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Axoplasmic Transport

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The Release of Acetylcholine from the Brain: An Approach to the Study of the Central Cholinergic Mechanisms

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AXOPLASMIC TRANSPORT

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1. Introduction

Neurons have unique problems maintaining themselves because they have a relatively large surface area and enormously elongated processes, the axons whose volume can exceed by several fold that of the nerve cell body. This morphology poses considerable problems in supply and maintenance of essential metabolites to the axon and its terminal which may be a considerable distance from the nuclear regulatory genes. When nuclear control is exerted over protein synthesis there are three possible sources of proteins found in the axon and axon terminal: (1) proteins are synthesized within the nucleated part of the neuron and transported along the axon by axoplasmic flow to the terminals; (2) proteins may be synthesized locally within regions of the axon and axon terminal; (3) proteins may be synthesized at some extra-neuronal site and transported into the axon. All available evidence suggests that the majority of neuronal components would seem to be synthesized in the cell body and transported to the terminals. These newly synthesized proteins provide the enzymes involved in energy production and the metabolism of various transmitters required for synaptic transmission. They must also account for the repair and maintenance of the axonal plasma membrane and axon terminals as well as providing the components of the mechanism for transport.

Three main approaches are commonly used in elucidating the phenomenon of axoplasmic transport: (a) transport of radioactive substances along nerves; (b) accumulation of substances in constricted and severed nerves; and (c) direct observations of the movements of organelles and axoplasm in cultured neurons. While the concept of axoplasmic transport is unequivocally established, the ultrastructural basis of transport is still not understood. This review attempts to bring together the many facets of axoplasmic flow including the anterograde and retrograde flow of various cellular components in the peripheral and central nervous system; the involvement of flow in regenerating and degenerating nerves and the mechanisms for axoplasmic flow.

The first systematic study of axoplasmic flow was carried out by Weiss and Hiscoe in 1948. Based on constriction studies on peripheral nerves in rats, rabbits, chickens and monkeys they postulated that a column of axoplasm flowed from the cell body at a rate of 1 mm per day. The fluid nature of axoplasm in the sciatic nerve of both warm- and cold-blooded animals was investigated by Ochs (1965). The fibres after stretching appeared in a beaded

configuration with constrictions spaced along the nerves at regular intervals. Components within the axon were distributed in a manner consistent with that of a dynamic unidirectional stream. Subsequent work on the movement of axoplasm in nerves indicated that there are at least two rates, that described by Weiss and Hiscoe (1948), which corresponds to the relatively slow rate of growth and regeneration, and a faster rate which operates for the transport of various organelles (Grafstein, 1967, 1969; Lasek, 1968 a, b).

2. Axoplasmic Transport of Cellular Components

Protein has been the most commonly used vehicle for the study of axoplasmic flow because of its ease of labelling and isolation. The early studies of Weiss and Hiscoe (1948) have been extended and refined by a large number of investigators working with different experimental models and techniques. Intravenous and intraperitoneal injections of various labelled precursors (Samuels *et al.*, 1951), ^{14}C-labelled amino acids (Waelsch, 1958; Lajtha, 1961; Weiss, 1961), ^{35}S-methionine (Droz and Verne, 1960; Verne and Droz, 1960) have shown the proximo-distal flow of radioactive protein in nerves. The radioautographic studies of Droz and Leblond (1962, 1963), after intraperitoneal injection of ^{3}H-leucine, showed that most of the protein transported in the axon was previously synthesized in the neuronal cell body.

Methods which employ intraperitoneal or intravenous injection of label suffer from the limitation that some label is invariably taken up by surrounding satellite cells, especially glial and Schwann cells, thus casting doubt on the origin of labelled components detected in the fibres. In an attempt to overcome this problem, techniques have been developed for the injection of radioactive tracers into regions rich in perikarya. Three systems where these problems have been overcome include: (1) injection of label into the spinal cord at the level of the motoneurons of sciatic nerve fibres in chicken (Austin *et al.*, 1966) and cat (Ochs *et al.*, 1967; Ochs and Burger, 1958; Ochs *et al.*, 1962); (2) injection of label into the posterior chamber of the eye and measurement of the rate of movement of protein into the optic nerve (Taylor and Weiss, 1965); (3) insertion of a pad (wad of gel foam) impregnated with the labelled precursor into the nostrils of toads and mice and the subsequent transport within axons of the olfactory system (Weiss and Holland, 1967).

These early studies showed that incorporation of amino acids into protein occurred in neuronal cell bodies and that a peak of radioactively labelled protein subsequently moved into and along the axon at a rate of 1–5 mm/day.

2.1 SLOW AND FAST TRANSPORT OF PROTEINS

Most early estimates of the rate of flow in nerve fibres were of the order of 1–5 mm/day (see Lubińska, 1964 a, b). Although the experiments of Miani (1962, 1963) and Goldberg and Kotani (1967) had indicated the possibility of a more rapid rate of transport it was left to Lasek (1966) and Grafstein (1967) to show that transport occurred at two distinct rates in fibres. Rather than go into an involved discussion of the slow and fast rates, we have summarized the results in Tables 1 and 2 where Table 1 shows experiments indicating slow rates of transport for various systems and Table 2 those with fast rates. The subcellular distribution of radioactivity between fast and slow rates of axoplasmic flow has been investigated by various workers in several systems (McEwen and Grafstein, 1968; Bray and Austin, 1969; Kidwai and Ochs, 1969; Elam and Agranoff, 1971a; Cuénod and Schonbach, 1971; Sjöstrand and Karlsson, 1969). The particulate/soluble ratio of labelled protein

TABLE 1. SLOW RATES OF AXOPLASMIC FLOW OF PROTEIN

Authors	System	Animal	Radioactive label	Rate
Weiss and Hiscoe (1948)	peripheral nerves	rats, rabbits, chickens, monkey	visual examination at various times after constriction	1 mm/day
Grafstein (1967)	optic system	goldfish	^{3}H-leucine	0.4 mm/day
Lasek (1966, 1967, 1968 a, b)	motor neurons, sensory neurons	adult rat cat	^{3}H-leucine	1 mm/day
McEwen and Grafstein (1968)	optic system	goldfish	^{3}H-leucine	0.4 mm/day
Droz and Leblond (1962, 1963)	intravenous injection into peripheral nerve	rats	^{3}H-leucine	1–2 mm/day
Austin *et al.* (1966) Bray and Austin (1968)	sciatic nerve	fowls	^{14}C-leucine	2–3 mm/day
Koenig (1958)	sciatic nerve	cat	^{14}C-glycine	7–11 mm/day
Ochs *et al.* (1967) Ochs *et al.* (1968) Ochs and Johnson (1969)	motor neurons of sciatic nerve	cat	^{3}H-leucine	1–2 mm/day
Taylor and Weiss (1965)	optic system	mouse	^{3}H-leucine	1 mm/day
Karlsson and Sjöstrand (1968)	optic system	rabbit	^{3}H-leucine	1–2 mm/day
Weiss and Holland (1967)	olfactory nerve	toad	^{3}H-leucine	1 mm/day
Weiss (1967)	excised spinal ganglia and associated nerves	mice	^{3}H-leucine	1 mm/day
Sjöstrand (1969, 1970)	vagus and hypoglossal nerves	rabbit	^{3}H-leucine	26 mm/day vagus 5 mm/day hypoglossal
Fernandez and Davison (1969) Fernandez *et al.* (1970)	abdominal ganglia	crayfish procambarus *Clarkii girard*	^{3}H-leucine	1.1 mm/day
Smith (1971a)	abdominal ganglia	cockroach *Periplaneta americana*	^{3}H and ^{14}C-labelled amino acids	1–2 mm/day possibility of faster rate of approx 40 mm/day
Chou (1970)	optic system	monkey	^{3}H-leucine	1 mm/day
Schonbach and Cuénod (1971a) Cuénod and Schonbach (1971)	optic system	pigeon	^{3}H-leucine	1 mm/day
Heslop (1971)	cerebro-visceral connective, intercerebral and cerebropedal connective	swan mussel *Anodonta cygnea*	^{14}C-valine	18.5 mm/day

TABLE 2. FAST RATES OF AXOPLASMIC TRANSPORT OF PROTEIN

Authors	System	Animal	Radioactive label	Rate
Lasek (1968a,b)	dorsal root ganglion sciatic nerve	cat rat	^{3}H-leucine ^{3}H-leucine	500 mm/day > 100 mm/day
McEwen and Grafstein (1968)	optic system	goldfish	^{3}H-leucine	40 mm/day
Elam and Agranoff (1971a)	optic system	goldfish	^{3}H-proline ^{3}H-asparagine	70–100 mm/day 20°, temperature dependent
Karlsson and Sjöstrand (1968)	optic system	rabbit	^{3}H-leucine	110–150 mm/day
Ochs *et al.* (1969)	sensory neurons of sciatic nerve	cat	^{3}H-leucine	410 mm/day
Ochs *et al.* (1968) Ochs and Johnson (1969)	motor neurons of sciatic nerve	cat	^{3}H-leucine	930 mm/day
Ochs and Ranish (1969) Ochs *et al.* (1970)	sensory and motor neurons of sciatic nerve	cat	^{3}H-leucine	400 mm/day (recalculated)
Livett *et al.* (1968a,b)	splenic nerve	cat	^{14}C-leucine	96–120 mm/day
Bray and Austin (1969)	sciatic nerve	fowl	^{14}C-leucine	250–350 mm/day
Sjöstrand (1969) (1970)	vagus and hypoglossal nerves	rabbit	^{3}H-leucine	hypoglossal 240–360 mm/day vagus 384–408 mm/day
Fernandez, Burton and Samson (1971)	abdominal ganglia	crayfish procambarus *Clarkii girard*	^{3}H-leucine	10 mm/day
Edström and Mattsson (1972)	sciatic nerve (*in vitro*)	frog	^{3}H-leucine	60–90 mm/day 17°
Chou (1970)	optic system	monkey	^{3}H-leucine	100 mm/day
Schonbach and Cuénod (1971a,b) Cuénod and Schonbach (1971)	optic system	pigeon	^{3}H-leucine	20–500 mm/day
Heslop (1971)	cerebro-visceral, intercerebral and cerebropedal connectives	swan mussel *Anodonta cygnea*	^{14}C-valine	66.5 mm/day 12–14°C
Heslop and Howes (1972)	cerebro-visceral connective	swan mussel *Anodonta cygnea*	^{3}H-tryptophan ^{14}C-valine	41 mm/day 44 mm/day 15°C

decreased with time after injection of labelled precursor (Ochs *et al.*, 1967; Ochs *et al.*, 1969; Kidwai and Ochs, 1969; Sjöstrand and Karlsson, 1969). In studies in the chicken sciatic nerve (Bray and Austin, 1969) and the goldfish optic system (McEwen and Grafstein, 1968) it has been found that the soluble proteins within the slow component were preferentially labelled whereas in the rapid flow proteins, a particulate fraction showed preferential label.

The distribution of labelled proteins in the slow component were 40% particulate and 60% soluble, whereas they were 85% and 15% respectively for the fast flow. Thus the proteins of rapid axoplasmic transport are predominantly membrane-bound and the soluble proteins constitute only a minor fraction of these proteins. Soluble proteins transported at both fast and slow rates have received attention from this laboratory (James and Austin,

1970) and by Kidwai and Ochs (1969). Kidwai and Ochs found that the soluble proteins of cat ventral roots could be separated into two fractions on Sephadex G-100 columns, the first containing soluble protein and the second polypeptide. The relative amounts of labelled activity in the two peaks showed a shift with time. Shortly after isotope injection, activity was in the second peak but after 1 day the bulk of the radioactivity was found in the soluble protein of the first peak. On Sephadex G-200 columns, the first peak could be separated into two groups, the first and largest contained proteins with molecular weights of approximately 450,000 and the second with molecular weights of 68,000. These soluble proteins were acidic with pIs in the range of 3.5–5.0. A more comprehensive study of labelled axoplasmic proteins in chicken sciatic nerve has been carried out by James (1970) and James and Austin (1970) utilizing Sephadex G-200 chromatography, isoelectric focusing and polyacrylamide gel electrophoresis. More than 90% of the labelled soluble proteins in both fast and slow transport samples were retarded on Sephadex G-200 columns and could be fractionated further by isoelectric focusing. Two prominent peaks of labelled protein were isolated from both fast and slow flow components with pI values of 4.6–4.8 and 4.9–5.1. The binding of the anti-mitotic agent, colchicine, was examined and it appeared to associate most strongly with one protein group, of pI value 4.9–5.0, which is the major ^{14}C-labelled component of slow-transport protein. Other fractions also bound colchicine but not as strongly. No major qualitative differences were apparent between the labelling patterns of the soluble proteins of the two phases of transport after polyacrylamide gel electrophoresis, except that the level of labelling in soluble proteins of the slow rate were usually 10-fold higher than soluble proteins of the fast rate.

The administration of labelled precursor by intraocular injection was first carried out by Taylor and Weiss (1965) in mouse. This technique has been used in the goldfish by Rahmann (1968a); McEwen and Grafstein (1968) and Elam and Agranoff (1971 a, b); in the monkey by Hendrickson (1969); in the pigeon by Cuénod and Schonbach (1971) and in the rabbit by Karlsson and Sjöstrand (1968). In the optic system of the adult rabbit, Karlsson and Sjöstrand (1971b, 1971f) have shown axonal transport of protein from the perikarya of the retinal ganglion cells to the nerve terminals in the lateral geniculate body occurred at four different rates: 150, 40, 6–12 and 2 mm/day, respectively. The relative amounts of radioactivity in the phases migrating at 150, 40, 6–12 and 2 mm/day were 1, 1.8, 1.5 and 8.5. The two most rapid transport systems were involved with the transport of light particulate fractions which had relatively rapid turnover rates at the nerve terminal. The intermediate rate (6–12 mm/day) was possibly associated with the transport of mitochondria and lysosomes. The slow rate (2 mm/day) carried predominantly soluble proteins down to the nerve terminals in the lateral geniculate body where they were degraded with a half-life of approximately 10 days (Sjöstrand and Karlsson, 1969; Karlsson and Sjöstrand, 1971b). Fucose containing glycoproteins were also shown to be components of the rapidly transported systems (Karlsson and Sjöstrand, 1971d). The microtubular protein subunit, which is thought to be involved in the mechanism of axoplasmic flow, was a constituent of the slow rate of flow (Karlsson and Sjöstrand, 1971c), confirming the results of James and Austin (1970). Most of the soluble proteins in the axon were transported with the slow-rate of axonal flow in this system. Some of the rapidly transported proteins were solubilized from particulate fractions with non-ionic detergents (Karlsson and Sjöstrand, 1971a) and were shown to consist of polypeptides with high molecular weights (Karlsson and Sjöstrand, 1971e). The major labelled component had a molecular weight of approximately 100,000–110,000 in the optic nerve, tract and in the microsomal fraction from the lateral geniculate

body. Most of the rapidly transported glycoproteins had molecular weights of 40,000 and higher (Karlsson and Sjöstrand, 1971d). The axons of the retinal ganglion cells bifurcate and send one branch to the lateral geniculate body and the other to the superior colliculus. In the rabbit the superior colliculus received somewhat more of the rapidly transported proteins from the retinal ganglion cells than the lateral geniculate body (Karlsson and Sjöstrand, 1972).

Similar results have been obtained in the pigeon optic system by Schonbach and Cuénod (1971a), Cuénod and Schonbach (1971), Marko *et al.* (1971) and Cuénod *et al.* (1972) in respect to flow and protein composition of flow. After sodium dodecyl sulphate solubilization of synaptosomal fractions and polyacrylamide gel electrophoresis, the subunits of the labelled proteins detected one day after injection have higher molecular weights than those proteins labelled 14 days after intraocular injection.

The movement of the brain specific protein S-100 has been followed by Miani (1971) and Miani *et al.* (1972). Although it is generally assumed that S-100 is a glial protein in the CNS (Cicero *et al.* 1970; Perez *et al.* 1970), in the peripheral nerves it seems to be primarily an axonal protein (Perez and Moore, 1968). The S-100 protein was isolated by chromatographic and electrophoretic procedures and determined by radioimmunoassay techniques. The rate of proximodistal migration was 25 and 20 mm/day in the vagus and glossopharyngeal nerves and approximately 4 mm/day in the hypoglossal nerve. The transport rates are comparable to those calculated for AChE, ChAc and protein in the vagus and hypoglossal nerves of rabbit (Frizell *et al.* 1970). Radioautographic evidence was also presented for the transynaptic transfer of S-100 protein after labelling the salivatory nucleus from the nerve endings of the otic ganglion to the nuclei of the post-synaptic neurons (Miani, 1971). Although S-100 protein has no known enzymic activity, it may play a role in the control of Ca^{++} metabolism in neural processes or be involved as a carrier of transneuronal signals.

The intra-axonal location of the transported protein in both the fast and slow rates of axoplasmic transport has been shown in the cat sciatic nerve (Ochs *et al.*, 1967; Ochs *et al.*, 1969); in the chicken sciatic nerve (Peterson *et al.*, 1968); in the rabbit optic nerve and tract (Sjöstrand and Karlsson, 1969) and in the pigeon optic nerve (Schonbach and Cuénod, 1971b) using radioautographic methods.

2.1.1 *Site of protein synthesis*

The most fruitful approach to this problem has been to use a combination of electron microscopy and radioautography after administration of radioactive amino acids. Droz and his colleagues have made extensive studies in the central nervous system (Droz *et al.*, 1968; Droz and Koenig, 1969, 1970, 1971; Droz, 1969; Droz and Barondes, 1969).

These studies showed the movement of protein from the rough endoplasmic reticulum of the neuronal cell body into the axon. They also showed a very rapid appearance of label over the nerve endings; although whether this was due to rapid flow in very short axons or to local protein synthesis remains an open question. In one series of experiments, Droz and Koenig (1969, 1970, 1971) examined neurons of the mouse spinal cord, chick ciliary ganglion and rat and leech nervous systems. As can be seen in Fig. 1, proteins making up the neurofilament and microtubule subunits were synthesized in perikaryal ribosomes and bypassed the Golgi apparatus to be added to pre-existing structures in the cytoplasm. By contrast, proteins which were involved in the formation of lysosomes, smooth endoplasmic reticulum and multivesicular bodies migrated to the Golgi apparatus, where the addition of glucosamine and galactose took place for the synthesis of glycoproteins. Most of the label, when radioactive

amino acid was used as precursor, was distributed in that part of the axoplasm rich in neurofilaments and neurotubules, though smaller amounts were also found in multivesicular bodies, smooth endoplasmic reticulum, mitochondria and in the axolemmal region (Fig. 2). Another proposal for the origin and growth of microtubules (neurotubules) has been put forward by Weiss and Mayr (1971b) who favour the idea that the tubular subunits are preformed in the nucleus.

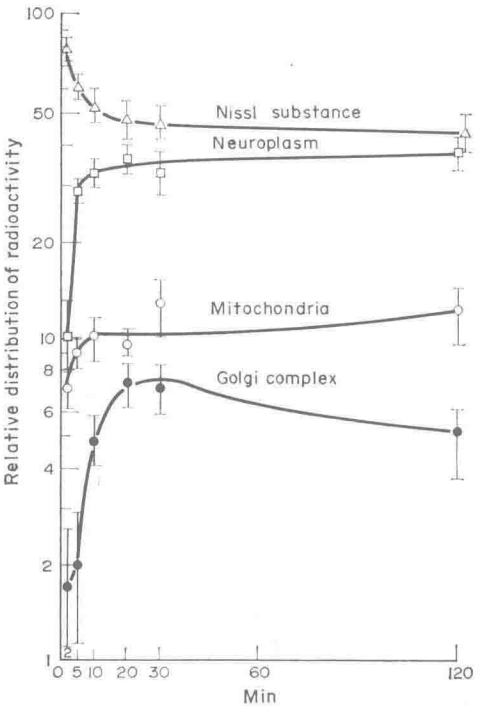

FIG. 1. Distribution of radioactivity in various cell organelles of the perikaryon of spinal ganglion cells in rats intravenously injected with ^{3}H-leucine, as estimated from electron-microscope radioautographs. The Nissl substance appears to be the first and main site of incorporation of the label, which rapidly declines, probably by transfer to other organelles. In the Golgi complex the early uptake of label is exceedingly low. A peak of radioactivity is reached in the Golgi region at 20–30 minutes, presumably by transfer from the Nissl substance. Both neuroplasm and mitochondria show a moderate incorporation very soon after injection. Then the label increases progressively in these organelles, probably by migration from the Nissl substance. (From Droz and Koenig, 1969. Reproduced from *Symposia of the International Society for Cell Biology*, Vol. 8, by permission of the copyright holder.)

In a given time the total amount of protein transported at the fast rate is greater than that transported by the slow rate even when the higher specific activity of the slow component is taken into account. This is because the differences in rates is between 10- and 100-fold whereas the difference in specific activity is only of the order of 5–6-fold. The particulate localization of the labelled protein in the fast rate of flow coupled with radioautographic studies has shown that this rate of flow is supplying components of the mitochondria, smooth endoplasmic reticulum and the synaptic vesicles by pathways located mainly at the periphery of the axon and possibly the axolemma. The slow rate of flow is now seen as

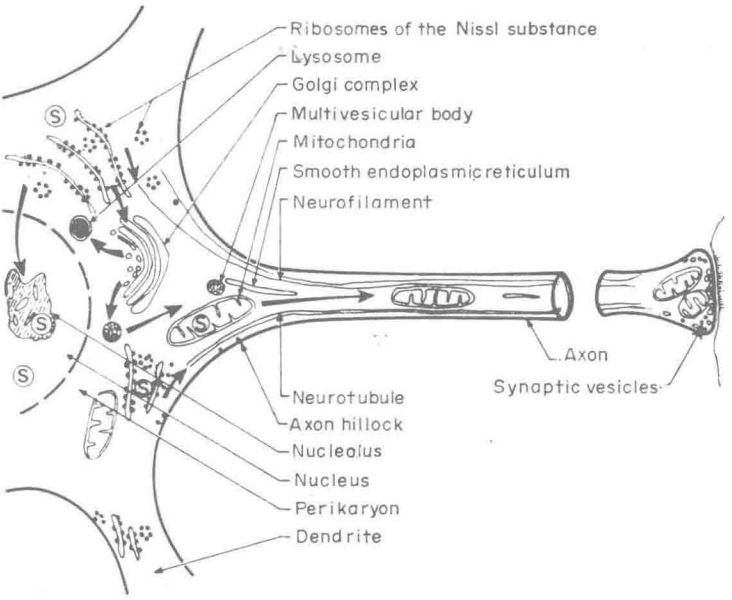

FIG. 2. Diagrammatic representation of the sites of synthesis and of the pathways of transfer of protein in the neuron. The main sites of protein synthesis (S) are the ribosomes of the perikaryon. Mitochondria also seem able to edify protein. In the nucleus both chromatin and nucleolus incorporate early amino acids into proteins. Protein elaborated in the ribosomes may follow various pathways; a first fraction would migrate to the nucleus and would account for the late wave of labeled protein appearing in this structure; a second fraction would share in the elaboration of mitochondrial components, such as enzymes. Mitochondria may be engulfed into the axon and transported toward nerve endings; a third fraction, probably the greatest one, would be responsible for the edification of the subunits making up neurofilaments and neurotubules; it is speculated that most of the newly produced protein replaces and repairs neurofilaments and neurotubules worn out by proteolytic enzymes; a fourth fraction is transferred to the Golgi complex. In the course of this migration sugars (glucosamine, galactose, etc.) may be added to a protein moiety and would give rise to glycoprotein. From the Golgi complex protein would move in the neuron associated to lysosomes, multivesicular bodies, elements of the smooth-surfaced endoplasmic reticulum, and "coated vesicles". Some of these organelles enter the axon and move along its length. Thus the axon is submitted to an incessant traffic of various types of proteins which move to nerve endings. (From Droz and Koenig, 1970. Reproduced from *Protein Metabolism of the Nervous System*, edited by A. Lajtha, by permission of the copyright holder.)

movement of the bulk of the axoplasm, where it progresses as a column involving the microtubules (neurotubules) and perhaps the neurofilaments.

While most studies on axoplasmic flow have focused on the movement of protein, recent studies have shown that a variety of cellular components are transported in a similar manner.

2.2 AMINE STORAGE VESICLES

More information is available on the movement of adrenergic vesicles than that of any other transmitter. The most commonly used technique for studying flow of adrenergic vesicles is to constrict a sympathetic nerve bundle causing an accumulation of noradrenaline

in granulated vesicles which can be visualized by fluorescence microscopy (Eränko, 1955; Falck *et al.*, 1962). Dahlström and her co-workers have used this technique to study the flow of amine storage vesicles, and have calculated their turnover in peripheral adrenergic nerves (Dahlström and Fuxe, 1964; Dahlström, 1965; Dahlström and Häggendahl, 1966; Dahlström, 1967 a, b, c; Dahlström, 1971a). Evidence for retrograde flow of noradrenaline after ligation of adrenergic nerves was initially claimed from these results, although the flow appeared very small in comparison to anterograde flow. No evidence for retrograde flow was found in intact or ligated splenic nerves of cat (Geffen *et al.*, 1969) or in cat hypogastric nerves constricted at two points (Banks *et al.*, 1969). Whereas noradrenaline and granular vesicles accumulated proximal but not distal to constrictions, mitochondria and cytochrome oxidase accumulated on both sides of constrictions, indicating a bi-directional movement of mitochondria (Banks *et al.*, 1969). Thus retrograde flow of noradrenaline possibly occurs only in ligated peripheral adrenergic nerves as a result of axonal injury, and then only over short distances.

Livett *et al.*, (1968 a, b) have shown the transport of both noradrenaline and protein in the splenic nerve following injection into the coeliac ganglion of the cat. Radioactivity of both protein and noradrenaline rapidly accumulated above a constriction of the splenic nerve. Geffen *et al.* (1971) have extended these studies and demonstrated that the accumulation of radioactivity was intra-axonal and restricted to portions dilated with organelles. The results also suggested a close relationship between intra-axonal noradrenaline and large granulated vesicles. This intra-axonal transport of noradrenaline contributes little to the rapid turnover of transmitter stores in sympathetic nerve terminals, which is primarily dependent on local synthesis and reuptake of noradrenaline (Dahlström and Häggendahl, 1966; Livett and Austin, 1968; Geffen and Rush, 1968). (For a general review of synaptic vesicles in sympathetic neurons, see Geffen and Livett, 1971.) Table 3 shows the rates of transport of various adrenergic components in several systems.

2.3 ENZYMES

The nature of protein flow in axons has been extensively studied but this tells us little about the fate of individual proteins. Different proteins can be followed if they have indentifying characteristics such as enzymic activity. Thus investigations have been carried out on the flow of various enzymes. Ideally the enzymes chosen for study should be confined only to the nerve cell and its axon and although there are none that conform entirely to this criterion, those involved in neurotransmitter biosynthesis or degradation clearly are more suitable than others which perform a more general function. Enzymes have also been used as markers for organelles such as mitochondria and catecholamine-containing vesicles.

Acetylcholinesterase (AChE) activity has been used extensively to study movement of an enzyme in the axon. There are several reasons for this. It is found in all cholinergic axons; it is easily measured and a wide range of inhibitors, particularly those which inhibit the enzyme irreversibly, are available for these studies. Much of the early work on the flow of AChE was carried out by Lubińska and her colleagues and this has been reviewed by Lubińska (1964 a, b). More recently these workers, Skangiel-Kramska, Niemierko and Lubińska (1969), have compared the distribution of this enzyme with that of phosphoglucose isomerase (PGI) in nerves with a double ligation. The latter is an enzyme located in the cytosol whereas the former occurs attached to membranes and tubules within the axon (Kasa, 1968). When a double tie is attached to a nerve, the segment between the ties is

isolated from both the cell body and nerve endings. Within this segment the AChE redistributed, accumulating at both the proximal and distal ties, with a corresponding fall in the central portion. The total enzyme activity remained constant within the segment. The increment in AChE activity was much sharper than that of PGI and continued to grow with time whereas the increase in PGI developed fully within the first few hours after transection and did not change thereafter. The rise was independent of the length of segment. In addition, the total enzyme activity increased. These differences in transport behaviour between the two enzymes suggested to the authors that particulate material may flow in both direc-

TABLE 3. AXOPLASMIC TRANSPORT RATES IN LIGATED SYMPATHETIC NERVES
Modified by permission of the copyright holder
(From Geffen and Livett, 1971)

Constituent	Nerves	Rate mm/day	Reference
Noradrenaline	cat sciatic	240	Dahlström and Häggendal (1966)
	rat sciatic	120	Dahlström and Häggendal (1966)
	rabbit sciatic	72	Dahlström and Häggendal (1967)
	guinea pig hypogastric	48	Kapeller and Mayor (1967)
	cat splenic	34	Geffen and Rush (1968)
	cat splenic (also decentral)	84	Geffen and Rush (1968)
	chicken sciatic	62.5	Jarrott and Geffen (1972)
^{14}C-noradrenaline	cat splenic	120	Livett *et al.* (1968b)
^{14}C-protein	cat splenic	120 and 1	Livett *et al.* (1968b)
Dopamine-β-hydroxylase	rat sciatic	70–100	Dairman *et al.* (1973)
	rat sciatic	40	Coyle and Wooten (1972)
	rat sciatic	110	Brimijoin (1972)
	dog splenic	72	Laduron and Belpaire (1968a)
	chicken sciatic	82	Jarrott and Geffen (1972)
Tyrosine hydroxylase	dog splenic	0	Laduron and Belpaire (1968b)
	rat sciatic	20–30	Thoenen *et al.* (1970)
	chicken sciatic	46	Jarrott and Geffen (1972)
	rat sciatic	38	Coyle and Wooten (1972)
Dopa decarboxylase	dog splenic	<24	Laduron and Belpaire (1968a)
	rat sciatic	<24	Dahlström and Jonason (1968)
	rat sciatic	36	Dairman *et al.* (1973)
Monoamine oxidase	rat sciatic	2	Dahlström *et al.* (1969)
	dog splenic	0	Laduron and Belpaire (1968a)
Cytochrome oxidase	cat hypogastric	14.4	Banks *et al.* (1969)

tions and is blocked at blind ends or ties, whereas the soluble component ebbs and flows like a tide or may be a feature of a peritraumatic reaction.

Transport of newly synthesized AChE to the nerve ending was demonstrated by Austin and James (1970). In these studies rats were treated with di*iso*propyl phosphorofluoridate (DFP), an irreversible inhibitor of AChE. Subcellular fractions, including synaptosomes, were isolated from the brain and the recovery of AChE activity was measured. Newly formed enzyme first appeared in the microsomal fraction, derived partly from the cell bodies, and there was a delay of 24 hours before it could be detected in the synaptosomes. This strongly suggested that the enzyme was synthesized in the cell body and subsequently transported to the terminals. Koenig (1967) also demonstrated a flow of newly formed

AChE but he suggested that about half of the total enzyme is synthesized in the axon. Lubińska also concluded that there are two populations of AChE, one stationary and the other which flows in a cellulifugal direction at 260 mm/day and in a cellulipetal direction at 134 mm/day (Niemierko and Lubińska, 1967; Lubińska and Niemierko, 1971; Lubińska, 1971). Kahn *et al.* (1971) found that in the cat sciatic nerve the anterograde rate of flow was 430 mm/day and the retrograde flow, 220 mm/day. Thus both groups find that the rate of retrograde flow is half that of anterograde flow. A fast rate of flow of AChE was also shown in frog sciatic nerve *in vitro* (Partlow *et al.*, 1972). Here the anterograde rate was 99 mm/day, and the retrograde rate 19 mm/day. Choline acetyltransferase moved only at a slow rate, 0.34 mm/day. This is a soluble enzyme and again reflects the different pattern of movement of enzymes in axons. AChE was also transported in the CNS at a rapid rate and evidence was presented that it was incorporated into synaptic membranes (White *et al.*, 1972). Rates of movement of acetylcholine of 1–1.5 mm/day in rabbit sciatic nerve (Evans and Saunders, 1967) and 17 mm/day in the giant nerve of the Aplysia (Koike *et al.*, 1972) have been reported. Data was also presented that the acetylcholine was membrane enclosed in vesicles.

The enzymes involved in catecholamine metabolism have also been studied for flow characteristics. Dopamine-β-hydroxylase (DBH) has been widely studied particularly as a marker for granulated vesicles which transport and store noradrenaline in sympathetic neurones. The use of DBH as a marker for this purpose could be misleading since mixed populations of vesicles have been found in various systems, some of which are rich in DBH but lacking in noradrenaline and vice versa (De Potter *et al.*, 1972; Bisby *et al.*, 1973). DBH transport has been shown using various methods. Enzymic measurement of material accumulating above a tie suggests a fast flow of the enzyme (Laduron and Belpaire, 1968a; Coyle and Wooten, 1972; Brimijoin, 1972; Jarrott and Geffen, 1972; Dairman *et al.*, 1973). These various authors found rates between 1.6 and 5 mm per hour. DBH has been shown to accumulate rapidly at a constriction by immunofluorescence (Livett *et al.*, 1969). Tyrosine hydroxylase, unlike DBH, appears to be a soluble enzyme which can associate with subcellular particles, depending on ionic strength. Theonen *et al.* (1970) concluded that this enzyme flows at a slow rate whereas others have shown that the rate is faster (Coyle and Wooten, 1972; Jarrot and Geffen, 1972). The discrepancy could occur because the first group measured the activity in a low-speed supernatant, and thus discarded much of the enzyme activity, while the latter used a total homogenate of each nerve segment. Tyrosine hydroxylase may exist in two distinct physical forms *in vivo*, a soluble and a membrane bound form, with the membrane bound or particulate form predominating in the striatum, an area enriched with catecholamine nerve endings and associated with a specific membrane fraction (Kuczenski and Mandell, 1972).

Aromatic amino acid decarboxylase, another enzyme involved in catecholamine biosynthesis, also appears to flow at a fast rate. Following nerve transection its activity fell off rapidly in distal nerve segments (Andén *et al.*, 1965) and accumulated at a rate one-third of that of DBH at the proximal side of a ligature on rat sciatic nerve (Dairman *et al.*, 1973). These discussions are summarized in Table 3.

Less attention has been paid to enzymes involved in the metabolism of other transmitters. Meek and Neff (1972) have measured transport of tryptophane 5-hydroxylase, an enzyme component in the pathway for 5-hydroxytryptamine synthesis, in the spinal cord. They measured the flow rate both after spinal cord transection and colchicine treatment and found it to be 5–7 mm per hour. In lower animals such as the toad, the accumulation of