J. Physiol. (1969), **200**, *pp.* 745–762
With 6 plates and 6 text-figures
Printed in Great Britain

THE RE-DISTRIBUTION OF CYTOCHROME OXIDASE, NORADRENALINE AND ADENOSINE TRIPHOSPHATE IN ADRENERGIC NERVES CONSTRICTED AT TWO POINTS

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(*Received* 14 *August* 1968)

SUMMARY

1. The experiments correlate certain changes in the ultrastructure of cat hypogastric nerves constricted at two points with the distribution of a mitochondrial enzyme (cytochrome oxidase), noradrenaline (stored in some of the vesicles with an electron dense core, i.e. granular vesicles) and adenosine triphosphate (ATP) (present in noradrenaline storage granules, mitochondria and the soluble fraction of the axon).

2. Noradrenaline (NA) and granular vesicles accumulated proximal but not distal to both constrictions. The total amount of NA and the concentration of granular vesicles above the first constriction was greater than that present in a similar piece of normal nerve, indicating that the cell body was continuing to produce the transmitter despite injury to its axon. The granular vesicles proximal to the first constriction were found in swollen or distorted axons and in new axonal outgrowths. It was concluded that the movement of NA in these constricted nerves was only centrifugal in direction.

3. Mitochondria and cytochrome oxidase accumulated on both sides of the two constrictions, indicating a bi-directional movement of mitochondria in the damaged axons. The possibility that some of the increase in the cytochrome oxidase could be related to an increase in the number of mitochondria in cells other than neurones is considered.

4. The adenosine triphosphate content increased on both sides of the two constrictions. This increase developed more slowly and was less marked than that of the other two substances.

5. It was concluded that (*a*) there was a close correlation between the behaviour of noradrenaline and granular vesicles and between cytochrome oxidase and mitochondria, (*b*) the dense cored vesicles and the mitochondria moved independently of one another and at different rates after

constriction of non-myelinated axons, (*c*) while some of the changes may be attributed to an obstruction to the free movement of axoplasm others may be due to an active reaction to axonal injury, and (*d*) localized intra-axonal synthesis of noradrenaline and cytochrome oxidase did not occur between the two constrictions.

INTRODUCTION

Previous studies on post-ganglionic sympathetic nerves constricted at one or two points have shown a close correlation between the location of fluorescent material derived from noradrenaline (NA), as demonstrated with the technique described by Falck (1962), and vesicles with an electron-dense core, i.e. granular vesicles (Kapeller & Mayor, 1967; Mayor & Kapeller, 1967). In these experiments the fluorescent material and the granular vesicles generally accumulated proximal but not distal to the constrictions. Similar accumulations of fluorescent material proximal to a lesion in post-ganglionic adrenergic nerves have been described by other workers (Dahlström & Fuxe, 1964; Blümcke & Niedorf, 1965; Dahlström, 1965; Eränkö & Härkönen, 1965; Hamberger & Norberg, 1965; Dahlström, 1966, 1967). Quantitative estimations of the amount of NA accumulating in these nerves have also been carried out by Dahlström (1966, 1967), Dahlström & Häggendal (1966, 1967) and Laduron & Belpaire (1968). In recent electron microscopic studies of non-myelinated axons constricted at one or two points striking accumulations of mitochondria were seen on both sides of the constrictions (Mayor & Kapeller, 1967; Kapeller & Mayor, 1969*a*, *b*).

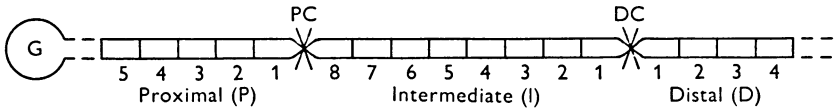
The present investigation was undertaken to correlate the relevant ultrastructural changes, namely the accumulation of granular vesicles and mitochondria, with the distribution of cytochrome oxidase, noradrenaline and adenosine triphosphate in post-ganglionic sympathetic nerves constricted at two points and particularly to compare the behaviour of cytochrome oxidase with that of noradrenaline.

METHODS

Altogether eighty-eight hypogastric nerves from forty-four cats of both sexes weighing between 1·5 and 4 kg were studied. Under Nembutal anaesthesia the nerves were constricted tightly at two points with fine silk ligatures which were left *in situ* (see Mayor & Kapeller, 1967, for further operative details). The first, or proximal, ligature was placed approximately 1 cm distal to the inferior mesenteric ganglion and the second, or distal, ligature was placed 0·5–1 cm further along the nerve as illustrated diagrammatically in Text-fig. 1. The nerves from different animals were excised 4 hr, 16–18 hr, 24 hr, 2, 3 and 4 days after operation.

Electron microscopy

The tissue was fixed at 4° C for 1½–2 hr in 1% osmium tetroxide adjusted to pH 7·4 with veronal acetate buffer. Whilst in the fixative the nerves were divided into segments 0·7–1·2 mm long; the length of each segment was measured after embedding. After dehydration in graded ethanols the tissue was passed through epoxypropane and embedded in epoxy resin (Araldite, CIBA, England). The pieces of nerve were orientated horizontally in the block so that longitudinal sections could be obtained. Ultra-thin sections were stained with lead citrate (Reynolds, 1963) and examined in a Philips EM 200 electron microscope.



Text-fig. 1. Diagrammatic representation of the segmentation of the hypogastric nerves proximal (P) to the first (proximal) constriction (PC), distal (D) to the second (distal) constriction (DC) and intermediate between the two constrictions (I). G represents the inferior mesenteric ganglion. Each segment for the biochemical studies was 0·8 mm long.

Biochemical studies

Mesenteric tissue was carefully cleaned from the nerves during their removal. The nerves were then laid out straight on a clean card and drawn out to their full length without tension. Using a stainless-steel ruler the nerves were carefully cut into segments $\frac{1}{32}$ in., i.e. 0·8 mm, long with a sharp razor blade (Text-fig. 1).

Cytochrome oxidase activity was estimated using single segments from one nerve or two corresponding segments from each hypogastric nerve from a single cat. The segments were homogenized by hand with a glass rod in a polyethylene tube, in 0·1 ml. 150 mM-KCl containing 1 mM ethylenediaminetetra-acetic acid (EDTA), pH 7·4. The cytochrome oxidase activity of a sample of this homogenate was measured by a modification of the spectrophotometric method described by Polakis, Bartley & Meek (1964). The assay was carried out at room temperature and the microcuvette contained 0·2 ml. 75 mM potassium phosphate buffer pH 7·0, 0·01 ml. 15% bovine plasma albumin, 0·1 ml. 65·6 μM reduced cytochrome *c*. The extract was made up to a final volume of 0·4 ml. with glass-distilled water. The reaction was started by the addition of the extract and the optical density (*E*) at 550 mμ was recorded. After a suitable time, which depended upon the activity of the sample and was usually between 5 and 30 min, 25 μl. saturated $K_3Fe(CN)_6$ were added to oxidize cytochrome *c* completely. (E_{550} at various times) − (E_{550} after complete oxidation) was plotted against time on semi-log. paper. This yielded a straight line. The first-order rate constant (*K*) for the oxidation of reduced cytochrome *c* is given by the slope of the graph and 16·4 *K* gives the activity of the sample in μmoles oxidized per minute since the initial concentration of reduced cytochrome *c* was 16·4 μM (the molecular weight of cytochrome *c* was taken as 12,500).

Cytochrome *c* was reduced by diluting 0·5 ml. of a stock solution, containing 4 mg/ml., with 0·5 ml. glass-distilled water and adding a little KBH_4. The solution was subsequently neutralized to pH 7·0 with acetic acid and made up to 2·5 ml. with water. This solution was then used to provide the reduced cytochrome *c* for the estimations.

Noradrenaline present in corresponding segments taken from both hypogastric nerves from two cats (four pooled segments) was measured by a fluorimetric method (Häggendal, 1963). The four segments were homogenized in 0·1 ml. ice-cold 1% perchloric acid, centrifuged and neutralized with 0·07 ml. 0·1 M-K_2CO_3. The resulting $KClO_4$ was spun down and the supernatant was applied to a column of Dowex AG 50W-X8 cation exchange resin pre-

pared and eluted as described by Häggendal (1963). In the present experiments 3·5 ml. of eluate was collected after applying 1 N-HCl to the column. Control experiments, in which 10, 50 and 100 mμg NA were applied to the columns, showed that the recovery of NA in the eluate was $62 \pm 9{\cdot}2\%$.

The eluate was neutralized to pH 6·5 with 2 M-K_2CO_3 before being used for the NA assay. Faded blanks (see Häggendal, 1963) were used for each sample. In order to characterize the fluorescent material present after oxidation with $K_3Fe(CN)_6$ and subsequent treatment with 2-3-dimercapto-propanol (BAL) and NaOH, the emission spectrum of each sample was measured in an Aminco Bowman spectrofluorimeter over the range 450–600 mμ using an excitation wave-length of 400 mμ. In all instances the spectra corresponded with those given by similarly treated samples of standard NA. The difference in the intensity of the fluorescence at 515 mμ between samples and their faded blanks was directly proportional to the NA content of the samples.

Adenosine triphosphate was measured in corresponding segments from both hypogastric nerves from a single cat. The two segments were homogenized in 0·2 ml. ice-cold 1 N-H_2SO_4 and centrifuged. 20 μl. samples of the supernatant were added to 0·5 ml. 0·1 M sodium arsenate buffer at pH 7·4 plus 0·63 ml. H_2O in a fluorimeter tube. The ATP content of the samples was estimated by injecting 0·5 ml. of a firefly tail extract through a light-proof lid into the fluorimeter cell housed in a Farrand fluorimeter. The output from the photomultiplier (anode load 2 MΩ) in response to the flash of light was fed into a Vibron electrometer and thence to a recording potentiometer. The maximum output of the electrometer in millivolts immediately after injecting the firefly tail suspension was directly proportional to the ATP content of the samples over the range 0·05–0·8 mμ moles.

The firefly tail extract was made by grinding 50 mg of tails (Sigma Chemical Co.) in 1 ml. ice-cold 0·1 M sodium arsenate buffer at pH 7·4 and subsequently making the volume up to 7·5 ml. with the buffer. Shortly before use, 50 mg $MgSO_4.7H_2O$ was added to the extract. This preparation of firefly tails was insensitive to ADP and creatine phosphate.

RESULTS

Electron microscopy

The ultrastructure of normal non-myelinated post-ganglionic sympathetic axons in the cat hypogastric nerve has been described previously (Mayor & Kapeller, 1967; Kapeller & Mayor, 1969*a*). In addition to filamentous and tubular structures they contain mitochondria and occasionally vesicles with an electron-dense core, commonly referred to as granular vesicles. The mitochondria are irregularly distributed along individual axons either singly or in small groups. The granular vesicles are very few in number and sparsely scattered along the length of each axon.

The segments used for electron microscopy were generally slightly longer than those employed for the biochemical estimations. However, it was possible to orientate them accurately with respect to the site of constriction (see Kapeller & Mayor, 1969*a*, *b*). Thus the distance of the various ultrastructural features from the constrictions could be estimated and correlated with the biochemical changes. For convenience the segmentation referred to in this section will correspond to that utilized for the biochemical studies (see Text-fig. 1).

Following operation large numbers of organelles accumulated on both sides of the two constrictions in the non-myelinated axons which became progressively more swollen or distorted (see Plates 1–6). This accumulation of organelles was always greatest adjacent to the constrictions and was associated with varying degrees of degenerative changes, e.g. with myelin figures. There was considerable variation in the size and organelle content of adjacent axonal profiles and also along the length of individual axons at all times.

In addition to the swollen and distorted axons, numerous very small axons, considered to be new axonal outgrowths, were found proximal to the first constriction from about 2 days onwards (Pl. 2). Initially they were seen in segment P1 but at longer time intervals they also occurred in segment P2. These very small new axons were not seen distal to the first constriction nor on either side of the second constriction.

The accumulation of organelles started next to the constricted zones and extended both proximally and distally away from these zones with the passage of time. By 4 days it was evident 1–2 mm above the first constriction (i.e. in segments P1–P3) and approximately 0·5–1 mm below the second constriction (in segment D1). It only rarely extended into segment D2. In the intermediate piece of nerve the intra-axonal accumulation of organelles was confined mainly to the first 0·5 mm immediately adjacent to the two constrictions (i.e. within segments I1 and I8). In the other intermediate segments (I2–I7) the axons were generally narrow and devoid of their normal complement of organelles. Alternating narrow regions and swollen axonal profiles, filled with an amorphous debris, were sometimes seen.

An increase in the amount of Schwann cell cytoplasm and in its content of endoplasmic reticulum, ribosomes and mitochondria was seen from 2 days onwards after operation. This was most noticeable in the intermediate segments (Pl. 4, fig. 2). Similar changes in the Schwann cells could be identified occasionally above the first and below the second constriction.

Further consideration of the ultrastructural changes will be limited to the behaviour of mitochondria and granular vesicles which can be correlated with cytochrome oxidase and NA respectively.

Mitochondria. Within a few hours of operation they were more numerous than normal in many of the non-myelinated axons on both sides of the two constrictions. Proximal to the first constriction the number of mitochondria increased rapidly. At first they were confined to segment P1 (Pl. 1) but at longer intervals after operation they accumulated in some axons in segments P2 and P3. They were found in both swollen axons (presumably the original constricted axons), where they were frequently

densely packed together (Pl. 1) and also in many of the new axons (Pl. 2).

By contrast the accumulation of mitochondria distal to the second constriction was fairly well localized to segment D 1. In this segment the types of mitochondria and the pattern of their accumulation in swollen axons (Pl. 6) were similar to that seen in segment P 1. Only occasionally did they accumulate in axons in segment D 2 or more distally.

In the intermediate segments more mitochondria than normal were found in many axons in segments I 1 and I 8 (Pls. 3, 4, fig. 1 and Pl. 5). In the other intermediate segments the non-myelinated axons contained fewer mitochondria than normal; usually no mitochondria were found in the majority of these axons (Pl. 4, fig. 2).

The mitochondria situated very near to the constrictions were generally swollen and many showed degenerative changes, e.g. excessive swelling, broken cristae and transformation into myelin figures (Pl. 5). Further from the constriction they resembled normal mitochondria; they were relatively electron-dense, compact and frequently elongated (see Pls. 1, 4, fig. 1 and Pl. 6).

Similar accumulations of mitochondria were present in the few myelinated axons present in these nerves. Schwann cells and other connective tissue cells also contained more mitochondria than normal from about 2 days onwards after operation (Pl. 4, fig. 2). However, although these would undoubtedly contribute to the total number of mitochondria in any one segment of nerve, by far the most prominent accumulation of these organelles was seen in non-myelinated axons.

Vesicles with an electron dense core (granular vesicles). These vesicles started to accumulate in non-myelinated axons proximal to both constrictions, i.e. in segments P 1 and I 1 very soon after operation. Their over-all diameter varied but most were within the range 600–900 Å. There was also considerable variation in the size, shape and electron density of their electron-dense core.

Granular vesicles continued to accumulate proximal to the first constriction throughout the four day period (Pls. 1 and 2). Initially they were confined to segment P 1 but as time progressed they were seen further from the constriction in segment P 2 and sometimes in P 3. They were located in swollen or deformed axons, with or without associated mitochondria (Pl. 1), and also in many of the new axonal outgrowths (Pl. 2).

During the first 4 hr after operation the accumulation of granular vesicles in segment I 1 (Pl. 3, fig. 1) resembled that seen in segment P 1. This particular plate is typical of both segments P 1 and I 1 at this time interval. Thereafter, there did not seem to be any further increase in the number of granular vesicles in segment I 1. However, they were

still evident in segment I 1 from 2–4 days after operation (Pl. 3, fig. 2).

Granular vesicles were exceedingly rare in segment I 8; they did not accumulate in this region of the nerve (Pl. 5, figs. 1 and 2). They were sometimes found distal to the second constriction, but they were few in number and difficult to identify amongst other structures and degenerative changes. There was no obvious accumulation of these vesicles in segment D 1 or more distally placed segments at any time after operation. However, from the electron micrographs it was impossible to ascertain whether or not the total number of granular vesicles in this part of the nerve exceeded the number present in a similar segment of normal nerve.

Biochemical studies

Cytochrome oxidase activity. The normal control value for cytochrome oxidase activity per single 0·8 mm segment of nerve was calculated from estimations carried out on pieces of nerve several millimetres long taken from the same region of the hypogastric nerve as that used for the experiments. The normal range was found to be $0{\cdot}47 \pm 0{\cdot}24$ (S.D.) μ-moles cytochrome *c* oxidized per minute.

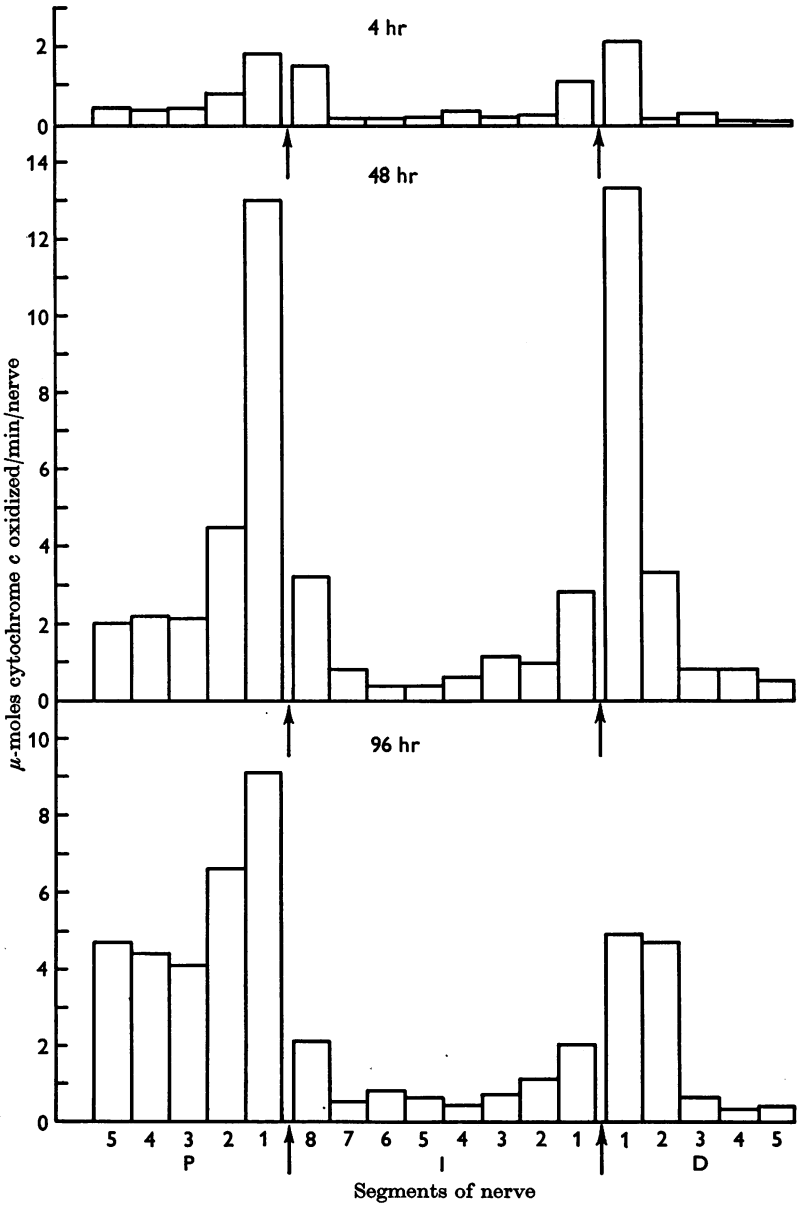
After the nerves had been tied for 4 hr there was a noticeable increase in the cytochrome oxidase activity proximal to the first (proximal) constriction and distal to the second (distal) constriction. Between the two constrictions cytochrome oxidase activity was considerably higher in segments I 1 and I 8 than in similar 0·8 mm segments of normal unconstricted hypogastric nerves (Text-fig. 2). In the intervening segments (I 2–I 7) the activity was generally less than normal.

In the three segments proximal to the first constriction (P 1-P 3) and in the three segments distal to the second constriction (D 1–D 3) the cytochrome oxidase activity continued to rise until about 72 hr after operation. Thereafter it began to decrease (Text-fig. 3).

At all times the total activity of cytochrome oxidase in segments P 1–P 3 was greater than in segments D 1–D 3. From 24 hr onwards the activity of cytochrome oxidase in the nerve proximal to segment P 3 rose above the control value whilst in the segments distal to D 3 it fell below the normal control values.

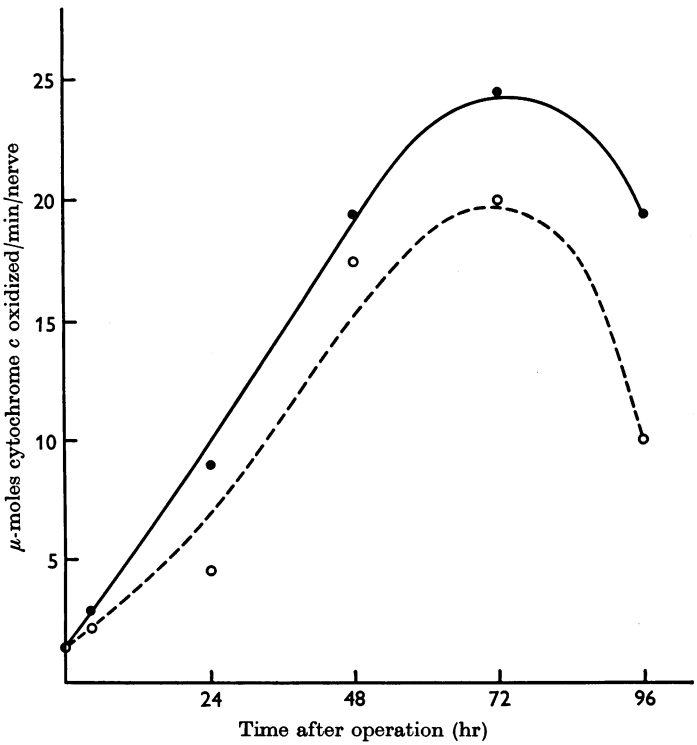
In segments I 1 and I 8 cytochrome oxidase activity increased during the first 24 hr operation; thereafter no further significant changes were observed.

Noradrenaline. From estimations performed on 2–3 cm lengths of normal hypogastric nerve the mean NA content of a single 0·8 mm segment was found to be $0{\cdot}01 \pm 0{\cdot}004$ (S.D.) mμ-moles. In the experiments on ligated nerves NA would not have been detected in an 0·8 mm segment if the amount present was less than 0·01 mμ-moles (i.e. 1·6 mμg).



Text-fig. 2. Histograms showing the distribution of cytochrome oxidase activity in hypogastric nerves constricted at two points (indicated by the vertical arrows) 4 hr, 48 hr and 96 hr after operation. The enzyme activity in each 0·8 mm segment is expressed as μ-moles cytochrome *c* oxidized per minute per nerve. The segments P are proximal to the first constriction, segments D distal to the second constriction and segments I are between the two constrictions. Cytochrome oxidase activity in an 0·8 mm segment of normal nerve was $0{\cdot}47 \pm 0{\cdot}24$ (S.D.) μ-moles.

An increase in the NA content of segments P1 and P2 was detected as early as 4 hr after operation (Text-fig. 4). By 24 hr this accumulation proximal to the first constriction had extended into segment P3. The total NA in these three segments continued to rise in a roughly linear manner during the 4-day period (Text-fig. 5). The greatest quantity of NA was usually found in segment P1, but sometimes in P2, at the longer times after operation, as seen in Text-fig. 4 at 96 hr.



Text-fig. 3. Cytochrome oxidase activity in the first three segments proximal to the first constriction and in the first three segments distal to the second constriction at different time intervals after operation. ●——● sum of activity in P1+P2+P3; ○----○ sum of activity in D1+D2+D3.

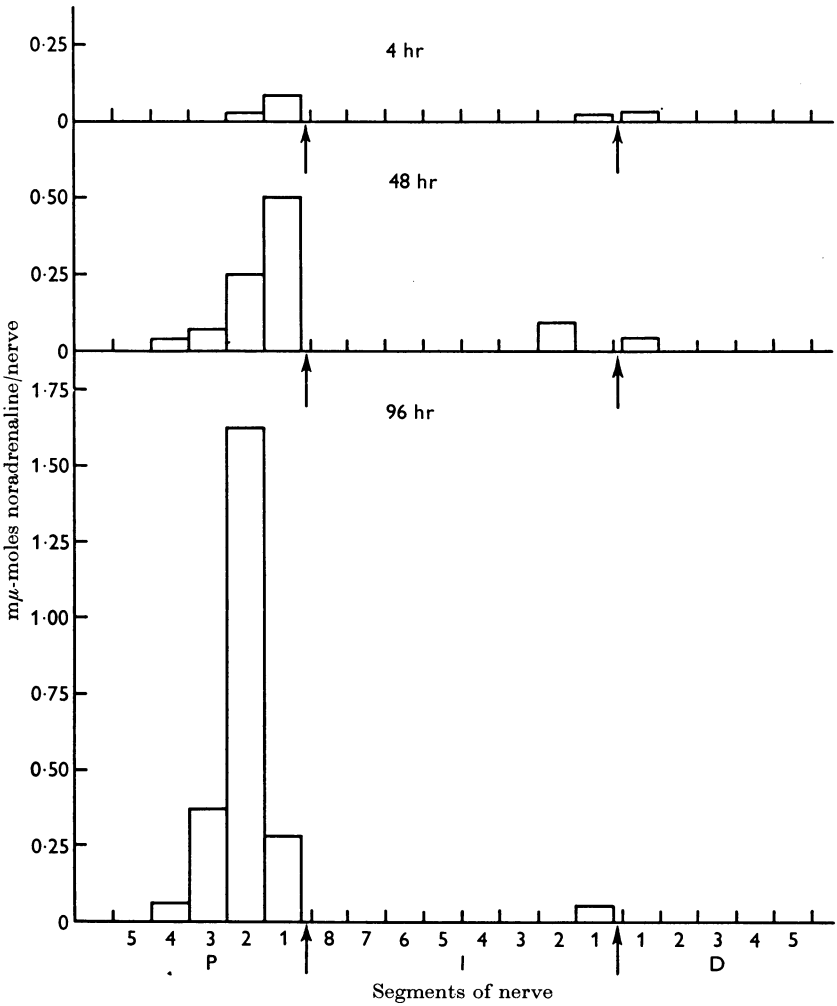
A small amount of NA, equivalent to the amount present in approximately two segments (rarely as many as four) of normal nerve was usually detected in segment D1. It exhibited no significant increase after the first 4 hr after operation; on the contrary it frequently declined. Sometimes, as in Text-fig. 4, there was no detectable NA in this segment 96 hr after operation. There was no detectable NA in the more distally placed segments, i.e. beyond D1 at any time afer constriction.

By 4 hr after operation it was just possible to detect an accumulation

of NA in segment I 1. From 24 hr onwards this segment, or segment I 2 in some experiments (see Text-fig. 4), contained 0·09 mμ-moles NA, i.e. equivalent of approximately eight segments of normal nerve. NA was never detected in segment I 8.

In two experiments, 3 and 4 days after the operation, traces of NA were found in segments I 3 and I 7 in addition to the usual accumulation in I 1. The possible significance of this finding will be considered in the Discussion.

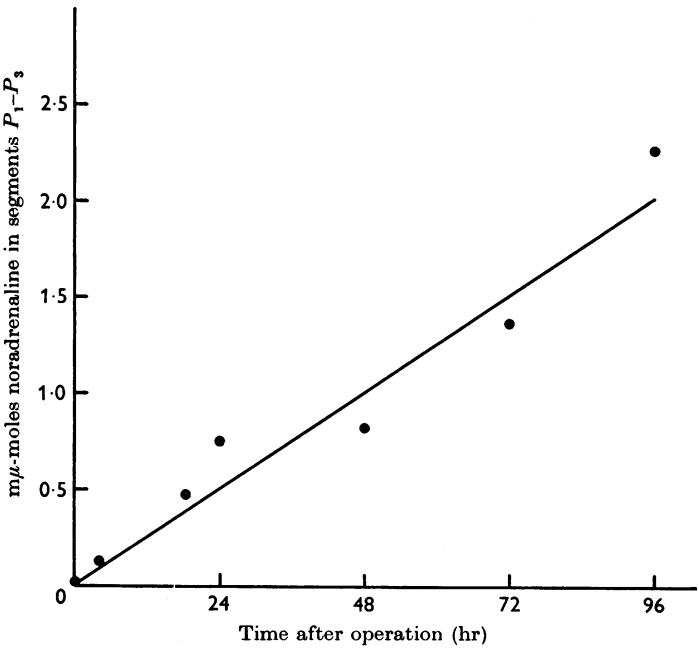
Adenosine triphosphate. From estimations performed on pieces of nerve



Text-fig. 4. Histogram showing the distribution of noradrenaline in hypogastric nerves constricted at two points (indicated by the vertical arrows) 4 hr, 48 hr and 96 hr after operation. Segmentation as in Text-figs. 1 and 2. NA content of an 0·8 mm segment of normal nerve was 0·01 ± 0·004 (S.D.) mμ-moles.

2–3 cm long the mean normal content ATP of a single 0·8 mm segment was found to be 0·15 ± 0·07 (S.D.) mμ-moles.

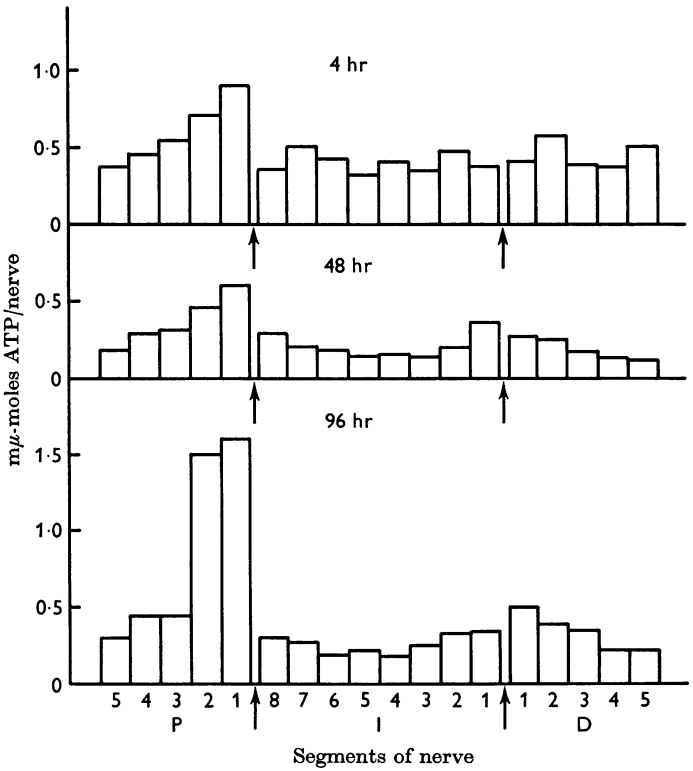
Four hours after ligating the nerves there was a noticeable increase in the amount of ATP present in segments P 1 and P 2 (see Text-fig. 6). The ATP content of the nerve proximal to the first constriction continued to increase gradually so that by 4 days after operation the total amount in segments P 1 to P 3 was about ten times greater than the control value for three segments of normal nerve.



Text-fig. 5. Total noradrenaline content in segments P 1 + P 2 + P 3 proximal to the first constriction at different time intervals after operation.

By 24 hr after constriction the ATP content of segment D 1 was greater than normal and was significantly higher than that of the more distally placed segments. It remained at a similar level for the succeeding 3 days.

Initially there was little change in the ATP content of any individual segments between the two constrictions (Text-fig. 6). The variation seen in these intermediate segments at 4 hr was identical to that observed in normal control nerves. From 24 hr onwards the two segments adjacent to constrictions (i.e. I 1 and I 8) contained more ATP than normal whilst in the other intermediate segments it commonly fell below normal levels.



Text-fig. 6. Histogram showing the distribution of ATP in hypogastric nerves constricted at two points 4 hr, 48 hr and 96 hr after operation. Segmentation as in Text-figs. 1, 2 and 4. ATP content of an 0·8 mm segment of normal nerve was $0{\cdot}15 \pm 0{\cdot}07$ (S.D.) mμ-moles. (The nerves used for the 4 hr experiment were thicker than average. This accounts for the apparent higher concentration of ATP compared with other nerves.)

DISCUSSION

Very short segments of nerve were necessary for electron microscopy, principally for technical reasons, but also because the most marked morphological changes have been located very near to the constrictions (Kapeller & Mayor, 1969*a*, *b*). Small segments were also desirable for the biochemical studies since the greatest changes in the enzyme content of constricted nerves are confined to narrow zones on either side of the lesion (see Lubińska, 1964).

The present results have demonstrated a close correlation between the distribution of granular vesicles and NA and between mitochondria and cytochrome oxidase. Proximal to the first constriction the pattern of the accumulation of mitochondria and cytochrome oxidase was very similar

to that of the concomitant accumulation of granular vesicles and noradrenaline. The piece of nerve between the ganglion and the first constriction, approximately equal to twelve segments, would normally contain $5{\cdot}64 \pm 2{\cdot}88$ μ-moles cytochrome oxidase and $0{\cdot}12 \pm 0{\cdot}048$ mμ-moles of NA. However, by 72 hr after operation the total cytochrome oxidase activity in segments P1–P3 increased to 25 μ-moles and the total NA in these three segments was 1·50 mμ-moles. Thus the increase in amount of the enzyme and NA seen in segments P1–P3 was more than could be accounted for by the simple re-distribution of the amount normally present between the ganglion and the site of constriction. These results suggest that during the first 3 days after operation the cell bodies continued to produce both the transmitter and mitochondria (carrying cytochrome oxidase) which then moved along the axons and piled up above the constriction.

The increase in the NA content of segments P1–P3 over normal values was $1{\cdot}5-(3 \times 0{\cdot}01) = 1{\cdot}47$ mμ-moles by 72 hr after operation, i.e. it was accumulating at the rate of 0·02 mμ-moles per hour. This amount is equivalent to the NA content of about 2 mm of normal nerve; thus the rate of the proximo-distal movement of NA after constriction was 2 mm per hour. This is similar to the 1·7 mm per hour calculated by Laduron & Belpaire (1968) for the rate of displacement of NA in constricted dog splenic nerves but it is slower than the rate of NA transport in the adrenergic fibres in the sciatic nerves of several animals (cat 9–10 mm, rat 5–6 mm and rabbit 3 mm per hour) according to Dahlström & Häggendal (1966, 1967).

By 72 hr after operation the cytochrome oxidase content of segments P1–P3 had increased by $25-(3 \times 0{\cdot}47) = 23{\cdot}59$ μ-moles, i.e. at the rate of 0·33 μ-moles per hour. This is equivalent to the cytochrome oxidase content of about 0·6 mm of normal nerve. Thus the rate of movement during the first 3 days after operation was 0·6 mm per hour. By 4 days (Text-fig. 3) the rate of accumulation of this enzyme was declining. It is clear, therefore, from the present results that NA moves some three times faster than cytochrome oxidase (and presumably mitochondria) in the portion of nerve above the proximal constriction. Despite this difference both substances moved very much faster than the 1 mm per day described by Weiss & Hiscoe (1948) for the rate of the proximo-distal displacement of axoplasm in constricted myelinated axons. The proximo-distal migration of radioactively labelled proteins in cranial and spinal nerves has also been found to be slow, of the order of one to a few millimetres per day (see Weiss, 1961; Droz & Leblond, 1963; Taylor & Weiss, 1965; Ochs, 1966; Korr, Wilkinson & Chornock, 1967; Weiss, 1967 and Weiss & Holland, 1967). On the other hand, in addition to slowly moving radioactively labelled phospholipids, Miani (1964) described some phospholipids moving

at the rate of 72 mm per day in the cervical vagus nerve and 39–41 mm per day in the hypoglossal nerve. These facts, together with the present observations, suggest that several translocating systems may co-exist in non-myelinated adrenergic axons. Those involving granular vesicles and mitochondria may be considered as providing a relatively rapid means of movement whilst the proximo-distal progression of axoplasm is a slower process, more akin to axonal growth as discussed by Weiss & Hiscoe (1948) and Weiss (1961, 1963).

Elsewhere in the constricted nerves the re-distribution of mitochondria and cytochrome oxidase differed from that of the granular vesicles and NA. Between the two constrictions this re-distribution was apparently achieved without any significant change in the total amount of each substance present. Furthermore, the cytochrome oxidase and NA content of this piece of nerve remained approximately equal to that of a similar length of normal nerve during the 4-day period. The enzyme moved both centrifugally and centripetally, falling below the normal control values in the middle of the isolated region of nerve; on the other hand the NA moved only centrifugally. This pattern of re-distribution again indicates that the mitochondria and the granular vesicles are able to move independently of one another. In segment D 1 cytochrome oxidase accumulated at about the same rate as in segment P 1, whereas NA did not rise significantly above its control value; in some experiments it was less than normal. Thus a unidirectional movement of NA can take place at a time when cytochrome oxidase is moving bi-directionally. Since these differential movements can continue in segments of axons isolated from the influence of their cell bodies they cannot be entirely dependent upon the continued production of either axoplasm or their own related axonal organelles.

However, in two experiments in the present series (3 and 4 days after operation) small accumulations of NA, about three times normal, were observed in segment I 7, i.e. just below the first constriction. On both these occasions an increased amount of NA was also found in segment I 3 or I 4. These findings could be due to the presence of aberrant ganglion cells (or possibly chromaffin cells) which are known to exist along the course of the hypogastric nerves in several species (see Vanov & Vogt, 1963; Zaimis, 1964; Mayor & Kapeller, 1967). The axons from such ganglion cells may pass along the hypogastric nerve in either a distal or proximal direction. In the latter case they could be obstructed by a constriction and give rise to an apparent retrograde accumulation of NA below the lesion (Mayor & Kapeller, 1967).

Dahlström (1965, 1967) found small amounts of fluorescent material, indicative of NA, distal to constrictions applied to sciatic nerves. This was interpreted as indicating a retrograde flow of NA in adrenergic nerve

fibres. However, the amount of NA which could be detected quantitatively in this region was never very large (Dahlström, 1966); in some experiments it was less than normal (Dahlström & Häggendal, 1967). The present results indicate that, compared with cytochrome oxidase, the retrograde movement of NA in adequately constricted adrenergic nerves is insignificant.

Although a proportion of the cytochrome oxidase activity in any given segment undoubtedly stemmed from mitochondria present in extra-axonal sites, e.g. Schwann cells and connective tissue cells, the greatest increase in the mitochondrial population was within axons in segments P1–P3 and segments D1 and D2. This was also evident in segments I1 and I8 during the first 48 hr after operation. By 4 days there was a definite subjective increase in the number of mitochondria in extra-axonal sites, which at this time could make a significant contribution to the total amount of cytochrome oxidase in any given segment of nerve. However, the present experiments have shown that mitochondria can move both centrifugally and centripetally in constricted non-myelinated axons. This raises the question of whether mitochondria move bi-directionally under normal circumstances, or whether the present findings represent a response to the possible increased energy requirements at the sites of axonal injury. Using time-lapse cine-microphotography Pomerat, Hendleman, Raiborn & Massey (1967) have shown that individual mitochondria can move forwards and backwards along axons in tissue culture preparations. The presence of numerous mitochondria in axons at synapses and neuromuscular junctions together with their tendency to congregate at nodes of Ranvier in normal and damaged myelinated axons (see Webster, 1962), may be indicative of the relatively high energy requirements of these various regions. Thus both a normal bi-directional movement and an active response of the mitochondria to the effects of axonal injury may be responsible for the re-distribution of mitochondria seen in the present experiments.

The change in the distribution of ATP following ligation was much less marked than that of either NA or cytochrome oxidase. In segments P1–P3 at 3 days it was only two to three times greater than normal. In contrast, at the same time interval, the NA content of these segments was fifty times greater than normal and cytochrome oxidase was twenty times higher than in three control segments. Assuming a 4:1 molar ratio of NA:ATP in granular vesicles (see Stjärne, 1966), their redistribution would account for only 20% of the extra ATP present in segments P1–P3, 4 days after operation. The failure of the ATP content to parallel the cytochrome oxidase activity may be expected if the increased number of mitochondria adjacent to the lesion is a response to increased energy demands. If these

demands are high more mitochondria may well be required to maintain the ATP concentration at normal levels.

The present results indicate, therefore, that the interpretation of the rate and direction of movement of different materials along axons is not a simple matter. Non-myelinated post-ganglionic sympathetic axons are particularly instructive in this context since they contain two organelles whose biochemical parameters can be investigated independently. It must be borne in mind, however, that the rates of movement obtained from experiments involving axotomy or some other traumatic procedure refer to injured axons. Therefore, these results should not be applied to normal nerves without some reservations.

We are grateful to Professors W. Bartley and R. Barer for their helpful advice during the course of this work. We thank Mr R. Hardy and his staff and Mr B. Christian for their skillful technical assistance. We acknowledge with thanks assistance from S.R.C. and M.R.C. in support of this work. Mr D. Mangnall is in receipt of a Post-graduate Research Studentship from M.R.C.

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EXPLANATION OF PLATES

All the electron micrographs are from material fixed in osmium tetroxide. The sections were stained with lead citrate and the segmentation refers to that used in Text-fig. 1. In all plates the magnification mark represents 1 μ, A indicates non-myelinated axons, S indicates Schwann cell cytoplasm.

PLATE 1

Section taken from the middle of segment P 1, 48 hr after operation, illustrates the accumulation of slightly swollen mitochondria (m) and granular vesicles (g) in several non-myelinated

axons. Myelin figures (mf), indicative of degenerative changes, are very common. Note the variation in the organelle content of adjacent axons and the densely packed mitochondria in axon A 1.

PLATE 2

Section from the proximal part of segment P 1, 4 days after operation showing numerous very small axons considered to represent regenerating axonal outgrowths (arrowed) alongside swollen axons (SA). The majority of the new axons contain granular vesicles and some contain mitochondria. Note the variation in the diameter and organelle content of axon (A 1) at different points along its length, and the variation between adjacent axonal profiles.

PLATE 3

Sections from segment I 1. Granular vesicles (g) and mitochondria (m) are more numerous than normal.

Fig. 1, 4 hr after operation from the middle of the segment.

Fig. 2, very near to the constriction, 48 hr after operation. This also demonstrates the considerable tissue disruption common immediately adjacent to the constrictions, consequently axons cannot be differentiated from Schwann cell cytoplasm.

PLATE 4

Figure 1 shows the accumulation of mitochondria (m) in a swollen axon from the middle of segment I 1, 48 hr after operation, i.e. slightly proximal to Pl. 3, fig. 2. Vesicles with an electron dense core (g) are slightly more numerous than normal.

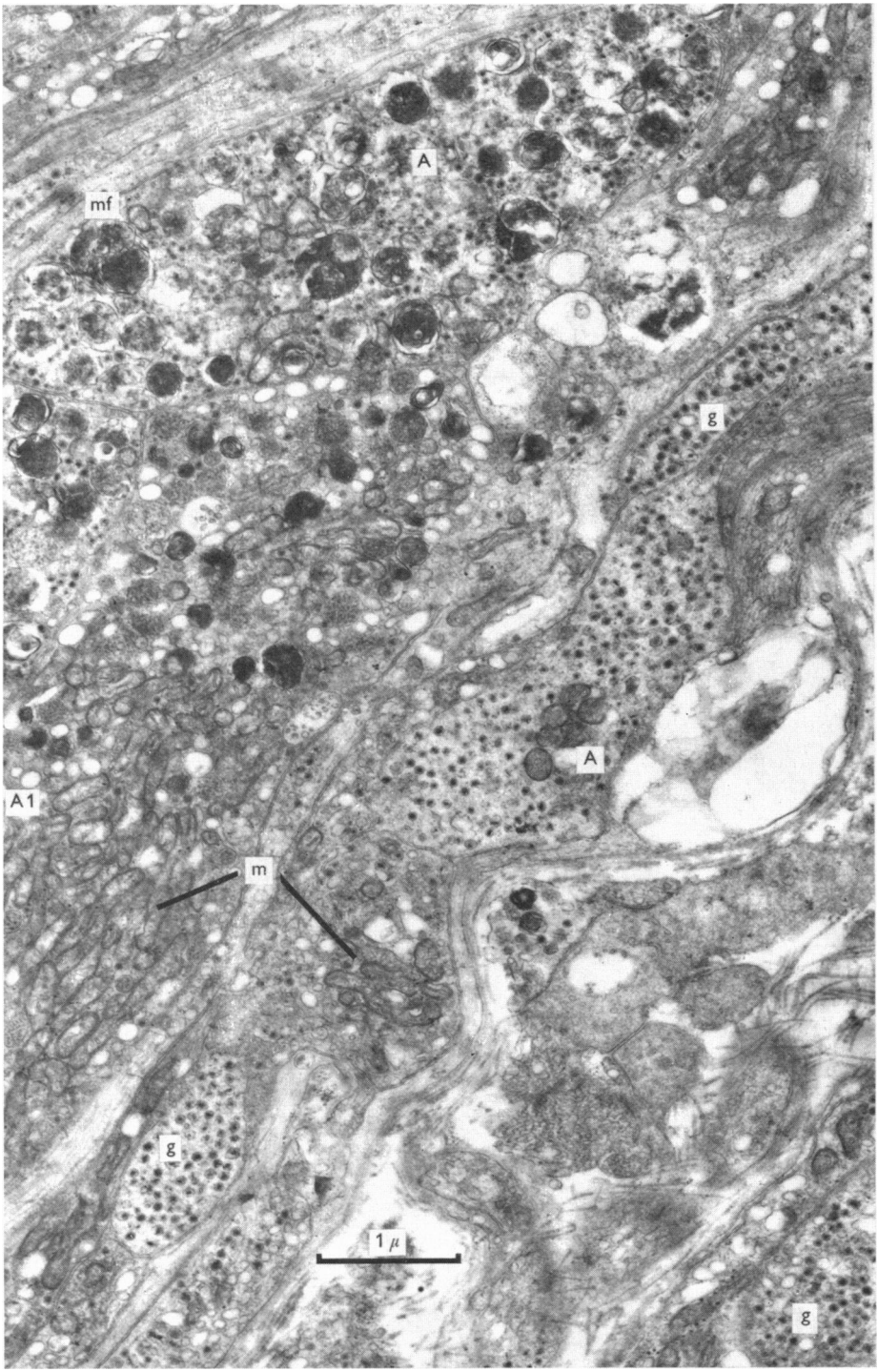
Figure 2 illustrates the appearances of axons and Schwann cells in the intermediate segments remote from the constriction. The axons (A) are narrow and contain fewer organelles than normal. The Schwann cell contains more mitochondria (m) than normal and the connective tissue cell (C) is more prominent than in normal nerves. Segment I 3, 48 hr after operation.

PLATE 5

Two examples of the different appearances of axons in segment I 8, 48 hr after operation. Figure 1 shows the accumulation of swollen mitochondria (sm) in two enlarged axons very near to the constriction. (This appearance of the mitochondria was also seen in segment I 1). Figure 2 is from the middle of segment I 8. The enlarged axon (A) contains a few swollen mitochondria (sm), numerous agranular vesicles (a) and myelin figures (mf). Occasional granular vesicles (g) can be seen.

PLATE 6

Section from the middle of segment D 1, 4 days after operation showing the very dense packing of electron dense mitochondria (m) which was common in many axons. Note the marked variation between adjacent axons. Axon A 1 contains fewer mitochondria, but still more than normal; numerous myelin figures (mf) and multivesicular bodies (mvb) are present in this axon.



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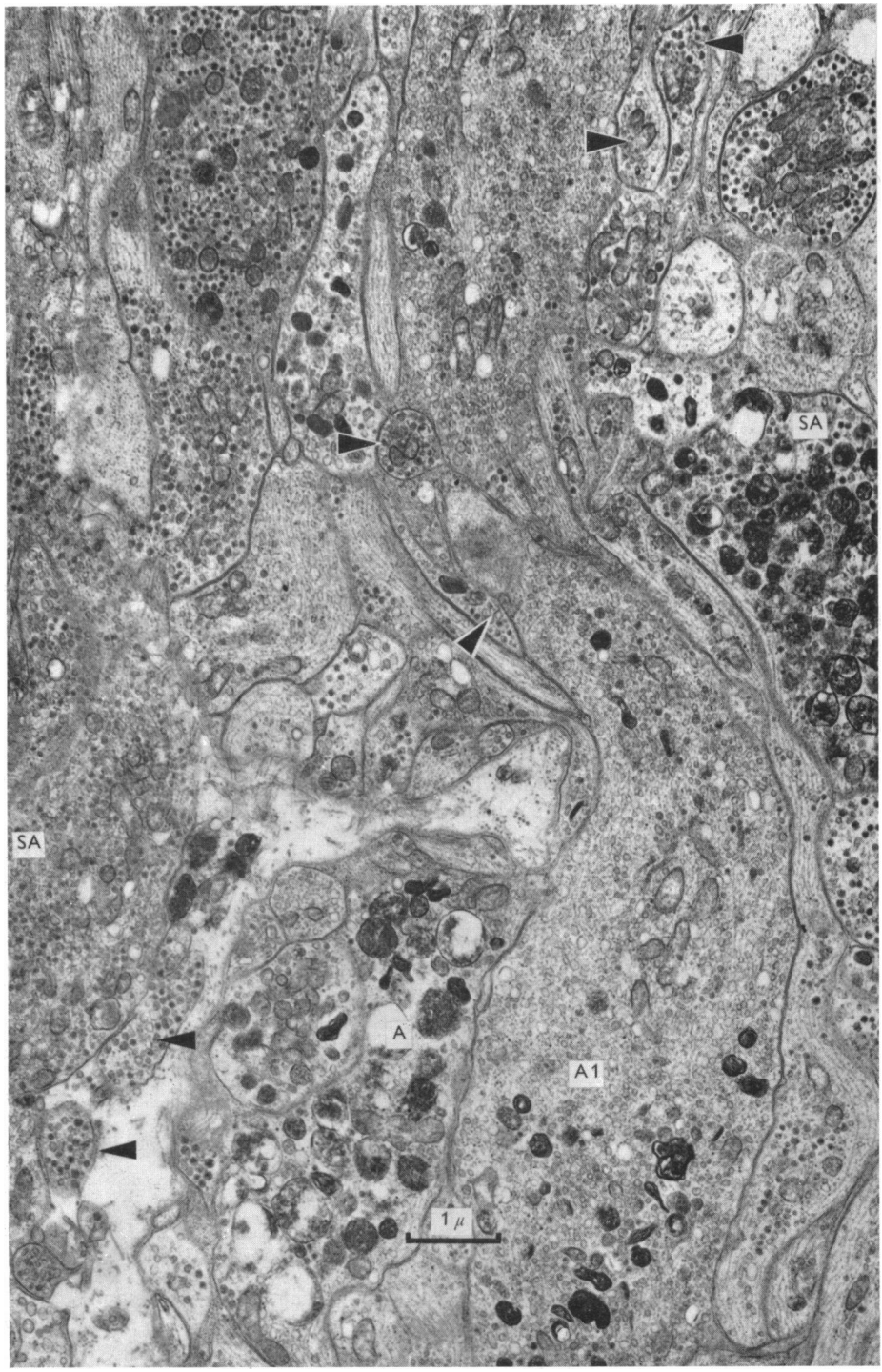

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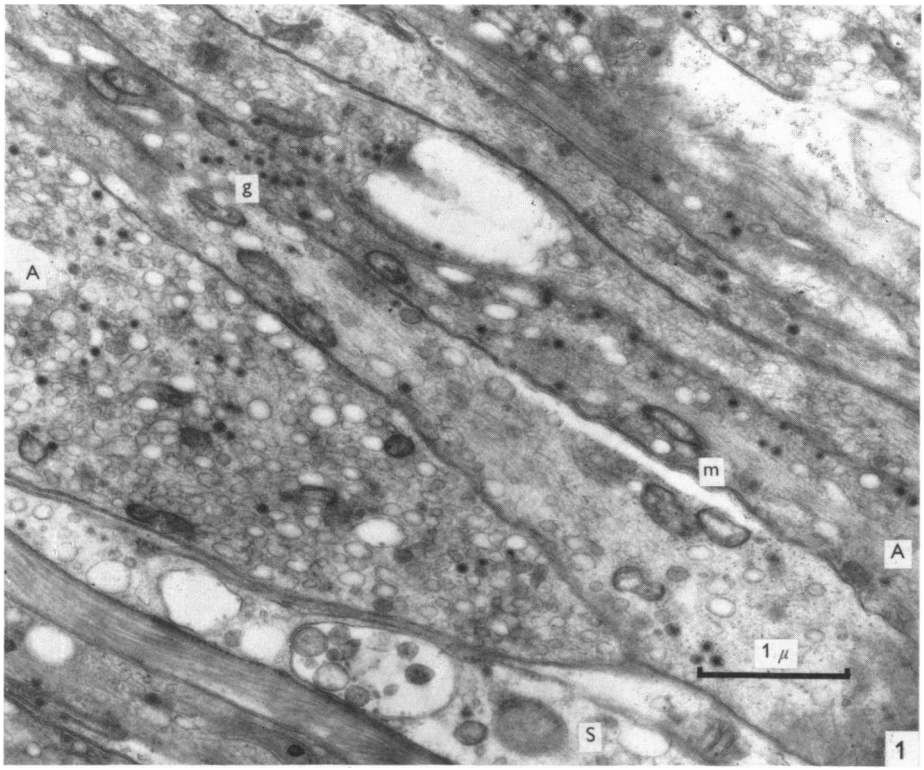

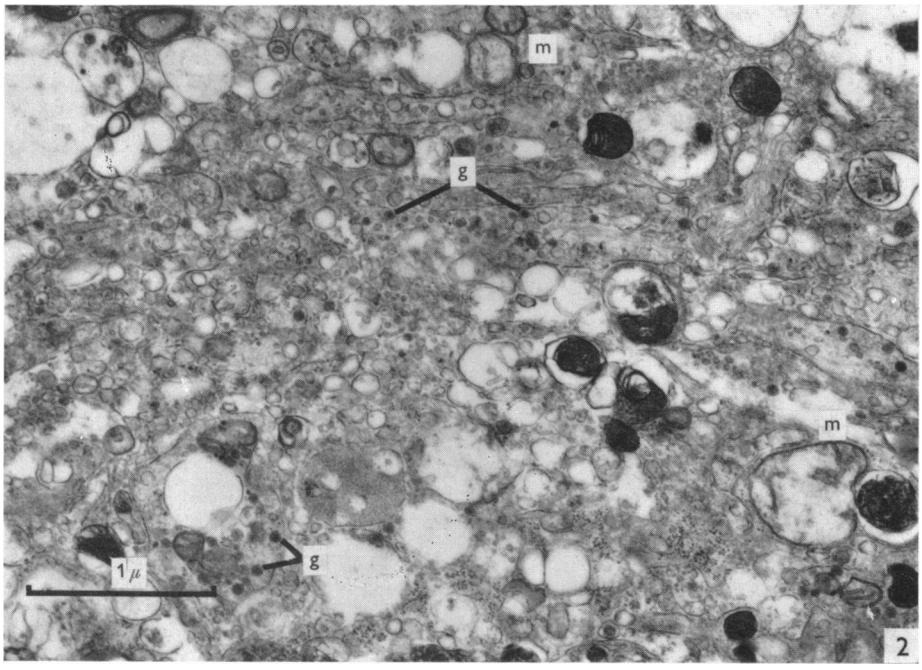

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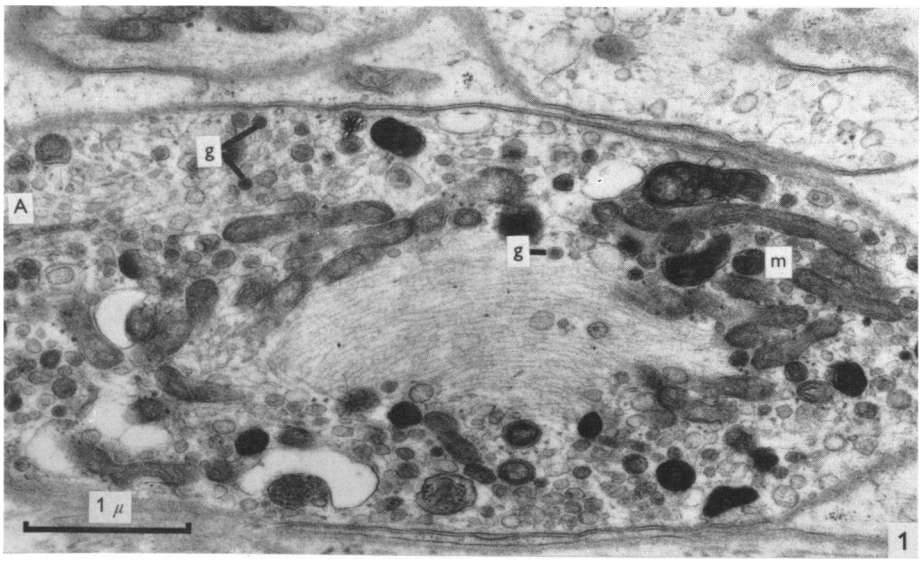
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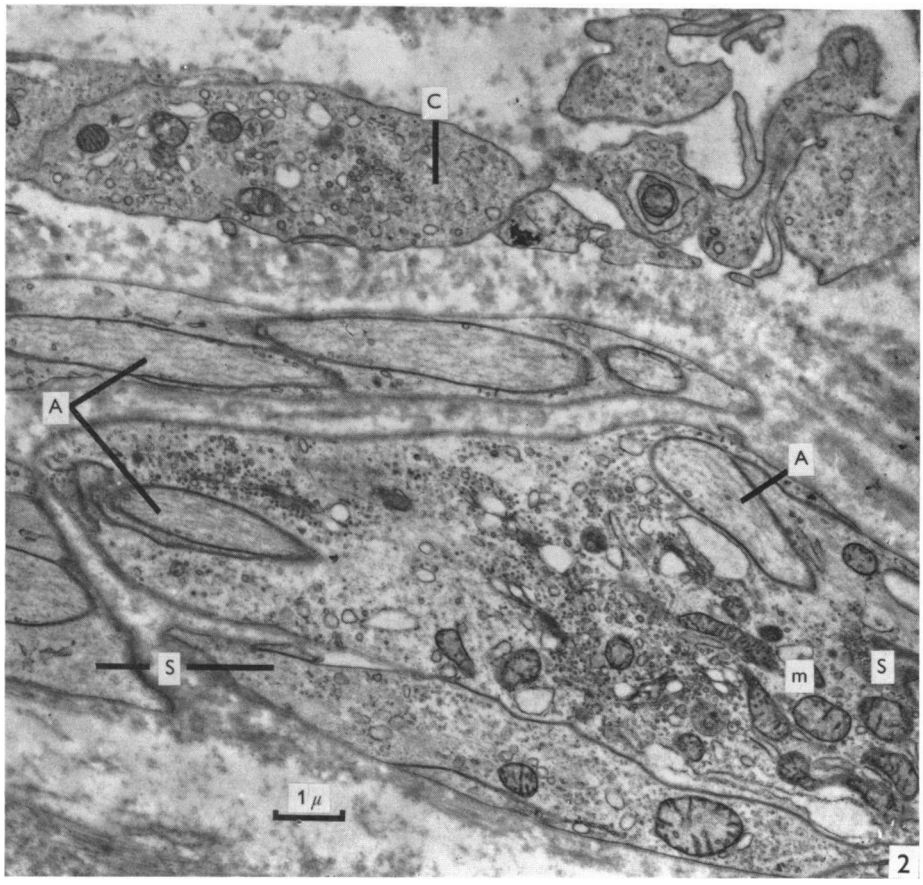
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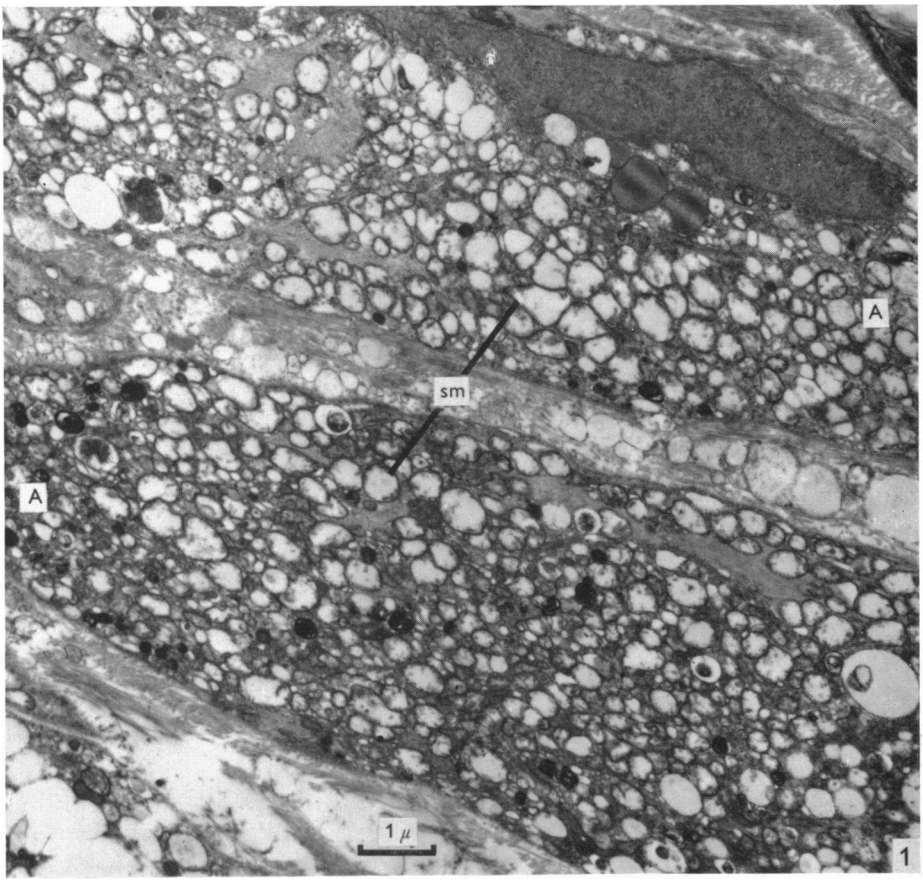

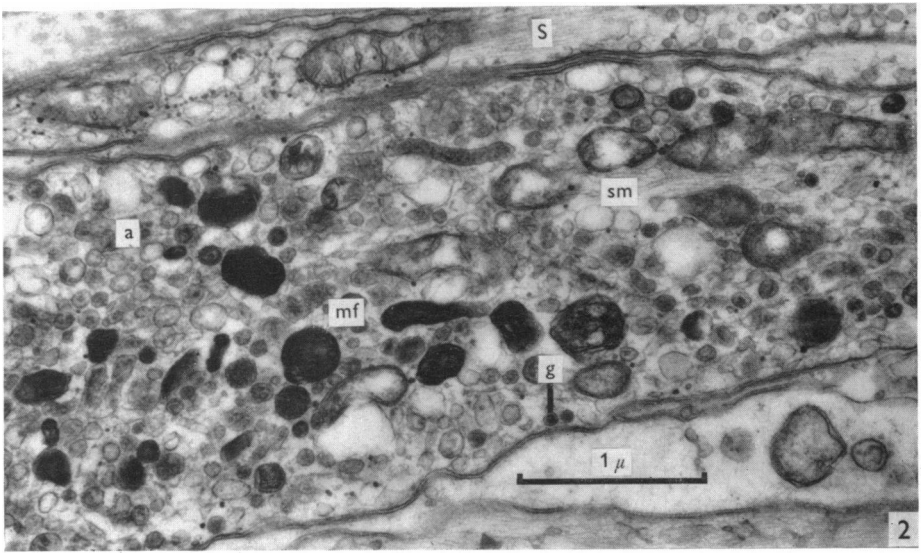

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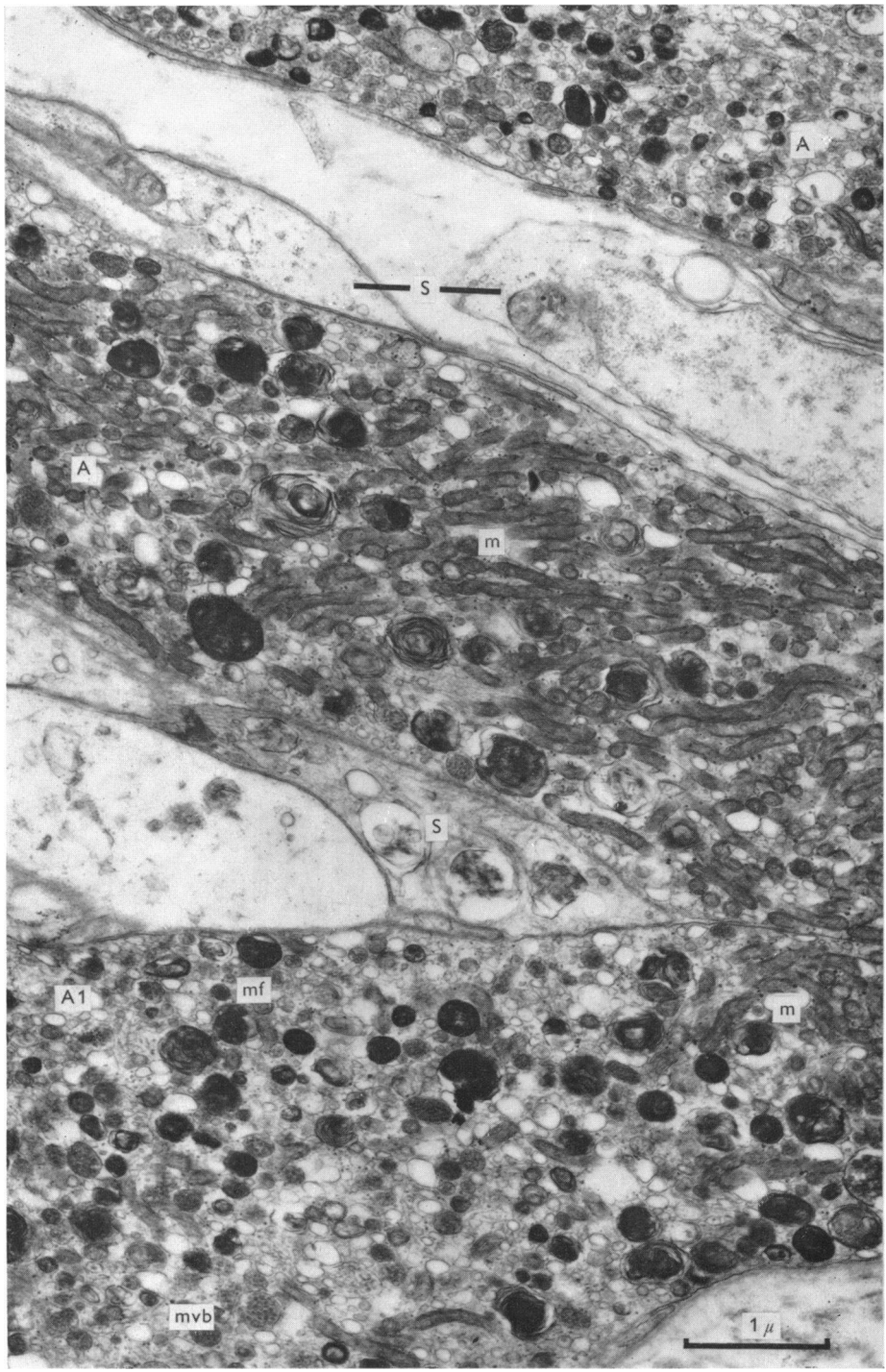

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