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Axoplasmic Transport (with Particular Respect to Adrenergic Neurons)

Annica Dahlstrom

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By Annica Dahlström
Institute of Neurobiology, Department of Histology, University of Göteborg, Göteborg, Sweden

[Plate 61]

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The article presents a review and summary on the development of studies in the field of axoplasmic transport in non-adrenergic and adrenergic neurons. Results obtained by, for example, histochemical, biochemical, electronmicroscopical, autoradiographical and radiological studies are discussed. Two main types of axoplasmic transport appear to operate in neurons; the slow axonal flow of 1 to 2 mm/day, and the fast transport exceeding 100 mm/day. However, also intermediary rates have been observed. The influence of nerve activity and drugs on axoplasmic transport is discussed, in relation to the possible mechanisms for axoplasmic transport which may operate in the neuron.

Introduction

In living cells, of both animal and plant origin, intracellular transport of cell organelles occurs. Such a transport is essential for the metabolism of the cell. Whilst neurons often have extremely long processes the protein synthesizing machinery is concentrated in the cell body. The very low concentrations of RNA which have been found in axons (Edström, Eichner & Edström 1962; Koenig 1965), are probably insufficient for synthesizing the protein needed by the axons and the nerve terminals. Efficient systems for intraneuronal transport of materials, particularly from the cell body into the nerve processes, therefore appear necessary.

The first author to introduce ideas of a transport of material from the cell body down the axon, was probably Scott, in 1906. In his experiments he cut the dorsal roots of frogs close to their ganglia, stimulated the central end and studied the effect of cutting and varied impulse frequencies on the fatiguability of the reflex arch. In cut roots the reflex permanently disappeared earlier when a large number of stimuli was given than when a small number was given. Roots connected to their ganglia could recover after a period of rest in contrast to cut roots. The difference between cut and intact roots was probably not due to degeneration of the cut roots since cut roots, left unstimulated, were effective for far longer periods than stimulated cut roots. Scott also ruled out muscle fatigue and fatigue of the motor neurons, and proposed instead that the ineffectiveness of stimulated, cut roots was the result of a using up of 'all the substance which passes between the root and the cells in the cord'. This sentence in fact suggests chemical transmission. He states in his summary: '...it seems to me simpler to suppose that the nerve cells secrete a substance the passage of which from the nerve endings is necessary to stimulation. The recovery of effect after transient fatigue I attribute to the passage of a portion of this substance down the nerve fibre to the nerve ending.' It is amazing to read these speculative sentences some 60 years later when it has been found that the amine storage granules, essential for normal transmission in adrenergic nerves, are formed in the cell body and transported down the axon to the nerve terminals (see, for example, Dahlström & Häggendal 1970).

The first direct observations which indicated a proximodistal movement of axoplasm were made on regenerating nerves (for review see, for example, Cajal 1928; Young 1942; Gutmann 1958). The outgrowth of regenerating fibres must involve a process which causes axoplasm to move down into the regenerating tip. In the experiments of Weiss & Hiscoe (1948) nerves were partially obstructed. They found that the diameter of the axons was reduced, not only within the area of obstruction, but also in all parts of the axons distal to the obstruction. Proximal to the obstruction the fibres were dilated, with balloon-like distortions of the axons, separated by 'bottlenecks'. When the chronic obstruction was removed, the diameter of the distal parts widened and that of the proximal parts shrank, the axons thus gradually regaining normal appearance (figure 1). These results were interpreted to demonstrate a steady transport of axoplasm, from the cell body and down the axons. Based on more direct observations, the concept of axoplasmic flow was formulated.

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This concept initially referred to the slow (1 to 2 mm/day) steady movement of axoplasm down the axon, the 'perpetous nerve growth'. Later, however, it was demonstrated that certain substances, e.g. acetylcholine esterase (AChE) and vesicles, accumulated faster than 1 to 2 mm/day (see below and review by Lubińska 1964). In addition to axoplasmic *flow* an active axoplasmic *transport* of certain substances and particles has during the last few years been demonstrated to take place in neurons. Recently it has been stated (Martinez & Friede 1970) that 'the theory of axoplasmic flow, as originally stated, is unacceptable'. One may agree on this, but it should also be pointed out that the axoplasmic flow concept, when introduced, represented a very important step forward in biological sciences. The recent discoveries of fast axoplasmic transport is not opposed to, but rather extends the concept of, axonal flow. As Weiss (1967) states: '. . . the continuous outgrowth of the axon from its root in the cell body and its cellulifugal propulsion as a semisolid column in no way precludes the presence within that carrier system of separate routes or channels for express traffic'.

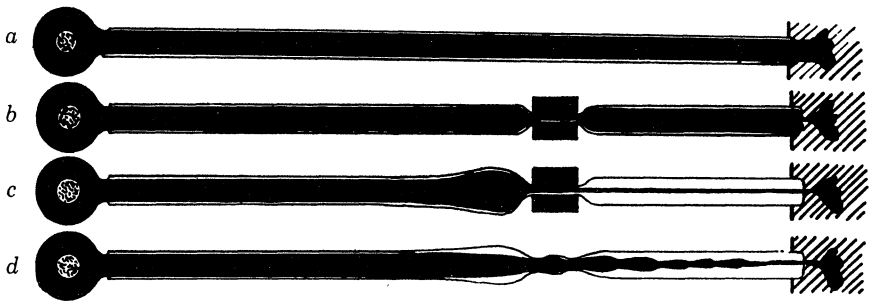

FIGURE 1. Schematic representation of single nerve fibres subject to chronic constrictions: (*a*) normal mature fibre; (*b*) same fibre as *a* immediately after application of constricting cuff; (*c*) same fibre as *b* after assuming new stationary asymmetry: damming of neuroplasm on the proximal side of the 'bottleneck' coupled with reduction of caliber distally; (*d*) same fibre as *c* after removal of chronic construction: downflow of dammed-up neuroplasm. (From Weiss 1963.)

At present, it may appear convenient to differentiate between the slow type of flow (1 to 2 mm/day) and fast transport of material (100 to 700 mm/day), but intermediary rates have also been described (see, for example, Koenig 1958, Miani 1962, Frizell, Hasselgren & Sjöstrand 1970).

This article is not intended to give a complete review of the literature in a field, which has expanded greatly in the last few years. For work done before 1964 a complete review has been published by Lubińska (1964). Also Friede (1966) has written a review on axoplasmic flow, and the reader may, in addition, consult Barondes's (1969) article on, for example, methods for the study of axonal transport. The aim of this article is rather to give an introduction to, and a summary of, some interesting experiments and results on axoplasmic transport of different neuronal components in myelinated and unmyelinated, particularly adrenergic axons. I have chosen to divide the topic into non-adrenergic and adrenergic neurons, and to collect the material mainly according to the type of method and approach used by the different investigators.

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1. Axoplasmic flow in non-adrenergic neurons

(*a*) *Accumulation of enzymes in ligated nerves*

(i) *Oxidative enzymes*

The first publication concerning an enzyme in interrupted nerve fibres was probably the one by Marinesco (1924). He found an increased activity of oxidative enzymes in the enlarged parts of sciatic nerve axons (dog) just above the lesion. The same was found for the dog spinal cord. The part distal to the injury contained very little activity. Friede (1959) found, in rat sciatic nerve, that NAD-diaphorase and succinic dehydrogenase had accumulated in the proximal nerve part by 12 h after the cut. No increase was observed in the distal part. In contrast to this, Kreutzberg (1963), using the same preparation, found an increase of NAD-diaphorase, succinic, lactic, and malic dehydrogenases, not only in the proximal parts, but also in the distal stump up to 48 h after ligation. These studies were performed by histochemical methods. Since oxidative enzymes may be regarded as markers for mitochondria, we may compare these results with those of electronmicroscopical studies on mitochondria (§1 *b*(ii)). By either technique, increases were found on both sides of the lesion, the proximal accumulation being larger and longer lasting than the distal accumulations (Kreutzberg 1963; Zelená 1969). The results seem to indicate that this organelle can move bidirectionally in axons, at least after ligation. Evidence for a bidirectional movement of organelles in living nerves has also been obtained by microcinematography (see, for example, Burdwood 1965).

(ii) *Acetylcholinesterase* (*AChE*)

The accumulation of AChE in ligated nerves has been subject to many studies. Sawyer (1946) found, by a biochemical approach, an increase in enzyme activity in the proximal end of guinea-pig's sciatic nerve 2 to 38 days after interruption. In the distal part the enzyme activity fell. L. Lubińska *et al.* (1961) observed similar changes 4 to 6 day safter ligation of dog's phrenic nerve. When studied at shorter periods after transsection (4 h), a clear-cut accumulation of

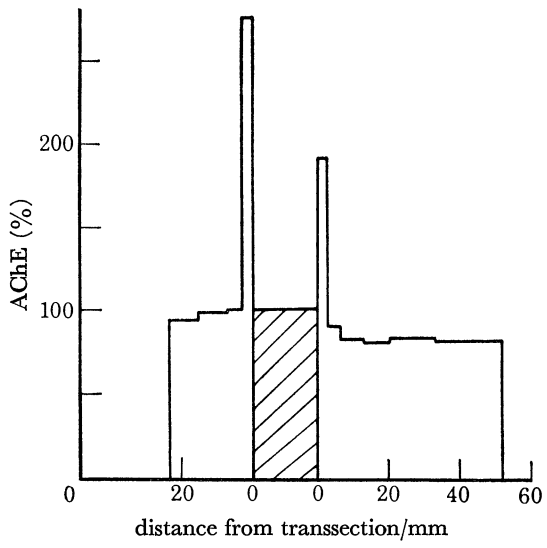

Figure 2. Changes in AChE activity in the peroneal nerve of dog near the site of transsection 4 h after cutting (as percentage of normal activity). The line shows the length of segments used for analyses and the activity in each segment. Hatched, normal activity in the segment removed at the time of operation. (From Lubińska *et al.* 1964.)

enzyme activity was also disclosed in the 2 to 3 mm just distal to transsection (dog peroneal and tibial nerves). The increase was clearly smaller in the distal part than in the proximal part (see also figure 2).

Using a histochemical technique Zelená & Lubińska (1962) have studied the distribution of AChE in nerve parts (dog, rat, and rabbit sciatic nerve) separated by double ligations that were performed simultaneously. The enzyme activity increased at both ends of the nerve segment, being more pronounced at the distal than at the proximal end. In the middle part the activity decreased markedly. A shift of AChE had occurred, especially towards the distal end, but also upwards. The results indicate that AChE may be transported bidirectionally, at least in ligated nerves.

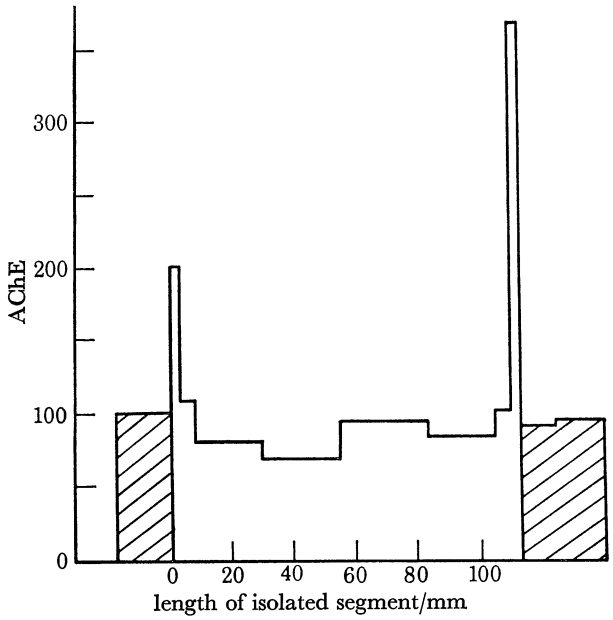

FIGURE 3. Longitudinal distribution of AChE activity (as percentage of normal) in the isolated peroneal nerve segment of dog (in percentage of normal activity) 5 h after cutting. Hatched, normal activity in pieces removed at the time of transsection. The line shows the lengths of pieces of nerve used for analyses and the activity of each piece. (From Lubińska *et al.* 1964.)

In a quantitative biochemical study, Lubińska, Niemierko, Oderfeld-Nowak & Szwarc (1964) found that although a shift of AChE had occurred in the nerve between two ligations, the total enzyme activity of the nerve was unchanged (Lubińska *et al.* 1964, see figure 3). It was concluded, therefore, that the accumulation of AChE activity observed above ligations was probably not due to local synthesis but to axoplasmic transport. The amount of AChE activity in the proximal 2 mm nerve above an 18 h ligation was about 6.5 times that in a normal piece of similar length (figure 4). From these data it was evident that the migrating portion of AChE in axons was transported at a comparatively high rate, several mm/day (see also Table IV in Lubińska 1964). The transport velocities for AChE in rat vagus and hypoglossal nerves after ligation have been studied by Fritzell, Hasselgren & Sjöstrand (1970). Local synthesis of AChE above the ligation was excluded by local injections of cycloheximide, a potent inhibitor of protein synthesis. No effect on AChE accumulation was observed after such injections. The authors arrived at figures of 15 and 5 mm/day in vagal and hypoglossal nerves, respectively. These figures are in good agreement with the results of Lubińska *et al.* (1964). In a recent study, Jankowska, Lubińska & Niermierko (1969) have calculated the minimal rate of transport of

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migrating AChE in dog peroneal nerve to be 5 mm/h, i.e. belonging to the category of fast transport. Still higher rates for proximodistal transport of AChE have recently been calculated (about 260 mm/day, Lubińska & Niemierko 1971).

The AChE which is transported along axons is most probably not bound to the axon membrane but to the membrane of an intra-axonal organelle (see, for example, Niemierko & Lubińska 1967). The endoplasmic reticulum has been suggested to be the carrier of the enzyme (see, for example, Brzin, Tennyson & Duffy 1966, and §1*b*(iv)).

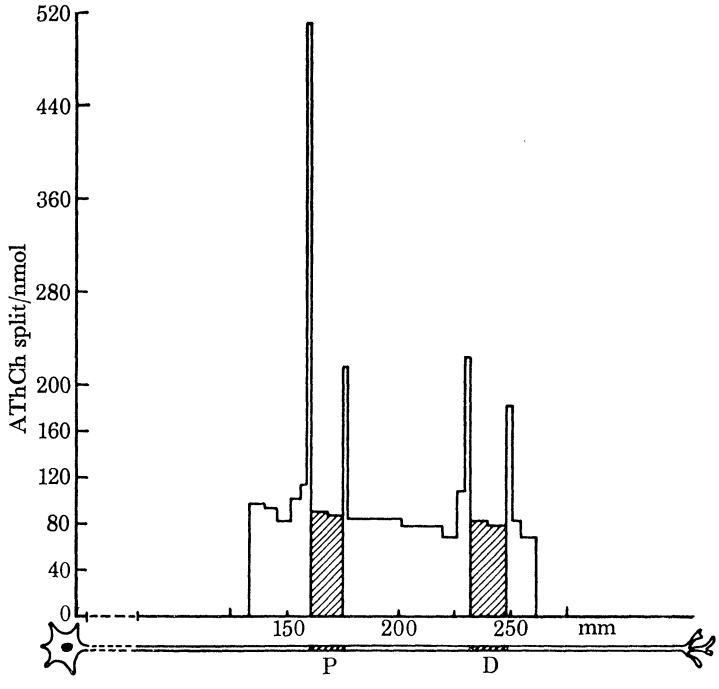

FIGURE 4. AChE activity in the peroneal nerve of dog cut in two places 18 h before dissection. Distance between cuts about 60 mm. P, proximal transsection; D, distal transsection. The line shows the length of pieces of nerve used for analyses and their AChE activity. Ordinate: AChE activity in nanomoles of AThCh split by 1 mm of nerve in 2 h. Abscissae: distance from cell bodies in millimetres. Hatched: normal activity of pieces removed at the time of operation. An increase of AChE activity is seen on both sides of each lesion. (From Lubińska *et al.* 1964.)

(iii) *Soluble enzymes*

Both phosphoglucoisomerase (PGI) and cholinacetylase (ChAc) are considered to be soluble enzymes, not bound to any particular organelle. These enzymes may therefore be expected to be carried distally with the slow flow of axoplasm. In accordance with this view PGI was found to behave differently from AChE in transected nerves. A small increase of PGI activity was observed in the proximal stump, but, in contrast to AChE activity, which continued to increase with time, the PGI ceased to accumulate after 4 h (Skrangiel-Kramska, Niemierko & Lubińska 1969). ChAc activity, on the other hand, which has earlier been demonstrated to accumulate in transsected nerves of the goat (Hebb & Silver 1961, 1963), has been found to accumulate approximately in parallel with AChE activity in rat vagal and hypoglossal nerves (Fritzell *et al.* 1970), and calculations performed by these authors revealed similar rates of transport for both AChE and ChAc activities in the nerves studied (see above). The results thus appear to be conflicting, since both PGI and ChAc, if localized freely in the axoplasm, should both behave differently from AChE, being membrane bound. It must be borne in mind, however, that

estimations of enzyme activity may not necessarily reveal the amount of enzyme protein. An enzyme may possibly change from an active into an inactive form, and thus escape detection with ordinary methods. Also, although ChAc is considered soluble in nerve endings isolated from brain tissues (see, for example, Fonnum 1967) the possibility remains that the enzyme in axons may be bound to an organelle, which is transported distally at fairly high rates. ChAc in peripheral axons (ventral roots and sciatic nerve of goat, dog and rabbit) has been reported to be present mainly in a particulate fraction (Hebb, Krause & Silver 1959). This may, however, be explained by an artificial binding of the positively charged ChAc to negatively charged membranes (see discussions in Fonnum 1967, 1970).

(*b*) *Accumulation of cell organelles after axotomy* (*studied by electronmicroscopy*)

With the introduction of electronmicroscopy the nerve fibre was subjected to numerous investigations on the redistribution of cell organelles after axon interruptions. In the sciatic nerve of different species marked accumulations of vesicles, mitochondria, multivesicular bodies, membranes, tubules and endoplasmic reticulum profiles were observed (see, for example, Estable, Acosta-Ferreira & Sotelo 1957, guinea-pig; Van Breemen, Anderson & Reger 1958, frog; Wechsler & Hager 1962 and Weiss, Taylor & Pillai 1962, rat). Similar findings were made by Scholte & Hager (1960) in sectioned spinal cord of rat, and by Wettstein & Sotelo (1963) in crushed dorsospinal nerve of mouse. Most investigators interpreted their results as due to local synthesis (local retrograde reactions) of vesicles, for example, in the end of interrupted fibres. Wechsler & Hagar (1962) put forward this idea, since the accumulations of organelles at the cut end occurred too rapidly to be explained by axonal flow, which at that time was considered not to exceed 1 to 2 mm/day. However, Van Breemen *et al.* (1958) suggested that the vesicles, mitochondria and membranes, observed above a ligation of frog's sciatic nerve, originated in the perikarya. These authors also examined the perikarya and found synaptic vesicles here, mostly in close vicinity to the Golgi complex. They drew the conclusion that synaptic vesicles were formed in the region of the Golgi complex and were transported down the axon, to accumulate above the ligation.

(i) *Neurosecretory granules*

These were found to accumulate above injuries in the hypothalamo-hypophyseal tracts of, for example, dog (Scharrer & Wittenstein 1952; Hild & Zetler 1953), frog (Hild 1951) and rabbit (Christ 1962). Most investigators soon accepted the view that neurosecretory granules are formed in the cell bodies and transported distally down the axons (cf. Palay 1943; Bargmann & Scharrer 1951; Scharrer & Scharrer 1963). The rate of movement of neurosecretary granules has been reported to reach enormous figures in stimulated neurons. Jasinski, Gorbmann & Hara (1966) have mentioned rates of 2800 mm/day.

(ii) *Mitochondria*

The movement of mitochondria in ligated axons (rat sciatic) has been thoroughly studied by Zelená (1968, 1969). She found that mitochondria accumulated to a similar extent on both sides of a ligation during the first 18 h after the lesion. This may indicate that the organelle is transported bidirectionally in axons. Between two ligations on the same nerve, the mitochondria moved in both directions, accumulating near the ligated ends and leaving a clear zone in the middle part of the nerve piece. Zelená also reported that the accumulated mitochondria (in the proximal part of the nerve found mainly just above a zone occupied by vesicular or tubular

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profiles) were arranged irregularly, in contrast to the linear arrangement in normal nerves. This has also been pointed out by Weiss & Pillai (1965). The mitochondria were considered to originate mainly in the perikarya. No figures for the rate of transport were given.

(iii) *Synaptic vesicles*

With further development and refinement of electronmicroscopical techniques, an increasing number of investigations has been performed on changes in the fine structure of lesioned axons. The results are, however, varying. No doubt, this is primarily due to different interpretations of the pictures obtained. For instance, the difference between smooth endoplasmic reticulum and microtubules may sometimes appear obscure. As pointed out by Martinez & Friede (1970) the most reliable way of morphologically identifying these organelles depends upon the presence or absence of a unit membrane. The smooth endoplasmic reticulum has a complex membrane containing lipid and protein, but the microtubules are organized in a different pattern, having a one-layered membrane composed of protein subunits (cf. review by Schmitt 1968). High magnifications are, however, needed for identification on this basis. Thus, synaptic vesicles have been suggested to arise from endoplasmic reticulum tubules above ligation (Blümke, Niedorf & Rode 1966). Also, it has been proposed that synaptic vesicles arise by budding off from microtubules (de Iraldi & de Robertis 1968). However, no identification of microtubules as discussed by Martinez & Friede (1970) was performed in this study. The suggestion that the synaptic vesicles or granules, which accumulate in ligated axons, are formed locally from neurotubules, seems less reasonable in view of some recent findings. The accumulation of vesicles is rapid (see above) but the growth, or rate of transport, of microtubular protein in axons is very slow, in the order of 1 to 2 mm/day (Droz 1967; Sjöstrand & Karlsson 1971). Therefore, the vesicles are unlikely to accumulate in such high numbers if there origin was the slowly growing microtubules. Also the structure of the membrane of the two organelles is quite different, the vesicles having a unit-membrane but the microtubules a single protein-subunit wall. The origin of synaptic vesicles is thus a matter for discussion. However, as mentioned by Breemen *et al.* (1958) the perikarya may possibly be the site of formation of this organelle, or a precursor of it.

(iv) *Smooth endoplasmic reticulum*

The tubules and vesicles of the smooth endoplasmic reticulum in axons are markedly increased in the nerve proximal and distal to a crush or cut (see, for example, Martinez & Friede 1970). As pointed out by Lubińska (1964) and Martinez & Friede (1970) the distribution of endoplasmic reticulum profiles in crushed nerves appears similar to the distribution of AChE. Brzin *et al.* (1966) observed in frog nerves a high AChE activity in endoplasmic reticulum tubules and vesicles, and Niemierko & Lubińska (1967) therefore suggested that the AChE accumulating in ligated nerves was confined to this particular organelle, and not to the axon membrane. Recently, Kaśa (1968) was able to demonstrate electronmicroscopically that the AChE activity in the axon membrane of ligated nerves was unchanged, while the endoplasmic reticulum profiles, with high AChE activity were markedly increased in number. Thus, the smooth endoplasmic reticulum in cholinergic nerves appears to be transported in the axon and to accumulate near cut ends of nerves causing the increase in AChE activity.

In a recent study Martinez & Friede (1970) have demonstrated a close relation between changes in axoplasmic composition and the degree of axonal swelling above a nerve transection. They state that 'the theory of local formation of organelles in the axon stump is unlikely in

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view of the magnitude of the increase in organelle volume'. The more than tenfold increase in cell organelles measured by these authors within 48 h would require a multiplication rate which is in disagreement with the low RNA concentrations found in axons (Edström *et al.* 1962). The authors consider a mechanism of 'selective, active redistribution of axoplasm and axoplasmic organelles' to operate within the axons.

(*c*) *Transport of isotopically labelled material*

With the introduction of radioactive markers the transport of axoplasmic constituents could be studied in uninjured fibres. The ligation technique is a useful tool in studies on axoplasmic flow, but the possibility of pathological changes in the flow due to the axonal trauma can never really be ruled out (cf., however, page 335, para. 2).

The first experiments on axoplasmic flow with the use of labelled material (^{32}P) were started in 1949 (Samuels *et al.* 1951; Shepherd 1951). A general shift of ^{32}P-labelled material in nerves was observed, later confirmed by Ochs & Burger (1968) and Ochs *et al.* (1962). Waelsch (1958) introduced ^{14}C-labelled amino acids and demonstrated a cellulifugal transport of labelled proteins in the axon (see also Miani 1960). ^{32}P-labelled orthophosphate introduced into the fourth ventricle of rabbit was used to study the migration of phospholipids, which were synthesized in the perikarya of hypoglossal and vagal nerves (Miani 1962). In these early studies the recorded rates of migration of the labelled substances were mainly of the slow type, i.e. 1 to 2 mm/day. However, somewhat faster rates were also recorded. Koenig (1968) found, after injection of [^{35}S]methionine and [^{14}C]glycine into the cisternal system of cat sciatic nerves peaks of radioactivity travelling at rates of 4 to 5 and 7 to 11 mm/day in the ulnar and sciatic nerves, respectively. In the study of Miani (1963), a comparatively fast-moving phospholipid was observed, which was travelling at 72 mm/day in the vagal nerve, and at about 40 mm/day in the hypoglossal nerve. These rates may, thus, now be classified as intermediary.

With the introduction of autoradiography clear evidence was obtained that the transport of labelled material really occurred *inside* axons, and not in extraneuronal channels (Droz & Leblond 1962, 1963). These authors used systemic injections of [^{3}H]amino acids.

More clear-cut results, due to lower background activity, could be obtained if the amino acids were administered locally, e.g. by micro-injections, instead of systemically. A useful system for local administrations is the eye with the retinal neurons. [^{3}H]Leucine, injected into mouse eyes unilaterally, was rapidly taken up by the retinal cells (Taylor & Weiss 1965) and the peak of radioactivity estimated in the optic nerve was found to be transported with the slow phase. The same system has been used by Grafstein (1967) and McEwen & Grafstein (1968) in goldfish, and by Sjöstrand & Karlsson (1969) in rabbit. In both these studies a rapidly moving peak of radioactivity (estimated by liquid scintillation counting) was found, in addition to the slowly moving portion. Nostrils of mice have also been exposed unilaterally to labelled amino acids with excellent results, showing transport of labelled proteins in the olfactory nerves towards the olfactory bulb (Weiss & Holland 1967).

Axonal transport of the fast type was first reported in 1965 (Burdwood (1965), cinemicrographical studies; Dahlström (1965), suggested on the basis of histochemistry of adrenergic neurons, see also Dahlström & Häggendal (1966) and pp. 337–338). In 1967 reports on the fast transport of labelled material in sensory and motor neurons of mammals were published. Lasek (1967, 1968) injected labelled amino acids into the ventral horn of rat spinal cord and into sensory ganglia of the cat. In rat motor neurons a transport rate of 100 mm/day was observed

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(liquid scintillation counting of labelled protein along the nerve), which occurred inside the axons (controlled by autoradiography). In cat sensory nerves Lasek found rates as high as 500 to 700 mm/day. Also, a slowly migrating peak of radioactivity occurred. Kidwai & Ochs (1967), with a similar approach, found, in addition to a slow flow, a rate of transport of labelled proteins of 800 mm/day in the ventral roots of the cat. In cat sensory nerves rates of 410 mm/day were

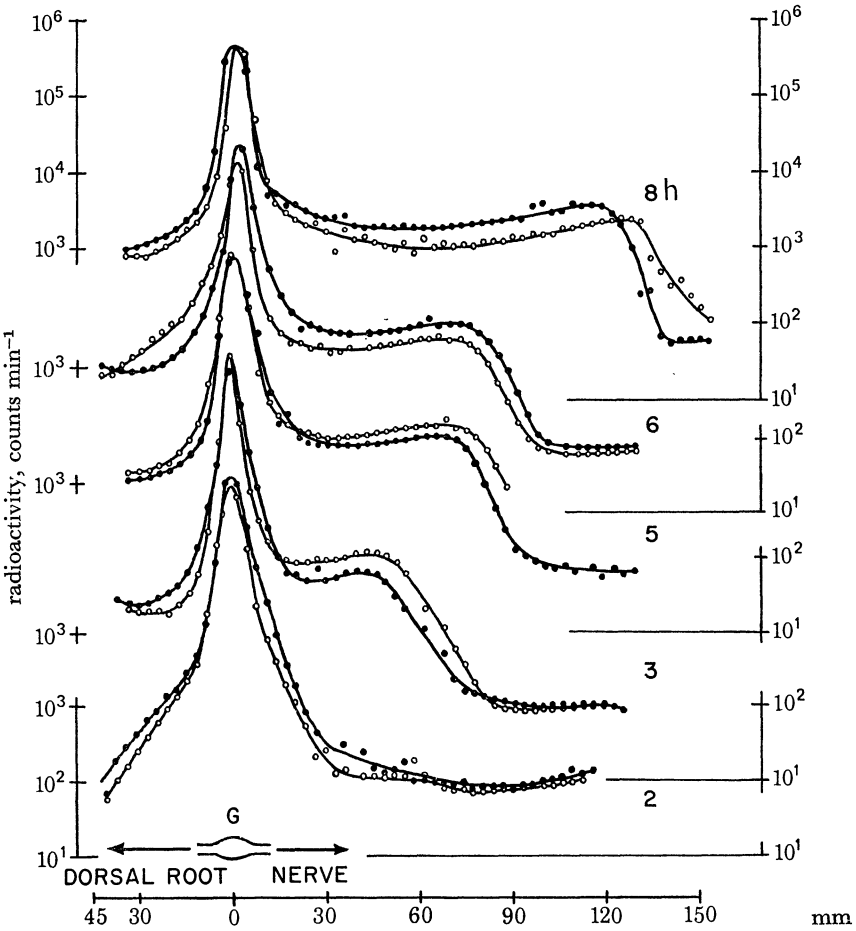

FIGURE 5. The transport of [^{3}H]proteins, indicated from the distribution of activity in the dorsal roots and sciatic nerves of five cats at different times from 2 to 8 h after injection of [^{3}H]leucine into the L7 ganglia (G). The symbols (•, ○) represent the activity in roots and fibres of each side. The ordinate scales are shown only in part. That for the 2 h roots and nerves is given at the bottom left, showing divisions from 10 to 1000 counts/min. At bottom right, only the lowest amount (10 counts/min) is shown. Above it on the right is the scale for the 3 h nerves. Other scales are indicated only by the scale from 10 to 1000 on the right and 1000 counts/min on the left. At the top on the right, a full scale is given for the 8 h nerves and roots. (From Ochs *et al.* 1969.)

found (figure 5) (Ochs, Sabri & Johnson 1969). Freezing of the nerve, which causes a rapid degeneration and occlusion of the axon without the derangement of axonal content obtained after ligation, inhibited the transport to the nerve parts distal to the point of freezing. Instead, the labelled material accumulated in the part proximal to the point of freezing (Ochs *et al.* 1969). Administration of the protein synthesis inhibitor puromycin into the ventral horn before injecting the [^{3}H]leucine could prevent the downtransport of labelled material into the axons, indicating that preferentially labelled material incorporated into protein was transported (Ochs, Johnson & Ng 1967).

Kidwai & Ochs (1969) have investigated the types of radioactively labelled material which are transported down the axon in the two phases. They found that the particulate fraction was more heavily labelled in the fast phase. Some free leucine, polypeptides and soluble protein were also found in the fast phase. The slow phase consisted mainly of soluble proteins, with a smaller ratio of leucine and peptides. In order to find out whether protein synthesis occurred in the axons, 'pulse' experiments were carried out. A low ligation was made early after [^{3}H]leucine injection in the cord, and the components of fast flow were allowed to pass into the nerve. Then a high ligation was made, which prevented the components of the slow flow to enter the nerve part; 14 to 22 h later the nerve was taken out and analysed. The components of the fast flow, locked up between the ligations, were essentially unchanged in composition. Obviously, no protein synthesis had occurred from the leucine and polypeptides in the axon (cf. for example, Ochs 1970). This is considered indicative of, but not proof for, a lack of protein synthesis in axons.

Another observation by Ochs & Ranish (1969), of great importance for all studies on axonal transport that have included ligation experiments, was that the fast rate of transport of labelled proteins was *the same in ligated nerves as in unligated nerves*.

Barondes (1966) studied the reappearance of monoamine oxidase (MAO) in brain nerve endings after irreversible inhibition of the enzyme. He also studied the distribution of radioactivity in whole brain mitochondria and nerve ending mitochondria after the intracerebral injection of [^{14}C]leucine. His results indicated a transport of mitochondria from nerve cell bodies to nerve endings, and a turn-over of mitochondria in the nerve endings of at least 10 days. However, his results did not exclude a local synthesis of mitochondrial protein in the nerve endings.

[^{3}H]Orotic acid (precursor of RNA) and [^{3}H]leucine were injected into spinal cords of fowls in experiments by Austin, Bray & Young (1966). They found no significant transport of free leucine along the sciatic nerve; only [^{3}H]leucine incorporated into proteins were observed in the nerve. However, both [^{3}H]orotic acid and some [^{3}H]RNA were found in the sciatic nerve. The results were interpreted to indicate a slow cellulifugal flow of proteins, orotic acid and perhaps some RNA from perikarya into the axons. No transport of RNA in axons was observed by Rahmann (1966) in the fish brain.

(*d*) *Transmitter substances in injured axons*

(i) *Acetylcholine (ACh)*

The distribution of ACh in crushed nerves has been studied in cat ventral roots by Diamond & Evans (1960), who found an increase in ACh central to the crush, and a decrease in the distal part of the nerve roots. In rabbit sciatic nerve the ACh content increased to about 4.5 times the control level by 5 days after ligation (Evans & Saunders 1967). In the nerve part below the lesion, the ACh content declined to about 25 % in the same time period. The results seem to indicate a cellulifugal flow of ACh, and are in agreement with the view that transmitter vesicles, or precursor stages of vesicles, containing ACh are transported distally in cholinergic axons (compare Van Breemen *et al.* 1958; Martinez & Friede 1970). The situation in cholinergic neurons may thus be similar to that in adrenergic neurons, where the noradrenaline (NA) containing amine granules are transported from the perikarya, down the axons to the nerve terminals (see below). This similarity may be further accentuated in view of very recent results demonstrating a clearcut accumulation of ACh-like substances above but not below short-term

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ligations (3–24 h) of rat sciatic nerves. In both single and double ligated nerves ACh was found to be redistributed in a pattern qualitatively similar to that of NA in ligated nerves (Häggendal, Saunders & Dahlström 1971).

(ii) *Glutamate*

The transmitter candidate glutamate has been studied in snail neurons. After application of radioactive glutamate to brain cells a rapid transport of this substance down the nerve trunk to the muscle was observed (Kerkut, Shapira & Walker 1967). The rate of transport was considered to be greatly enhanced by stimulation of the brain cells, rates of 2 to 3 cm/h were estimated (see below, § 3*a*). The autoradiographic pictures obtained indicated an intra-axonal location of the labelled glutamate.

Glutamate has also been studied in the spinal cord. After transection (21 days) of the spinal cord of cat at the thoracic level, Rizzoli (1968) found a significant decrease in glutamate concentrations in the dorsolateral funiculus at the lumbar region. He suggested that this indicated the presence of glutamate within descending axons. Glycine and γ-aminobutyric acid were also studied, but no clear results were obtained (Rizzoli 1968). In the thoracic part of the cord above the transections, a considerable increase in glutamate, together with protein, was noticed, but whether this was clear evidence for axoplasmic transport of the substances, or due to other reasons was not investigated (A. A. Rizzoli, personal communication).

(iii) *Substance P*

Another proposed transmitter substance, substance P, accumulates in rabbit auricular and sciatic nerves proximal to a section. In the distal part of the sectioned nerves the level of the substance fell markedly (Holton 1960). Substance P in peripheral nerves thus behaves like a molecule which is transported along the nerve with axonal flow. Evidence for the localization of substance P in intraneuronal particles has been given (Euler & Lishajko 1961).

(iv) *Catecholamines and serotonin* (5-*HT*)

Within the central nervous system, evidence for axoplasmic flow of transmitters has been obtained for serotonin (5-HT), dopamine (DA) and noradrenaline (NA). After cutting the spinal cord marked increases in 5-HT and NA were observed histochemically (Dahlström & Fuxe, 1964*a*, 1965) and biochemically (NA, J. Häggendal & T. Magnusson 1964, unpublished). The two amines accumulated in axons in the white matter in descending bulbo-spinal 5-HT and NA neurons, respectively. In the cord below the transsection the levels of the amines fell, approaching zero during the third day (Andén, Häggendal, Magnusson & Rosengreen 1964).

The DA-containing nigro-neostriatal neurons in the brain behave similarly after lesioning their axons. In rats with brain lesions in the upper part of the mesencephalon, DA rapidly accumulates in swollen axons caudal to the lesion. Rostrally to the lesion, in the neostriatum, the fluorescent DA nerve terminals disappear, and the DA levels, estimated biochemically, decrease (see, for example, Andén *et al.* 1966).

All these observations in the mammalian brain are in agreement with the view that amine granules, which store the respective amines, are transported distally in the axons to the nerve terminals of the 5-HT, NA or DA containing neurons, respectively.

An attempt has been made to calculate the rate of transport of NA in central NA neurons. The rate obtained in this study was 0.7 mm/h (Häggendal & Dahlström 1969), which is clearly

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lower than in peripheral adrenergic nerves (see below). However, for technical reasons, the rats were given reserpine 3–4 days before death. As mentioned below (§ 3*b*(i)) such treatment may possibly have effects on the rate of transport of amine granules. The figure of 0.7 mm/h will therefore probably have to be somewhat adjusted in the future.

2. Axoplasmic flow in adrenergic neurons

(*a*) *Amine storage granules*

(i) *Noradrenaline (NA)*

The first experiments to indicate axoplasmic flow of amine granules in sympathetic adrenergic neurons were published in 1964 (Dahlström & Fuxe 1964*b*). Several reports on axoplasmic flow in cholinergic neurons had earlier appeared and when the histochemical fluorescence method of Hillarp, Falck and co-workers (for references and description see Corrodi & Jonsson 1967), for the demonstration of catecholamines and 5-HT, was developed, it appeared natural to see if the adrenergic transmitter, NA, was transported in adrenergic axons. The first experiments were carried out in Hillarp's department in Stockholm, when Dr Zelená was a temporary guest of Professor Hillarp's. Dr Zelená demonstrated to us the type of ligation technique, used by Professor Lubińska and herself, for interrupting nerve fibres (see, for example, Lubińska 1959).

In the great splanchnic and the sciatic nerves of rats large amounts of strongly green fluorescent material (the reaction product between NA or DA and paraformaldehyde—later confirmed to be NA by spectrophotofluorimetric methods by Ritzén and biochemical assays by Häggendal; acknowledged in Dahlström 1965) were observed to accumulate above the ligation within 12 h after the ligation (splanchnic nerve, Dahlström & Fuxe 1964*b*). During the days following ligation, the accumulations increased in amount, the diameter of the swollen axons and the length of the accumulations gradually increasing (figure 6, plate 61). In the great splanchnic nerve the accumulation of NA in the axons could sometimes be traced back to the ganglion cells, in the lower thoracic ganglia, from which the axons originated (Dahlström & Fuxe 1964*b*).

Since reserpine could deplete the accumulated NA (see § 2*c*, para. 3), indicating that the accumulated NA was stored in amine granules, it was argued that a rapid accumulation had occurred of amine storage granules, formed in the nerve cell bodies, and transported along the axons to the nerve terminals (Dahlström & Fuxe 1964*b*).

Similar studies on NA accumulations in ligated adrenergic nerves were independently started in England by Kapeller & Mayor (1966*a*, *b*). The results obtained, both regarding NA accumulations and the depletion of the accumulated NA by reserpine treatment, were in accordance with the results obtained in Sweden.

The suggestion that the amine granules are formed and transported down the axon by an active process at high rates was put forward in a subsequent paper (Dahlström 1965), when the accumulations of NA were studied in more detail. Already 15 min after ligation, the accumulation of NA was evident in many axons. Also it was pointed out that different adrenergic perikarya probably vary in the rate of production of amine granules, since the amount of fluorescent material accumulated above the ligation varied considerably in the individual axons (Dahlström 1965).

Together with Dr Häggendal, who quantitated accumulations of NA in transected spinal

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cords of rabbit (J. Häggendal & T. Magnusson 1964, unpublished), the time course of NA accumulation in ligated sciatic nerves was studied (Dahlström & Häggendal 1966, 1967). In all animals studied (rat, cat and rabbit), the amount of NA in the proximal 1 cm part just above the ligation increased approximately linearly with time (figure 7*a*, *b*) (see also Banks, Magnall & Mayor 1969). The rapid onset of the accumulations, and their linearity in time indicated that the ligation had interfered with a process, occurring in the intact axons, probably the axoplasmic transport of amine granules. By comparing the amount of NA in 1 cm of the nerve at different times after ligation, with the amount of NA present in the same length of nerve before ligation, the rate of transport of NA in adrenergic axons of the sciatic nerve was calculated to be about 5, 10 and 3 mm/h in rat, cat and rabbit respectively (Dahlström & Häggendal 1966, 1967).

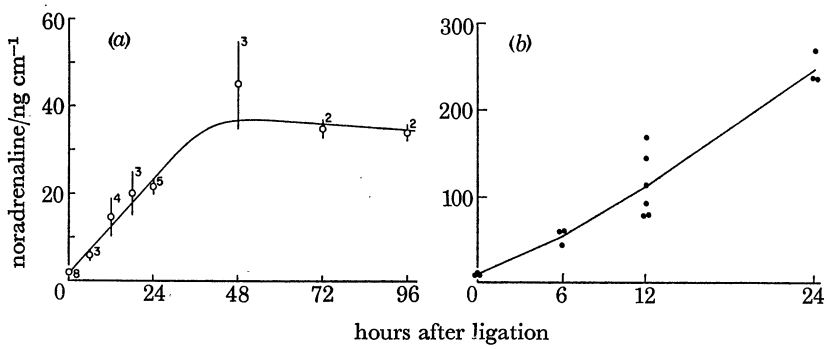

FIGURE 7. The accumulation of noradrenaline (NA) in the sciatic nerve after ligation. The NA content in the proximal 1 cm part of nerve just above the ligation is shown. (*a*) Rat sciatic nerve. Mean values ± s.e.m. are indicated (small figures represent numbers of observations). (*b*) Cat sciatic nerve. The individual values (.) are indicated. (From Dahlström & Häggendal 1966.)

These publications were among the first to report such high transport rates in mature axons *in vivo*. Independently, Lasek (1966, 1967) and Kidwai & Ochs (1967) reported a rapid transport of material in mature axons of rat and cat sensory and motor fibres, and later studies have given further information about fast transport (see § 1*c*, para. 5).

DESCRIPTION OF PLATE 61

FIGURE 6. Longitudinal section of sciatic nerve of rat, 8 h after ligation. Above the ligation (→) accumulations of strongly fluorescent material (the fluorescent product of NA and formaldehyde) can be seen within distorted and bulgy axons. Below the ligation small retrograde accumulations of material can be observed. Fluorescence microphotograph, magn. × 180.

FIGURE 16. The effect of vinblastine on lumbar sympathetic ganglia of rat. (*a*) The ganglion was treated locally for 10 min with saline. Ganglion cells with weak to medium NA fluorescence are seen. The intraganglionic axons are of weak fluorescence intensity. (*b*) The ganglion was treated with a 0.01 mol/l solution of vinblastine, applied to small cotton pellets, for 10 min 24 h before death. The fluorescence intensity of the nerve cells is generally increased, although variations in intensity are observed. In many cells the fluorescence is concentrated to the periphery (→). The intraganglionic axons are swollen, and have a strong fluorescence intensity (↦). Fluorescence microphotographs, magn. × 320.

FIGURE 17. Spinal cord, thoracic level, of a rat, treated with vinblastine (0.01 mol/l solution) locally on the spinal cord 24 h before death. The vinblastine solution was put on a small cotton pellet, which was then carefully applied on the cord, exposed by laminectomy. After 10 min the cotton pellet was removed. Longitudinal section of the lateral funiculus, with enlarged and bulgy NA (→) and 5-HT (↦) containing axons. Normally these fibres are thin and of extremely weak fluorescence intensity. Note the bulgy and irregular appearance of the non-fluorescent myelinated fibres. Fluorescence microphotograph, magn. × 320.

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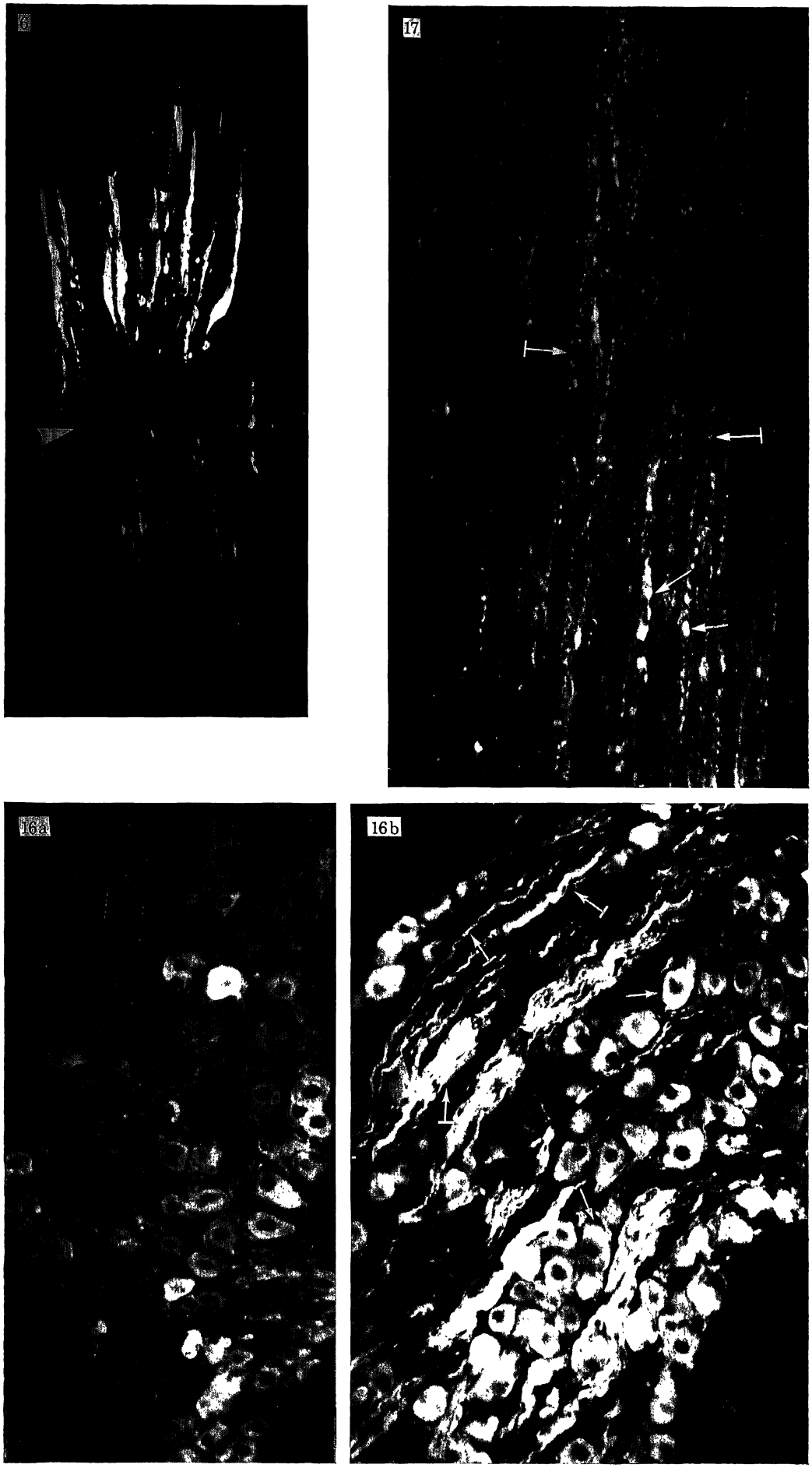

FIGURES 6, 16 AND 17. For legends see facing page.

(*Facing p.* 338)

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(ii) *Dense-cored vesicles*

The histochemical and biochemical studies of NA accumulations gave, together with pharmacological studies, indirect evidence for the view that the NA transported down the adrenergic axons was stored in amine granules. More direct evidence for this view has been given by Kapeller & Mayor (1966*c*, 1967, 1969*a*) and by Geffen & Ostberg (1969) in electron-microscopic studies. A pronounced increase in the number of dense-cored vesicles, considered to represent amine storage granules, was found in the nerve proximal to the ligation (also observed by Dahlström & T. Hökfelt 1966, unpublished).

(iii) *Labelled noradrenaline and proteins*

Livett, Geffen & Austin (1968*a*, *b*) studied the transport of [^{14}C]NA and ^{14}C labelled protein in cat splenic nerves. They injected [^{14}C]NA and [^{14}C]leucine into the coeliac ganglion, and

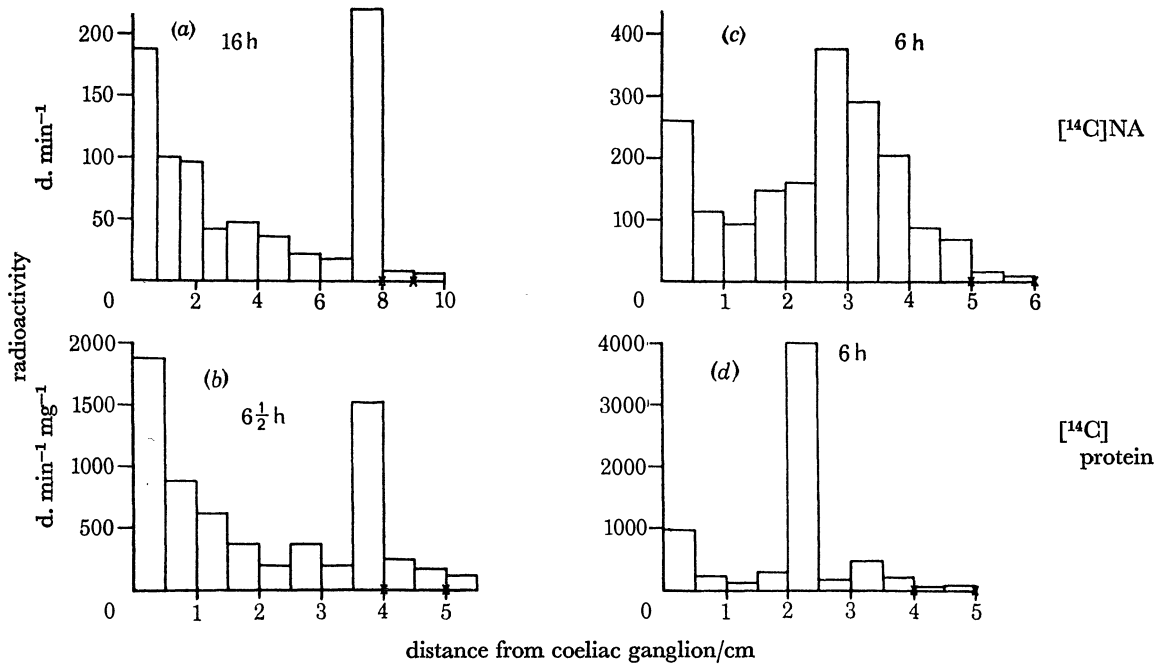

FIGURE 8. The transport of [^{14}C]noradrenaline (*a* and *c*) and [^{14}C]protein (*b* and *d*) in cat splenic nerves. ×, site of constriction. The distances shown are the minimum as measured *in situ* and are subject to an error of up to 5 mm. (From Livett *et al.* 1968*a*.)

estimated the levels of the amine and labelled protein both in intact and constricted nerves. A peak of radioacitivity, both of [^{14}C]NA and [^{14}C]protein, travelled down the splenic nerve at about 5 mm/h. In constricted nerves, both protein and [^{14}C]NA accumulated proximal to the constriction. In addition to the rapid flow of protein, a slowly migrating peak was observed, with an estimated rate of 1 mm/day (figure 8). As discussed by the authors, *the rapidly* moving protein may possibly be localized to the amine granules, since the same rate of transport was found for [^{14}C]NA.

A similar study, where a mixture of ^{3}H-labelled leucine, lysine, tyrosine and phenylalanine was locally applied on rat lumbar sympathetic ganglia, was carried out in 1967 in Weiss's

laboratory during a short term visit (A. Dahlström, unpublished). Although only pilot experiments were performed, the results were similar to those reported by Livett *et al.* (1968*b*). Figure 9 (right) shows the amount of radioactivity in 5 mm segments of the right sciatic nerve 26 h after soaking the ganglia L2 to L3. Two peaks may be observed, one apparently moving rapidly and one moving more slowly. By comparing these values with values, obtained from the left nerve of the same rat (figure 9, left), dissected out 8 h earlier than the right nerve (i.e. 18 h after soaking) it may be suggested that the fast peak had moved at a speed of 2 to 3 mm/h.

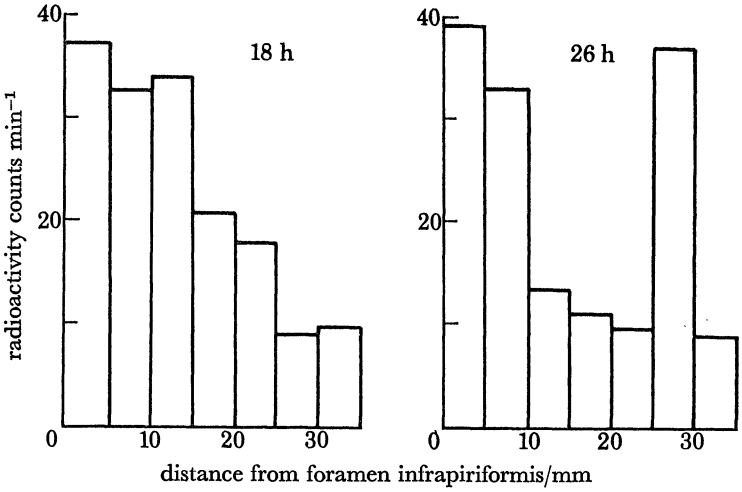

FIGURE 9. The transport of isotope-labelled material (probably proteins) in the sciatic nerves of a rat, after local treatment of the lumbar sympathetic ganglia with a mixture of [^{14}C]leucine, [^{14}C]lysine, [^{14}C]phenylalanine, and [^{14}C]tyrosine. The ganglia were soaked with the solution, applied on gel-foam, for 30 min. The left nerve was taken out at 18 h after treatment of the ganglia, and the right nerve at 26 h. 5 mm pieces were used.

(iv) *Dopamine β-hydroxylase and chromogranin*

Another marker for amine granules is the enzyme DA β-hydroxylase (cf. Kaufmann & Friedmann 1965; also N. and A. G. Kirshner, this volume, p. 279). By an immuno-fluorescence technique, Livett, Geffen & Rush (1969) and Geffen, Livett & Rush (1969*a*) have demonstrated the presence of DA β-hydroxylase and another protein component of the granules, chromogranin, in cell bodies, axons and nerve terminals of adrenergic neurons. Both these proteins accumulated proximal to a ligation. Laduron & Belpaire (1968*a*) have measured the DA β-hydroxylase activity in ligated splenic nerves of dogs; it accumulated approximately linearly, and in parallel to NA. The rate of transport of amine storage granules in dog splenic nerves, calculated from these studies, was about 3 mm/h (Laduron & Belpaire 1968*a*). The accumulating DA β-hydroxylase was bound to a sedimentable particle (Laduron 1968); thus, the results are consistent with a flow of amine granules.

(v) *Effect of long-term ligations*

As indicated in figure 7*a* the NA in rat sciatic nerve accumulated linearly up to about 48 h after ligation; after a plateau there followed a slow decrease in the amounts of NA (Dahlström & Häggendal 1966). Two alternative reasons were suggested for this decrease: (*a*) retrograde cell body changes, resulting in a decreased formation and transport of amine granules, or (*b*) outgrowth of axons into the distal nerve part, with subsequent losses of accumulated

material (Dahlström & Häggendal 1966). If the second alternative was operative, then a second ligation, tied above the initial ligation at a time when NA accumulation had decreased (e.g. 2 to 3 days), should result in normal accumulations of NA above this high, second ligature. Boyle & Gillespie (1968) found that no NA accumulated above a second tie, and suggested that the synthetic activity of the cell body declines after a constriction of the axons.

Recently, a more detailed study of this phenomenon has been undertaken (Karlström & Dahlström 1970, 1971). Rat sciatic nerves were exposed to different types of axonal trauma at a low level: cut, crush, or ligation with the ligature left *in situ*. Sham-operated rats served as

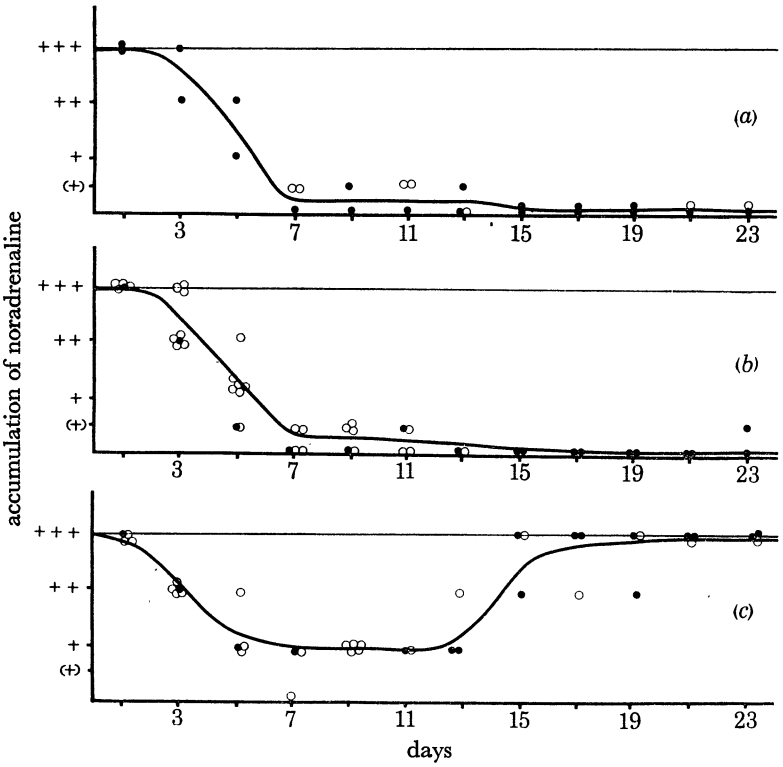

FIGURE 10. The accumulation of noradrenaline (expressed as intensity of fluorescence, recorded by eye), as compared to the accumulation observed in sham-operated controls (straight lines) above the second, high crush (2 h before death) of sciated nerves operated at a low level 1 to 23 days beforehand: (*a*) nerves which were *cut* at the first operation; (*b*) nerves which were *ligated* initially; and (*c*) nerves which were *crushed* at the initial operation. ○, bilaterally operated rats; ●, unilaterally operated rats. (From Karlström & Dahlström 1971.)

controls. At different time intervals after the initial operation, a crush (ligation type Lubińska 1959) was made 2 cm proximally. Two hours after the last operation the nerves were dissected out, and examined by fluorescence microscopy. The results are schematically indicated in figure 10. After cut (*a*), or ligation (*b*) with remaining ligature, the NA accumulating above the high ligature disappeared for the time period studied (23 days), while in crushed nerves (*c*) the NA accumulation after the initial decline reappeared, to approach control levels around 3 weeks after the initial operation. The results seem to demonstrate that axonal trauma causes a depression in the synthesis and axonal transport of amine granules, and that the duration of this depression is dependent on the type of axonal trauma (Karlström & Dahlström 1970, 1971).

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(vi) *Retrograde accumulations*

After ligation of adrenergic nerves according to Lubińska (1959), retrograde accumulations of NA are frequently observed, both histochemically and biochemically (Dahlström 1965, 1967; Dahlström & Häggendal 1966). These accumulations develop somewhat later than the proximal accumulations, and disappear 18 to 24 h after ligation. Histochemically, the accumulations are of weak fluorescence intensity, and rarely exceed 0.5 mm below the ligation (figure 6), in contrast to the proximal accumulations, which are of very strong intensity and can develop up to 7 to 10 mm above the lesion. It has been suggested that these retrograde accumulations

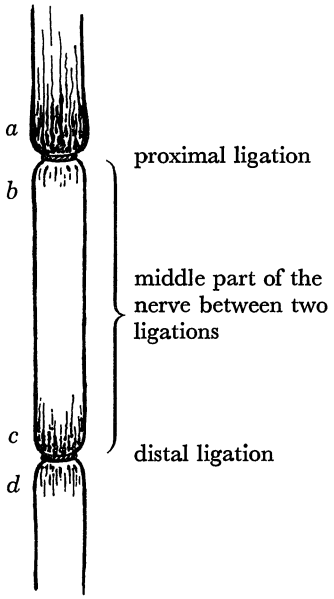

FIGURE 11. Schematic illustration of the fluorescence microscopical appearance of sciatic nerve of rat or cat 12 h after the application of double ligatures. The accumulations at (*a*) are considerably larger than those at (*c*), while the retrograde accumulations are larger at (*d*) than (*b*). (From Dahlström & Häggendal 1966; Dahlström 1967*a*.)

occur in axons w ich originate from cell bodies located in the distal part of the nerve (Mayor & Kapeller 1967). This, however, is unlikely for the following reasons: (*a*) In the rat sciatic nerve, aberrant ganglion cells have been observed only very rarely, while the retrograde accumulations are regularly observed histochemically and (*b*) in longitudinal sections the retrograde accumulations, below the dark area of ligation, have sometimes been observed to have a pattern mirroring that of the much stronger accumulations proximal to the ligation. Thus, the same axons seem to develop both anterograde and retrograde accumulations.

It can also be argued, that with this type of ligation, NA can have leaked through the ligation from the proximal accumulations. This is also unlikely. In figure 11 the relations between proximal and distal accumulations in double ligated nerves are schematically indicated. The retrograde accumulations appear almost always to be smaller and weaker in intensity in the separated nerve part, below the high ligation, than below the low ligation. The proximal accumulations, however, show the reverse picture. If the retrograde accumulations were due to leakage through the ligated area, the proximal and distal accumulations should reasonably be proportional to each other in double ligated nerves. A similar picture has been observed for AChE distribution in double ligated nerves (figure 4) (Lubińska *et al.* 1964).

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The nerve part just distal to ligations has been studied electronmicroscopically (Kapeller & Mayor 1969*b*) for the presence of dense cored vesicles. No such dense vesicles were observed, but empty vesicles were present. Since OsO_4 fixation was used in this study and since $KMnO_4$ fixation is a better preserver of the dense core of the vesicles (cf. Richardson 1966; Hökfelt 1968), some of the empty vesicles observed in the distal part by Kapeller & Mayor (1969*b*) might have been shown up as dense-cored in $KMnO_4$-fixed tissues. As indicated by the disappearance of the specific NA fluorescence after reserpine treatment, the NA in retrograde accumulations is probably located to amine granules.

Thus, if seems documented that in ligated nerves a retrograde flow of NA, and also of AChE, occurs. As regards NA, this flow appears *very* small in comparison to the anterograde flow.

In cholinergic nerves the retrograde accumulations of AChE seem to be of higher magnitude, more similar to the proximal accumulations. This is indicated in figure 4 (from Lubińska *et al.* 1964). Recently, Lubińska & Niemierko (1971) studied the velocity of both cellulifugal and cellulipetal transport of AChE in ligated peroneal nerves of dog. On the basis of the rates of anterograde and retrograde accumulations of AChE in the nerves, the velocities of migration were calculated to be 260 mm/day (cellulifugal) and 134 mm/day (cellulipetal), respectively, in ligated nerves (Lubińska & Niemierko 1971).

The question arises as to whether such a retrograde flow exists in normal, unligated, nerves or only in ligated nerves. A retrograde flow of [^{14}C]glutamate has been reported to occur in intact snail neurons (Kerkut *et al.* 1967). Geffen, Hunter & Rush (1968, 1969) made an effort to answer this question for the adrenergic neuron. They injected [^{14}C]NA into the spleen intra-arterially, and measured the content of the isotope in the splenic nerve and the coeliac ganglion. No signs of a retrograde flow of [^{14}C]NA from the nerve terminals back into the axons were observed, neither in intact, nor ligated nerves. It may thus be that a retrograde flow of NA occurs only in ligated peripheral adrenergic nerves, and then only from a short distance within the nerve. Friede (1964) has pointed out that electrophoretical forces within the nerve can cause the appearance of beaded fibres with accumulation of substances. Therefore the type of ligation used may possibly create alterations of the potential along the nerve in such a way that an electrophoretic field appears, which causes the negatively charged amine vesicles to move towards the ligation. At present, no direct evidence for a retrograde transport of NA in intact, mature peripheral adrenergic nerve fibres, appears to have been reported.

However, in unligated central NA neurons the phenomenon of retrograde flow has recently been observed by Ungerstedt *et al.* (1969). They studied the uptake of DA in the nerve terminals of the nigro-neostriatal DA neuron system in rats pretreated with reserpine and nialamide. After injection of small amounts of DA into the caudate nucleus the DA nerve terminals, depleted of all fluorescence due to the pretreatment, took up the injected DA and became strongly fluorescent. The DA fluorescence also increased in the non-terminal axons, which could be traced back more than half-way to the cell bodies in the substantia nigra. The DA taken up by the nerve terminals was probably transported in a retrograde direction in the axons (Ungerstedt *et al.* 1969) at a fairly high rate (several millimetres per hour) as judged from the results obtained by these authors. In these studies the DA may have been located extragranularly, since reserpine was given beforehand, and the results do therefore not necessary indicate that the storage granules were transported centripetally. In the studies on peripheral adrenergic nerves, however, the [^{14}C]NA was probably taken up into the amine granules, since no reserpine-pretreatment was undertaken (Geffen *et al.* 1968, 1969).

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(*b*) *Extragranular enzymes*

(i) *DOPA decarboxylase*

This enzyme takes part in the synthesis of DA from DOPA, and is considered to be located extragranularly. This is clearly indicated by its behaviour in ligated nerves, which is quite different from that of NA and DA β-hydroxylase. In the rat sciatic nerve, no clear change in enzyme activity was found in the 1 cm nerve above a 6, 12, 18 or 24 h ligation. Two and four days after ligation, however, a marked increase in enzyme activity had occurred (Dahlström & Jonason 1968). In lumbar sympathectomized animals, such an increase was not observed,

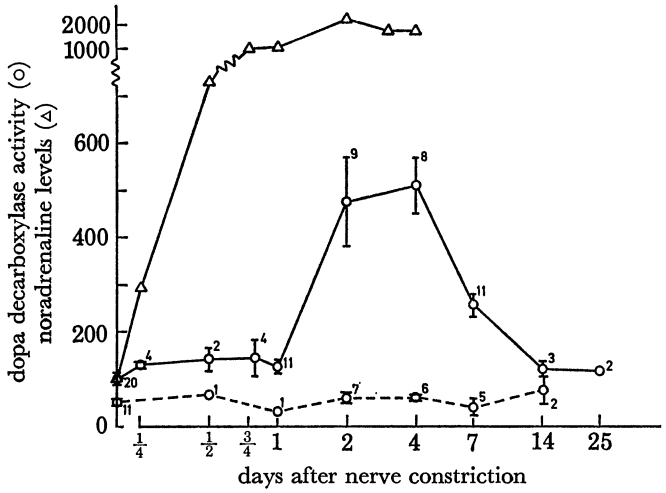

FIGURE 12. Dopa decarboxylase activity and noradrenaline levels in the rat sciatic nerve above a ligature. The figure shows the dopa decarboxylase activity in the 1 cm part of the rat sciatic nerve just above the ligature at different times after constriction of the nerve. The enzyme activity of a normal nerve is $28.3 \pm 3.6 \times 10^{-2} \mu g$ DA formed per hour and 1 cm nerve ($100 \pm 12.7\%$). All values are expressed as percentage of this normal value (mean ± s.e.m.). The small figures represent the number of experiments performed. Furthermore, the NA level in the 1 cm part of the rat sciatic nerve just above the ligature at different times after constriction of the nerve is presented. These values are calculated from the data of Dahlström & Häggendal (1966) and are presented as the NA content in percentage of normal (mean values). ○—○, constricted sciatic nerve of normally innervated rats; ○---○, constricted sciatic nerves of lumbar sympathectomized rats; △—△, accumulation curve for noradrenaline (from Dahlström & Häggendal 1966). (From Dahlström & Jonason 1968.)

indicating that the DOPA decarboxylase was confined to the adrenergic axons of the sciatic nerve (figure 12). In the part distal to the ligation, a much smaller increase of activity than above the ligature was initially observed, followed by a decrease.

If DOPA-decarboxylase is normally transported down adrenergic axons, one would expect an onset of accumulation of the enzyme above the ligature very soon. In the study by Dahlström & Jonason (1968) increases in enzyme activities were observed not earlier than 2 days after ligation, in spite of a fairly large number of experiments performed at 1 day. This suggests that the ligation procedure may have influenced and increased a normally rather slow production and transport rate for DOPA decarboxylase. However, in recent experiments by Laduron (1970), an increase in enzyme activity was estimated as early as 1 day after ligation of rat sciatic nerves. Earlier intervals after the ligation were not studied. The results do, however, suggest that axonal flow of DOPA decarboxylase activity occurs in adrenergic nerves. The rates of such

a flow may possibly be in the order of 1 to 2 mm/h during the second day after ligation, as shown by the results of Dahlström & Jonason (1968).

(ii) *Tyrosine hydroxylase*

Subcellular fractionation studies on adrenal medulla (Laduron & Belpaire 1968*b*), showed that both DOPA decarboxylase and tyrosine hydroxylase are soluble, and located free in the cytoplasm. Since this also is true in axons of the splenic nerve (Smith, De Potter & De Schaepdryver 1969) both enzymes should behave in a similar pattern above ligations. In a study by Laduron & Belpaire (1968*c*) the activity of tyrosine hydroxylase above a ligation was in the splenic nerve somewhat decreased 48 h after operation as compared to the activity in intact nerves. This is in contrast to the observations on DOPA-decarboxylase accumulations in ligated nerves (see above). With the usual reservation for interpretations regarding enzyme studies (i.e. the question of whether the enzyme activity is equal to the amount of enzyme protein) the data available at present seem to indicate different compartments for the enzymes. M. Goldstein (personal communication) has suggested that DOPA decarboxylase in living nerves may be bound to the endoplasmic reticulum. If this is true, further studies on DOPA decarboxylase behaviour in ligated nerves should be considered in view of the results obtained on the distribution of AChE, since part of the axonal AChE is bound to the endoplasmic reticulum.

(iii) *Mitochondrial enzymes*

In adrenergic nerves, monoamine oxidase (MAO) and cytochrome oxidase activities after ligation have been studied. After an initial decrease in activity during the first day, MAO

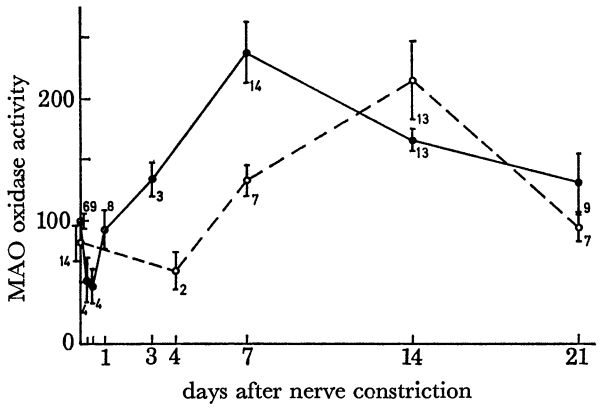

FIGURE 13. MAO activity in the proximal 1 cm part of ligated sciatic nerves of normal (●——●) and lumbar sympathectomized (o– – –o) rats at different time intervals after the nerve constriction. The values are expressed as a percentage of the MAO activity in normal non-ligated nerves per centimetre of nerve. The small figures indicate the number of experiments. The vertical bars represent the s.e.m. (From Dahlström, Jonason & Norberg 1969.)

accumulated slowly in rat sciatic nerves, reaching a peak of 2.5 times the normal activity one week after ligation (Dahlström, Jonason & Norberg 1969). Lumbar sympathectomy delayed the development of the peak by 1 week, and also the onset of the accumulation (figure 13). In the nerve distal to the ligation no clear changes were observed. Histochemical studies were in agreement with these biochemical results; clear accumulations above, but no accumulations

below, the ligation. The mitochondria in adrenergic axons were suggested to accumulate somewhat faster than in non-adrenergic nerves.

Banks *et al.* (1969) studied the redistribution of cytochrome oxidase in hypogastric nerves of cat. This biochemical study was paralleled by electron-microscopical studies on mitrochondria. The nerves were ligated in two places with about 6 to 7 mm distance between the two ties. Cytochrome oxidase was found to accumulate to a similar degree both proximal to the high, and distal to the low ligation, the activity in the proximal part of the nerve always being somewhat larger. Electronmicroscopically, accumulations of mitochondria were found at both locations. In the intermediate part between the two ligations, the total amount of cytochrome oxidase was unchanged, but was redistributed towards both ends of the piece of nerve, leaving the middle part with lower levels than normally. The results indicated that mitochrondria in adrenergic nerves move bidirectionally, at least after ligation, and that the rate of transport may be around 0.6 mm/h (Banks *et al.* 1969).

(*c*) *Axoplasmic transport of amine granules and functions of the adrenergic neuron*

Earlier calculations on the transport and life-span of amine granules were based on the assumption that the granules contained about the same amount of NA in all parts of the neuron

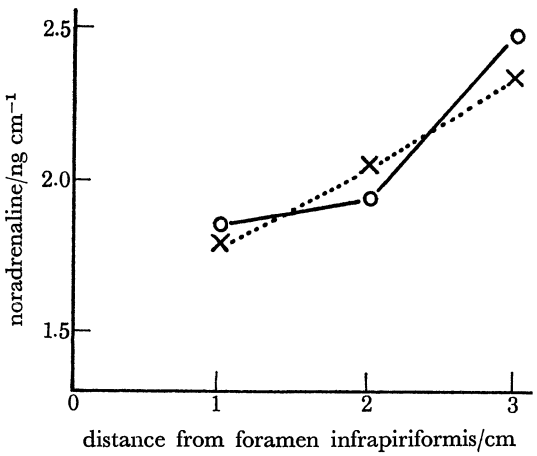

FIGURE 14. The gradient of noradrenaline content along the sciatic nerve of rat. Three consecutive nerve parts of 1 cm length were dissected from the foramen infrapiriformis and distally. Nerve parts from six rats were collected from each determination. Results from two experiments are given. (From J. Häggendal and A. Dahlström, unpublished.)

(Dahlström & Häggendal 1966). Recent observations indicate, however, that this assumption must be reconsidered. Assays of consecutive segments of rat sciatic nerves indicate that lower parts contain more NA/cm than higher levels (figure 14; J. Häggendal & Dahlström, unpublished). Similar gradients in AChE have also been observed (Lubińska *et al.* 1961, 1962), but in contrast to NA this enzyme decreased in more distal parts of the nerves. An increased NA content in lower segments suggests either that the number of granules per centimetre of nerve increases, or that the amine granules, during their passage down the axons increase their content of NA. The latter alternative is supported by recent findings in sciatic nerves with 'delayed double ligations' (Dahlström, J. Häggendal, K. Larsson & T. Magnusson, unpublished). Six hours after a low ligation, a second high ligation was performed on the same nerve. The nerve part between the two ligations was dissected out at different time intervals

after the second ligation, and the NA content was assayed. A gradual increase was observed having a maximum of about 150 %, 9 h after the second ligation. This suggests that the amine granules, which had accumulated above the low ligation during the initial 6 h, had increased their NA content by about 50 % during the 9 h when they were locked up between the two ligations. Treatment with reserpine removed the NA, indicating that the increase was confined to the amine granules. It is interesting that Evans & Saunders (1967) observed a gradient of ACh, similar to the NA gradient, i.e. an increase in more distal nerve parts. One may speculate that the cholinergic vesicles are also transported proximo–distally in cholinergic nerves and gradually build up their ACh content during the transport.

Using these new results which suggest different degrees of NA loading of the amine granules, the figures for life-span of the granules have been re-calculated. The life-span, according to the latest calculations, is 3 to 4 weeks (see, for example, Dahlström & Häggendal 1970) as compared with 5 weeks (Dahlström & Häggendal 1966).

The life-span of 3 to 4 weeks for rat amine granules relates to their capacity to store endogenous NA. Recently it has been suggested that the properties of young, newly formed granules may be changed with increasing age (Dahlström & Häggendal 1970; Häggendal & Dahlström 1970, 1971*a*, *b*). Reserpine administration depletes the NA stores, and both transmission and retention of exogenous [^{3}H]NA fail. After reserpine treatment (10 mg/kg i.p.) (which causes a long-lasting block of the NA storage mechanism in the amine granules, cf. Carlsson 1966) the recovery in the nerve terminals of endogenous NA levels, transmission and [^{3}H]NA uptake capacity, proceeds at different rates. The recovery of endogenous NA to normal levels needs several weeks (cf. Häggendal & Dahlström 1971*a*, *b*), while transmission and [^{3}H]NA retention capacity appears to be normalized within only a few days (see, for example, Andén, Magnusson & Waldeck 1964, Häggendal & Dahlström 1970). Both the recovery of NA, and of [^{3}H]NA retention, could be delayed by axotomy. It was concluded that axoplasmic flow of newly synthesized granules was necessary for the recovery in the nerve terminals of these functions. Consequently, the different time courses for recovery to normal levels of endogenous NA and of [^{3}H]NA retention, may imply that young and older granules have different properties. The young granules may be particularly active in incorporating [^{3}H]NA (and possibly also in releasing NA at nerve stimulation), and this capacity may decline after some days. The rapid recovery to normal of [^{3}H]NA retention may thus be due to a rapid refillment by axoplasmic transport of the normal fraction of *young* granules, which is then maintained by the transport. Although the young granules may soon loose their capacity of [^{3}H]NA retention, the capacity of the terminals to store [^{3}H]NA therefore remains. The capacity to store endogenous NA may, however, remain in the ageing granules for several weeks (the life-span), and a complete refillment of the large fraction of *old* granules in the nerve terminals from the younger amine granules would be necessary to normalize the endogenous NA levels. For a more detailed discussion the reader is referred to the following publications by Dahlström & Häggendal (1970) and Häggendal & Dahlström (1971*a*, *b*).

Electronmicroscopical observations and biochemical studies lend support to the hypothesis that young and old amine granules may have different properties. The size of the electron dense vesicles appears to vary in the different parts of the adrenergic neuron. In the nerve terminals the vesicles are mainly of the small type (diameter around 50 nm), only a small percentage of large or intermediate vesicles being found. In the cell bodies, the proportion of large vesicles is higher than in the nerve terminals (Hökfelt 1969; Geffen & Ostberg 1969). With $KMnO_4$ fixation, which gives a better preservation of the dense core (Richardson 1966;

Hökfelt 1968), a moderate number of small vesicles is also observed in the cell bodies (Hökfelt 1969). In ligated axons, the accumulated vesicles are mainly of the large type (Geffen & Ostberg 1969), although small vesicles are also observed in $KMnO_4$ fixed material (Dahlström & T. Hökfelt, unpublished, Hökfelt & Dahlström 1971). The large dense cored vesicles, observed in the cell body and particularly in ligated nerves, may possibly represent young amine granules. The small vesicles in the nerve terminals may represent later stages, i.e. older amine granules (see discussion in Häggendal & Dahlström 1971*a*).

By sucrose gradient centrifugation studies Roth, Stjärne, Bloom & Giarman (1968) discovered two types of NA storage particles: a less dense type, found only in tissue containing adrenergic nerve terminals, and a heavier type, present particularly in adrenergic axons, but also in nerve terminals. Similar observations have been reported by De Potter (1968) and De Potter, Chubb & De Schaepdryver (1970), in the dog spleen and splenic nerves. DA β-hydroxylase activity was present in the heavy granule population from the splenic nerve axons (Smith, De Potter & De Schaepdryver (1969), but was low or absent from the light particles population from the spleens (De Potter *et al.* 1970).

It has recently been shown that both chromogranin and DA β-hydroxylase are released together with NA during nerve stimulation (De Potter, De Schaepdryver, Moerman & Smith 1969; Geffen, Livett & Rush 1969*a*), although the ratio of protein/NA in the perfusate appears to be very small. The results concerning light and heavy particles, together with the observations on the recovery of nerves after reserpine, could possibly be interpreted as follows: the young amine granules are fairly large, and contain a maximum amount of protein (chromogranin and DA β-hydroxylase). When the granules leave the perikarya, their NA content is not fully built up, but during their transport along the nerve to the nerve terminals, the NA store is increased by synthesis in the axons. This young granule would thus correspond to the heavy particles in, for example, the splenic nerve. In the nerve terminals the granules are exposed to nerve impulses, and release NA, together with a small fraction of this protein content, at each impulse. The repeated losses of protein will result in a shrinkage of the granule, which becomes smaller and gradually lighter. The properties of the granules are likely to change with the changes in their chemical composition. [^{3}H]NA uptake may perhaps require a greater amount of proteins in the granules, and if the mechanism for NA release also requires the proteins, then both [^{3}H]NA retention and NA release can occur only from young granules, possessing large quantities of the proteins. The storage of endogenous NA may perhaps not require such large amounts of proteins; a precipitation of NA together with only ATP has been shown to occur *in vitro* by Berneis, Pletscher & Da Prada (1969).

Discussions regarding the possible differences between new and old granules have been published earlier (see, for example, Häggendal & Dahlström 1971*a*, *b*). The possibility of two distinct types of amine storage particles must, however, also be considered, as pointed out in the last-mentioned article.

3. Experimentally induced changes in axonal transport, and possible mechanisms for transport

(*a*) *The influence of nerve activity on axonal transport*

The rapid cytoplasmic streaming in plant cells can be inhibited by electrical stimulation (see, for example, Kishimoto & Akabori 1959; Vorobyev & Vorobyeva 1963). By contrast, the

axoplasmic transport in nerves appears to be unaffected, or even increased, by electrical stimulation. The effect of stimulation on the transport of neurosecretory granules has been mentioned above (§ 1*b*(i)). Jasinski *et al.* (1966) found, in the neurosecretory neurons in the preoptic nucleus of goldfish, a rate of transport of neurosecretory granules of 2 mm/min, i.e. about 2800 mm/day, after electrically stimulating the olfactory tracts. This rate was considerably faster than at rest, but no figure for the rate in normal animals was given (Jansinski *et al.* 1966).

In snail neurons and in frog ventral roots [^{14}C]glutamate is transported along the nerve in a proximo-distal direction. The rate of transport of glutamate from the CNS, down the nerve to the muscle part was markedly increased (up to 30 mm/h) after a low-frequency stimulation. Administration of xylocain or Nembutal to the nerve, or cooling of the nerve trunk, delayed the appearance of [^{14}C]glutamate in the muscle bath, indicating a transport of less than 10 mm/h in this situation (Kerkut 1967, Kerkut *et al.* 1967). In this frog preparation (spinal cord–sciatic nerve–muscle) evidence for a retrograde flow of glutamate was also obtained (see also above, § 2*a*(v)). The rapid appearance of glutamate in the muscle bath after stimulation may, however, have explanations other than an increased rate of transport of glutamate. An increased release of glutamate from the nerve terminals of the muscle during stimulation would give the same results, whereas in the unstimulated preparation perhaps the nerve terminals do not lea any glutamate until certain concentrations are reached, or special binding sites are saturated.

Geffen & Rush (1968) investigated the problem in adrenergic nerves. In intact splenic nerves of cat, the rate of NA transport was calculated to be 1.4 mm/h, whereas preganglionically denervated nerves exhibited a flow rate of 3.3 mm/h. The difference was not statistically significant, but the results demonstrated that nerve impulse activity is not necessary for transport of NA in adrenergic nerves.

The accumulation of NA in rat sciatic nerve has been studied after stimulation (Dahlström & J. Häggendal, unpublished). Under Nembutal anaesthesia the sciatic nerves were ligated bilaterally and the lumbar sympathetic chain was stimulated 6 to 7 times per second (biphasic, 6 V, duration 2 ms) for 6 h. Control rats were ligated and sham-stimulated. The amount of NA, accumulating in the sciatic nerves above the ligature was on every experimental occasion (6) somewhat higher in the stimulated rats than in the control rats, but the difference was not statistically significant. Thus, nerve impulse activity does not appear to significantly alter the rate of NA accumulation.

The transport of AChE in dog peroneal nerves has been studied after electrical stimulation by Jankowska *et al.* (1969). In isolated nerve segments, the rate of translocation of AChE towards the two ends was similar, both in stimulated and unstimulated nerves. Also, the effect of the direction of the nerve impulses was tested. The pattern of AChE translocation was the same in distal-proximally stimulated nerves as in proximo-distally stimulated ones (Jankowska *et al.* 1969).

The conclusions drawn from the above studies are that the nerve activity of the neuron seems to have little or no influence on the axoplasmic flow in most mammalian nerves, at least in short-term experiments, and that the two processes of nerve conduction and axoplasmic transport are independent, being dependent upon different mechanisms in the neuron.

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(*b*) *The influence of drugs on axonal transport*

(i) *Reserpine*

A high dose of reserpine depletes the NA stores in adrenergic neurons, and also causes a disappearance of the NA which has accumulated above a ligation. This depletion is probably due to a long-lasting or permanent block of the NA storage sites in the amine granules (see Carlsson 1965; Dahlström & Häggendal 1970; Häggendal & Dahlström 1971*b*). During the initial 12 to 15 h after reserpine, no NA accumulates above a short-term (1 to 6 h) ligation (see, for example, Dahlström 1967*b*; Dahlström & Häggendal 1969). This, however, does not imply that amine granules, empty of NA, are not transported along the axons in this time interval. More studies are required to elucidate this question.

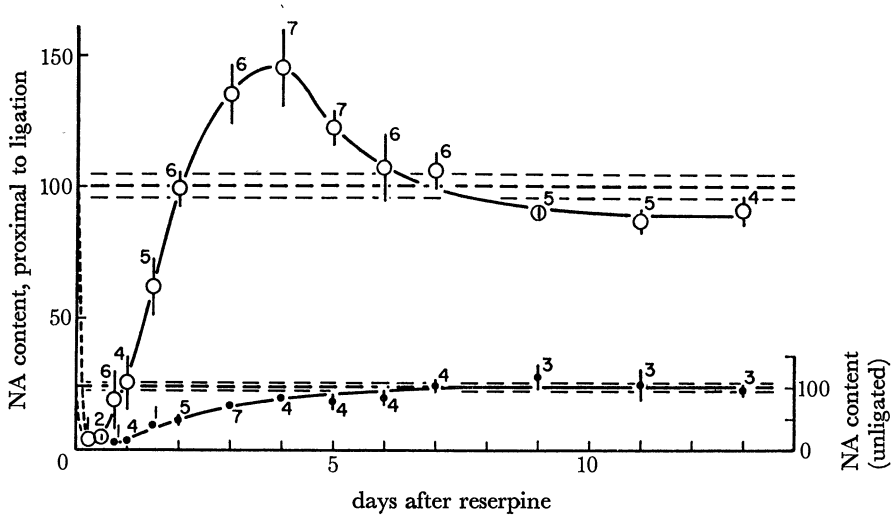

FIGURE 15. The noradrenaline (NA) content in sciatic nerve of normal and reserpine-treated rats (one single dose of 10 mg/kg i.p.). The upper curve (○——○) indicated the accumulation of noradrenaline in the 1 cm part of nerve above a ligation performed 6 h before death. The values are expressed as a percentage of the mean value for the noradrenaline amount found to accumulate in normal rat nerves above a 6 h ligation on every experimental occasion. 100% corresponds to 7.99 ng ± 0.36 (mean ± S.E., $n = 26$). The lower curve (●——●) indicates the noradrenaline content in unligated nerves, expressed as a percentage of the content found in normal unligated nerves at every experimental occasion. The amount of noradrenaline per centimetre of normal unligated nerve (100%, ordinate to the right) corresponds to 1.91 ng/cm ± 0.08 (mean ± S.E., $n = 24$). The vertical bars represent the S.E., and the numerals indicate number of experiments. Left ordinate: NA content (expressed in % of normal) in the 1 cm part of nerve just proximal to the ligation, performed 6 h before death. Right ordinate: NA content per centimetre of unligated nerve (expressed as % of normal). (From Dahlström & Häggendal 1969.)

Fifteen hours after reserpine, NA accumulations reappeared above short-term ligations (Dahlström 1967*b*). The NA amounts, probably intragranular, then increased, and reached normal levels around day 2, and supranormal levels between days 3 and 5 (figure 15) (Dahlström & Häggendal 1969). Since the NA levels in the unligated nerves were subnormal whereas supranormal amounts of NA had accumulated in 6 h ligated nerves, the rate of transport of NA granules appeared to have increased, as compared to the normal rate. Also, the number of amine granules (as judged from the NA amounts) transported distally in the nerves was increased, and the maximal increase, occurring during the third and fourth days, was estimated to be about 50 to 60% above normal. Consequently, the formation of amine granules in the cell bodies must also be increased during this time period after reserpine. Reserpine thus appears to increase both the formation of amine granules in the perikarya and

the rate of transport of the granules in the axons. (For more detailed discussions, see Dahlström & Häggendal 1969, 1970.)

The mechanisms behind these actions of reserpine in the adrenergic neuron are so far not clarified. Iggo & Vogt (1960) observed an increased impulse activity in the preganglionic neurons to the cat cervical sympathetic ganglion after reserpine. Such an increased impulse activity may induce an increased protein synthesis in the postganglionic adrenergic perikarya (cf. Hydén 1960), and thereby an increased rate of formation of granules. Thoenen, Mueller & Axelrod (1969) reported an increase in tyrosine hydroxylase activity in sympathetic ganglia after reserpine, and also a gradient of tyrosine hydroxylase activity that travelled distally in the sciatic nerve. These authors could prevent this increase in tyrosine hydroxylase after reserpine by pretreatment with ganglionic blocking agents. Thus, the mechanism behind the increased production of granules after reserpine may be a generally increased protein formation, due to increased preganglionic nerve activity. Also, an increase in RNA (Mytilineou 1969) has been found in sympathetic ganglion cells after reserpine (C. Mytilineou & J. J. Jarlstedt, personal communication).

(ii) *Mitosis inhibitors*

Both colchicine and vinblastine, applied locally on adrenergic neurons, inhibit the axoplasmic transport of amine storage granules (Dahlström 1968, 1970*a*, *b*). In fluorescence histochemical studies a marked increase in NA fluorescence was observed in ganglion cell bodies and axons, after soaking sympathetic ganglia with saline solutions of colchicine or vinblastine (figure 16, plate 61). Injections of the solutions (3 to 5μl) under the epineurium of the sciatic nerve caused pronounced accumulations of NA both within and above the area of the injection blister. Controls, injected with saline, showed no, or occasionally a few, weakly fluorescent accumulations. Soaking the spinal cord of rats with colchicine or vinblastine resulted in accumulations of NA and 5-HT in the descending NA and 5-HT neurons (figure 17, plate 61), respectively (Dahlström 1970*b*). The results indicate that the two mitosis inhibitors, when applied locally to sympathetic adrenergic, or monoamine containing neurons, cause an inhibition of the transport of amine granules.

The increased NA fluorescence could be abolished by treatment with reserpine, which indicated that the NA was stored in amine granules. Electronmicroscopical studies have shown that a large number of dense cored vesicles are accumulated in those locations where an increased NA fluorescence was observed (Hökfelt & Dahlström 1970, 1971). When using two different fixation techniques, $KMnO_4$ fixation for demonstration of dense cored vesicles, and glutaraldehyde–OsO_4 fixation for demonstration of neurofilaments and axonal microtubules, results were obtained suggesting that in axons with heavy accumulations of vesicles few microtubules were present, but instead—in the case of vinblastine-treated nerves—a large number of densely packed neurofilaments were present (compare Seil & Lampert 1968). The results may support the view that microtubules present in the adrenergic axons (see, for example, Cravioto 1965), are involved in the fast transport of amine storage granules.

The concentrations of colchicine needed to obtain effects were higher than those of vinblastine. Colchicine was effective in 0.03 mol/l solutions, while vinblastine solutions of 0.001 mol/l caused pronounced effects. Both substances have been shown to influence the organization of microtubules, and their effect on the transport of amine granules may be related to this capacity. The difference in effective concentrations may possibly be related to the fact that vinblastine binds

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to a site which also binds guanosine triphosphate (GTP) (Weisenberg 1968), while colchicine has another site of binding. GTP appears to stabilize the microtubules and the ratio between GTP sites and colchicine sites appears to be 2:1 (Weisenberg, Borisy & Taylor 1968).

Colchicine injected into peripheral nerves causes degeneration of axons (see, for example, Angevine 1957) and inhibits the proximo-distal transport of AChE in rat sciatic nerves (Kreutzberg 1969). In fact, the first to demonstrate the inhibitory effect of colchicine on axonal transport in mammalian nerves was G. W. Kreutzberg in 1968 (personal communication). Injected into the rabbit eye, the drug has a pronounced inhibiting effect on the last phase of transport of labelled proteins, while the slow phase seems affected to a smaller degree (Karlsson & Sjöstrand 1969). In contrast, James, Bray, Morgan & Austin (1970) reported that colchicine had a more pronounced blocking effect on the slow flow (2 mm/day) than on the fast transport of proteins in the chicken sciatic nerve. In the crayfish spinal cord, colchicine blocked the slow flow of proteins (1.1 mm/day, Fernandez & Davison 1969, 1970).

Intravenous injections of vinblastine (Keen & Livingstone 1970) (3 mg/kg) appear to give results somewhat different from those obtained by local administration. A decrease in tissue NA was observed, but sciatic nerve ligations resulted in accumulations of NA, which may indicate an uninterrupted flow of amine granules in the axons. The reason why intravenous vinblastine has these mixed effects is so far not clear. The intravenous route was chosen by these authors because of the low pH of vinblastine solutions (P. Keen, personal communication). However, citrate buffer solutions of the same pH as 0.01 mol/l solutions of vinblastine in saline (pH = 4.5) appear to produce no specific effects as regards transport of amine granules in the adrenergic neurons (A. Dahlström, unpublished). The transport inhibitory effect of locally administered vinblastine is therefore unlikely to be due to the low pH of the solutions. Since intravenous vinblastine effects the mitosis in other tissues, the local treatment may appear preferable, the effects observed being largely confined to the treated tissue, i.e. the cell bodies or axons.

(*c*) *Possible mechanisms for axonal transport*

As indicated in the preceding section the mechanism for axoplasmic transport may be linked to the microtubules, as suggested by Schmitt and co-workers (figure 18, cf. Schmitt 1968). The metabolic dependence of fast axonal transport has been demonstrated by Ochs & Ranish (1970). The rapid transport of radioactive material in the cat sciatic nerve, following injection of [^{3}H]amino acids in the L_7 ganglion, ceased within 15 min of killing the animals by bleeding. The removal of the nerves immediately after death and their incubation in oxygen at body temperature resulted in a maintained fast transport. Incubation in 100% nitrogen rapidly blocked the transport. The mechanism by which the axonal microtubules may participate in fast transport is, thus, dependent upon oxidative metabolism.

This article has discussed the two main types of axonal transport, the fast and the slow type. Such a division is, however, an oversimplification of the situation. Karlsson & Sjöstrand (1971) recently studied the migration of proteins in the rabbit retinal neurons. They found *four* different rates of transport in this system: 150 mm/day (I), 40 mm/day (II), 6 to 12 mm/day (III) and 2 mm/day (IV) (figures 19*a*, *b*). The two rapid phases (I and II) referred to proteins which were bound into a light particle fraction. These two phases may represent the transport of transmitter vesicles and endoplasmic reticulum. The intermediate phase (III) is suggested to be associated with the migration of mitochondria, whereas soluble proteins were predominant in the slow phase (IV) (Karlsson & Sjöstrand 1971).

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It would be very convenient if these four rates could be considered as compartments, into which the results from investigations on axonal flow could be fitted. This appears, unfortunately, to be impossible. For instance, the rate of transport of one particular organelle, the amine storage granule, appears to be about 5 to 10 mm/h, i.e. 120 to 240 mm/day, in cat sciatic and splenic nerves (Dahlström & Häggendal 1966; Livett *et al.* 1968), and thus belongs to phase I, while the transport in rabbit sciatic nerve appears to be slower, 2 to 3 mm/h, i.e. 48 to 72 mm/day, and more in line with phase II. Species differences must thus be taken into consideration.

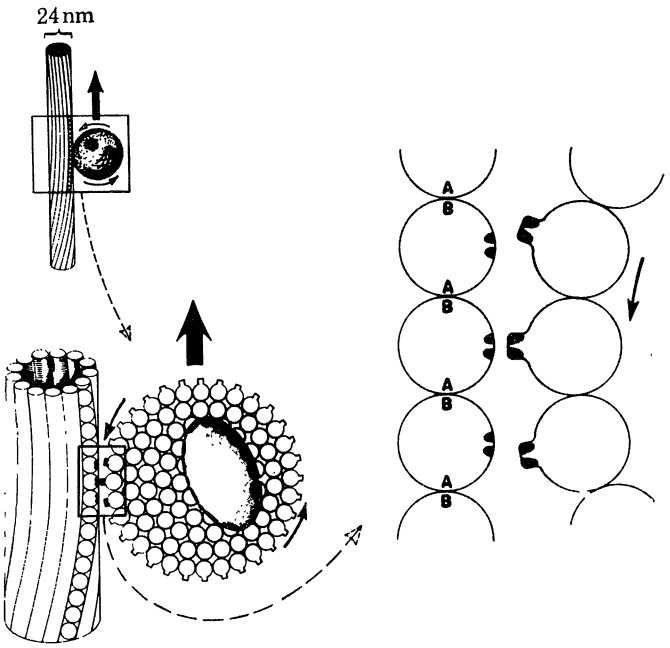

FIGURE 18. Hypothetical sliding-vesicle model of mechanism of fast specific transport of vesicles by (saltatory?) interaction with microtubules; principle of mechanochemical coupling based on sliding filament hypothesis of muscle contraction. Binding between subunits of microtubule and of vesicle wall at one of paired binding sites; other site relates to binding between nucleotide of microtubules with ATPase (GTPase) of vesicle subunit. (From Schmitt 1968.)

Also, different neuron systems within the same species may differ. Thus the transport of NA granules in rat sciatic nerve has been calculated to be about 80 to 120 mm/day (Dahlström & Häggendal 1966, 1970), but only of the order of about 15 mm/day in the rat bulbo-spinal neurons (Häggendal & Dahlström 1969). The migration rate of AChE and ChA in vagal nerves of rat has been estimated to be 15 mm/day, while the rate in hypoglossal nerves of the same animal was 5 mm/day (Fritzell *et al.* 1970).

Local injections of colchicine in peripheral nerves block the transport of NA granules (Dahlström 1968), AChE (Kreutzberg 1969; Sjöstrand, Fritzell & Hasselgren 1970) and ChAc (Sjöstrand *et al.* 1970). Vinblastine blocks the transport of NA granules (Dahlström 1970*a*; Hökfelt & Dahlström 1970). These two mitosis inhibitors destroy the microtubules, and the transport of the mentioned particles and enzymes may therefore possibly involve the microtubules. The rates of transport of the different substances which may be transported via the microtubules are very different, and it is possible that the configuration of an organelle, or the number of sites reacting with the microtubule wall, may influence the transport rates. Alternatively, more than one basic transport mechanism related to the microtubules may exist. In this connexion an interesting observation regarding the transport of virus in nerves may be

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mentioned. Herpes simplex virus, inoculated intradermally to suckling mice, was transported along the sciatic nerve towards the spinal cord. The spread upwards of the infection was blocked not only by ligation of the nerve, but also by freezing and local treatment of the nerve with colchicine. The results indicated that the virus was transported centripetally in axons, by a mechanism possibly connected to the microtubules (Kristensson, Lycke & Sjöstrand 1970).

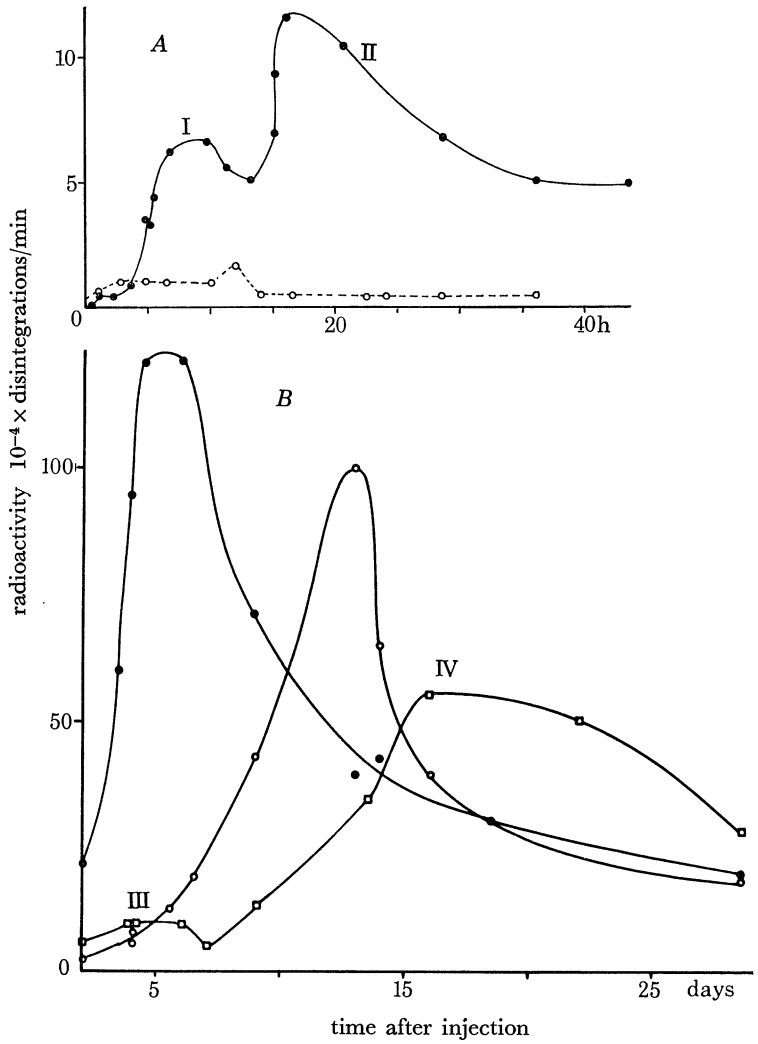

FIGURE 19. (*a*) Radioactivity in the lateral geniculate bodies at different intervals following intraocular injections of [^{3}H]leucine. Each symbol represents the average of 2 to 4 animals. I and II show the two rapid phases of axonal transport that arrived to the lateral geniculate body. ●, protein; ○, TCA supernatant. (*b*) Protein-bound radioactivity in the optic pathway at different intervals following intraocular injections of [^{3}H]leucine. Each symbol represents the average of two animals. III and IV show the two slow phases of axonal transport which reach maximum in the lateral geniculate bodies at about 5 and 16 days respectively. ●, optic nerves. ○, optic tracts. □, lateral geniculate bodies. (From Karlsson & Sjöstrand 1971.)

The view that microtubules are involved in axoplasmic transport has gained support from electronmicroscopical studies by Järlfors & Smith (1969, see also D. S. Smith, this volume, p. 395), who found a close association between axonal microtubules and transmitter vesicles in the lamprey nerve cord.

As regards mitochondrial movements, the mechanism seems to be different. Treatment with colchicine appears to have little or no effect on the transport of the mitochondria, neither in

myelinated (Kreutzberg 1969) nor in unmyelinated adrenergic nerves (Banks & Mayor, personal communications). As yet, the specific mechanism by which mitochondria are transported is entirely unknown. It has been reported that mitochondria contain an actomyosin-like protein (Onishi & Onishi 1962), and if this is so, the mechanism of movement of mitochondria may be localized to this organelle itself. In other words, they could have the property amoeboid movement, or creeping.

The slowest rates of transport of 1 to 2 mm/day, may be related to the growth of the axoplasmic bulk (see, for example, Weiss 1961). The growth of microtubules and filaments probably constitutes the major part of the slowly growing axoplasm (Droz 1967). A certain part of the soluble proteins is transported in the slow phase (see, for example, Karlsson & Sjöstrand 1971). The mechanism behind this transport may be a simple pushing down of the axoplasmic column or of the fibrous proteins, by the addition of newly formed subunits in the perikaryon. The interesting observation that colchicine also has an inhibitory effect—partial (Sjöstrand *et al.* 1970) or total (James *et al.* 1970)—on the slow transport, may perhaps not be so peculiar in view of the fact that the microtubules and filaments constitute such a prominent part of the growing axoplasm. If the microtubules are depolymerized, or reaggregated in different forms, one may expect that the subunits, or the re-formed elements, behave differently from the original tube with regard to growth, or axoplasmic flow.

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References (Dahlström)

Andén, N.-E., Dahlström, A., Fuxe, K., Larsson, K., Olson, L. & Ungerstedt, U. 1966 *Acta physiol. scand.* **67**, 313–326.
Andén, N.-E., Häggendal, J., Magnusson, T. & Rosengren, E. 1964 *Acta physiol. scand.* **62**, 115–118.
Andén, N.-E., Magnusson, T. & Waldeck, B. 1964 *Life Sci.* **3**, 19–25.
Angevine, J. B. 1957 *J. exp. Zool.* **136**, 363–391.
Austin, L., Bray, J. J. & Young, R. J. 1966 *J. Neurochem.* **13**, 1267–1269.
Banks, P., Magnall, D. & Mayor, D. 1969 *J. Physiol., Lond.* **200**, 745–762.
Bargmann, W. & Scharrer, E. 1951 *Am. Scient.* **39**, 255–259.
Barondes, S. H. 1966 *J. Neurochem.* **13**, 721–727.
Barondes, S. H. 1969*a* In *Handbook of neurochemistry*, vol. II (ed. A. Lajtha), pp. 435–446. New York, London: Plenum Press.
Barondes, S. H. 1969*b* In *Cellular dynamics of the neuron* (ed. S. H. Barondes), pp. 351–364. New York, London: Academic Press.
Berneis, K. H., Pletscher, A. & Da Prada, M. 1969 *Nature, Lond.* **224**, 281–283.
Blümke, S., Niedorf, H. R. & Rode, J. 1966 *Acta Neuropath.* **7**, 44–61.
Boyle, F. & Gillespie, J. 1968 *J. Physiol., Lond.* **195**, 27–28*P*.
Breemen, V. L. van, Andersson, E. & Reger, J. F. 1958 *Expl Cell Res. Suppl.* **5**, 153–167.
Brzin, M., Tennyson, V. M. & Duffy, P. E. 1966 *J. Cell Biol.* **31**, 215–242.
Burdwood, W. O. 1965 *J. Cell Biol.* **27**, 115A.
Cajal, S. R. y, 1928 In *Degeneration and regeneration of the nervous system.* Oxford University Press.
Carlsson, A. 1966 In *Handbuch der experimentellen Pharmakologie*, vol. 19 (ed. V. Erspamer), pp. 529–592. Berlin–Heidelberg–Göttingen: Springer-Verlag.
Christ, J. F. 1962 In *Neurosecretion* (eds. H. Heller and R. B. Clark), pp. 125–147. London: Academic Press.
Corrodi, H. & Jonsson, G. 1967 *J. Histochem. Cytochem.* **15**, 65–78.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Cravioto, H. 1966 *J. Comp. Neurol.* **126**, 453–461.
Dahlström, A. 1965 *J. Anat., Lond.* **99**, 677–689.
Dahlström, A. 1967*a* *Acta physiol. scand.* **69**, 158–166.
Dahlström, A. 1967*b* *Acta physiol. scand.* **69**, 167–179.
Dahlström, A. 1968 *Europ. J. Pharmacol.* **5**, 111–113.
Dahlström, A. 1970*a* In *New aspects of storage and release mechanisms of catecholamines*. Bayer Symposium II. Berlin–Heidelberg–New York: Springer-Verlag, 20-36.
Dahlström, A. 1970*b* *Acta physiol. scand. Suppl.* **357**, 6.
Dahlström, A. & Fuxe, K. 1964*a* *Acta physiol. scand.* **60**, 293–294.
Dahlström, A. & Fuxe, K. 1964*b* *Z. Zellforsch.* **62**, 602–607.
Dahlström, A. & Fuxe, K. 1965 *Acta physiol. scand.* **64**, Suppl. 247, 1–36.
Dahlström, A. & Häggendal, J. 1966 *Acta physiol. scand.* **67**, 278–288.
Dahlström, A. & Häggendal, J. 1967 *Acta physiol. scand.* **69**, 153–157.
Dahlström, A. & Häggendal, J. 1969 *J. Pharm. Pharmacol.* **21**, 633–638.
Dahlström, A. & Häggendal, J. 1970 In *Biochemistry of simple neuronal models* (eds. E. Costa and E. Giacobini). New York: Raven Press, 65–93.
Dahlström, A. & Jonason, J. 1968 *Europ. J. Pharmacol.* **4**, 377–383.
Dahlström, A., Jonason, J. & Norberg, K.-A. 1969 *Europ. J. Pharmacol.* **6**, 248–254.
De Iraldi, A. P. & de Robertis, E. 1968 *Z. Zellforsch.* **87**, 330–344.
De Potter, W. P. 1968 Thesis, University of Ghent.
De Potter, W. P., Chubb, I. W. & de Schaepdryver, A. F. 1970 *Acta physiol. scand. Suppl.* **357**, 7–8.
De Potter, W. P., De Schaepdryver, A. F., Moerman, E. J. & Smith, A. D. 1969 *J. Physiol., Lond.* **204**, 102–104*P*.
Diamond, J. & Evans, C. A. N. 1960 *J. Physiol., Lond.* **154**, 69*P*.
Droz, B. 1967 *J. Microscopie* **6**, 201–228.
Droz, B. & Leblond, C. P. 1962 *Science, N.Y.* **137**, 1047–1048.
Droz, B. & Leblond, C. P. 1963 *J. comp. Neurol.* **121**, 325–346.
Edström, J.-E., Eichner, D. & Edström, A. 1962 *Biochim. Biophys. Acta* (*Amst.*) **61**, 178–184.
Estable, C., Acosta-Ferreira, W. & Sotelo, J. T. 1957 *Z. Zellforsch.* **46**, 387–399.
Euler, U. S. v. & Lishajko, F. 1961 *Proc. sci. Soc. Bosnia Herzegovina* **1**, 109.
Evans, C. A. N. & Saunders, N. R. 1967 *J. Physiol., Lond.* **192**, 79–92.
Fernandez, H. L. & Davison, P. F. 1969 *Proc. natn. Acad. Sci., U.S.A.* **64**, 512–519.
Fernandez, H. L. & Davison, P. F. 1970 *Proc. natn. Acad. Sci., U.S.A.* (in the Press).
Fonnum, F. 1967 *Biochem. J.* **103**, 262–270.
Fonnum, F. 1970 In *Drugs and cholinergic mechanisms in the CNS* (eds E. Heilbronn & A. Winter). FOA Repro, Sweden.
Friede, R. L. 1959 *Expl. Neurol.* **1**, 441–466.
Friede, R. L. 1964 *Acta Neuropath.* **3**, 217–228.
Friede, R. L. 1966 In *Topographic brain chemistry*. New York: Academic Press, 388–400.
Fritzell, M., Hasselgren, P. O. & Sjöstrand, J. 1970 *Expl. Brain Res.* **10**, 526–531.
Geffen, L. B., Hunter, C. & Rush, R. 1968 *Aust. J. exp. Biol. med. Sci.* **46**, 21.
Geffen, L. B., Hunter, C. & Rush, R. 1969 *J. Neurochem.* **16**, 469–474.
Geffen, L. B., Livett, B. G. & Rush, R. 1969*a* *J. Physiol., Lond.* **204**, 58–59*P*.
Geffen, L. B., Livett, B. G. & Rush, R. 1969*b* *J. Physiol., Lond.* **204**, 593–605.
Geffen, L. B. & Ostberg, A. 1969 *J. Physiol., Lond.* **204**, 583–592.
Geffen, L. B. & Rush, R. 1968 *J. Neurochem.* **15**, 925–930.
Grafstein, B. 1967 *Science, N.Y.* **157**, 196–198.
Gutmann, E. 1958 In *Die Funktionelle Regeneration der peripheren Nerven*. Berlin: Akademie Verlag.
Häggendal, J. & Dahlström, A. 1969 *J. pharm. pharmacol.* **21**, 55–57.
Häggendal, J. & Dahlström, A. 1970 *Europ. J. Pharmacol.* **10**, 411–415.
Häggendal, J. & Dahlström, A. 1971*a* In *Proc. Int. Symp. Soc. Endocrinol.* Bristol, April 1970.
Häggendal, J. & Dahlström, A. 1971*b* *J. Pharm. Pharmacol.* **23**, 81–89.
Häggendal, J., Saunders, N. R. & Dahlström, A. 1971 *Nature, Lond.* (in the Press).
Hebb, C., Krause, M. & Silver, A. 1959 *J. Physiol., Lond.* **148**, 69–70*P*.
Hebb, C. & Silver, A. 1961 *Nature, Lond.* **189**, 124–125.
Hebb, C. & Silver, A. 1963 *J. Physiol., Lond.* **169**, 41–42*P*.
Hild, W. 1951 *Virchows Arch. path. Anat.* **319**, 526–546.
Hild, W. & Zetler, G. 1953 *Pflügers Arch. ges. Physiol.* **257**, 169–201.
Hökfelt, T. 1968 *Z. Zellforsch.* **91**, 1–74.
Hökfelt, T. 1969 *Acta physiol. scand.* **76**, 427–440.
Hökfelt, T. & Dahlström, A. 1970 *Acta physiol. scand. Suppl.* **357**, 10–11.
Hökfelt, T. & Dahlström, A. 1971 *Z. Zellforsch.* (in the Press).
Holton, P. 1960 In *Polypeptides which affect smooth muscle and blood vessels*, pp. 192–194. Oxford–London–New York–Paris: Pergamon Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Hydén, H. 1960 In *The cell* (eds. J. Brachet and A. Mirsky), **4**, 215–323. New York–London: Academic Press.
Iggo, A. & Vogt, M. 1960 *J. Physiol., Lond.* **150**, 114–138.
James, K. A. C., Bray, J. J., Morgan, I. G. & Austin, L. 1970 *Biochem. J.* **117**, 767–771.
Jankowska, E., Lubińska, L. & Niemierko, S. 1969 *Comp. Biochem. Physiol.* **28**, 907–913.
Järlfors, U. & Smith, D. S. 1969 *Nature, Lond.* **224**, 710–711.
Jasinski, A., Gorbman, A. & Hara, T. J. 1966 *Science, N.Y.* **154**, 776–778.
Kapeller, K. & Mayor, D. 1966*a* *J. Physiol., Lond.* **182**, 44–45*P*.
Kapeller, K. & Mayor, D. 1966*b* *J. Physiol., Lond.* **182**, 21–22*P*.
Kapeller, K. & Mayor, D. 1966*c* *J. Anat., Lond.* **100**, 439–441.
Kapeller, K. & Mayor, D. 1967 *Proc. Roy. Soc. Lond.* B **167**, 282–292.
Kapeller, K. & Mayor, D. 1969*a* *Proc. Roy. Soc. Lond.* B **172**, 39–51.
Kapeller, K. & Mayor, D. 1969*b* *Proc. Roy. Soc. Lond.* B **172**, 53–63.
Karlsson, J.-O. & Sjöstrand, J. 1969 *Brain Res.* **13**, 617–619.
Karlsson, J.-O. & Sjöstrand, J. 1971 *J. Neurochem.* (in the Press).
Karlström, L. & Dahlström, A. 1970 *Acta physiol. scand. Suppl.* **357**, 12.
Karlström, L. & Dahlström, A. 1971 (Submitted for publication in *J. Neurochem.*)
Kaśa, P. 1968 *Nature, Lond.* **218**, 1265–1267.
Kaufmann, S. & Friedman, S. 1965 *Pharmacol. Rev.* **17**, 71–100.
Keen, P. & Livingston, A. 1970 *Nature, Lond.* **227**, 967–968.
Kerkut, G. A. 1967 In *Growth of the nervous system.* Ciba Foundation (eds. G. E. W. Wolstenholme and M. O'Connor), pp. 220–229. London: J. and A. Churchill Ltd.
Kerkut, G. A., Shapira, A. & Walker, R. J. 1967 *Comp. Biochem. Physiol.* **23**, 729–748.
Kidwai, A. M. & Ochs, S. 1967 *Physiologist* **10**, 220.
Kidwai, A. M. & Ochs, S. 1969 *J. Neurochem.* **16**, 1105–1112.
Kishimoto, U. & Akabori, H. 1959 *J. gen. Physiol.* **42**, 1167–1183.
Koenig, E. 1965 *J. Neurochem.* **12**, 357–361.
Koenig, H. 1958 *Trans. Am. Neurol. Ass.* **83**, 162–164.
Kreutzberg, G. W. 1963 *Naturwissenschaften* **50**, 96.
Kreutzberg, G. W. 1969 *Proc. natn. Acad. Sci. U.S.A.* **62**, 722–728.
Kristensson, K., Lycke, E. & Sjöstrand, J. 1970 *Acta physiol. scand. Suppl.* **357**, 13–14.
Laduron, P. 1968 *Arch. int. Pharmacodyn.* **171**, 233–234.
Laduron, P. 1970 *Arch. int. Pharmacodyn.* **185**, 200.
Laduron, P. & Belpaire, F. 1968*a* *Life Sci.* **7**, 1–7.
Laduron, P. & Belpaire, F. 1968*b* *Biochem. Pharmacol.* **17**, 1127–1140.
Laduron, P. & Belpaire, F. 1968*c* *Nature, Lond.* **217**, 1155–1156.
Lasek, R. J. 1966 *Anat. Rec.* **154**, 373–374.
Lasek, R. J. 1967 *Neurosci. Res. Progr. Bull.* **5**, 314–317.
Lasek, R. J. 1968 *Brain Res.* **7**, 360–377.
Livett, B. G., Geffen, L. B. & Austin, L. 1968*a* *Nature, Lond.* **217**, 278–279.
Livett, B. G., Geffen, L. B. & Austin, L. 1968*b* *J. Neurochem.* **15**, 931–939.
Livett, B. G., Geffen, L. B. & Rush, R. A. 1968 *Biochem. Pharmacol.* **18**, 923–924.
Lubińska, L. 1959 *J. comp. Neurol.* **113**, 315–335.
Lubińska, L. 1964 In *Mechanisms of neural regeneration* (eds. M. Singer and J. P. Schadé) *Progr. Brain Res.* **13**, 1–66. Amsterdam: Elsevier.
Lubińska, L. & Niemierko, S. 1971 *Brain Res.* (in the Press).
Lubińska, L., Niemierko, S. & Oderfeld-Nowak, B. 1961 *Nature, Lond.* **189**, 123–124.
Lubińska, L., Niemierko, S., Oderfeld-Nowak, B. & Szwarc, L. 1962 *Science, N.Y.* **135**, 368–369.
Lubińska, L., Niemierko, S., Oderfeld-Nowak, B. & Szwarc, L. 1964 *J. Neurochem.* **11**, 493–503.
Marinesco, G. 1924 *Ann. Anat. pathol. méd. chir.* **1**, 121–162.
Martinez, A. J. & Friede, R. L. 1970 *Brain Res.* **19**, 183–198.
Mayor, D. & Kapeller, K. 1967 *J. R. microsc. Soc.* **87**, 277–294.
McEwen, B. S. & Grafstein, B. 1968 *J. Cell Biol.* **38**, 494–508.
Miani, N. 1960 *Nature, Lond.* **185**, 541.
Miani, N. 1962 *Nature, Lond.* **193**, 887–888.
Miani, N. 1963 *J. Neurochem.* **10**, 859–874.
Mytilineou, C. 1969 *Biol. Psychiatry* **1**, 61–72.
Niemierko, S. & Lubińska, L. 1967 *J. Neurochem.* **14**, 761–769.
Ochs, S. 1970 In *Protein metabolism of the nervous system* (ed. A. Lajtha). Plenum Press, 291–302.
Ochs, S. & Burger, E. 1958 *Am. J. Physiol.* **194**, 499–506.
Ochs, S., Dalrymple, D. & Richards, G. 1962 *Expl. Neurol.* **5**, 349–363.
Ochs, S., Johnson, J. & Ng, M.-H. 1967 *J. Neurochem.* **14**, 317–331.
Ochs, S. & Ranish, N. 1969 *J. Neurobiol.* **1**, 247–261.
Ochs, S. & Ranish, N. 1970 *Science, N.Y.* **167**, 878–879.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ochs, S., Sabri, M. I. & Johnson, J. 1969 *Science, N.Y.* **163**, 686–687.
Ohnishi, Ts. & Ohnishi, To. 1962 *J. Biochem.* **52**, 230–231.
Palay, S. L. 1943 *J. comp. Neurol.* **79**, 247–276.
Rahmann, H. 1966 *Experientia* **22**, 762–763.
Richardson, K. C. 1966 *Nature, Lond.* **210**, 756.
Rizzoli, A. A. 1968 *Brain Res.* **11**, 11–18.
Roth, R. H., Stjärne, L., Bloom, F. E. & Giarman, N. J. 1968 *J. Pharm. exp. Ther.* **162**, 203–212.
Samuels, A. J., Boyarski, L. L., Gerard, R. W., Libet, B. & Brust, M. 1951 *Am. J. Physiol.* **164**, 1–15.
Sawyer, C. H. 1946 *Am. J. Physiol.* **146**, 246–253.
Scharrer, E. & Scharrer, B. 1964 In *Handbuch der mikroskopischen Anatomie des Menschen* (eds. W. von Möllendorf and W. Bargmann). Berlin: Springer-Verlag.
Scharrer, E. A. & Wittenstein, G. J. 1952 *Anat. Rec.* **112**, 387.
Schlote, W. & Hager, H. 1960 *Naturwissenschaften* **47**, 448–451.
Schmitt, F. O. 1968 *Neurosci. Res. Progr. Bull.* **6**, 119–144.
Scott, F. H. 1906 *J. Physiol., Lond.* **34**, 145–162.
Seil, F. J. & Lampert, P. W. 1968 *Expl. Neurol.* **21**, 219–230.
Shepherd, E. H. 1951 Ph.D. Dissertation, University of Chicago.
Sjöstrand, J., Frizell, M. & Hasselgren, P.-O. 1970 *Exp. Brain Res.* **10**, 526–531.
Sjöstrand, J. & Karlsson, J.-O. 1969 *J. Neurochem.* **16**, 833–844.
Skrangiel-Kramska, J., Niemierko, S. & Lubińska, L. 1969 *J. Neurochem.* **16**, 921–926.
Smith, A. D., De Potter, W. & de Schaepdryver, A. F. 1969 *Arch. int. Pharmacodyn. Ther.* **179**, 495–496.
Taylor, A. C. & Weiss, P. 1965 *Proc. natn. Acad. Sci. U.S.A.* **54**, 1521–1527.
Thoenen, H., Mueller, R. A. & Axelrod, J. 1969 *Nature, Lond.* **221**, 1264.
Ungerstedt, U., Butcher, L. L., Butcher, S. G., Andén, W.-E. & Fuxe, K. 1969 *Brain Res.* **14**, 461–471.
Waelsch, H. 1958 *J. nerv. ment. Dis.* **126**, 33–39.
Wechsler, W. & Hager, H. 1962 *Acta Neuropathol.* **1**, 489–506.
Weisenberg, R. C. 1968 Ph.D. Thesis, University of Chicago.
Weisenberg, R. C., Borisy, G. G. & Taylor, E. W. 1968 *Biochemistry* **7**, 4466–4479.
Weiss, P. 1961 In *Regional neurochemistry* (eds. S. S. Kety and J. Elkes), pp. 220–242. London: Pergamon Press.
Weiss, P. 1963 In *Symposium on the effect of use and disuse of neuromuscular functions* (ed. E. Gutmann), pp. 171–183. Prague: Czechoslovak Acad. Sci.
Weiss, P. 1967 *Neurosci. Res. Progr. Bull.* **5**, 371–400.
Weiss, P. & Hiscoe, H. B. 1948 *J. exp. Zool.* **107**, 315–395.
Weiss, P. & Holland, Y. 1967 *Proc. natn. Acad. Sci. U.S.A.* **57**, 258–264.
Weiss, P. & Pillai, A. 1965 *Proc. natn. Acad. Sci. U.S.A.* **54**, 48–56.
Weiss, P., Taylor, A. C. & Pillai, P. A. 1962 *Science, N.Y.* **136**, 330.
Wettstein, R. & Sotelo, J. R. 1963 *Z. Zellforsch.* **59**, 708–730.
Vorobyev, L. N. & Vorobyeva, I. A. 1963 *Biofizika* **8**, 575–578.
Young, J. Z. 1942 *Physiol. Rev.* **22**, 318–374.
Zelená, J. 1968 *Z. Zellforsch. mikrosk. Anat.* **92**, 186–196.
Zelená, J. 1969 In *Cellular dynamics of the neuron* (ed. S. Barondes), pp. 73–94. New York–London: Academic Press.
Zelená, J. & Lubińska, L. 1962 *Physiologia bohemoslov.* **11**, 261–268.

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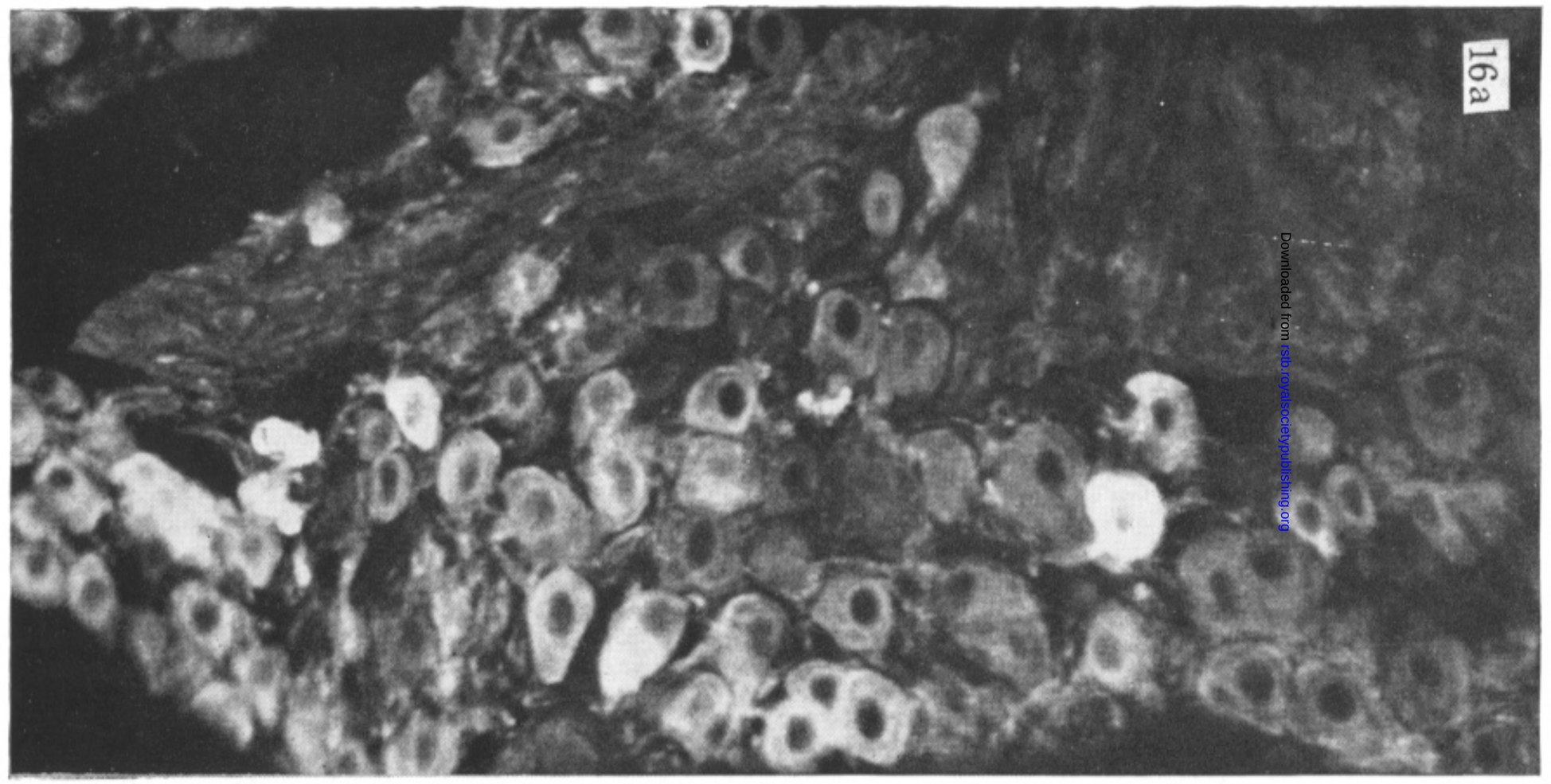

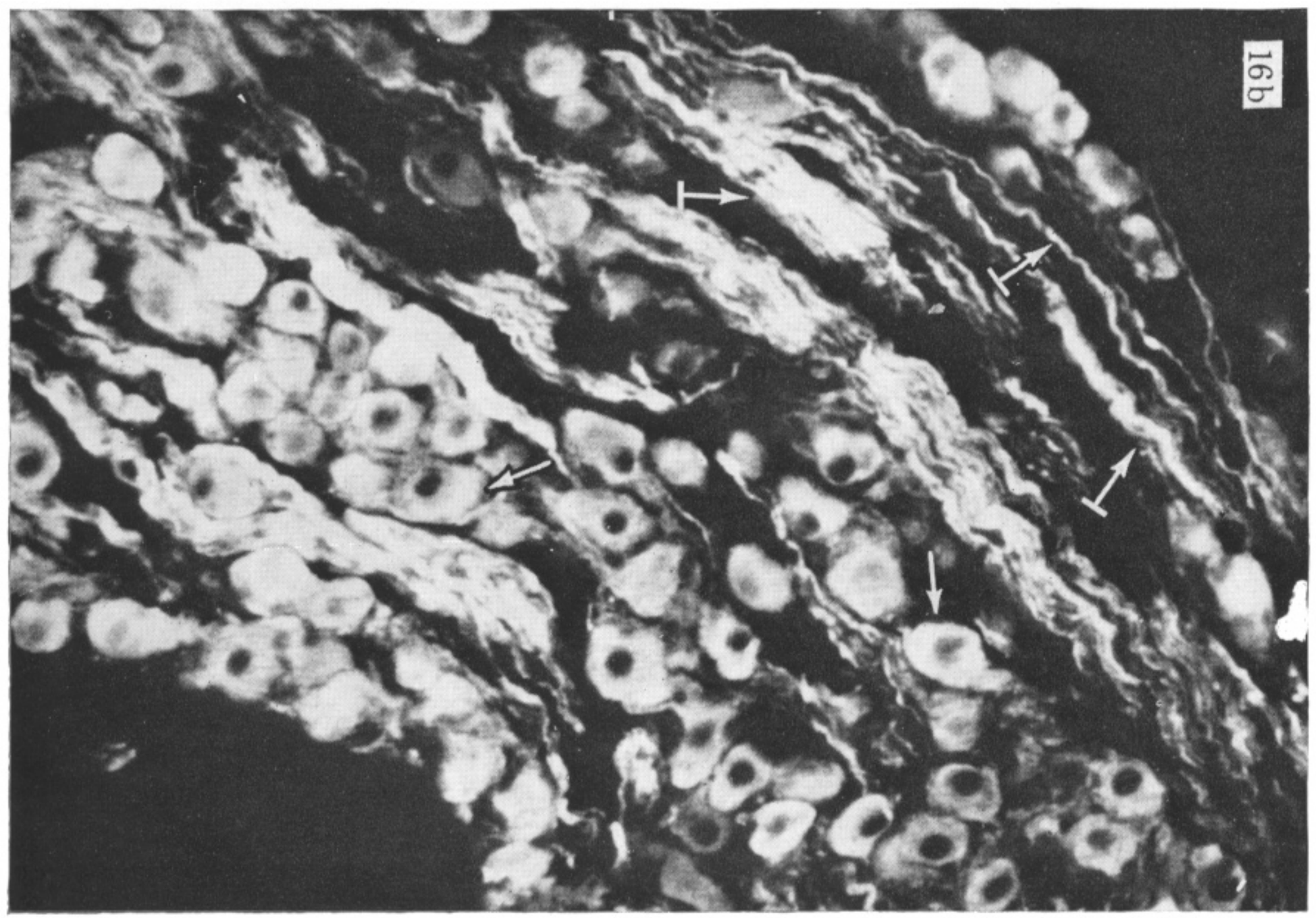

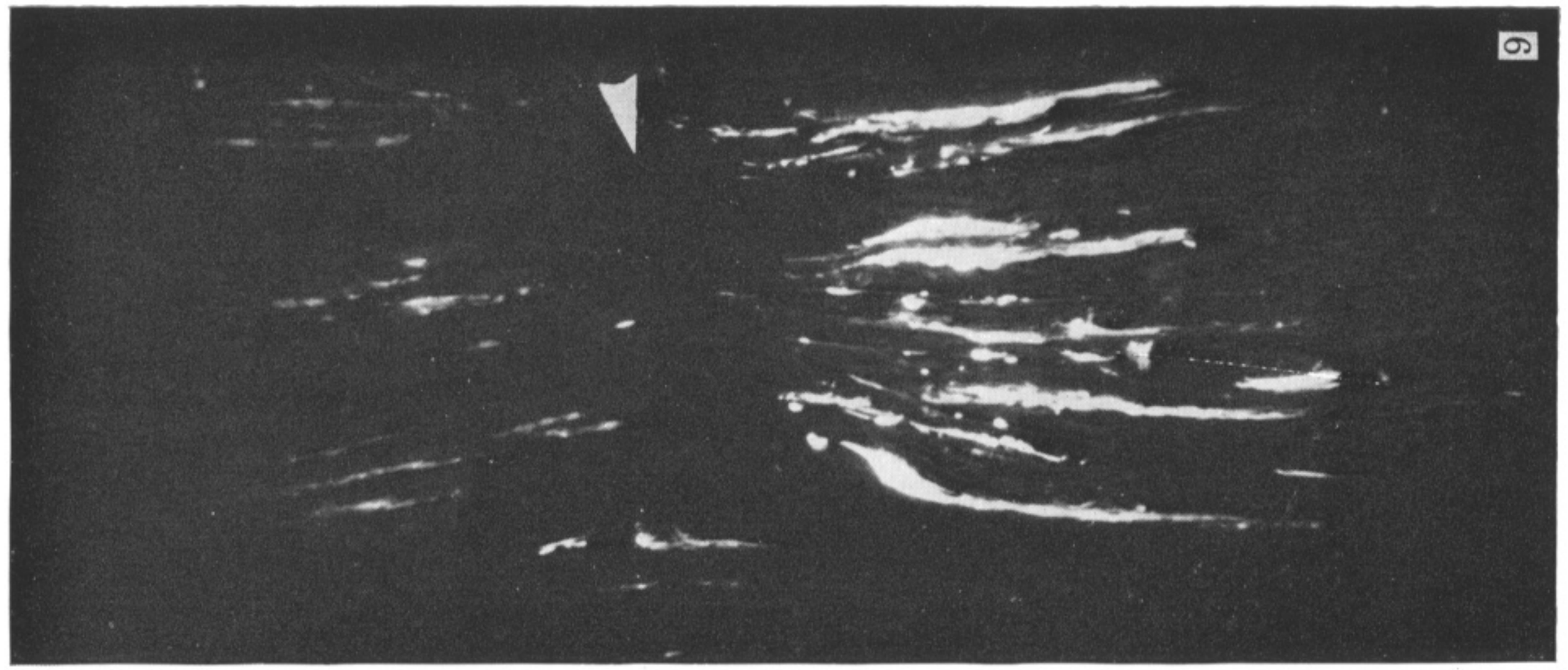

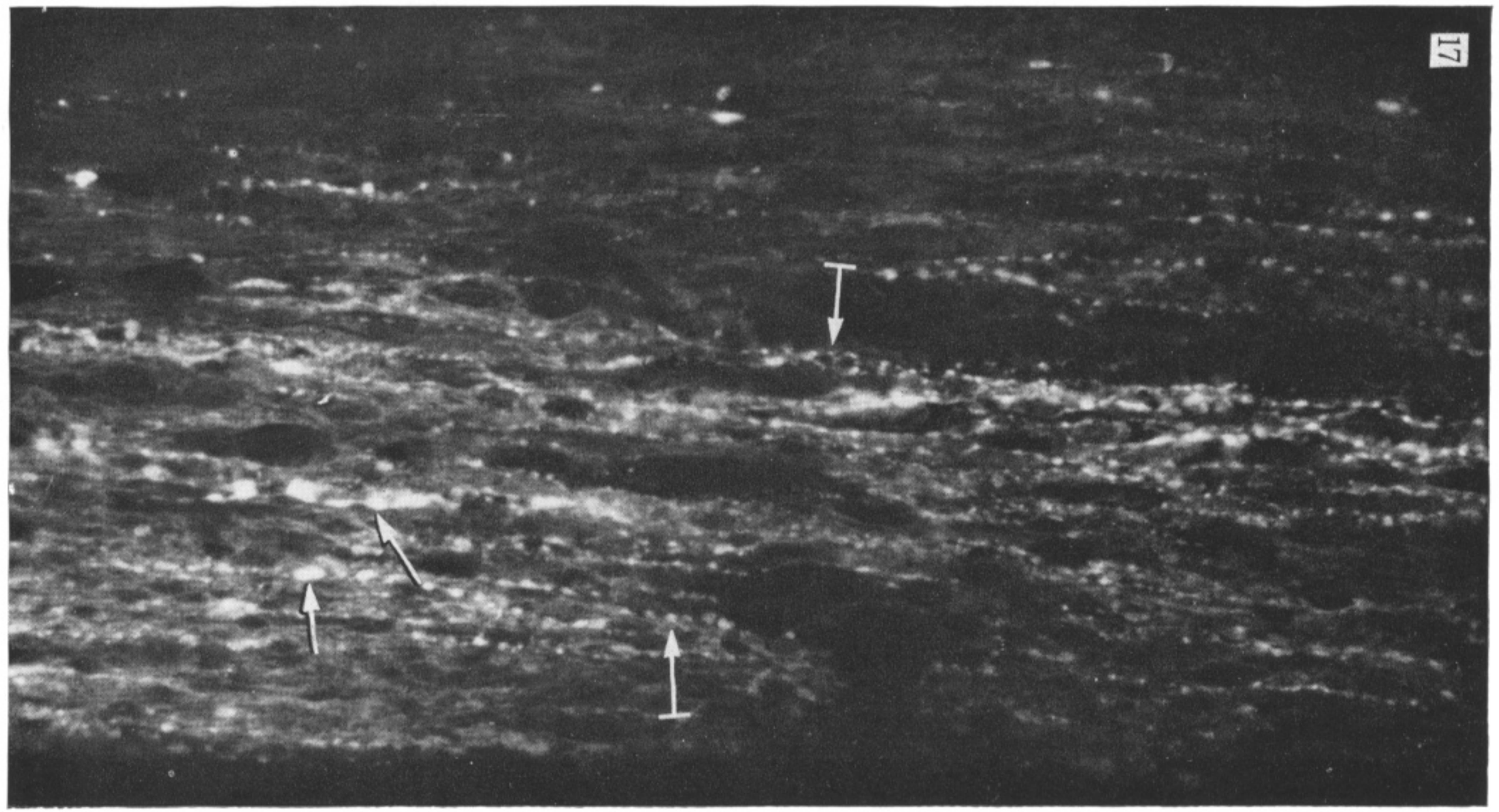

FIGURES 6, 16 AND 17. For legends see facing page.