another or to disembodied persons. They are not the kind of thing that can be localized and manipulated for such experiments.

The anesthesiologist who is interested not only in how anesthesia works, but also in why it works, rightfully examines the effects of anesthetics on organic functions. But his scientific conclusions, which I do not claim any competence to challenge, must be tempered by the realization that they are concerned with only one aspect of the person, his physical body, and that there is a two-way relationship between that person's physical states and his mental events. Neither of these aspects of the person has been proven totally determinative of the other. If we wish to conclude that thought is not free because physical energy is needed for the production of mental events, then we must also conclude that the body is determined by the mind because it is causally affected by thoughts. Rather than settle for such a self-contradictory mutual determinism, we would do better to acknowledge that neither the mind nor the body seems totally determined by the other. To conclude that thought is not free is to deny the very source of the curiosity and imagination that makes such a conclusion possible, human thought as it exists, reasons, and causes us to act.

CYNTHIA B. COHEN, PH.D.
*Chairman, Department of Philosophy*
*University of Denver*
*Denver, Colorado 80210*

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# *"Axoplasmic Transport"—The Catering and Communication System within Nerve Cells*

WHAT IS axoplasmic transport—and what significance does this phenomenon have in clinical practice? Quite simply, the intracellular transport of organelles and macromolecules that occurs in the cytoplasm of all living cells is called "axoplasmic transport" (or more properly, "intra-axonal" transport) when it occurs inside the long processes—axons—of nerve cells. As in all cells, the purpose of this transport in nerve cell processes (nerve fibers) is to convey substances that have been manufactured by the cell's synthesizing machinery to areas of the cell in need of the synthesized molecules or organelles. Also, communication between the center of the cell and its peripheral parts is provided. The special form of transport that is of interest in this context is not passive diffusion, it is rapid (several hundred mm/day), requires energy, and probably is linked to a specific organelle, the microtubule. This is a tubule 270 Å in diameter, made up of regularly arranged protein subunits—tubulin (fig. 1). Microtubules are found in all cells, and are concentrated in areas where motion or transport is most evident (*e.g.*, the flagella of protozoa, the tail of the sperm, and the processes of melanocytes). No other organelle is as circumstantially connected with transport and motion as the microtubule. (For references to articles on microtubules in biologic systems, see reference 1.)

From the structure of a nerve cell it is evident that there must be a well-developed transport system inside. The cell body contains the machinery for synthesizing macromolecules and organelles. The nerve endings (which release the transmitter substance) are located at the other end of the often very long axon (nerve fiber) (see fig. 1*B*) and do not have the capacity (or have very little capacity) to produce the macromolecules they need to function properly. Therefore,

Supported by the Swedish Medical Research Council (projects 14X-2207 and 04P-4173).

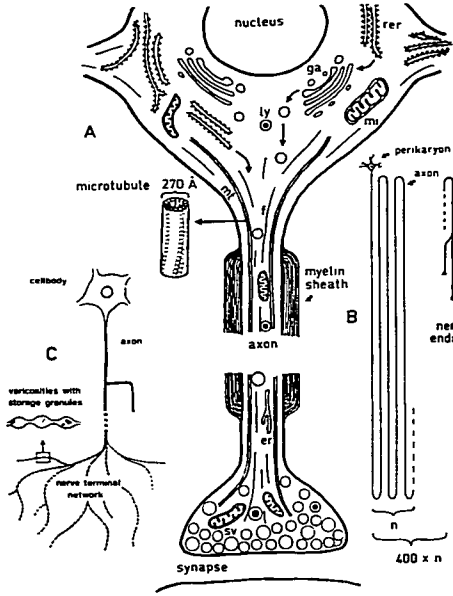


FIG. 1. *A*, very schematic drawing of a neuron. In the cell body (perikaryon, soma) the proteins are synthetized on the ribosomes of the rough endoplasmic reticulum (rer). They are then transferred to the Golgi apparatus (ga), where they are modified and packed into vesicles (see arrows on the right). The proteins may also bypass the Golgi complex to become incorporated into tubules and filaments, or be transported into the axon as soluble proteins (see arrow on the left). Mitochondria (mi) and lysosomes (ly) (which are formed by the Golgi complex) are also present. Extending down into the axon are microtubules (mi) and filaments (f). The microtubules consist of protein (tubulin) subunits, as indicated on the left.

The axons of peripheral motor and sensory fibers are surrounded by a myelin sheath, formed by the Schwann cells. In the axons mitochondria, smooth endoplasmic reticulum (er), lysosomes, and vesicles are present, in addition to microtubules and filaments. The nerve endings of efferent (motor) nerve fibers contain numerous synaptic vesicles (sv).

*B*, relative proportions of a peripheral nerve cell body (perikaryon) and its axon in man. The cell body synthetizes substances that have to travel approximately 400 times the indicated length of the axon (n) to reach the nerve endings.

*C*, peripheral adrenergic neuron. The multipolar cell body gives rise to a thin, nonmyelinated axon wrapped in Schwann cell cytoplasm. In the innervated organ the axon branches several times to form a nerve terminal network with numerous arborizations. These terminal arborizations expand to form bag-like structures ("varicosities") at regular intervals. The varicosities contain large numbers of amine storage granules that have, in all probability, been transported to the terminals via intra-axonal transport. The transmitter, norepinephrine, is considered to be released from the varicosities upon neural activity in the adrenergic neuron. The norepinephrine itself is synthesized locally in the varicosities before release.

the cell body must supply at least most of this necessary material, and an efficient transport mechanism—for carrying the material from the site of synthesis to the site of use—must be present.

The drawing on the right in figure 1(*B*) shows the relative proportions of the cell body and axon length of an ordinary peripheral neuron in man. The cell body is about 100 $\mu$ (= 0.1 mm) in diameter and must supply material to the whole length of the nerve fiber (as long as about 1 m or 1,000,000 $\mu$) plus the nerve endings. It is indeed an impressive distance that the material must travel before reaching the nerve endings. In the case of the peripheral adrenergic neuron (fig. 1*C*), the unmyelinated nerve fiber gives rise to numerous arborizations of nerve terminals with regularly occurring "bags," so-called "varicosities." All these varicosities may release the transmitter, norepinephrine, when the neuron is excited. Several thousand such "boutons en passage" are probably present on the arborizations of a single adrenergic neuron.

## Perikaryal Synthesis

Many classes of macromolecules are formed by the cell body for export into the axon, but most work so far has been done on the proteins. We now know that following the linking together of amino acids to form protein molecules in connection with ribosomes, a protein molecule may proceed in any of

at least three ways[2]: 1) it may be transferred to the Golgi apparatus, where additional molecules (*e.g.*, carbohydrates) are hooked on or where the proteins are concentrated and packed into vesicles; 2) it may pass out in the cytoplasm and incorporate into fibrils, microtubules, or mitochondria; 3) it may be carried out directly into the axon, travel distally and become incorporated into structures along the axon or be used mainly in the nerve terminals. Various types of lipids are likewise transported distally in axons, but so far we have less information about this group of macromolecules than about proteins.

## Rates of Proximo-distal Transport

When a radioactively labelled amino acid is introduced near the nerve cell body, it is taken up into the cell and incorporated into proteins. Some of these proteins are made for export into the axon. Therefore, hot proteins soon appear in the axons as a peak of radioactivity that can be traced more and more distally the longer after the isotope injection the nerve is dissected out. The rate of progress of such a peak can thus be calculated. (This method is used by Aasheim *et al.*, page 549, this issue). In most mammalian nerves the proximo-distal transport of labelled material occurs at two rates.[3,4] One peak of radioactivity travels at a rapid rate of 200–400 mm/day. In addition, a lot of proteins seem to travel very slowly—2–5 mm/day.[5] Intermediate rates have also been described. The interpretation of these findings has been that the slow transport corresponds to the slow growth of the axoplasm[6]; within the axoplasm at least one more transport mechanism which is rapid and probably connected to the microtubules is operating. This theory of two distinct transport mechanisms in the axon may be revised in the future; it is quite possible that there is only one mechanism for transport. The various rates described may depend on the time that each substance "hangs on" to the "bandwagon" during each session of transport. A substance that "jumps off" the "bandwagon" frequently and stays behind for long periods will travel at a net rate which is much slower than that of a substance that stays on continuously.

## Importance of Proximo-distal Transport in Intact Nerves

Rapid proximo-distal transport is very important to the structure and function of the nerve terminals. *Glycoproteins*, necessary for maintenance of the nerve membrane,[7] *enzymes*, necessary for synthesis of the transmitter and metabolism of terminals,[8] *transmitter storage organelles* (in the adrenergic[9,10] and possibly also the cholinergic motor[11,12] neurons), necessary for synthesis, storage and release of the transmitter,[13] and *smooth endoplasmic reticulum*,[14,15] providing both structural proteins and enzymes, are among the rapidly transported substances. If axoplasmic transport is interrupted by, *e.g.*, cutting the axon either near or far from the nerve terminals, the morphology, the chemical composition, and the ability to release the transmitter upon stimulation begin to deteriorate earlier the closer to the terminals the cut is made. If a long nerve stump is left attached, each 5–15 mm of nerve will delay the degeneration process by approximately an hour.[16] Rapid transport also appears to carry trophic substances to the innervated muscle fibers. Denervation changes of the sarcolemma are observed later with a long nerve stump than if axotomy is performed close to the muscle.[17]

## Retrograde Transport

This picture of intraneuronal dynamics, which so far has appeared rather simple and straightforward, is, however, a little more complicated. The axoplasmic flow does not only go *from* the cell body towards the nerve terminals, but also moves in the opposite (retrograde) direction. Exogenous protein molecules (*e.g.*, Evans blue–albumin or horseradish peroxidase, injected near motor nerve endings) are taken up into the nerve terminals and carried up the axon to accumulate in the perikaryon.[18] Also, virus particles injected near motor or sensory nerve endings may enter the endings and travel up the axon to the cell body.[19] This retrograde axoplasmic flow (suggested to occur also with certain endogenous, intraneuronal substances[15,20]) may constitute a communication system by which the cell body obtains information from the peripheral nerve segments and the innervation area.

## Mechanism of Transport

Rapid intra-axonal transport occurs via some mechanism that is confined to the axon itself. When a nerve segment is isolated from both perikarya and nerve endings by freezing,

cutting or ligating, the flow continues at the same rate as before cutting.[21] At the distal end of the isolated segment, the transported substances accumulate. This accumulation continues until the stores of transportable material in the segment are exhausted. The piling up of transported material, of course, occurs in the nerve attached to the cell body also, and the increase with time of the accumulation can be taken as a measure of the rate and amount of transport of a certain substance (as is done in the article by Ngai *et al.*, page 542 of this issue).

The transport is dependent upon temperature, oxidative metabolism, and the presence of ATP and glucose.[22,23] It is, however, *not* dependent upon nerve impulse conduction; in an isolated nerve segment rapid intra-axonal transport occurs, but there is *no* impulse conduction. Nor does excessive nerve stimulation seem to influence the *rate* of transport to any marked extent.[24] However, the *amounts* of transported material may increase after prolonged stimulation of a neuron.[25]

In order to test the hypothesis that microtubules are involved in axonal transport, *mitotic inhibitors* (colchicine and vinblastine) have been studied for their effects on axonal transport. Both drugs, if applied locally on nerve fibers, can block transport in a dose-dependent manner.[26–28] Morphologic studies of the ultrastructure have shown a parallel decrease in the number of axonal microtubules in peripheral nerves.[28,29] In other systems, *e.g.*, the hypothalamo-neurohypophyseal system of the rat, transport of neurohypophyseal proteins was blocked at doses which did not cause any reduction in the number of microtubules.[30] When *local anesthetic agents* have been used instead of mitotic inhibitors to arrest fast axoplasmic transport, the block of transport in some cases has been related to a loss of microtubules.[31] In other studies transport was blocked before the microtubules were morphologically influenced.[32] These observations indicate that microtubules probably participate in rapid intra-axonal transport. However, as important as their structural integrity may be the presence of some physicochemical, morphologically undetected, site on or near the tubule.

## Implications for Clinical Practice

As mentioned above, nerve impulse conduction and rapid intra-axonal transport are two separate phenomena in the neuron. The physiology of local anesthesia has been excellently reviewed by de Jong and Freund.[33] Colchicine can block intra-axonal transport before it interacts with impulse conduction.[29] Lidocaine and procaine, on the other hand, can block impulse conduction in doses which do not arrest intra-axonal transport.[34] However, as demonstrated earlier[21,22,24] and in the article by Aasheim *et al.* (this issue), local anesthetics in medium or high doses and after prolonged administration *do* block intra-axonal transport. In view of the demonstrated importance of rapid intra-axonal transport for the maintenance and function of nerve terminals, this "side-effect" of local anesthesia may be of clinical importance. Neurologic complications following regional block procedures are not rare.[35] The paresis or paralysis that sometimes occurs may in many instances be the result of the mechanical trauma of the injection procedure. However, it is also possible that block of rapid intra-axonal transport contributes to these undesired symptoms. In fact, degeneration of axons distal to the regional block may, in view of observations from animal experiments, be the result of irreversible arrest of intra-axonal transport caused by the anesthetic agent itself—without mechanical trauma. Since intra-axonal transport is essential for *regeneration* of nerves, local anesthetics should perhaps be avoided in situations where nerve repair is in progress.

A. DAHLSTRÖM, M.D., PH.D.
*Institute of Neurobiology*
*University of Göteborg*
*Göteborg, Sweden*

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