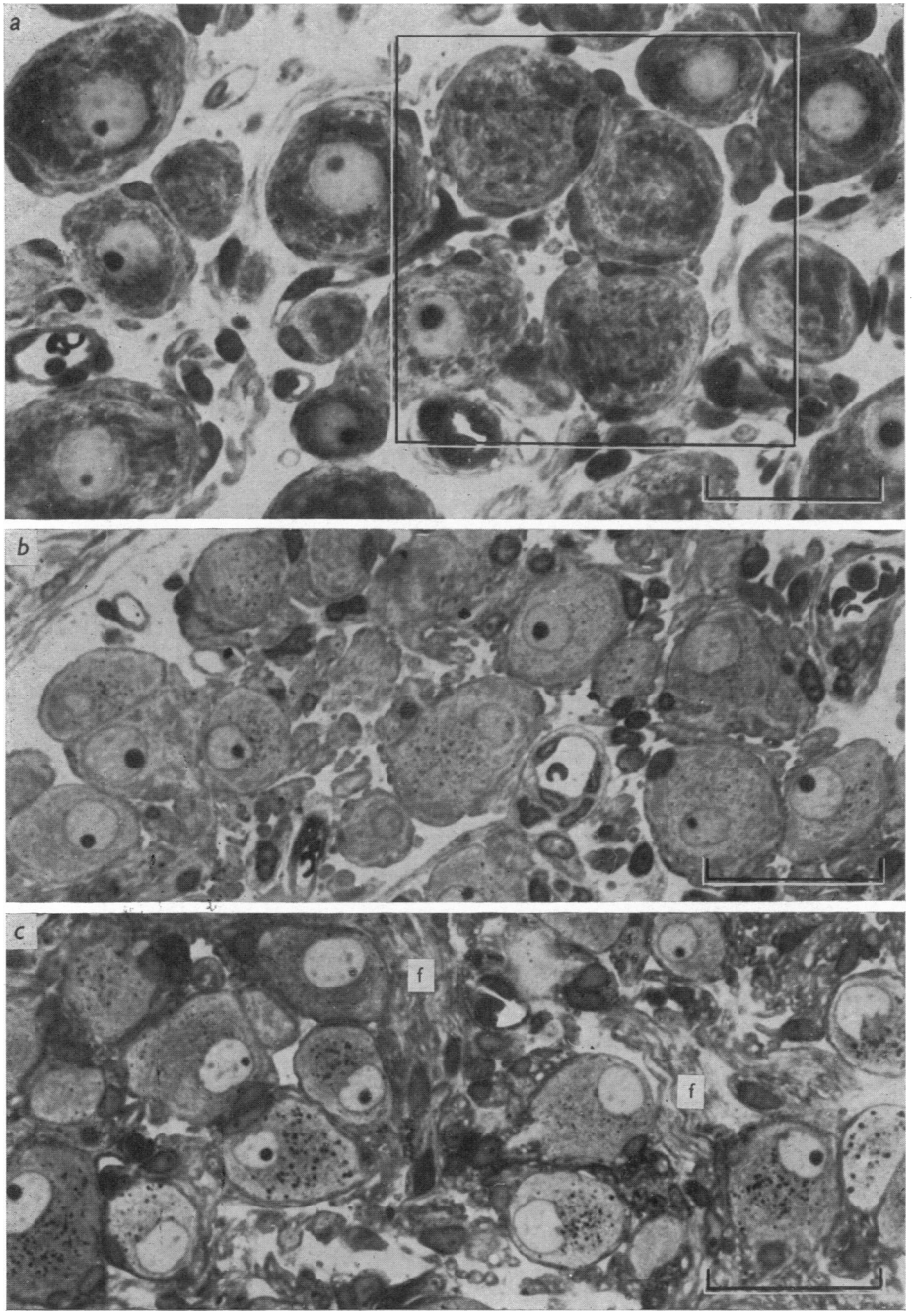
Plate 5

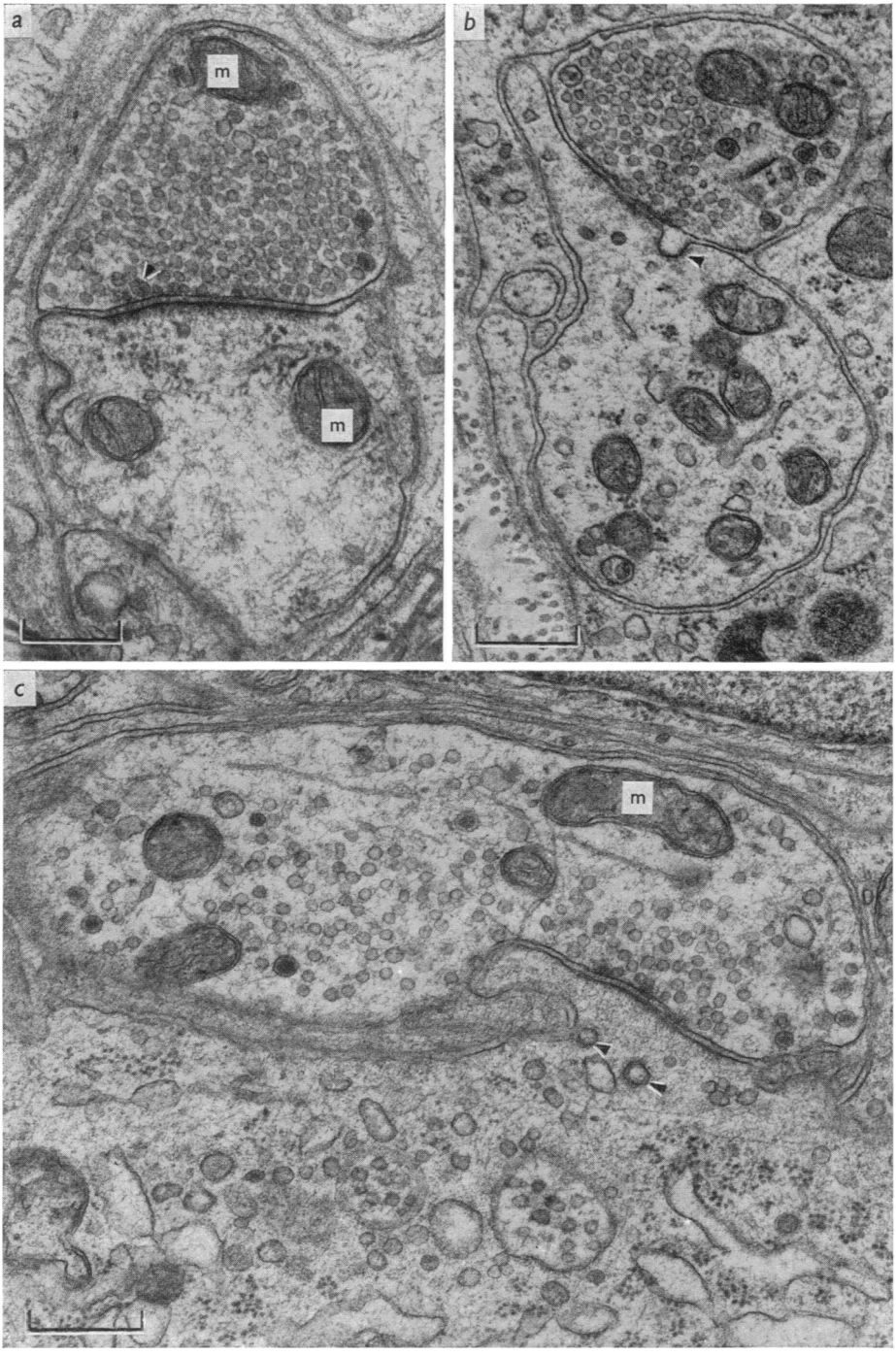
a, Area of ganglion 5 d after post-ganglionic axotomy showing, within the same satellite cell sheath, an intact synapse on to a small dendritic shaft (on the left), and a 'detached' presynaptic profile with unapposed synaptic specialization (on the right). In this example the length of the vacant presynaptic specialization is about half that of the presynaptic specialization at the synapse. Also shown are two vesicle-containing profiles of presynaptic type without obvious membrane specializations in the plane of section (left and centre). The larger of these contains a cytoplasmic dense body (arrow). Scale bar indicates 0·5 μm. *b–d*, Debris in satellite cells and satellite cell reactions. *b*, Lower left, rare instance of degenerating vesicle-containing profile, adjacent to one of normal appearance (above) in the same satellite cell; this is a ganglion 2 d after post-ganglionic axotomy, but similar degenerating profiles are sometimes seen in normal ganglia. Scale bar, 0·3 μm. Inset, irregularly stellate droplet of lipid-like material in satellite cell, 5 d post-operatively (cf. Table 1). Scale bar, 0·1 μm. *c*, intrusion of satellite cell cytoplasm (s) into the cytoplasm of a neurone (n), from a ganglion 17½ h after post-ganglionic axotomy. Scale bar, 0·5 μm. In the neuronal cytoplasm, various vesicles and sacs of endoplasmic reticulum are applied to the surface membrane bordering the intrusion. Within the satellite cytoplasm rings and arcs of vesicles partly encircle profiles of condensed-looking cytoplasm, almost certainly of neuronal origin; darkening of these profiles, compaction of their organelles and narrowing of the interval between their surface membranes and the enveloping satellite, relatively to that between the satellite and surrounding neurone, suggest that they are beginning to degenerate and to be digested by the active-looking satellite cytoplasm. Arrows indicate fragments in a more advanced stage of digestion. Degenerating fragments were especially numerous in this ganglion (see Table 1). *d*, Similar intrusion 2 d after axotomy showing continuity through region s with the enveloping layer of satellite cytoplasm which separates the neurone (n) from the connective tissue space of the ganglion. Scale bar, 0·5 μm. A second intrusion or a deeper level of the same intrusion (centre and right) surrounds and appears to be pinching off a spur-like fragment of cytoplasm from the neurone; this could be the manner of sequestration of fragments prior to digestion. The deeper intrusion contains a disorganized mass of cytoplasmic debris; a smaller item of debris, resembling a late phagosome, is seen just beneath the surface of the satellite cell, bordering on s.

Plate 6

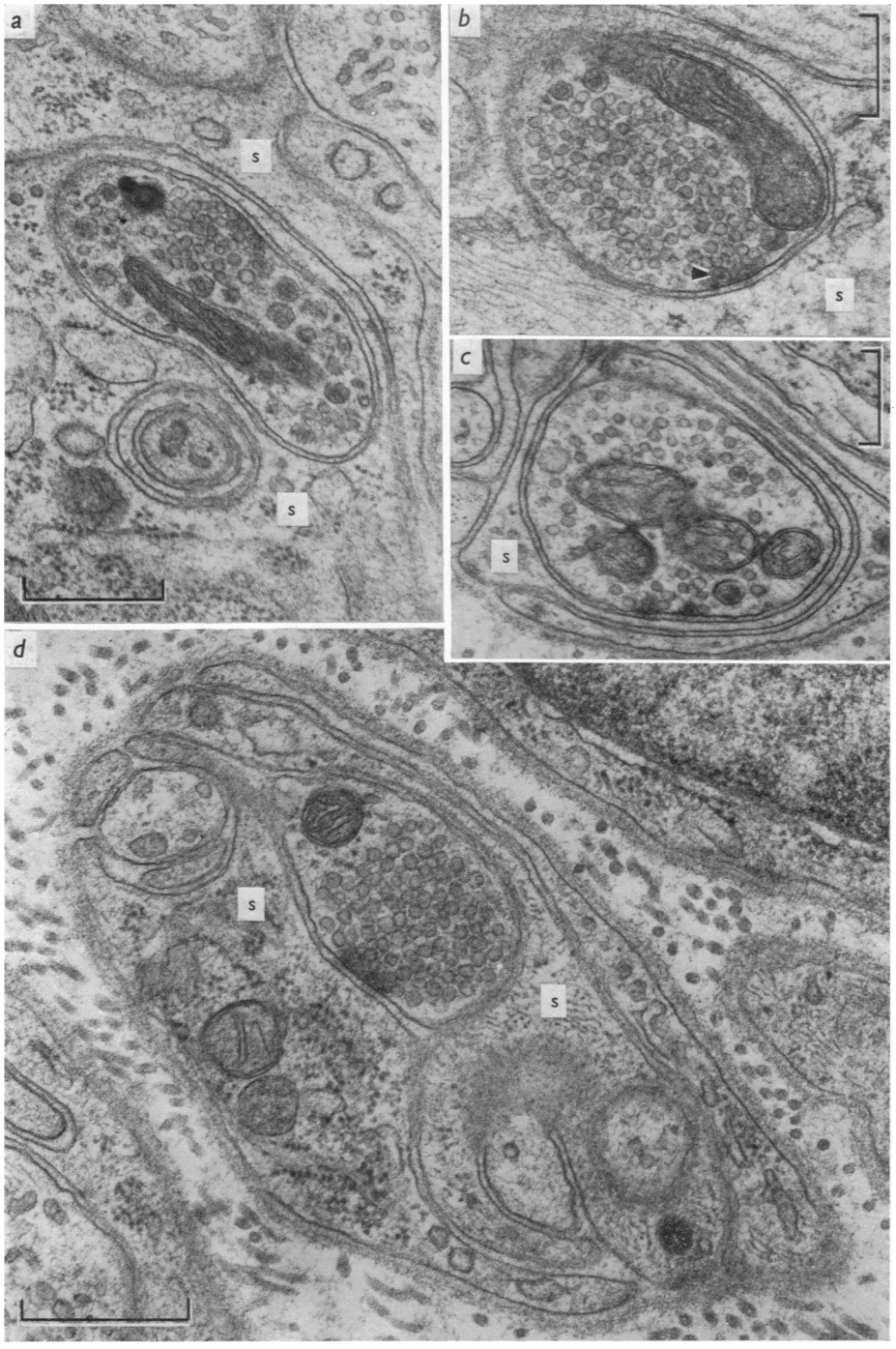
Desmosome-like attachment plaques between nervous profiles within the ganglion. *a*, 3 d control ganglion; *b*, *c*, normal ganglia. Scale bars 0·3 μm (*a* and *c*), 0·5 μm (*b*). These attachments involve symmetrical regions of aggregation of intracytoplasmic dense material along the apposed membranes, with further dense material between the membranes. About one third of them occur between the presynaptic type of vesicle-containing profile (VCP) and other profiles, which may be dendritic shafts (d, in *a*), dendritic spines (middle part at least of attachment to spine in *b*), cell bodies or sometimes other VCP (not shown). Desmosomes involving VCP would appear to be rather directly accessory to synaptic attachments. *c* Shows a desmosome-like attachment between a small nervous profile, probably a dendrite, and a neuronal soma, into which it is embedded; two-thirds of the neuronal desmosomes in the ganglion are of this general type, i.e. between apparently post-ganglionic profiles.

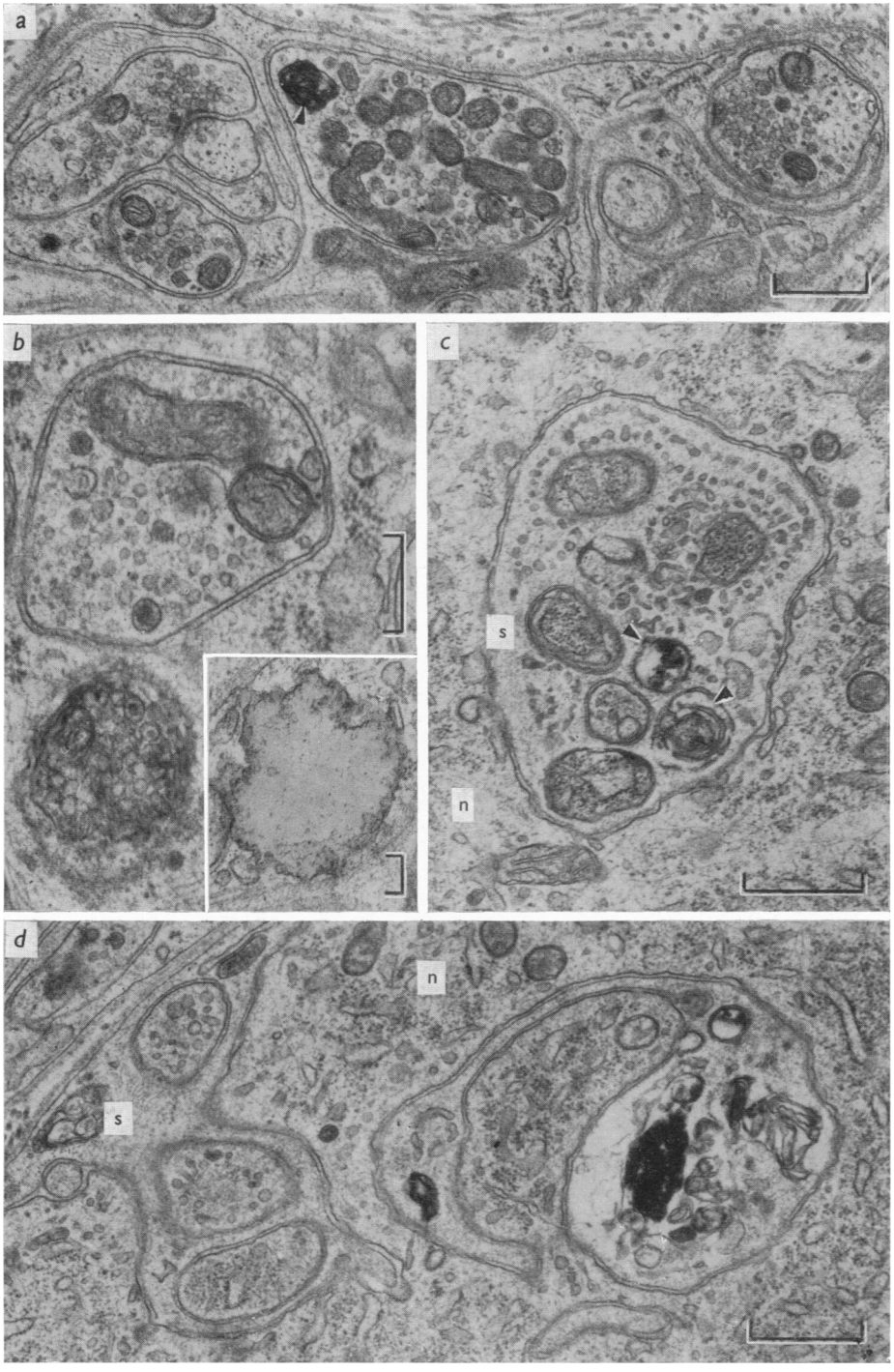

N
c
1 μm
a
c
x
1 μm
b


a
b
c
f
f

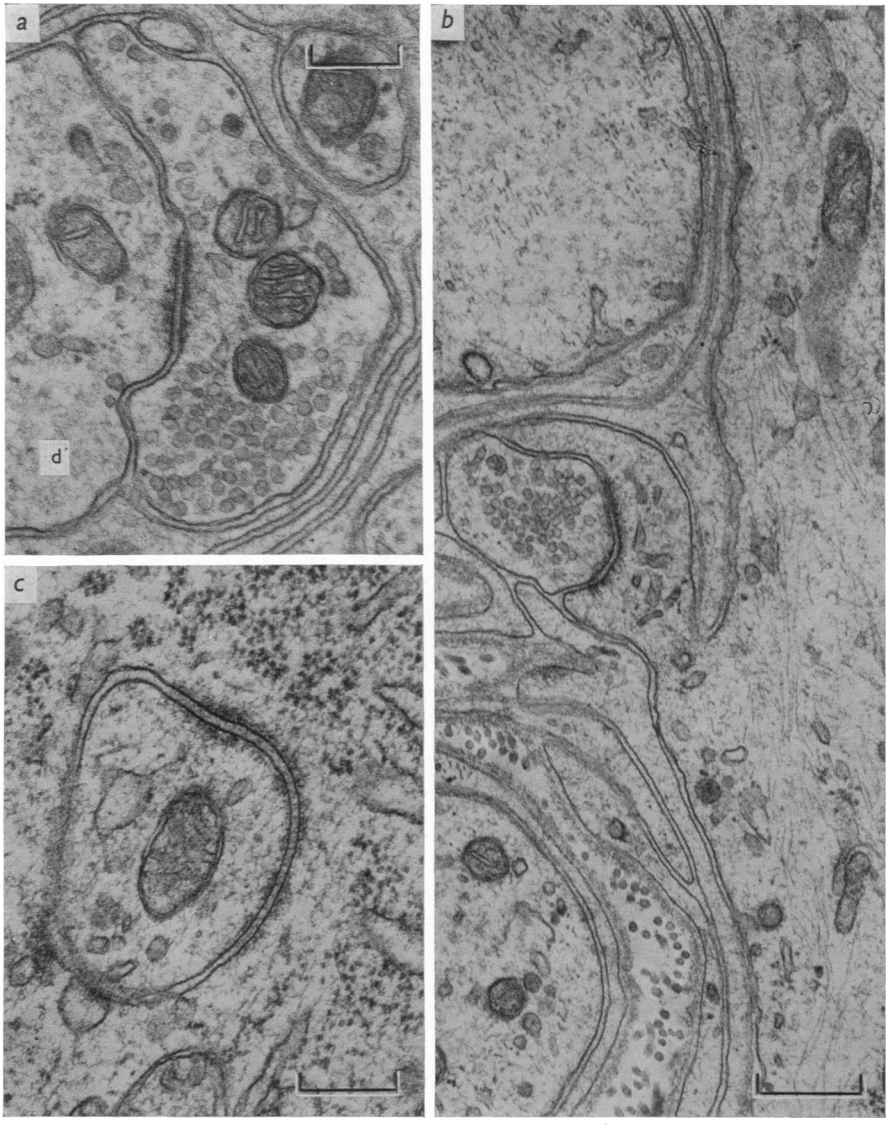


MARGARET R. MATTHEWS AND VICTORIA H. NELSON


a
s
s
b
s
c
s
d
s
s



MARGARET R. MATTHEWS AND VICTORIA H. NELSON


a
d
b
c

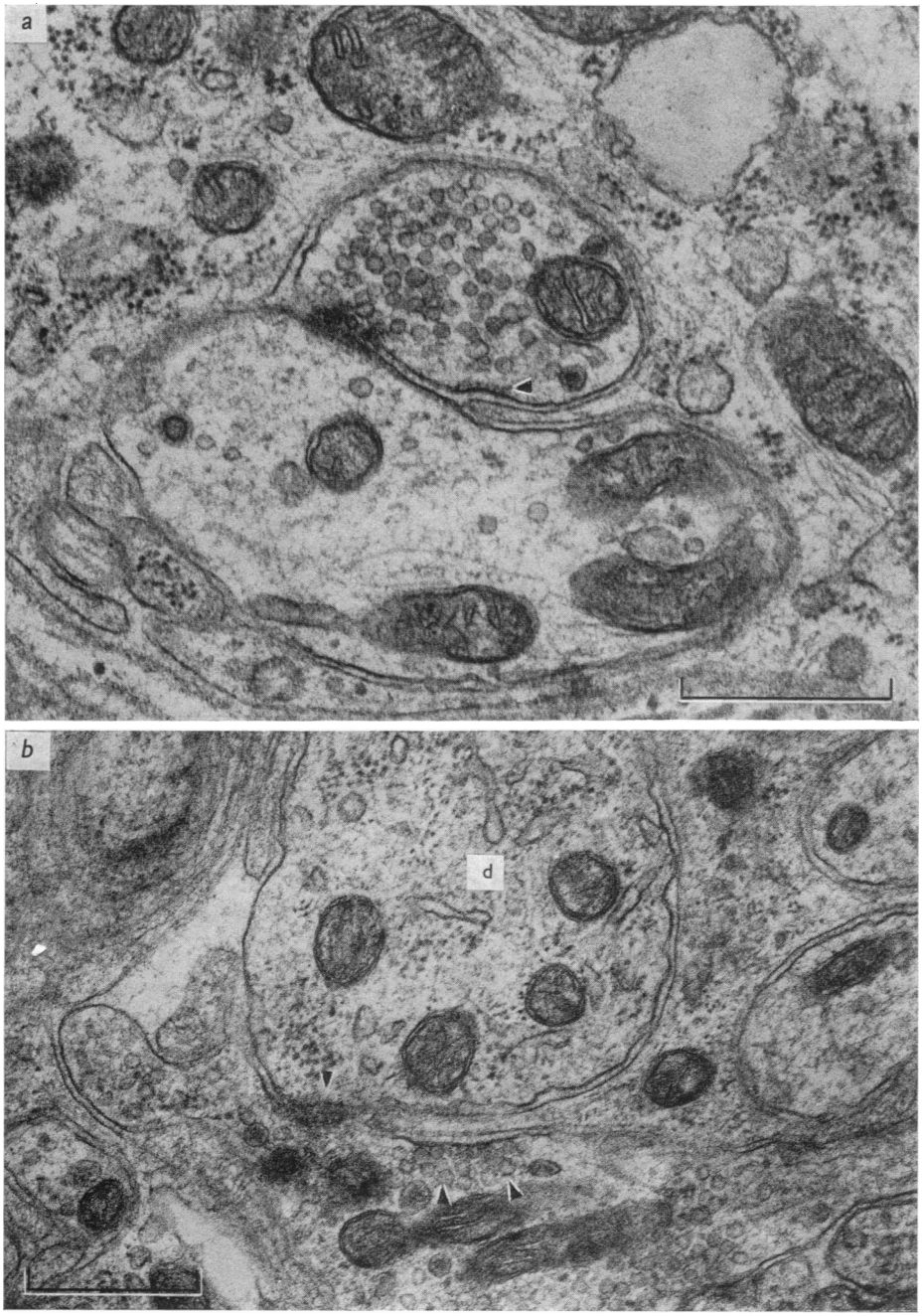

a
b
d

PLATE 7

Evidence suggesting that the detachment of synapses is preceded by loss of membrane specializations for attachment in the post-synaptic element, and that the final separation may be effected by the insinuation of a process of the satellite cell. Scale bars, 0·5 μm.

a, Synapse at which the presynaptic profile shows a band of dense material (arrow) applied to its membrane over the apex of a slender tongue of satellite cytoplasm, which intervenes between it and the post-synaptic profile (probably a dendrite). No corresponding band is seen in the post-synaptic element. This suggests a desmosome of which the post-synaptic half has disappeared, allowing the satellite cell to separate the pre- and post-synaptic profiles. This is from a ganglion 27 d after operation, but 71 % of its neurones were still chromatolytic. Possible alternative explanation: the membrane thickening in the presynaptic profile *might* be an incipient coated pit, but this seems unlikely as it shows very little evidence of indentation.

b, Synapse partly attached (single arrow, left-hand side), partly detached (two arrows, right-hand side); a slender tongue of satellite cytoplasm intervenes. Four d post-axotomy. Presynaptic element below. In the detached moiety of the synapse there is a well defined vacant presynaptic thickening, but there is no evidence of any vacated post-synaptic thickening in the corresponding zone of the post-synaptic profile (a large dendrite). The attached region however still shows some evidence of a post-synaptic thickening, i.e. dense material apposed to the post-synaptic membrane.