the band structure of crystals, the binding state of atoms in chemical compounds, surface chemistry and surface physics, particularly catalysis and adsorption phenomena, and elemental analysis and structural analysis of chemical substances, to mention a few. The use of monochromatized x-radiation results in improved resolution and the freedom from satellites. The reduced background improves the information content of the ESCA spectra significantly. Often, the actual shape of the ESCA line or the energy band becomes more clearly visible since it is less distorted by instrumental line shape. The signal-to-noise ratio is significantly improved and the instrumental background is purely statistical and independent of energy. Thus, the instrumental background does not introduce any structure into the ESCA spectrum which could be mistaken for actual information.

References and Notes

1. K. Siegbahn *et al., ESCA, Atomic, Molecular and Solid State Structure Studied by Means of Electron Spectroscopy* (Almqvist & Wiksells, Uppsala, 1967).
2. K. Siegbahn *et al., ESCA Applied to Free Molecules* (North-Holland, Amsterdam and London, 1970).
3. D. W. Turner, C. Baker, A. D. Baker, C. R. Brundle, *Molecular Photoelectron Spectroscopy* (Interscience, London, 1970).
4. L. G. Parratt, *Rev. Mod. Phys.* **31**, 616 (1959).
5. A. Fahlman and K. Siegbahn, *Ark. Fys.* **32**, 111 (1966).
6. E. M. Purcell, *Phys. Rev.* **54**, 818 (1938).
7. J. C. Helmer and N. H. Weichert, *Appl. Phys. Lett.* **13** (No. 8), 266 (1968).
8. Y. Baer, P. F. Hedén, J. Hedman, M. Klasson, C. Nordling, K. Siegbahn, *Phys. Scr.* **1**, 55 (1970).
9. N. V. Smith and M. M. Traum, paper presented at the International Conference on Electron Spectroscopy, Pacific Grove, Calif., 1971.
10. C. Gaehwiller and F. C. Brown, paper presented at the Conference on the Physics of Semiconductors, Cambridge, Mass., 17–22 Aug. 1970.
11. R. J. Archer, *J. Electrochem. Soc.* **104**, 619 (1957).
12. Many people have contributed significantly to the design of this instrument, among them R. Moody, H. Weaver, A. Melera, and C. Tyler.

Fast Transport of Materials in Mammalian Nerve Fibers

A fast transport mechanism for materials exists in nerve fibers, which depends on oxidative metabolism.

Sidney Ochs

Since Waller's demonstration in 1850 that nerve fibers distal to a transection undergo a typical sequence of degenerative changes, the suggestion has been advanced that materials needed to maintain the viability of axons are being continually supplied from their cell bodies (*1*). Within the last several decades evidence for a slow transport of material in nerve fibers at a rate of 1 to 10 millimeters per day has accumulated, initially on the basis of damming, and subsequently by the use of isotope tracer techniques (*2*). Only within the last several years have much faster transport rates been reported (*3*). If one considers that Wallerian degeneration makes its appearance only a few days after nerve section and that it occurs over relatively long lengths of nerve, a fast transport system would be required to provide a continuous supply of the substances postulated to keep the nerve fibers viable. However, there has been some uncertainty regarding the rate of fast transport, with reported values ranging from 50 to over 2000 mm/day (*3*). The values obtained depend in part on which technique is used for rate determination, whether it is by measurement of accumulated proteins labeled with radioisotopes, transmitter substances at a ligation or at the nerve ending, or by determination of the pattern of outflow of labeled materials in the nerve. The values also depend on whether mammalian or nonmammalian nerves are studied. In general, slower rates are found in invertebrate nerves. In any event, the variations found within a given species or even in the nerves of the same animal, have suggested to some the possibility that the rate might depend on the physiological state of the nerve or that more than one fast transport system is present in the nerve fiber.

Recently, a well-defined fast transport system was described with a rate close to 400 ± 35 (S.D.) mm/day (*4*, *5*). This was shown in cat sciatic nerve by the appearance of a crest of activity from labeled proteins passing down the axons after the amino acid precursor [^{3}H]leucine had been injected into the cell bodies of the lumbar 7th (L7) dorsal root ganglia or into the motoneurons of the L7 segment of the spinal cord. In this article the characteristic properties of this fast transport system in the mammal are described; reference to studies made in nonmammalian species can be found in recent reviews (*2*, *3*).

The fast transport system in the mammalian nerve indicated by the downward flow of a crest of radioactivity also occurs in vitro. This finding allowed further studies to be made of the nature of the underlying mechanism, and it was subsequently discovered that fast axoplasmic transport depends upon oxidative phosphorylation, with adenosine triphosphate (ATP) supplying energy to the transport mechanism locally in the axon. These results led to a "transport filament" model for fast axoplasmic transport which is described herein.

The Generality of Fast Axoplasmic Transport

Figure 1 shows the pattern of fast transport in sciatic nerves taken from cats at different intervals after injecting [^{3}H]leucine into the L7 dorsal root ganglia. The ganglia remain highly radioactive throughout, and after approximately 2½ to 3 hours, a plateau of activity appears in the nerve distal to the ganglia. More distally, the activity rises to a crest before falling abruptly to the baseline. As is described later, the

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radioactivity in the plateau and crest represents [^{3}H]leucine incorporated into protein components. As more time elapses between injection and nerve removal, the crest moves down the nerve in linear fashion. By measuring the distance from where the front of the crest intersects the baseline back to the peak of activity in the ganglion at different intervals after injection of the L7 ganglia, the rate at which the crest advances down the nerves was found to be around 400 to 410 mm/day (*4, 5*). A similar fast rate of axoplasmic transport occurs in the sciatic nerves of various other mammalian species such as the rabbit, monkey, dog, and rat, all of which show similar crests and transport rates of about 410 mm/day (*6*). Even in the goat, where the sciatic nerve is much longer, and the downflow of incorporated radioactive materials takes longer, there is a typical crest and a similar rate of fast transport after 22 hours.

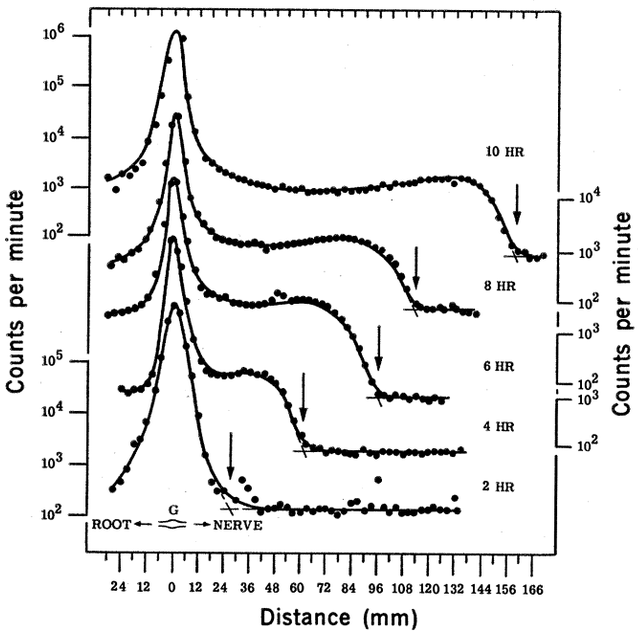
The cells of the dorsal root ganglia form two branches, with one fiber ascending into the dorsal column of the spinal cord (with a collateral to subnerve local reflex mechanism) and the other descending into the sciatic nerve. After injecting the L7 dorsal root ganglia with [^{3}H]leucine, a crest of radioactivity occurs in the dorsal columns at the same position as the crest in the sciatic nerves (*6*). While this represents only one fiber tract of the central nervous system (CNS) showing fast transport, it suggests that CNS fibers are not likely to differ in this respect from peripheral nerve fibers.

The same fast rate occurs in both motor and sensory fibers. This was first shown in the cat by injecting [^{3}H]leucine into the spinal cord near the motoneuron cell bodies of the L7 segment and finding the crest in the sciatic nerve at the distance that would have been expected for this rate of fast transport had the [^{3}H]leucine been injected into the L7 ganglion (Fig. 2). More recently, this was also shown to hold for the monkey in which the L7 dorsal roots are much longer and there is a greater displacement of the ganglia and motoneuron cell bodies (*6*).

The rate of fast transport is also independent of the diameter of the nerve fiber. After injection of [^{3}H]leucine followed by a period of downflow, nerve sections were removed at the calculated position of the crest and prepared by freeze-substitution for histological preparation (*7*) with cross sections cut and coated for radioautography. Grains indicating the presence of radioactivity were seen over the myelinated fibers, ranging in diameter from 23 micrometers down to approximately 3 to 4 μm, thus showing a downflow of labeled proteins at the same fast rate independent of the diameter of the axon (*6*). A similar rate of transport may also occur in nonmyelinated fibers: a fast transport system has been reported for the vagus nerve (*8*) which contains a high proportion of nonmyelinated fibers. There appears also to be a fast transport of material from the cell bodies into dendrites. This was shown by iontophoresis of [^{3}H]glycine into the cell bodies of the motoneurons in the spinal cord, killing the animals at different times thereafter and preparing the cord for radioautography. By this means a rapid spread of labeled materials into the dendrites of the injected cells was demonstrated (*9*).

Somal Synthesis Differentiated from Axonal Transport

When an amino acid precursor such as [^{3}H]leucine enters the body of a nerve cell, it becomes synthesized into a variety of protein components. Differential centrifugation of the sciatic nerves into which the labeled materials had been carried by fast transport, showed that there was a high proportion of activity in the small particulate fraction and a somewhat smaller amount in the proteins and polypeptides of the high speed supernatant (*10*). The soluble proteins in the high speed



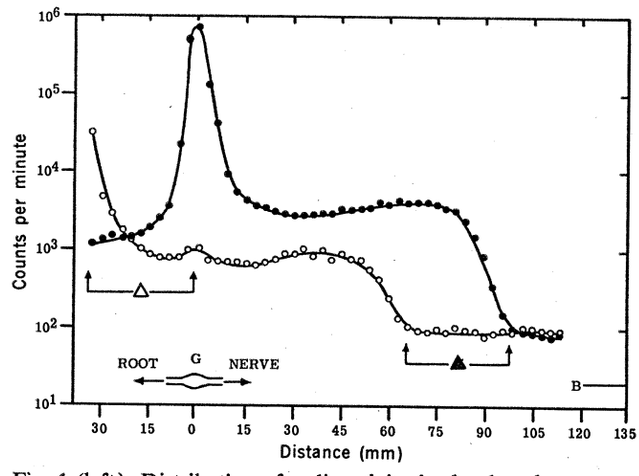


Fig. 1 (left). Distribution of radioactivity in the dorsal root ganglia and sciatic nerves of five cats taken between 2 to 10 hours after injecting [^{3}H]leucine into the L7 ganglia (*G*). The activity present in 5-mm segment of roots, ganglia, and nerves (abscissa) is given on the ordinate in logarithmic divisions. The ordinate scale for the nerve 2 hours after injection is given at the bottom left with divisions in counts per minute. At the top left a scale is given for the nerve taken 10 hours after injection. Only partial scales are shown at the right for the nerves taken 4, 6, and 8 hours after injection. Fig. 2 (right). Similarity of rate of fast transport in sensory and motor fibers shown by injection of [^{3}H]leucine into the L7 segment of the spinal cord near the motoneurons on the left side (○) and into the L7 dorsal root ganglion on the right (●). Nerves removed for sampling 6 hours later. The anteroposterior displacement (▲) of the fronts of the crests are comparable to the anatomical anteroposterior displacement of the L7 spinal cord motoneurons and L7 ganglion cell bodies (△). [From Ochs and Ranish (*5*)]

supernatant were separated by Sephadex gel filtration as: peak Ia proteins with a molecular weight of 480,000; peak Ib proteins with molecular weights from 60,000 to 100,000; and peak II polypeptides with molecular weights ranging from 4,000 to 10,000 (Fig. 3). These peaks could generally be matched with the proteins of high and low molecular weight similarly isolated from chicken peripheral nerves by Austin and his colleagues (*11*). The proteins of higher molecular weight were further characterized by peaks of radioactivity with isoelectric points (*p*I's) of approximately 4 to 5 (*10, 11*).

The radioactivity in the small particulate fraction represents a heterogenous class of materials. Some may include transmitter substances, as had been shown for the catecholamines transported in autonomic nerves (*12*). Others may be transmitter-related substances such as dopamine β-hydroxylase in adrenergic fibers, an enzyme involved in synthesis of the adrenergic transmitter in the terminals (*13*). In motor fibers, acetylcholinesterase (AChE) is present in the small particulate fraction and this enzyme has recently been reported to move down the nerve at the rate of 260 mm/day (*14*). In a similar study in this laboratory, the rate of transport of AChE was found to be 450 mm/day with a 95 percent confidence level of 380 to 578 mm/day (*15*). This range suggests that AChE is being transported down the fibers in the small particulate fraction by the fast transport system.

Some of the materials carried down the nerve fibers by the fast transport system, if not transmitter or transmitter-related substances in themselves, appear to be materials needed to maintain synaptic transmission at the motor nerve terminals. This was shown for the rat diaphragm by the failure of miniature end plate potentials (MEPP's) several hours after the phrenic nerve supply was cut (*16*). The failure of MEPP's occurred sooner when the nerves were cut closer to the muscle than when cut higher up from the muscle. The distance between the cuts and the difference in time between the onset of failure in the two cases was used to estimate a rate of 360 mm/day for the movement in the nerve of a material required to maintain MEPP activity; a rate reasonably close to the characteristic rate of 410 mm/day found for fast transport with [^{3}H]leucine injection.

The incorporation of amino acids into proteins in the cell bodies takes place rapidly. If either of the protein-synthesis blocking agents, puromycin or cycloheximide, are injected before the labeled amino acid precursor [^{3}H]leucine, there is a marked block in the synthesis and downflow of labeled proteins (*10, 17*). When these blocking agents are injected 10 to 30 minutes after the amino acid, little if any inhibition of incorporation occurs (Fig. 4). Even if the blocking agents are injected 5 minutes afterwards, only a partial degree of inhibition occurs. The results indicate that protein synthesis is probably completed in the neuronal cell bodies within 10 to 20 minutes after the entry of the precursor (*17*). Part of the rapidly synthesized protein is soon thereafter carried down the axon by the fast transport system. The bulk of the synthesized protein enters a "compartment" in the cell body and is then later removed from it to be carried down the nerve fibers by the slow axoplasmic transport system (Fig. 5).

Instead of depending on simple diffusion, the outflow of labeled materials from the cell bodies into the axons appears to be controlled by a "gate" which could be in or part of the Golgi apparatus. Using radioautography and electron micrography, Droz (*18*) has shown that soon after intravenous administration of [^{3}H]leucine, labeled proteins appear over the endoplasmic reticulum of the cell body; they appear later over the Golgi apparatus and still later over the axons. The control of outflow by a gate was also suggested by the finding that some materials are transported by the slow and others by the fast axoplasmic transport system (*17*). For example, materials that have incorporated [^{32}P]-orthophosphate, mainly as lipoproteins, are carried down the nerve fibers by the slow transport system while little if any are carried by the fast transport system (*19*). The slow system also carries proteins to which colchicine binds

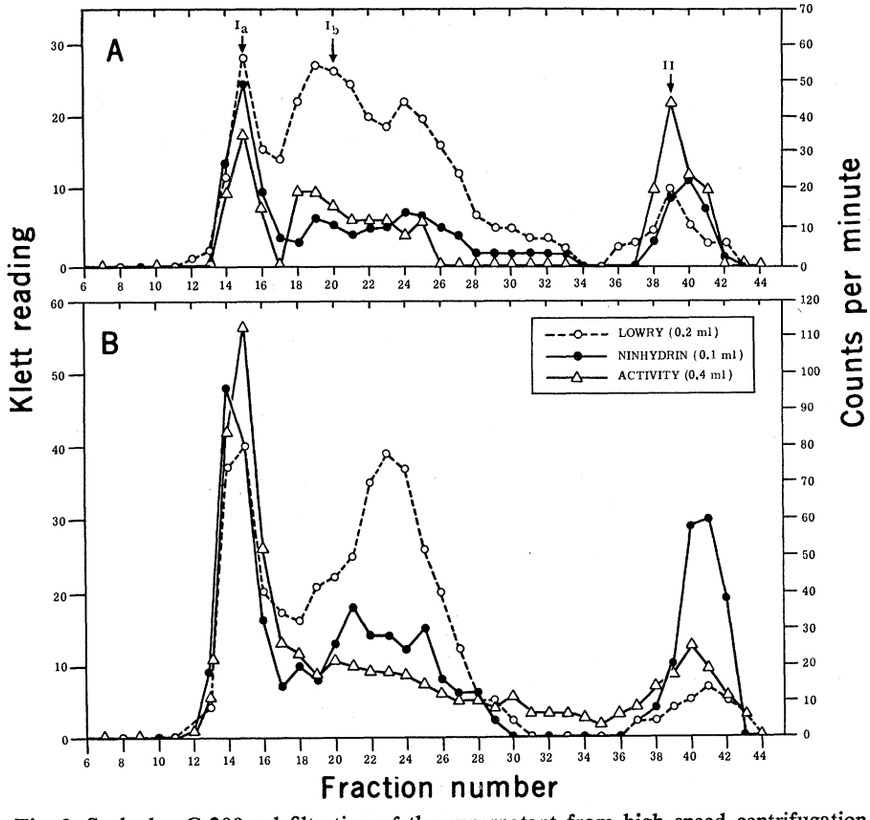


Fig. 3. Sephadex G-200 gel filtration of the supernatant from high speed centrifugation (105,000*g* for 60 minutes) of nerve homogenates taken from animals 6 hours (A) (fast transport) and 21 hours (B) (slow transport) after injection of the L7 dorsal root ganglia with [^{3}H]leucine. Peaks I_a and I_b represent higher molecular weight proteins; peak II represents polypeptides found in the eluate fractions. Ordinate given on left indicates protein, as determined by the method of Lowry (*60*) (○) and ninhydrin as determined by the method of Moore and Stein (*61*) (●), in Klett units with 100 units equal to 100 μg of bovine serum albumin (*LOWRY*) and 10 μg of L-leucine (*NINHYDRIN*). Right ordinate scale gives radioactivity (△) present in peaks in counts per minute. [From Ochs, Sabri, and Ranish (*17*)]

(*20*). A substance not normally incorporated, [^{3}H]cycloleucine, does not enter the nerve fibers when taken up by the cell bodies (*10*).

In addition to proteins, polysaccharides are carried down the nerve fibers by fast transport. This was first shown in nonmammalian species by using [^{3}H]-glucosamine, [^{3}H]fucose, or $^{35}SO_4$ as precursors, and in some studies by extracting labeled materials from the nerve which conformed to glycoprotein (*21*). In the cat, after [^{3}H]glucosamine was injected into the L7 dorsal root ganglia, a crest of activity was found to move down the sciatic nerves at 400 mm/day (*22*). The isolation and study of these materials and other unknown substances carried down the nerve fibers by the fast and slow axoplasmic transport systems provide an exciting challenge in neurobiology.

Once the materials gain entry to the axon, their subsequent fast transport down the fiber is independent of any motive force exerted by the cell body. This was shown by injecting [^{3}H]leucine into the L7 dorsal root ganglia, allowing a period of downflow into the fibers and then at various intervals destroying the L7 dorsal root ganglion on one side or making a ligation just distal to it to prevent further passage of labeled materials into the nerve fibers. The labeled materials which gained entry to the axons continued to move down the fibers at the usual fast rate of transport (*4, 5*). The materials entering the axons in the first hour after injection took up the most advanced position in the crest (Fig. 6). When the ligation was made after longer periods of downflow, additional labeled materials filled out the remainder of the crest as well as the plateau of activity behind the crest. The simile that comes to mind is of a conveyor belt with the labeled materials which first gain entrance to the transport system in the axon moving on ahead of the materials exiting later. A local mechanism of transport in the axon was earlier inferred from studies of double ligation in which enzymes or catecholamines accumulated at the distal ligation in the isolated nerve segment (*12, 14*). The studies of crest formation show that ligation of the nerve does not noticeably change the rate of transport in the axons; the results also remove the possibility that accumulations of enzymes are the result of damage, as might be suggested by the morphological changes found near the ligations (*23*).

Dependence of Fast Transport on Oxidative Metabolism

The close dependence of fast axoplasmic transport on oxidative metabolism was shown by transport experiments in vitro (*24*). The L7 ganglia were injected with [^{3}H]leucine as usual and a downflow of labeled materials into the nerve was allowed to take place for several hours before the animals were killed. The nerves were then removed, placed in chambers filled with 95 percent oxygen and 5 percent carbon dioxide, kept moist with a small amount of Ringer solution and maintained at a temperature of 38°C. Under such conditions the usual crest pattern moved down the fibers at a rate characteristic of fast transport (Fig. 7).

The addition of metabolic blocking agents such as sodium cyanide, dinitrophenol or azide to the nerve in vitro produced as rapid a block of fast axoplasmic transport as did an atmosphere of nitrogen (*24*). Dinitrophenol was of particular interest in this respect insofar as it uncouples phosphorylation; its effect on fast axoplasmic transport was to block it rapidly, thus indicating that the underlying mechanism responsible for transport requires a continued supply of ATP.

More direct evidence for the hypothesis that ATP is required to maintain fast axoplasmic transport comes from a determination of the amounts of ATP and creatine phosphate (CP) present in nerve. After a period of anoxic block, the amounts of ATP and CP decrease while there is a corresponding increase in the amount of inorganic phosphorus (*25*). The combined molar amount of ATP and CP falls to about half the control amount after 15 minutes of anoxia, at a time when fast transport is blocked. The remaining amount of ATP and CP is apparently unavailable to the fast transport mechanism; it is probably confined to some compartment less susceptible to anoxia.

The rapid block of fast axoplasmic

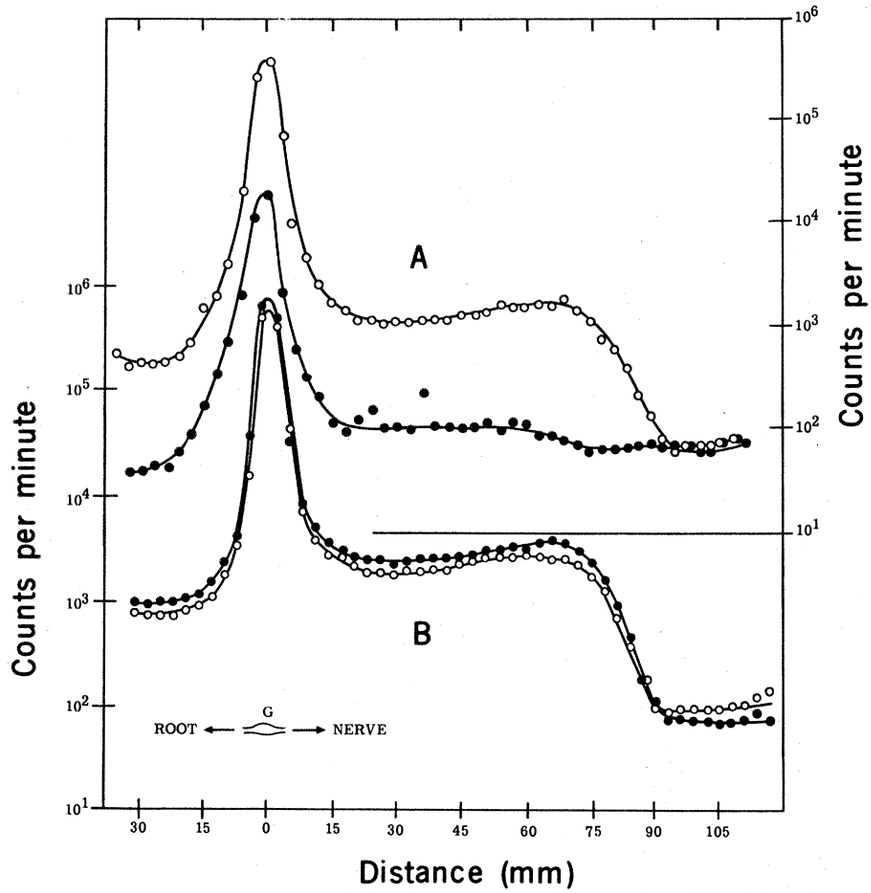


Fig. 4. (A) Block of protein synthesis shown with cycloheximide (17.8 m*M*) injected into the L7 dorsal root ganglion (●) on one side and Ringer solution into the right ganglion (○) 20 minutes before injection of [H^3]leucine into each ganglion. (B) Lack of effect with cycloheximide (17.8 m*M*) injected into the right L7 dorsal root ganglion (○) and Ringer solution into the left (●) 30 minutes after [^{3}H]leucine was injected. [From Ochs, Sabri, and Ranish (*17*)]

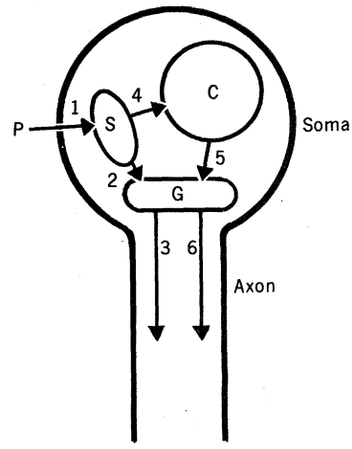


Fig. 5. Diagrammatic representation of uptake of amino acid, intrasomal synthesis, translocation, and axoplasmic transport. Precursor (P) entering the soma (1) is rapidly synthesized (S) into labeled components and then transferred (2) to a gate (G) for passage down the axon by the fast transport system (3). Another portion of synthesized materials is intrasomally translocated (4) to a compartment (C) from which materials are later released, mainly as higher molecular weight proteins (5) to be passed down the axon by the slow transport system (6). [From Ochs, Sabri, and Ranish (*17*)]

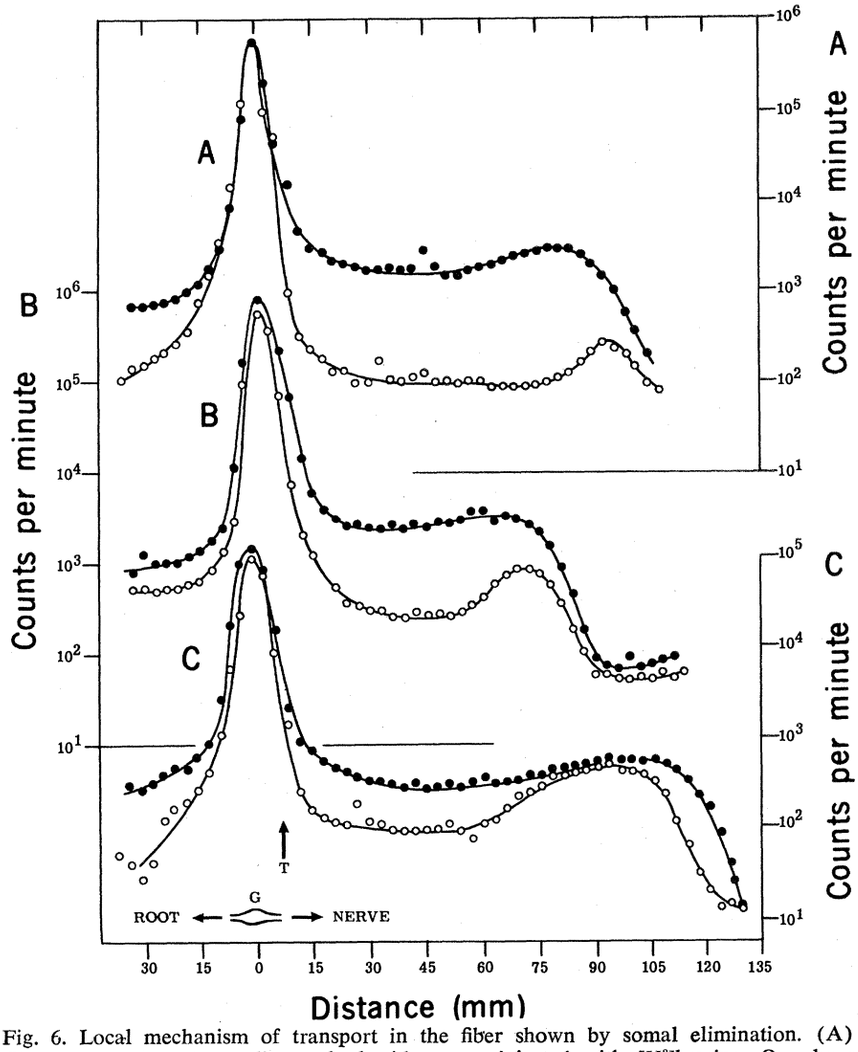


Fig. 6. Local mechanism of transport in the fiber shown by somal elimination. (A) The L7 dorsal root ganglia on both sides were injected with [H^3]leucine. One hour later the nerve on one side (○) was ligated just distal to the ganglion (*T*). After 6 hours of downflow the control (●) shows the crest typical of fast axoplasmic transport, the ligated nerve shows a smaller peak at the front of the crest position. (B) Two hours after ganglion injection the nerve on one side (○) was ligated just below the ganglion. Nerves removed 6 hours after injection show a larger peak again at the position of the crest in the control nerve (●). (C) Nerve ligation made just distal to the L7 dorsal root ganglion 3 hours after its injection. This nerve (○) shows a peak almost equal in size to the crest in the control nerve (●). [From Ochs and Ranish (*5*)]

transport that occurs during a block of oxidative metabolism is presumptive evidence that glycolysis cannot supply sufficient ATP to maintain transport. A study was made of the effect of blocking glycolysis with iodoacetic acid (IAA). When cat sciatic nerves were exposed to IAA in concentrations of 2 to 10 m*M*, sufficient IAA entered the fibers to completely block the enzyme phosphoglyceraldehyde-3-dehydrogenase within 5 to 10 minutes (*26*). The block of glycolysis so produced caused a characteristic decrement of fast axoplasmic transport which led to failure in about 1½ to 2 hours (Fig. 8).

The gradual decrement of transport caused by blocking glycolysis is in contrast to the abrupt cessation which occurs within 15 minutes of blocking oxidation and is considered to be caused by the utilization of endogenous metabolites, acetylcoenzyme A and α-ketoglutarate entering the tricarboxylic acid cycle below the site of glycolysis block (*27*). When these endogenous metabolites are eventually exhausted, the amounts of ATP and CP were reduced below the level necessary to maintain fast axoplasmic transport. To test this point, ATP and CP were measured at different times after exposure of nerves to IAA (*25*). The level of ATP and CP decreased to about half that present in controls in 1½ to 2 hours, the time at which fast transport failed. This represented the same decrease in ATP and CP which occurred after 15 minutes of anoxia and when fast transport failed. These results support the concept that ATP is required by the fast transport mechanism.

Relation of Nerve Membrane Activity to Fast Transport

The action potentials of mammalian nerves were reported to fail about 10 to 30 minutes after initiation of anoxia with nitrogen; in our studies the failure occurred in 10 minutes or less (*28*). The failure of both the action potentials and the fast axoplasmic transport at similar times after the initiation of anoxia suggests a common supply of ATP to the mechanisms underlying these two functions. In the giant axon of the squid a rapid cessation of the sodium pump was found when oxidative phosphorylation was blocked with azide, sodium cyanide, or dinitrophenol (*29*). However, the intra-axonal increase in sodium

ions and decrease in potassium ions subsequent to the block of the sodium pump take place slowly in the giant axon so that even 90 minutes after the initiation of anoxia, resting membrane potentials and action potentials remain fairly close to that of control axons. A much more rapid change in ionic concentrations would be expected in the smaller sized mammalian nerve fibers and this was indicated by the rapid depolarization found by Wright after initiation of anoxia (*28*).

A common pool of ATP supplying energy to the sodium pump and to the mechanism underlying fast axoplasmic transport suggests the possibility that a change of membrane activity and ATP utilization might in turn affect the rate of fast axoplasmic transport. However, this does not seem to be the case for the most part. Jankowska *et al.* (*30*) reported no effect of repetitive stimulation on the accumulation of AChE at a ligation and thus on the rate of transport. In studies in our laboratory, repetitive stimulation at 100 pulses per second was applied to 3- or 4-hour periods of downflow in vitro and a small, 10 percent, reduction in the rate of fast axoplasmic transport was found (*31*). Conversely, a block of membrane excitability produced by procaine or by tetrodotoxin had no effect on the rate of fast transport (*24*). In experiments now in progress, fast axoplasmic transport has been shown to be insensitive to radically changed ionic concentrations including low concentrations of sodium ions, depolarization with 140 m*M* potassium or wide *p*H changes from 6 to 9.

Blocking the sodium pump with ouabain could theoretically reduce the utilization of ATP by the sodium pump thereby making more ATP available to the transport mechanism and thus increasing the rate of transport. However, ouabain causes a decrease in the rate of fast transport. We are now investigating the possibility that there may be less obvious kinds of interaction between membrane processes and fast transport mechanisms other than a common supply of ATP.

Local Anoxia and Block of Transport

The localization of energy supply to the fast transport mechanism was studied by making a small region of the nerve anoxic just below the advancing crest of activity (*32*). This was done by covering the nerve with strips of plastic coated with petrolatum to prevent oxygen from diffusing into that region during a period of downflow in vitro. As a result, a sharp peak of activity became dammed up at the forward edge of such regions with just distally inside the covered region a sharp fall of activity to the baseline (Fig. 9). This pattern of damming is similar to that seen in ligated nerves or in nerves which have been frozen locally to seal off the fibers. However, the block produced by local anoxia was reversible and if the covering over the nerve was removed after periods up to approximately 1 hour, fast axoplasmic transport resumed its usual pattern of downflow (*32*).

The steep fall of activity just inside the local region of anoxia shows that oxygen, or the ATP produced by metabolism in the oxygenated region, does not diffuse very far into the anoxic portion of nerve. Furthermore, it indicates that ATP is supplied to the fast transport mechanism in a highly localized fashion all along the length of the nerve fiber (*32*). With no supply of energy to the transport mechanism, the labeled materials present in the nerve do not diffuse to any significant extent. Such findings are not in accord with some mechanisms invoked for transport, for example, a peristalsis of the nerve which squeezes materials along the inside of the fiber. Such a force would result in a facilitated-diffusion type of distribution shown by an exponentially declin-

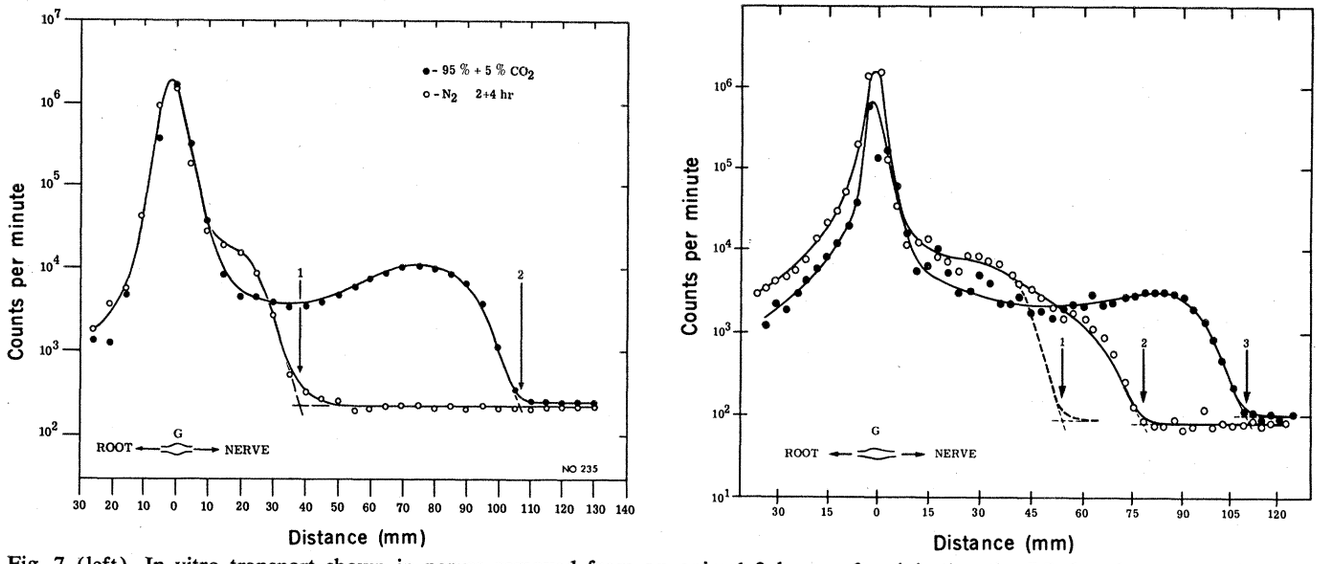


Fig. 7 (left). In vitro transport shown in nerves removed from an animal 2 hours after injecting the L7 dorsal root ganglia with [^{3}H]leucine. One nerve (●) was placed in a chamber containing 95 percent oxygen and 5 percent carbon dioxide for an additional 4 hours, kept moist with Ringer-lactate solution at 38°C. The rate of transport in vitro was that expected of fast transport in the animal (arrow 2). The other nerve (○) was similarly treated except that it was exposed to nitrogen while in the chamber for 4 hours; the crest advanced no further than that resulting from the 2 hours of downflow (arrow 1) which had taken place in the animal. Fig. 8 (right). Effect of IAA on transport in vitro shown in nerves removed 3 hours after injecting the L7 dorsal root ganglion with [^{3}H]leucine. One nerve was placed in a chamber with 4 m*M* IAA for 3 hours (○) and arrow 2 shows a diminished downflow compared to control nerve (●) without IAA present (arrow 3). The dashed line and arrow 1 indicate the pattern and extent of downflow expected for the 3-hour downflow which had taken place in the animal. [From Ochs and Smith (*27*)]

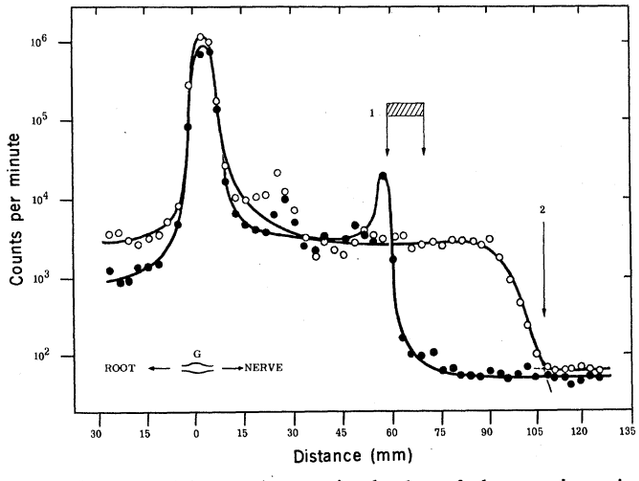
ing front entering the anoxic region and changing with time (see *19*), rather than the sharp and maintained drop of activity found.

"Transport Filament" Hypothesis

The linearly organized microtubules (neurotubules) present in axons have often been implicated in theories to explain axoplasmic transport (*33*). In one such theory Schmitt (*34*) considered a special matching of bonding sites along the microtubules to those present on the surface of vesicles which are assumed to be the only fast transported component. The bonds between them are thought to be made and broken as the vesicles roll down the microtubule, thus accounting for their transport. However, as noted previously, a varied complement of materials is transported down the axons and all at the same fast rate close to 410 mm/day. This led to another hypothesis for fast axoplasmic transport, one based closely on the sliding filament theory of striated muscle (*6, 32, 35, 36*). In this hypothesis the microtubules and the neurofilaments, or both, comprise the stationary member of the sliding filament pair, with a "transport filament" synthesized in the somas and entering the axons to become the moving member of the pair of sliding filaments. To this transport filament are bound somally synthesized small particulate substances, soluble proteins, and polypeptides as well as some free amino acids, all of which are carried down the axon on this common carrier at the same fast rate (Fig. 10). The cross bridges shown between the transport filament and the microtubules and neurofilaments in Fig. 10 are presumed to perform a similar cycle of ratchet propulsions as has been proposed for the sliding filaments of muscle (*37*). Adenosine triphosphate is required to supply energy to the meromyosin heads interacting with actin responsible for cross bridge activation and in turn the contraction of striated muscle. Our evidence showing a dependence of fast axoplasmic transport on ATP supply in the nerve is in accord with this key requirement of the theory. A temperature dependence with a Q_{10} of 2.5 has been found for the maximum rate of rise of tension in a muscle twitch (*38*), and it is of interest that a similar Q_{10} of 2 to 2.3 was found for fast axoplasmic transport (*31*) and for transport in the fish retino-tectal system (*39*). In our laboratory, the amounts of ATP and CP were found to be unchanged in cat sciatic nerves kept at low temperatures, thus suggesting that the effect of the lower temperature in blocking fast transport is to reduce the utilization of ATP.

Calcium is also required for actomyosin activity and muscle contraction. It appears possible that calcium ions are also required to activate the transport system in the nerves. Oxalate was recently found to block fast axoplasmic transport (*40*), presumably by entering the axons and binding the calcium ions, thus reducing the amount of the free calcium ions below that required for adenosine triphosphatase activity and fast transport. An entry of oxalate was indicated by the reduction in the amounts of ATP and CP found in the oxalate-treated nerves, although not down to the amounts associated with a block of fast axoplasmic transport. Further study is in progress to determine if the block of fast transport by oxalate can be ascribed to a reduction of free calcium ions in the axon or to a nonspecific action on metabolism.

Actomyosin is an adenosine triphosphatase, and it is significant that, while approximately one fourth of the adenosine triphosphatase present in giant nerve fibers was ascribed to Na^+, K^+–activated adenosine triphosphatase in the membrane, the larger part of the adenosine triphosphatase present was in the axoplasm (*41*). In experiments now in progress, a Ca^{2+}, Mg^{2+}–activated adenosine triphosphatase has been found in the cat sciatic nerve (*42*). Actomyosin-like proteins have been isolated from the brain (*43*), and a Ca^{2+}, Mg^{2+}–activated adenosine triphosphatase was reported in the microsomal fraction of crab nerve, though with another function ascribed to it (*44*). A variety of cells showing motility (streaming, cyclosis, and amoeboid movement) are found to contain actomyosin and to be sensitive to the action of ATP (*45*). Recently, it was shown that actin isolated from slime mold or amoeba can



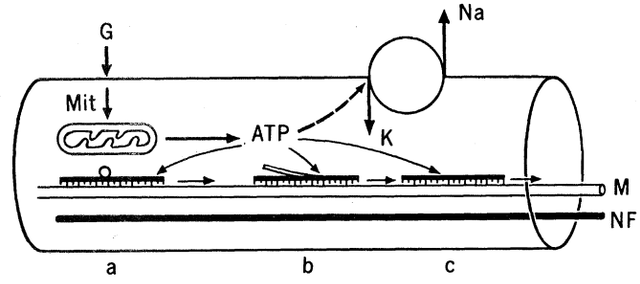


Fig. 9 (left). Local anoxic block of downflow shown after injection of the L7 dorsal root ganglion with [^{3}H]leucine. The nerve from one side (●) was removed after 2 hours of downflow in the animal and placed in a chamber containing 95 percent oxygen and 5 percent carbon dioxide for 3 hours of downflow in vitro. A short length of the nerve (arrow 1 and hatched bar) was covered with petrolatum. Damming occurred with the peak of radioactivity at the proximal edge of the anoxic region with a fall to the baseline a short distance inside the covered region. The control nerve [(○) and arrow 2] shows the usual fast transport in vitro. [From Ochs (*32*)] Fig. 10 (right). Transport filament hypothesis of fast axoplasmic transport. Glucose (G) enters the fiber and after glycolysis and oxidation in the mitochondrion (*mit*), the resulting ATP supplies the sodium pump (dashed arrow) controlling Na^+ and K^+ in the fiber. The ATP is also shown supplying energy to the "transport filament" which is indicated by a black bar to which particulates (*a*), soluble protein (*b*), and other undesignated substances (*c*) are bound; and in this way the various species are carried down the axon at the same fast rate. The cross bridges shown between the transporting filament and the microtubules or neurofilaments (or both) effect this downward movement and require ATP as a source of energy in analogy to cross bridge action in muscle. [From Ochs (36)]

combine with mammalian muscle myosin (*46*) and, in electron micrographs, these combinations show the usual arrowhead configuration. This suggests that the actomyosin from these lower forms has properties similar to those in the mammal and has evolved early in phylogeny as the means for mechanochemical transduction (*47*). This same actomyosin system could have been retained in the neuron early during evolution as the mechanism for material transport in its long fibrous extensions.

More information is required regarding the properties of microtubules and neurofilaments. Recently, these components were separately extracted from squid axoplasm and shown to have different protein compositions (*48*). Vinblastine binds microtubular protein, and microtubules isolated from HeLa cells with this agent were shown to have adenosine triphosphatase activity (*49*). Colchicine, the prototype of such microtubular binding agents, disrupts dividing cells, by causing a disassembly of microtubules into the dimeric subunits of colchicine; it has also been shown that colchicine, vinblastine, and vincristine all block axoplasmic transport (*50*). However, more recent evidence obtained by electron microscopy has indicated that the block of transport occurs by some other means than disassembly (*51*), possibly by binding to glycoproteins coating the microtubules (*52*). On the basis of the transport filament hypothesis we may consider several sites vulnerable to the action of such agents: the microtubules, the transport filament, and the cross bridges. The cross bridges with their related enzymes would be a most likely site of action. So far, however, projections extending laterally from the microtubules analogous to cross bridges have been seen only in lamprey nerve (*53*). A further study of the fine structure of the axon with various staining methods is required to test the reality of this aspect of the hypothesis.

Note should be made of the presence of a reverse or distal-proximal transport. Lubińska and Niemierko (*14*) reported a transport of AChE in the distal-proximal direction at a rate half that of the usual downward flow. In our studies of AChE transport where a proximo-distal rate closer to 410 mm/day was determined, a distal-proximal rate was also found and shown to be about half the downward flow—that is, 230 mm/day (*15*). This slower upward transport could, on the basis of the present hypothesis, be due to a different composition or number of cross-bridges on an upward moving transport filament. The ascending transport filament could originate in the nerve terminals, as suggested by evidence for a protein synthesis in the nerve terminal synaptosomes isolated from brain (*54*). The loss of an ascending substance may be the "signal" for the chromalysis seen in the somas of neurons after transection of their axons (*55*).

Conclusions

The transport filament hypothesis accounts for the heterogeneity of the materials, including small particulate substances, proteins, polypeptides, as well as some free leucine, which all move down the nerve fibers at the same rate thus implying a common carrier. The transport filament hypothesis, while it fulfills this requirement, is heuristic and the experimental attack designed to uncover its molecular mechanism is based on analogy to the sliding filament theory of muscle. Some of the expectations derived from the sliding filament theory have held for axoplasmic transport in nerve. The finding that transport in nerve depends on a supply of energy through ATP, while it might have been expected on general thermodynamic considerations, supports the hypothesis. The preliminary studies showing the presence of adenosine triphosphatase in mammalian nerve and a dependence of transport on calcium ions is in the direction of further support for the hypothesis. However, much remains unknown. There is a great gap between the information available on cross-bridges in striated muscle and the paucity of information concerning this structural element in the nerve. A somewhat similar difficulty occurs with respect to smooth muscle which does not show such cross bridges, although it is known to contain actomyosin as thick and thin filaments (*56*). It may be hoped that more studies of the fine structure of nerve by means of varied preparative techniques will be forthcoming. Additional information is also required concerning the chemical properties of these organelles.

In any event, the reality of a regular system of fast transport of materials in the nerve fibers offers a base for further advance. It also raises a number of related questions. What happens to those transported substances not used up along the way when they reach the nerve terminals? As noted, some supply of materials in the nerve is required to maintain synaptic transmission. There is also the little understood group of "trophic" substances carried from the nerve into the postsynaptic cell. In the case of motor nerves, such trophic materials act to control the spatial extent of receptors in the muscle membranes, the speed of contraction and, as well, the general metabolism of the muscle fiber (*57*). In the case of sensory nerve fibers, the trophic materials leaving the axon terminals act to control the structural integrity and function of the secondary cells of sensory organs (*58*). In the central nervous system such a trophic supply of materials could very well play an important role in the integration of neurons and behavior (*59*). Whatever form our understanding of the mechanism of fast axoplasmic transport eventually takes, there seems little doubt that further study of this protean system of intracellular supply will lead to new insights in our conception of neuronal function and its alteration in disease states.

References and Notes

1. A. V. Waller, *Phil. Trans. Roy. Soc. London* **140**, 423 (1850); *Arch. Anat. Physiol. Wissensch. Med. Berlin*, 392 (1852); S. Ramon y Cajal, *Degeneration and Regeneration of the Nervous System*, R. M. May, Transl. (Oxford Univ. Press, New York, 1928); G. H. Parker, *Quart. Rev. Biol.* **4**, 155 (1929); R. W. Gerrard, *Physiol. Rev.* **12**, 469 (1932).
2. L. Lubińska, *Progr. Brain Res.* **13**, 1 (1964); P. Weiss, in *Cellular Dynamics of the Neuron*, S. H. Barondes, Ed. (Academic Press, New York, 1969), p. 3.
3. S. H. Barondes, Ed., *Neurosci. Res. Program Bull.* **5**, No. 4 (1967); B. Grafstein, *Advan. Biochem. Psychol.* **1**, 11 (1969); S. Ochs, in *Protein Metabolism of the Nervous System*, A. Lajtha, Ed. (Plenum Press, New York, 1969), p. 291; R. Lasek, *Int. Rev. Neurobiol.* **13**, 289 (1970); A. Dahlström, *Phil. Trans. Roy. Soc. London Ser. B* **261**, 325 (1971).
4. S. Ochs, M. I. Sabri, J. Johnson, *Science* **163**, 686 (1969).
5. S. Ochs and N. Ranish, *J. Neurobiol.* **1**, 247 (1969).
6. S. Ochs, *Acta Neuropathol. Suppl.* **5**, 86 (1971); in preparation.
7. S. Ochs, *Exp. Neurol.* **12**, 84 (1965).
8. J. Sjöstrand, *Exp. Brain Res.* **8**, 105 (1969).
9. H. D. Lux, P. Schubert, G. B. Kreutzberg, A. Globus, *ibid.* **10**, 197 (1970).
10. S. Ochs, J. Johnson, M. H. Ng, *J. Neurochem.* **14**, 317 (1967); M. I. Kidwai and S. Ochs, *ibid.* **16**, 1105 (196→ B. S. McEwen and B. Grafstein, *J. Cell Biol.* **38**, 494 (1968).
11. K. A. C. James and L. Austin, *Biochemistry* **117**, 773 (1970).
12. A. Dahlström and J. Häggendal, *Acta Physiol. Scand.* **67**, 278 (1966).
13. L. B. Geffen and B. G. Livett, *Physiol. Rev.* **51**, 98 (1971); B. J. Livett, L. B. Geffen, R. A. Rush, *Biochem. Pharmacol.* **18**, 923 (1968).
14. L. Lubińska and S. Niemierko, *Brain Res.* **27**, 329 (1971).
15. M. I. Khan, N. Ranish, S. Ochs, *Abstr. Soc. Neurosci.* **1**, 144 (1971); N. Ranish and S. Ochs, in preparation.
16. R. Miledi and C. Slater, *J. Physiol.* **207**, 507 (1970).
17. S. Ochs, M. I. Sabri, N. Ranish, *J. Neurobiol.* **1**, 329 (1970).

18. B. Droz, *C. R. Acad. Sci. Paris* **260**, 320 (1965).
19. S. Ochs, D. Dalrymple, G. Richards, *Exp. Neurol.* **5**, 349 (1962); N. Miani, *J. Neurochem.* **10**, 859 (1963).
20. B. Grafstein, B. S. McEwen, M. L. Shelanski, *Nature* **227**, 289 (1970).
21. J. S. Elam, J. Goldberg, N. Radin, B. Agranoff, *Science* **170**, 458 (1970); J. S. Elam and B. W. Agranoff, *Trans. Amer. Soc. Neurochem.* **2**, 29 (1971); D. S. Forman, B. S. McEwen, B. Grafstein, *Brain Res.* **28**, 119 (1971); *Trans. Amer. Soc. Neurochem.* **2**, 28 (1971); B. S. McEwen, D. S. Forman, B. Grafstein, *J. Neurobiol.* **2**, 361 (1971).
22. S. Ochs, unpublished observations.
23. W. Schlote, *J. Ultrastruct. Res.* **16**, 548 (1966); A. Pellegrino de Iraldi and E. de Robertis, *Histochemie* **87**, 330 (1968).
24. S. Ochs and N. Ranish, *Science* **167**, 878 (197 → S. Ochs and D. Hollingsworth, *J. Neurochem.* **18**, 107 (1971).
25. M. I. Sabri and S. Ochs, *Trans. Amer. Soc. Neurochem.* **2**, 104 (1971); in preparation.
26. ———, *J. Neurochem.* **18**, 1509 (1971).
27. S. Ochs and C. Smith, *ibid.*, p. 833.
28. R. W. Gerard, *Physiol. Rev.* **12**, 469 (1932); J. E. Lehmann, *Amer. J. Physiol.* **119**, 111 (1937); E. Wright, *ibid.* **147**, 78 (1946); *ibid.* **148**, 174 (1947); J. Maruhashi and E. B. Wright, *J. Neurophysiol.* **30**, 434 (1967); S. Ochs, D. Hollingsworth, E. Helmer, *Biophys. Soc. Annu. Meet. Abstr.* **10**, 114a (1970).
29. A. L. Hodgkin and R. D. Keynes, *J. Physiol.* **128**, 28 (1955); P. C. Caldwell, *ibid.* **152**, 545 (1960); P. F. Baker, *ibid.* **180**, 383 (1965).
30. E. Jankowska, L. Lubińska, S. Niemierko, *Comp. Biochem. Physiol.* **28**, 907 (1969).
31. S. Ochs and C. Smith, *Fed. Proc.* **30**, 665 (1971).
→ S. Ochs, *Proc. Nat. Acad. Sci. U.S.* **68**, 1279 (1971).
33. K. Porter, in *Principles of Biomolecular Organization*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1966), p. 308.
→ F. O. Schmitt, *Proc. Nat. Acad. Sci. U.S.* **60**, 1092 (1968).
35. S. Ochs, Conference on Brain Research, 4th annual, Aspen, Colorado, 16 to 18 January 1971; *Trans. Amer. Soc. Neurochem.* **2**, 24 (1971).
36. ———, *J. Neurobiol.* **2**, 331 (1971).
→ H. E. Huxley, *Proc. Roy. Soc. London Ser. B* **178**, 131 (1971).
38. W. Hartree and A. V. Hill, *J. Physiol.* **55**, 389b (192 → A. V. Hill, *Proc. Roy. Soc. London Ser. B* **138**, 349 (1951).
39. B. Grafstein, D. S. Forman, B. S. McEwen, *Exp. Neurol.* **34**, 158 (1972).
40. S. Ochs, in preparation.
41. P. F. Baker and T. I. Shaw, *J. Physiol.* **180**, 424 (1965).
42. M. Khan and S. Ochs, *Trans. Amer. Soc. Neurochem.* **3**, 93 (1972).
43. S. Berl and S. Puzkin, *Biochemistry* **9**, 2058 (1970).
44. K. Bowler and C. J. Duncan, *Nature* **211**, 642 (1966).
45. T. L. Jahn and E. C. Bovee, *Physiol. Rev.* **49**, 793 (1969); see also N. Kamiya, *Protoplasmic Streaming in Protoplasmatologia* (Springer-Verlag, Vienna, 1959), vol. 8, No. 3a; R. D. Allen and N. Kamiya, Eds., *Primitive Mobile Systems* (Academic Press, New York, 1964); L. Wolpert, *Symp. Soc. Gen. Microbiol.* **15**, 270 (1965).
46. S. Hatano and F. Oosawa, *Biochim. Biophys. Acta* **127**, 488 (1966); M. R. Adelman and E. W. Taylor, *Biochemistry* **8**, 4964 (1969); *ibid.*, p. 4976.
47. V. T. Nachmias, H. E. Huxley, D. Kessler, *J. Mol. Biol.* **50**, 83 (197 → T. D. Pollard, E. Shelton, R. R. Weihing, E. D. Korn, *ibid.*, p. 91.
48. P. F. Davison and F. C. Huneeus, *ibid.* **52**, 429 (1970); F. C. Huneeus and P. F. Davison, *ibid.*, p. 415.
→ A. Nagayama and S. Dales, *Proc. Nat. Acad. Sci. U.S.* **66**, 464 (1970).
50. F. O. Schmitt and F. E. Samson, Jr., Eds., *Neurosci. Res. Program Bull.* **6**, No. 2 (1968); A. Dahlström, *Eur. J. Pharmacol.* **5**, 111 (1968); J. O. Karlsson and J. Sjöstrand, *Brain Res.* **13**, 617 (19(→ G. W. Kreutzberg, *Proc. Nat. Acad. Sci. U.S.* **62**, 722 (1969); K. A. C. James, J. J. Bray, I. G. Morgan, L. Austin, *J. Biochem.* **117**, 767 (1970).
51. H. L. Fernandez, F. C. Huneeus, P. F. Davison, *J. Neurobiol.* **1**, 395 (1970).
52. F. Sampson, *Trans. Amer. Soc. Neurochem.* **2**, 26 (1971); *J. Neurobiol.* **2**, 26 (1971).
→ D. S. Smith, U. Jarlfors, R. Beranek, *J. Cell Biol.* **46**, 199 (1970).
54. L. Austin and I. G. Morgan, *J. Neurochem.* **14**, 377 (1967); L. A. Autillo, S. H. Appel, P. Pettis, P. L. Gambeth, *Biochemistry* **7**, 2615 (1968).
55. S. Ochs, H. Booker, W. E. DeMyer, *Exp. Neurol.* **3**, 206 (1961); B. G. Cragg, *Brain Res.* **23**, 1 (1970).
56. J. Lowry and J. V. Small, *Nature*, **227**, 46 (1970).
57. A. J. Buller, J. C. Eccles, R. Eccles, *J. Physiol.* **150**, 399 (1960); *ibid.*, p. 417; E. Gutmann, *Progr. Brain Res.* **13**, 72 (1964); L. Guth, *Physiol. Rev.* **48**, 645 (1968).
58. J. Zelena, *Progr. Brain Res.* **13**, 175 (1964); L. Biedler, *Progr. Biophys. Mol. Biol.* **12**, 107 (1962); L. Guth, Ed., *Neurosci. Res. Program Bull.* **7**, No. 1 (1969).
59. S. Ochs, *Ann. N.Y. Acad. Sci.*, in press.
60. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
61. S. Moore and W. H. Stein, *ibid.* **176**, 367 (1948).
62. Supported by NSF grant 28664X; NIH grant 08706, and the Hartford Foundation, Inc.

NEWS AND COMMENT

The American Chemical Society: PEPing Up Its Rescue Efforts

The American Chemical Society (ACS) will ask its 100,000 members to donate a minimum of $10 apiece to a new "emergency" fund that the ACS is setting up to alleviate unemployment among the nation's chemists and chemical engineers. The ACS has not decided precisely how to spend the money it hopes will come pouring in, but the society's Washington staff is drawing up a shopping list of programs that range from direct financial assistance for down-and-out chemists to alerting legislators to the plight of unemployed scientists and engineers. Alan C. Nixon, the maverick president-elect of the ACS and the man who instigated the plan, says he hopes that the contributions will reach $1 million, and he says he'll be "very disappointed" if the total is under $500,000.

The society's 460-member representative council approved Nixon's unusual plan by a 4 to 1 margin during the national meeting of the ACS last week in Boston. The council's overwhelming consent provides some measure of the severe internal pressures that are forcing ACS to take a livelier interest in the job security of its members, what with 3000 of them out of work, another 6000 thought to be "malemployed," and even more June graduates than last year still scrabbling for jobs. Council approval for the solicitation of funds also amounts to a victory—and poses a new challenge—for Alan Nixon and the emerging populist party of worried chemists who have rallied around him under the banner of "professionalism," a term that connotes greater involvement by traditional scientific societies in employer-employee relations.

Nixon has been an amiable gadfly in ACS politics for years, known for needling the society's leadership for what he felt was a preponderance of influence by happily tenured academics and secure industrial managers and a dearth of representation for the industrial bench scientists like himself, who make up two-thirds of the ACS membership. In happier times, Nixon was at best a minor force in the society's affairs. But in the wake of more than a score of major industrial layoffs in 1969–71, his complaints have struck an increasingly responsive chord among a traumatized membership. Last year, Nixon got himself on the ACS presidential ballot by popular petition and, in the largest vote in the society's history, handily beat two academic candidates chosen by the traditional nominating committee.

Although he doesn't assume the presidency until next January, Nixon considers his mission too urgent to wait. Besides, his election gave him a 3-year term on the society's influential board of directors, along with considerable access to the time and energy of the Washington headquarters staff, and he seems anxious to take full advantage of it all. Thus, 8 months before his inauguration, he's in the difficult position of