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STUDIES ON THE TRANSLOCATION OF NORADRENALINE-CONTAINING VESICLES IN POST-GANGLIONIC SYMPATHETIC NEURONES *IN VITRO*. INHIBITION OF MOVEMENT BY COLCHICINE AND VINBLASTINE AND EVIDENCE FOR THE INVOLVEMENT OF AXONAL MICROTUBULES

By P. BANKS, D. MAYOR, MARY MITCHELL and D. TOMLINSON

From the Department of Biochemistry and Department of Human Biology and Anatomy, Sheffield University, Sheffield S10 2TN

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SUMMARY

1. Methods are presented for studying axonal transport mechanisms in preparations of constricted hypogastric nerve/inferior mesenteric ganglia maintained *in vitro* for periods up to 48 hr. Under these conditions the ultrastructure of the tissue is excellently preserved.

2. The proximo-distal movement of noradrenaline and noradrenaline storage vesicles in the non-myelinated axons of these preparations is inhibited by both colchicine (10 μg/ml.) and vinblastine sulphate (1 μg/ml.) whilst the movement of mitochondria appears to be unaffected.

3. Neither colchicine nor vinblastine sulphate depletes accumulated dense-cored vesicles of their stores of noradrenaline.

4. These drugs reduce the accumulation of noradrenaline and dense-cored vesicles against a constriction when they are in contact with the nerve trunks only, and are denied direct access to the nerve cell bodies in the ganglion.

5. The only other morphological change that can be attributed to the action of colchicine and vinblastine is a marked reduction in the number of axonal microtubules.

6. The experiments provide strong support for the view that the axonal system of microtubules is closely involved in the proximo-distal movement of noradrenaline storage vesicles within noradrenergic neurones. The microtubular system does not appear to be involved in mitochondrial movement.

INTRODUCTION

In recent years it has become evident that dense-cored vesicles containing noradrenaline are formed in the cell bodies of noradrenergic neurones and are then transported along the axons towards the sites of transmitter release in the terminal autonomic network (Banks, Mangnall & Mayor, 1969; Dahlström, 1969). This paper describes methods that allow the proximo-distal movement of noradrenaline storage vesicles (dense-cored vesicles) along post-ganglionic sympathetic axons to be studied *in vitro*. Besides enabling the effects of various metabolic inhibitors on the translocation process to be examined in the absence of more generalized side effects encountered *in vivo*, these preparations also make possible experiments that could not be performed with living animals. The problem investigated in the present series of experiments concerns the role of the axonal microtubules (neurotubules) in the translocation of dense-cored vesicles and the site of action of the antimitotic drugs colchicine and vinblastine, which are known to inhibit the movement of these visicles *in vivo* (Dahlström, 1970; Hökfelt & Dahlström, 1971).

METHODS

Ganglion/nerve preparation. The two hypogastric nerves from sixty cats of both sexes weighing 1·0–2·5 kg were used. Under intraperitoneal Nembutal anaesthesia, the hypogastric nerves were constricted 1·5–2·5 cm distal to the inferior mesenteric ganglion with fine silk ligatures; these were used to suspend the preparation *in vitro*. A similar ligature was placed on a group of preganglionic nerves. In some cases the colonic nerves were ligated 1–1·5 cm from the inferior mesenteric ganglion. The mesenteric and connective tissue was then dissected from the nerves and ganglion. The latter was frequently in several parts on either side of the inferior mesenteric artery. The ligated preganglionic nerves were cut proximal to their ligature, other preganglionic fibres were cut and the ganglion/ligated hypogastric nerve preparation was excised *en bloc*. In those cases in which the ganglion was astride the artery, part of the vessel was included in the ganglionic mass. The preparation was then either suspended in a test-tube or placed in a specially designed two compartment Perspex box.

In all the experiments the ganglion/nerve preparations were maintained in Eagles Minimal Essential Medium (Eagle's Medium MEM, Wellcome Research Laboratories, Beckenham, Kent) without added calf serum and containing the following antibiotics: penicillin (100 u./ml.); streptomycin (100 μg/ml.); polymixin (100 u/ml.) and nystatin (25 u./ml.).

Incubation in test-tubes. In these experiments the ganglion/nerve preparations were maintained at 37° C in large test-tubes (Text-fig. 1) containing Eagle's medium and gassed with 95% O_2 + 5% CO_2 saturated with H_2O by passage through Krebs bicarbonate saline at 37° C. In some experiments, after a 24 hr period of incubation, a second ligature was placed on the nerve trunks 0·5–1 cm proximal to the first ligature. The incubation was then continued for a further 24 hr.

Incubations in two compartment boxes. In some experiments it was desired to study the effects of colchicine and vinblastine on the axonal transport of noradrenaline when the drugs were in contact with the nerve trunks but were denied access to

the neuronal cell bodies in the ganglion. For this purpose a two compartment box was designed which allowed the ganglion to be isolated from a substantial length of post-ganglionic nerve trunk by a water-tight partition (Text-fig. 2). In setting up the preparation, the lower half of the partition was placed in position after applying silicone grease (M 494 I.C.I. Ltd.) to the narrow surfaces. The upper partition was similarly greased and the 'half' holes on both partitions were filled with grease. The nerve trunks were then laid upon the grease in the lower 'half' hole and the upper part of the partition was placed in position on top of the lower part; the threads were then secured to the Perspex posts at each end of the box. The nerve trunk thus passed through a grease seal in the Perspex barrier. In all experiments 1 μc of [^{14}C]sodium acetate or [^{14}C]valine was added to the Eagle's medium on the ligated nerve trunk side of the preparation so that any leakage through

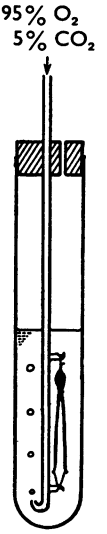


Fig. 1.

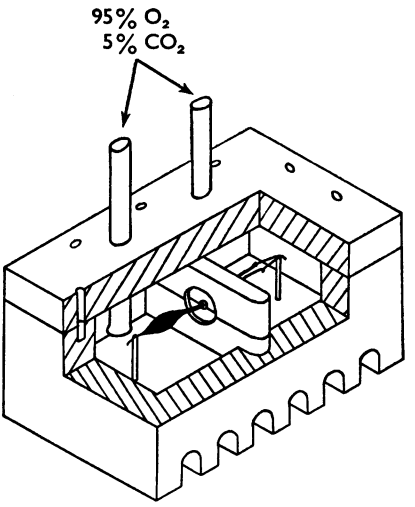


Fig. 2.

Text-fig. 1. Diagram of incubation test-tube. A single ganglion/nerve preparation was supported by silk threads tied to hooks attached to the gassing tube. The vessel contained 30 ml. Eagle's medium gassed with $O_2 + CO_2$ (95 : 5) and was maintained at 37° C in a water-bath. The thread tied to the post-ganglionic nerve trunk also provided the distal constricting ligature.

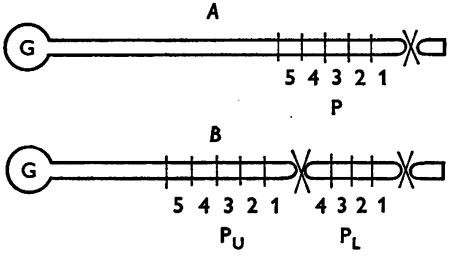
Text-fig. 2. Diagram of two-compartment Perspex box showing the positioning of the nerve/ganglion preparation. The preparation was mounted in the box with the nerve trunks passing through the silicone grease seal in the Perspex barrier as described in the text. The two compartments were then filled with Eagle's medium and 1 μC [^{14}C]valine or [^{14}C]acetate was added to the compartment containing the distal portion of the nerve trunk. Where appropriate colchicine or vinblastine sulphate was also added to that compartment. The lid was then screwed on and the box was submerged in a water-bath at 37° C and each compartment was gassed with 95% $O_2 + 5\%$ CO_2.

The dimensions of the box were 8·7 × 6·3 × 3·7 cm and each of the two compartments had a volume of 35 ml.

the barrier could be detected by the appearance of radioactivity in the compartment containing the ganglion. Each compartment of the box was gassed with 95% O_2 + 5% CO_2 saturated with H_2O by passage through Krebs bicarbonate saline at 37° C.

Segmentation of the nerves. After 24 or 48 hr incubation the nerves were placed on a dry postcard. The regions proximal to the ligature (and, where appropriate, adjacent to the situation of the water-tight partition) were cut into small segments 0·8 mm long (see Text-fig. 3 *a*, *b*).

Electron microscopy. In some of the incubation experiments in test-tubes, the colonic nerves were included in the ganglion/constricted nerve preparations. These colonic and/or the hypogastric nerves were used for electron microscopy. At the end



Text-fig. 3. Diagrams indicating the segmentation of the constricted nerves for chemical analysis and electron microscopy. G, inferior mesenteric ganglion. X, sites of constriction. A, Segments 1–5: proximal to single constriction from either test-tube or divided box experiments. *B*. Segments P_L1–4: above lower constriction applied when preparation excised from animal. Segments P_U 1–5: above second or upper constriction applied after initial 24 hr incubation.

of the incubation period the nerves were placed in a pool of ice-cold fixative and segments P1 to P3 proximal to the constriction were cut as indicated in Text-fig. 3*a*. A 1 cm piece of nerve, situated about halfway between the ganglion and segment P3, and the ganglia were also cut into small pieces for electron microscopy. Two methods of fixation were used: (1) 1% osmium tetroxide adjusted to pH 7·3–7·4 with veronal acetate buffer for 1½–2 hr, or (2) 3% glutaraldehyde in phosphate buffer at pH 7·3 for 4 hr. The tissue was then washed in frequent changes of the buffer for 24–48 hr and post-fixed in 1% aqueous osmium tetroxide for 2 hr. Following fixation by either method, the specimens were dehydrated in graded ethanols passed through epoxy propane and embedded in epoxy resin (Araldite, Ciba, England). The segments of nerve near to the site of constriction were orientated and cut longitudinally. The portions of the nerve remote from the site of constriction were cut transversely so that the number of microtubules in transverse or short oblique sections of non-myelinated axons could be counted. Ultra-thin sections were stained with lead citrate (Reynolds, 1963) and examined in either a Philips EM 200 or an A.E.I. 6B electron microscope. For quantitative studies on the microtubules, random fields were photographed at a primary magnification of 20,550×. From these standard prints were produced to give a final magnification of 51,375×. The number of microtubules cut either transversely or in short oblique section were counted in each non-myelinated axon. The axonal area was estimated by tracing their outline on to constant weight paper, cutting out the profiles and weighing the paper.

Noradrenaline present in corresponding segments from both hypogastric nerves of a single preparation was measured by the fluorimetric method of Häggendal (1963). The two segments were homogenized in 0·1 ml. ice-cold 1% perchloric acid and centrifuged. The supernatant was retained and the sediment was extracted with a further 0·1 ml. ice-cold 1% perchloric acid, before being recentrifuged. The two supernatants were pooled, diluted with 1 ml. H_2O, neutralized to pH 6·5 with 0·1M K_2CO_3 and then diluted to 2 ml. with H_2O. The $KClO_4$ was allowed to precipitate and a 1 ml. sample of the solution was used for the noradrenaline estimation without any further purification. 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 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 noradrenaline. The difference in intensity of the fluorescence at 515 mμ between samples and their faded blanks (see Häggendal, 1963) was directly proportional to the noradrenaline content of the samples. A standard curve was prepared for each set of noradrenaline determinations.

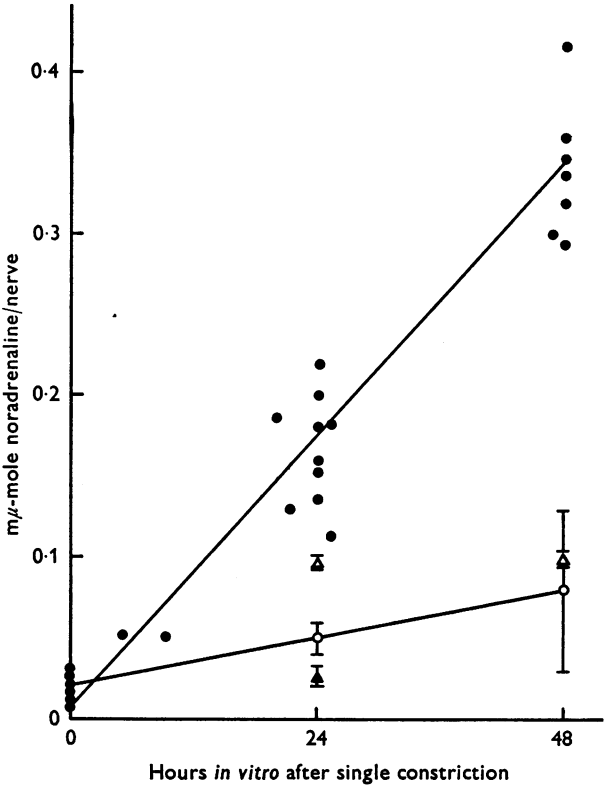
RESULTS

Single ligation experiments in test-tubes

The ultrastructure of the ligated non-myelinated axons and the neuronal cell bodies in the ganglion was well preserved despite 48 hr incubation *in vitro*. The changes immediately above the constriction and at more proximal levels in the nerve were identical with those seen in similar situations in ligated cat hypogastric nerves *in vivo*, and the variation between adjacent axons was equally evident (see Kapeller & Mayor, 1969; Banks *et al.* 1969). Large numbers of granular vesicles, mitochondria and myelin figures accumulated in non-myelinated axons, many of which were extremely swollen and distorted (Pl. 1, fig. 1). There was also an increase in the tubulovesicular components of the axonal endoplasmic reticulum and microtubules were considerably more prominent than usual, especially in the less swollen and in the more normal looking axons some distance from the constriction (see Pl. 1, fig. 2). The cytology of the neuronal cell bodies was similar to that seen in both normal ganglia and in ganglia where the hypogastric and colonic nerves had been ligated for 2 days *in vivo* (Tomlinson, Mayor, Mitchell & Banks, 1971).

In ganglion/nerve preparations where the hypogastric nerves had been ligated at one point, the amount of noradrenaline accumulating in Segments P1 + P2 + P3 (see Text-fig. 3*a*) increased linearly during the first 48 hr of incubation in the test-tubes (Text-fig. 4). The amount of noradrenaline accumulating in this part of the nerve was approximately half the amount found in a similar region in previous experiments on ligated hypogastric nerves *in vivo* (Banks *et al.* 1969). This may be related to the smaller size of the cats used in the present series of experiments or to the fact that the preganglionic input to the ganglion had been severed. The

method of least squares was used to calculate the best straight line fitting the experimental points (correlation coefficient 0·97). These data indicated that the noradrenaline accumulated proximal to the constriction at the rate of 0·007 mμ-mole/hr) and that the noradrenaline content of an 0·8 mm segment of nerve at zero time was 0·003 mμ-mole. Assuming that

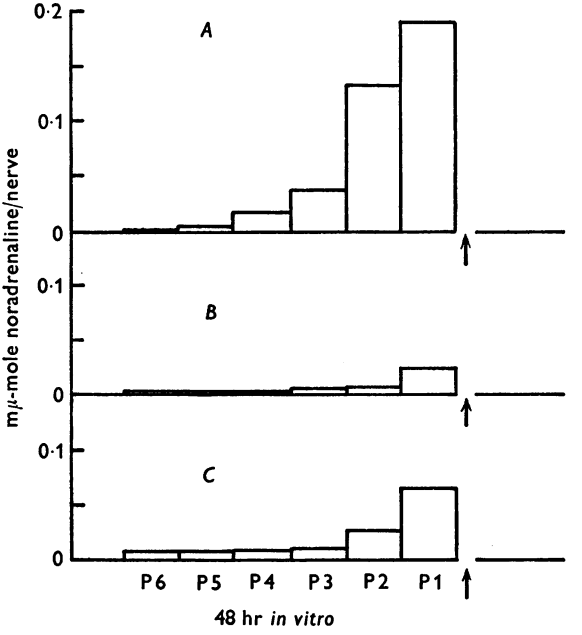


Text-fig. 4. Noradrenaline content of segments P1 + P2 + P3 proximal to a single constriction at different time intervals after incubation of a ganglion/ligated hypogastric nerve preparation in a test-tube. ●, Control incubation; ○, with added colchicine 10 μg/ml; ▲, with added vinblastine sulphate 10 μg/ml.; △, with added vinblastine sulphate 1 μg/ml.

the noradrenaline passes proximo-distally along non-myelinated axons (Mayor & Kapeller 1967; Banks *et al.* 1969) within granular vesicles, the rate of movement of the vesicles can be calculated to be 1·9 mm/hr. Direct analysis of 0·8 mm lengths of hypogastric nerves well away from the site of constriction showed that they contained 0·006 $\pm$ 0·001 mμ noradrenaline after 24 hr incubation *in vitro*. Using this value the rate of movement of the vesicles was found to be 0·9 mm/hr. Despite the discrepancy in the two

values they are both close to the value of approximately 2 mm/hr found in ligated cat hypogastric nerves in previous experiments *in vivo* (Banks *et al.* 1969).

Incubation of ganglion/hypogastric nerve preparations with colchicine (10 μg/ml.) caused a marked reduction in the accumulation of noradrenaline adjacent to the constriction at both time intervals studied (Table 1 and Text-fig. 5).



Text-fig. 5. Histograms illustrating the distribution of noradrenaline in constricted hypogastric nerve/inferior mesenteric ganglion preparations maintained for 48 hr *in vitro*. The site of constriction of the nerves is indicated by the vertical arrow. Segmentation as in Text-fig. 3*A*. *A*, Control incubation; *B*, with added colchicine 10 μg/ml.; *C*, with added vinblastine sulphate 1 μg/ml. Each of the histograms *A*, *B*, and *C* refers to a single experiment.

This was accompanied by a very obvious decrease in the number of dense-cored vesicles seen in axon profiles from segment P1 (Pl. 2). Furthermore the axons were frequently less swollen and appeared to contain fewer microtubules than those incubated without the drug. Mitochondria still accumulated in the axons close to the constriction despite the presence of colchicine. At a concentration of 1 μg/ml., colchicine showed only a slight tendency to inhibit noradrenaline accumulation.

Vinblastine sulphate (1 μg/ml.) was almost as potent in preventing the accumulation of noradrenaline and dense-cored vesicles as colchicine

(10 μg/ml.) and had similar effects upon the morphology of the axons close to the constriction (see Pl. 3). It did not prevent the accumulation of mitochondria immediately above the constriction.

These observations indicate that both colchicine and vinblastine inhibit the movement of noradrenaline in post-ganglionic noradrenergic nerves. However, they give no indication of the site of action of these drugs. They could, for example, inhibit the synthesis of dense-cored vesicles in the neuronal cell bodies, interfere with intra-axonal transport mechanisms or deplete dense-cored vesicles of their stored noradrenaline. Further experiments were designed to examine these possibilities.

TABLE 1. The effect of colchicine and vinblastine on the accumulation of noradrenaline in segments P1 + P2 + P3. Ganglion/hypogastric nerve preparations were incubated in test-tubes as described in the text for 24 or 48 hr in the presence or absence of drugs. Each hypogastric nerve carried a single ligature. Figures in parentheses indicate the number of observations

	Noradrenaline accumulating (mμ-mole/nerve ± S.E. of mean)	
Drug added	24 hr	48 hr
None	0·17 ± 0·01 (8)	0·34 ± 0·02 (7)
Colchicine 1 μg/ml.	—	0·22 ± 0·05 (4)
10 μg/ml.	0·06 ± 0·01 (4)	0·08 ± 0·03 (3)
Vinblastine 1 μg/ml.	0·10 ± 0·00 (2)	0·10 ± 0·01 (2)

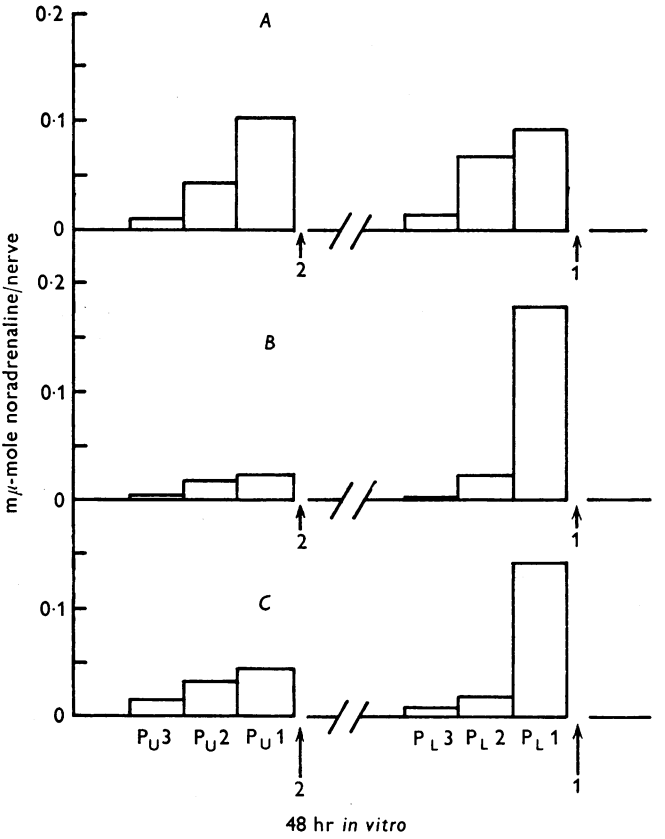
Asynchronous double ligation experiments in test-tubes

In these experiments ganglion/ligated nerve preparations were incubated in test-tubes for 24 hr before a second ligature was placed on each nerve 0·5 cm proximal to the first ligature. Then, in all except the control experiments, either colchicine or vinblastine sulphate was added to the incubation medium and remained there throughout the second 24 hr period of incubation. In the control experiments, after a total period of 48 hr incubation, the amount of noradrenaline immediately proximal to the second or upper constriction did not differ significantly from the amount adjacent to the first or lower constriction (Table 2 and Text-fig. 6*A*).

The presence of colchicine (10 μg/ml.) or vinblastine sulphate (1 or 10 μg./ml.) in the incubation medium during the second 24 hr period greatly decreased the amount of noradrenaline accumulating close to the second or upper constriction. However, these drugs did not diminish the amount of noradrenaline found proximal to the first or lower constriction (Table 4 and Text-fig. 6*B*, *C*).

These experiments indicate that neither colchicine (10 μg/ml.) nor vinblastine sulphate (1 or 10 μg/ml.) deplete stores of noradrenaline accumulated against a ligature before their addition to the incubation medium. They

also confirm the previous observations that incubation with colchicine (10 μg/ml.) or vinblastine (1 μg/ml.) prevents the accumulation of noradrenaline proximal to a ligature.



Text-fig. 6. Histograms illustrating the distribution of noradrenaline in hypogastric nerve/inferior mesenteric ganglion preparations following asynchronous double ligation. Initially applied ligature indicated by vertical arrow 1. The second ligature (vertical arrow 2) was applied after 24 hr incubation. Drug was present only during the second 24 hr period of incubation. Total incubation period was 48 hr. Segmentation as in Text-fig. 3*B*. *A*, Control incubation; *B*, colchicine, 10 μg/ml., added after first 24 hr incubation; *C*, vinblastine sulphate, 1 μg/ml., added after first 24 hr incubation. Each of the histograms *A*, *B*, and *C* refers to a single experiment.

Divided box experiments

In these experiments the ganglion/nerve preparations were set up as shown in Text-fig. 2. The grease seal in the Perspex barrier caused no obvious constriction of the nerve trunks but, as indicated by the lack of movement of radioactively labelled valine or acetate from one compart-

ment to the other, it prevented any mixing of the Eagle's medium in the two compartments. The presence of colchicine (10 μg/ml.) or vinblastine (1 μg/ml.) in the compartment containing the ligated distal portion of the nerve trunks caused a considerable diminution in the amount of noradrenaline accumulating proximal to the constriction.

This reduction in the amount of noradrenaline close to the constriction was frequently associated with an accumulation of noradrenaline in the

TABLE 2. The lack of effect of colchicine and vinblastine on stores of noradrenaline accumulated proximal to a constriction before the addition of these drugs. Ganglion/ligated hypogastric nerve preparations were incubated *in vitro* for 24 hr. Then a second ligature was applied to each nerve proximal to the first and colchicine or vinblastine was added to the Eagle's medium for a further 24 hr incubation at 37° C. Segments $P_L1+P_L2+P_L3$ were immediately proximal to the first or lower constriction and segments $P_U1+P_U2+P_U3$ were proximal to the second or upper constriction. Figures in parentheses indicate number of observations

	Noradrenaline accumulating (mμ-mole/nerve ± S.E. of mean)	
	Segments	Segments
Drug	$P_U1+P_U2+P_U3$	$P_L1+P_L2+P_L3$
None	0·16 ± 0·02 (4)	0·18 ± 0·02 (4)
Colchicine 10 μg/ml.	0·06 ± 0·01 (2)	0·18 ± 0·03 (2)
Vinblastine 10 μg/ml.	0·03 ± 0·00 (2)	0·19 ± 0·06 (2)
1 μg/ml.	0·10 ± 0·00 (2)	0·16 ± 0·01 (2)

TABLE 3. Effect of colchicine and vinblastine on the movement of noradrenaline in nerve trunks isolated from the inferior mesenteric ganglion by a grease barrier (see text for arrangement of experimental box). Colchicine and vinblastine were present only in the compartment containing the distal ligated portion of the nerve trunks. Figures in parentheses indicate the number of observations

	Noradrenaline accumulating (mμ-mole/nerve ± S.E. of mean)	
Drug in nerve trunk compartment	24 hr	48 hr
None	0·23 ± 0·04 (3)	0·29 ± 0·02 (3)
Colchicine 10 μg/ml.	0·10 ± 0·02 (3)	0·10 ± 0·04 (3)
Vinblastine 1 μg/ml.	—	0·12 ± 0·02 (3)

region of the nerve trunk either within or just proximal to the grease barrier. Such accumulations of noradrenaline in the vicinity of the barrier did not occur in control experiments.

These experiments indicate that when colchicine or vinblastine are in contact with the nerve trunk, but have no direct access to the neuronal cell bodies they are still able to inhibit the proximo-distal movement of noradrenaline and dense-cored vesicles.

Effect of colchicine and vinblastine sulphate on axonal microtubules

Electron microscopic examination of P1 segments (compare Pl. 1 with Pls. 2, 3) led to the subjective impression that colchicine (10 μg/ml.) and vinblastine sulphate (1 μg/ml.) caused a reduction in the number of axonal microtubules. To carry out a quantitative assessment, the number of microtubules in transverse sections of non-myelinated axons midway between the ganglion and constriction were counted in control and drug-treated ganglion/nerve preparations incubated in test-tubes for 48 hr. This region was chosen because, although the axons were not entirely normal, they showed no gross distortion characteristic of the region immediately adjacent to the ligation (Pls. 4–6).

TABLE 4. The effect of colchicine and vinblastine sulphate on the number of microtubules in non-myelinated axons from transverse sections of ligated hypogastric nerve attached to the inferior mesenteric ganglion and maintained for 48 hr *in vitro*. The figures in parentheses indicate the number of axonal profiles counted

Drug	Microtubules/axon ± S.E. of mean	Microtubules/μ^2 of axon ± S.E. of mean
None	16·5 ± 0·5 (91)	75·5 ± 3·1 (91)
Colchicine 10 μg/ml.	1·1 ± 0·05 (105)	5·3 ± 0·3 (105)
Percentage reduction	93·3	93·0
Vinblastine sulphate 1 μg/ml.	8·4 ± 0·15 (123)	29·0 ± 1·5 (123)
Percentage reduction	49·1	61·5

Data obtained from a total of 91 axons indicated that control axons incubated for 48 hr contained 16·5 ± 0·5 microtubules per axon. Axons from preparations incubated with colchicine (10 μg/ml.) or vinblastine sulphate (1 μg/ml.) for 48 hr contained only 1·1 ± 0·05 or 8·4 ± 0·15 microtubules per axon respectively (Table 4). The data in this Table suggest that the axons in control and colchicine-treated nerves were of a similar size whilst after treatment with vinblastine sulphate some axons were swollen.

The abundance and ultrastructural appearances of fine filamentous structures (neurofilaments) in both transverse and longitudinal sections of non-myelinated axons were not obviously changed by treatment with either colchicine or vinblastine sulphate. Furthermore no significant changes in the tubulo-vesicular components of the axonal endoplasmic reticulum were detected following treatment with either drug.

DISCUSSION

It is clear from these results that the production and transport of granular vesicles and noradrenaline can continue for up to 48 hr in constricted sympathetic neurones isolated from the body and maintained in an artificial environment. The preparations described have the advantage of eliminating many of the uncontrollable factors which arise in studying the effects of drugs on noradrenergic neurones in intact animals. This type of preparation should prove valuable for investigating various aspects of the cellular dynamics of the noradrenergic neurone. Furthermore, the double ligation experiments provide a novel method for studying the influence of drugs on noradrenaline storage granules in an intra-axonal environment which is more physiological than any that can be provided for granules isolated by traditional methods of tissue homogenization and centrifugation.

The present experiments suggest that colchicine and vinblastine prevent the accumulation of noradrenaline storage vesicles above a constriction by interacting with components of the post-ganglionic nerve trunk rather than by inhibiting the formation of the granular vesicles in the neuronal cell bodies, or by depleting the accumulated granular vesicles of their store of noradrenaline.

Since both colchicine and vinblastine are known to interact with the protein subunits of microtubules (Schmitt, 1969) it has been suggested that these fibrous protein elements are in some way involved in the rapid proximo-distal movement of granular vesicles within non-myelinated noradrenergic axons (Dahlström, 1970; Hökfelt & Dahlström, 1971). This view is supported by the present findings that low concentrations of colchicine or vinblastine, which greatly reduce the proximo-distal movement of granular vesicles and noradrenaline, also cause marked reductions in the number of microtubules present in the non-myelinated axons of constricted hypogastric nerves. The latter was the only ultrastructural change that could be unequivocally attributed to the action of colchicine and vinblastine, apart from the reduction in the number of granular vesicles accumulating against the constriction. There was no evidence to suggest that the microtubules were converted into neurofilaments (Hökfelt & Dahlström, 1971). Indeed such a conversion of microtubules to 'neurofilaments' is unlikely to occur in view of the very different amino acid compositions of their component proteins (Davison, 1970; Davison & Huneeus, 1970; Huneeus & Davison, 1970).

These results are consistent with the view that there is a close relationship between the structural integrity of the microtubules and the ability of noradrenergic axons to maintain the proximo-distal movement of granular vesicles.

Although a quantitative determination of the amount of cytochrome oxidase accumulating above a ligature has not been made in the present series of experiments, a subjective assessment of the number of mitochondria present in electron micrographs of segments P1 to P3 (Pls. 2, 3) suggests that colchicine and vinblastine have little, if any, effect upon the movement of mitochondria. This agrees with the observations of Kreutzberg (1969) on rat sciatic nerve and leads to the conclusion that microtubules are not involved in the movement of mitochondria. These findings support previous observations indicating that the mechanism responsible for the transport of granular vesicles differed from that involved in mitochondrial movement in constricted non-myelinated axons *in vivo* (Banks *et al.* 1969).

Although the experiments described in this paper are suggestive of a role for microtubules in the axonal transport of noradrenaline storage vesicles, the nature of the relationship between these two structural elements remains to be discovered. At the present time no close association between microtubules and vesicles, such as that observed by Smith, Järlfors & Beránek (1970) in lamprey nerves, has been found in noradrenergic neurones.

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EXPLANATION OF PLATES

PLATES 1–3

All electron micrographs are from nerves fixed in osmium tetroxide. The sections were stained with lead citrate. The magnification markers represent 1 μ. A indicates non-myelinated axons, S indicates Schwann cell cytoplasm.

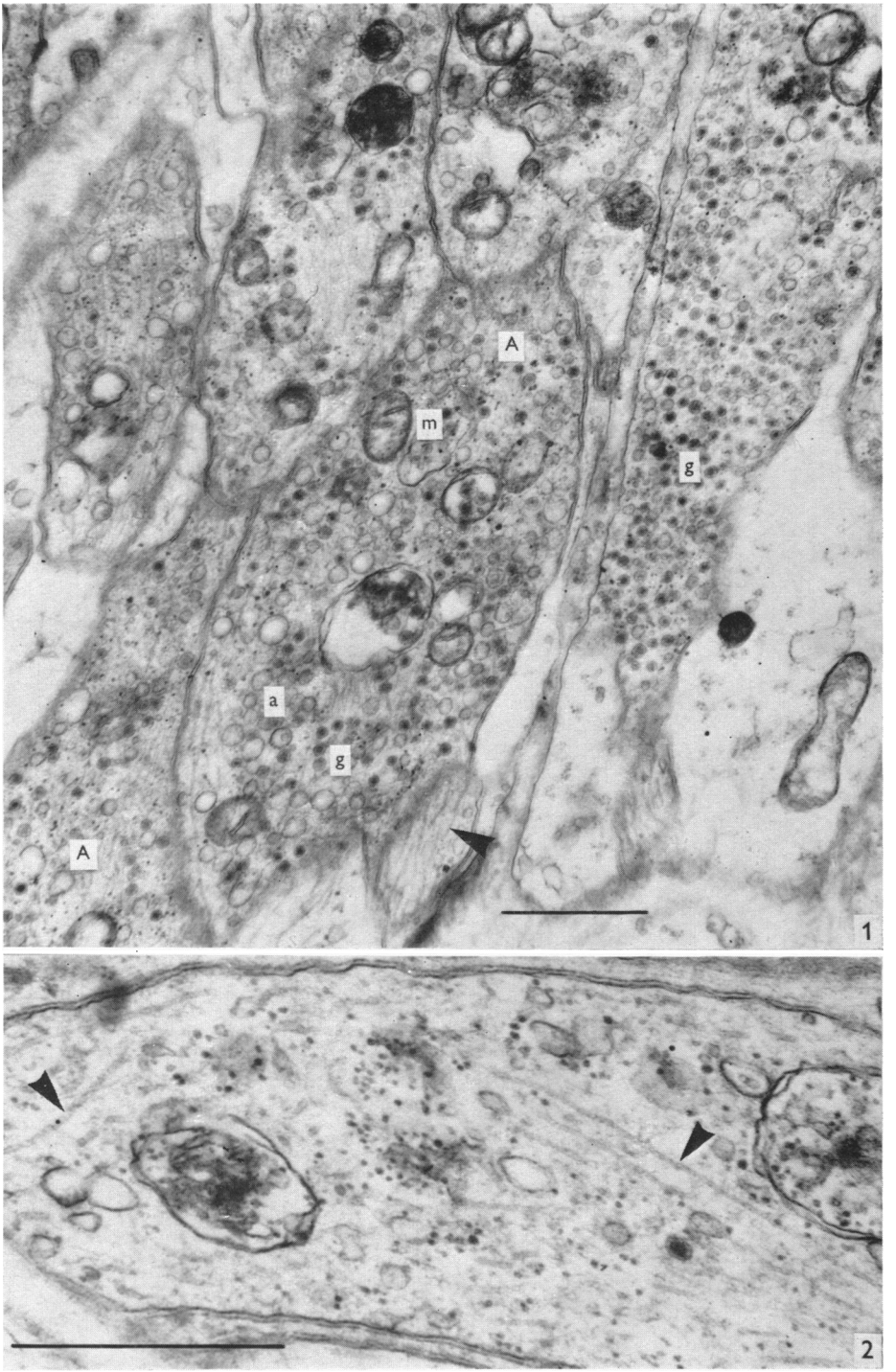
PLATE 1

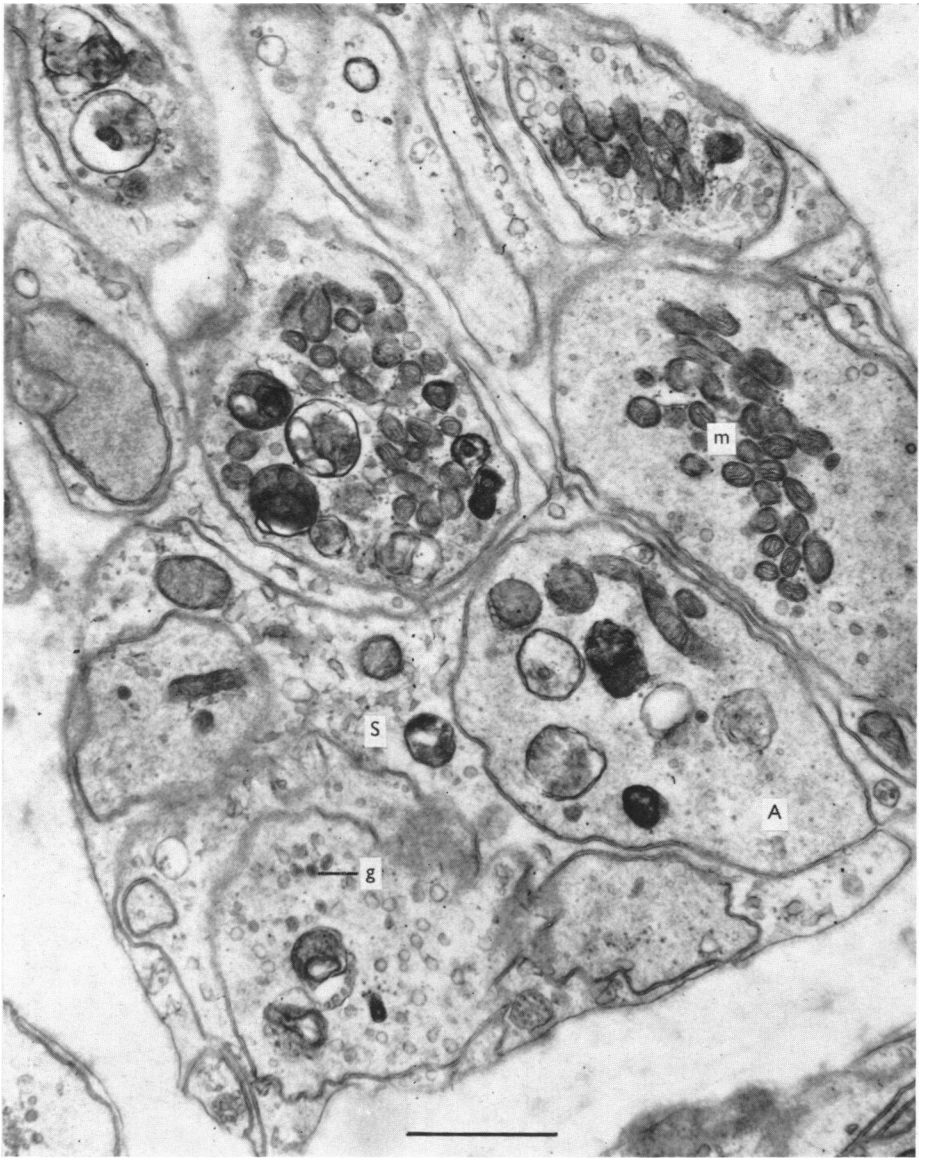
Electron micrographs from the middle of segment P1 (Text-fig. 3A) after 48 hr incubation in Eagle's medium only. Fig. 1 illustrates the accumulation of granular or dense-cored vesicles (g), agranular vesicles (a) in slightly swollen and deformed axons. Microtubules (arrowed) can be seen in the narrower axons. The mitochondria (m) are swollen.

Fig. 2 illustrates typical microtubules (arrowed) in a longitudinal section of a slightly swollen axon at higher magnification.

PLATE 2

Electron micrograph from the middle of segment P1 after 48 hr incubation in Eagle's medium with added colchicine (10 μg/ml). Several axons are swollen, mitochondria (m) have accumulated in several axons. Granular or dense-cored vesicles (g) are exceptionally rare and microtubules cannot be identified. The axoplasm has an amorphous appearance.


A
m
g
a
g
A
1
2


m
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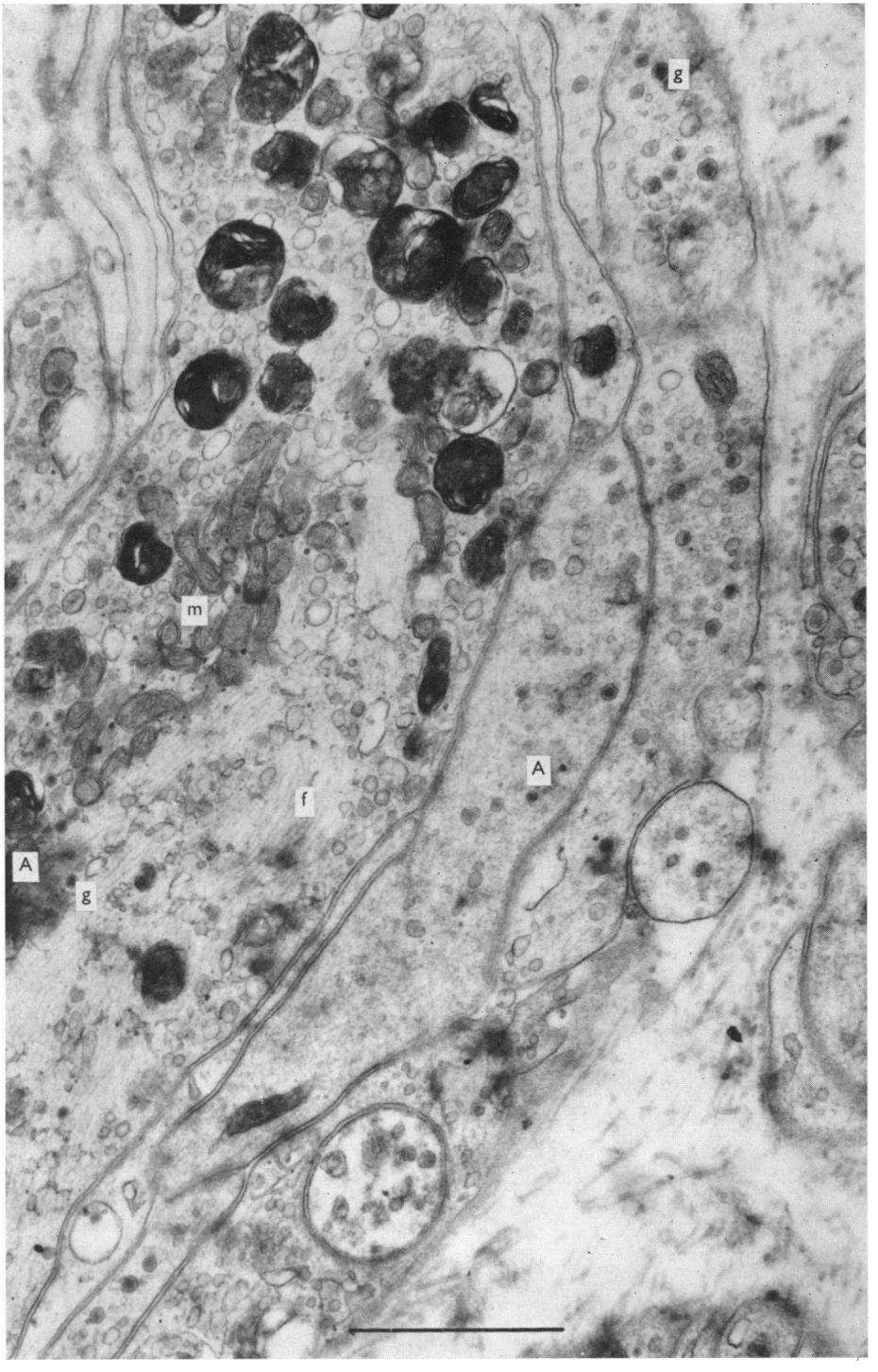
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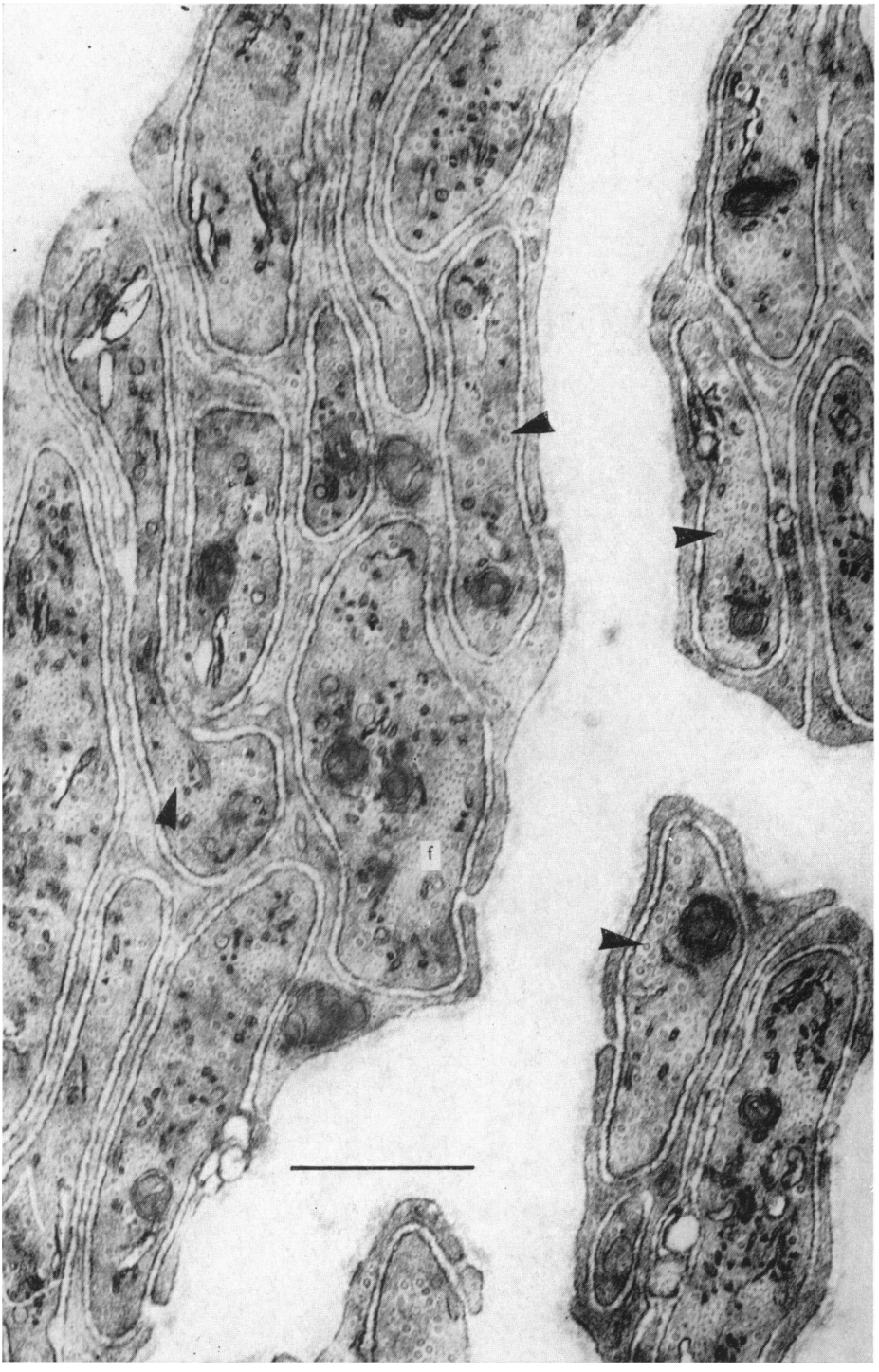
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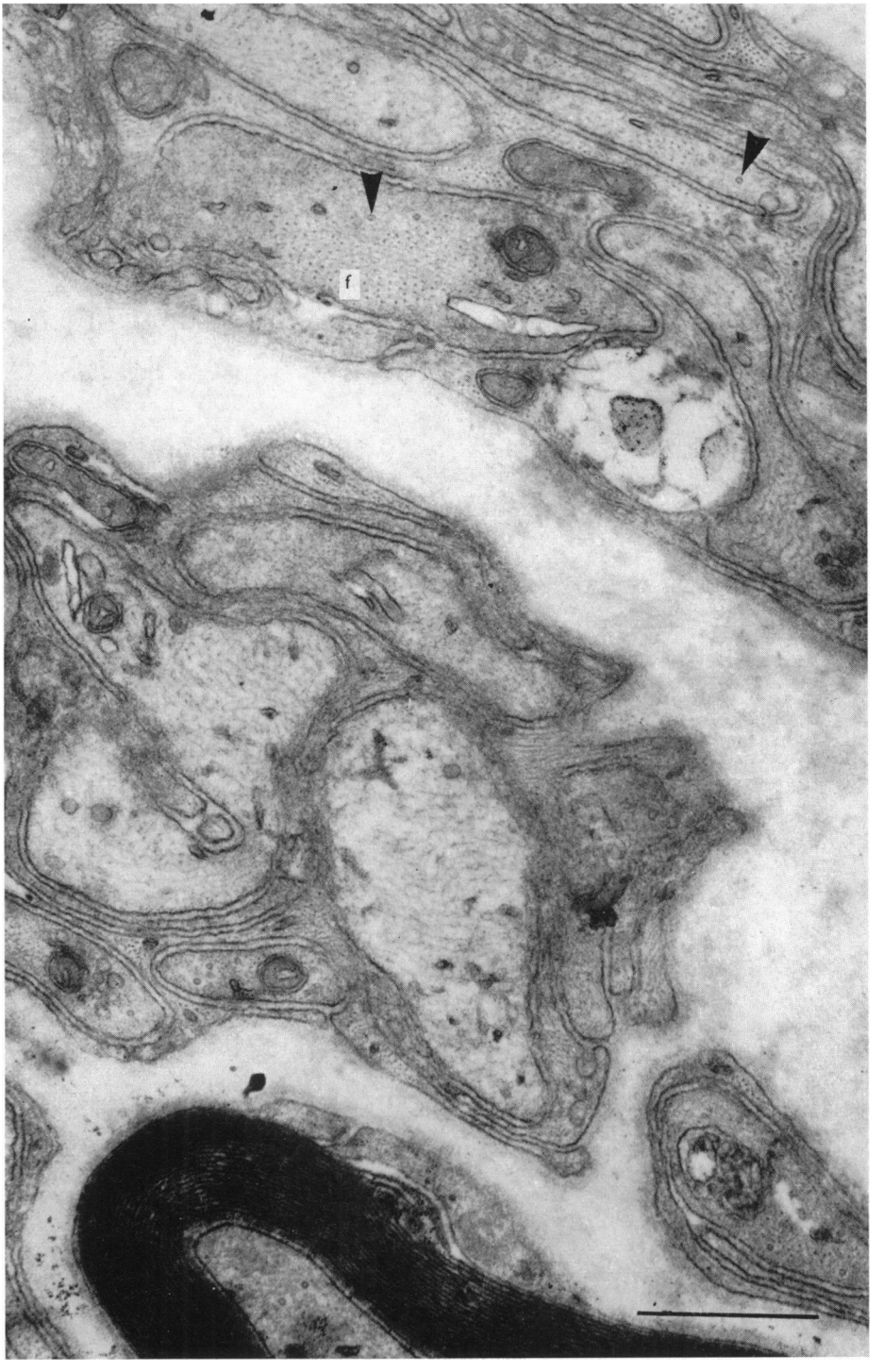
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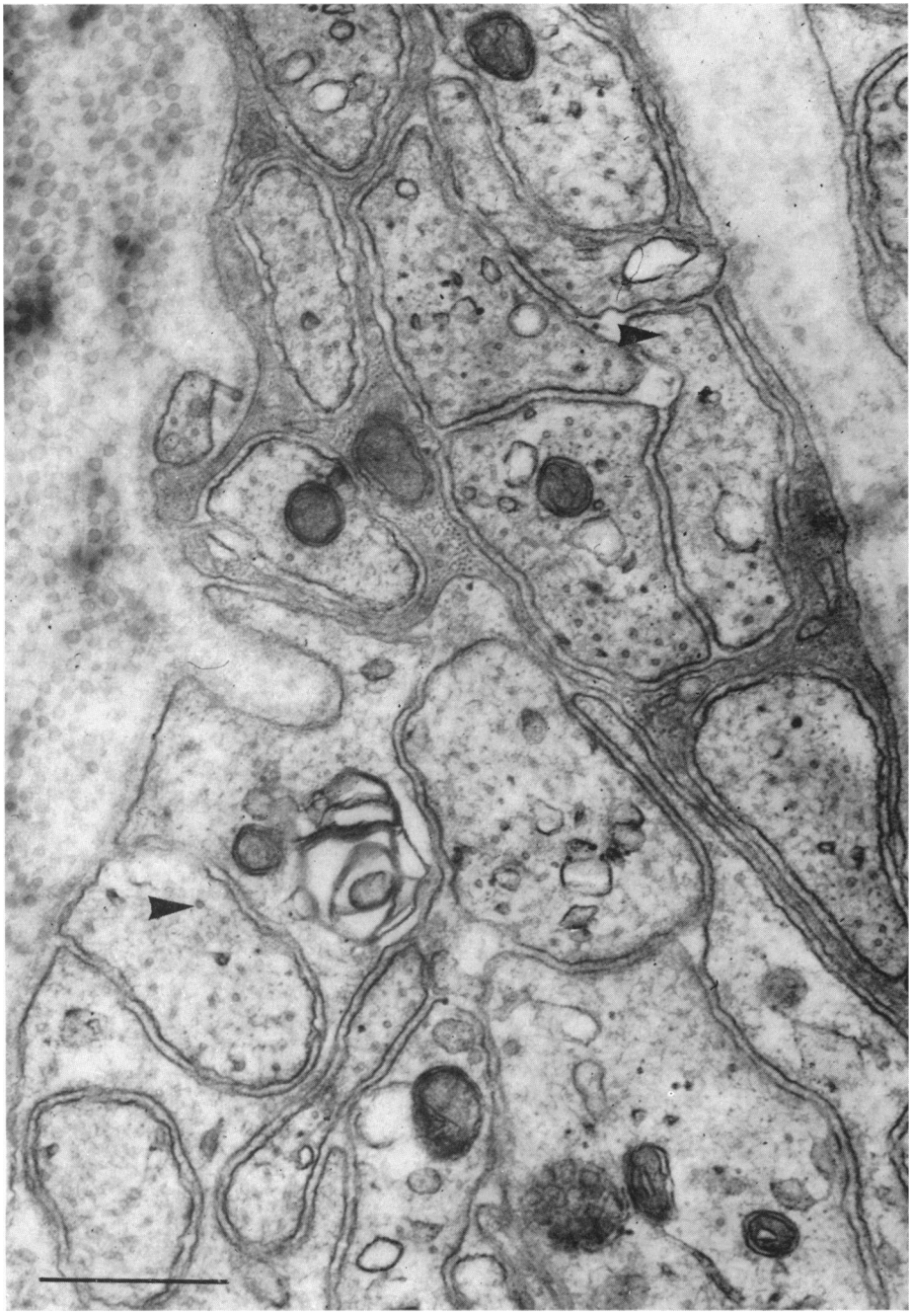

PLATE 3

Electron micrograph from middle of segment P1 after 48 hr incubation in Eagle's medium with added vinblastine sulphate (1 μg/ml). The more swollen non-myelinated axon contains several mitochondria (m) and a few granular vesicles (g). Microtubules cannot be identified but fine filaments (f) are present.

PLATES 4–6

In these electron micrographs from transverse sections of hypogastric nerves mid-way between segment P3 (see Text-fig. 3A) and the ganglion there is a considerable widening of the interstitial spaces and distortion of the non-myelinated axons cut in transverse or short oblique section due to the incubation for 48 hr in Eagle's medium *in vitro*. Glutaraldehyde followed by osmium tetroxide fixation. Magnification markers equal 0·5μ.

PLATE 4

From a control incubation, illustrates the abundance of microtubules (arrowed) with their typical clear halo when cut transversely. Fine filamentous structures (f) seen as dark gray or black dots in transvere sections are very common.

PLATE 5

From an experiment with colchicine 10μg/ml. added to the incubation medium. Note the paucity of microtubules (arrowed). Fine filaments (f) are just as common as in control incubation

PLATE 6

From an experiment with vinblastine sulphate 1 μg/ml. added to incubation medium. The non-myelinated axons contain fewer microtubules (arrowed) than control axons (Pl. 4). The amorphous nature of the axoplasm obscures the fine filaments in this micrograph.