lytic viruses uniquely tropic for endocrine-producing cells and may have also involved autoantibodies against hormone-producing cells.

If it is found that viruses can similarly impair other differentiated or specialized functions of cells that constitute the human immune, endocrine, nervous, and other systems, our findings could have important implications concerning several diseases of man currently of unknown origin.

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Fast Axonal Transport in Squid Giant Axon

Abstract. *Video-enhanced contrast–differential interference contrast microscopy has revealed new features of axonal transport in the giant axon of the squid, where no movement had been detected previously by conventional microscopy. The newly discovered dominant feature is vast numbers of "submicroscopic" particles, probably 30- to 50-nanometer vesicles and other tubulovesicular elements, moving parallel to linear elements, primarily in the orthograde direction but also in a retrograde direction, at a range of steady velocities up to ±5 micrometers per second. Medium (0.2 to 0.6 micrometer) and large (0.8 micrometer) particles move more slowly and more intermittently with a tendency at times to exhibit elastic recoil. The behavior of the smallest particles and the larger particles during actual translocation suggests that the fundamental processes in the mechanisms of organelle movement in axonal transport are not saltatory but continuous.*

Processes of fundamental functional importance in the nervous system include the action potential, synaptic transmission, and axonal transport. The last process conveys substances, organelles, and tubulovesicular structures in both directions, toward the synapse (orthograde direction) and toward the cell body (retrograde direction).

Large membranous organelles in neurites have been reported to exhibit bidirectional saltatory motion (*1*). These structures have instantaneous velocities consistent with subsequent measurements of the bidirectional transport rates of radioactively labeled substances (*2, 3*). However, the 30- to 50-nm vesicles and other submicroscopic structures known or suspected to move along axons have only recently been directly observed in living cells (*4*). By means of video-enhanced contrast–differential interference contrast (AVEC-DIC) microscopy (*5, 6*) it has become possible to observe microscopic events corresponding to fast axonal transport. AVEC-DIC microscopy has considerably increased the detectability of structures as small as one-tenth the resolution limit of the microscope (for example, microtubules 25 nm and intermediate filaments 10 nm in diameter). With this method, direct observation of axonal transport in the squid giant axon is possible despite the large amount of light scatter present.

Carefully dissected, well-oxygenated squid axons with the ganglia attached to establish polarity of the preparation were viewed by the AVEC-DIC method at a magnification of 12,000 with a ×100 oil-immersion planopochromatic objective (numerical aperture, 1.3). Each microscopic field displayed an optical section (21.3 by 21.3 μm and about 0.2 μm thick) of an axon 0.5 mm in diameter. In a typical field, small particles (0.1 to 0.2 μm in apparent diameter) moved in one direction or the other (never reversing) at velocities up to ±5 μm/sec (±432 mm/day) and averaging 2.5 μm/sec (216 mm/day) at 21°C, while some of the larger organelles exhibited more intermittent movements (Fig. 1). Although the visual impression was that of almost uniform velocities of continuous movement, detailed analysis (*7*) revealed a considerable range of velocities (Fig. 2).

Individual particles were tracked over distances from 5 to 20 μm. In the cortex, movements were mostly orthograde, while in the endoplasm, particles could be seen moving in both directions. Or-

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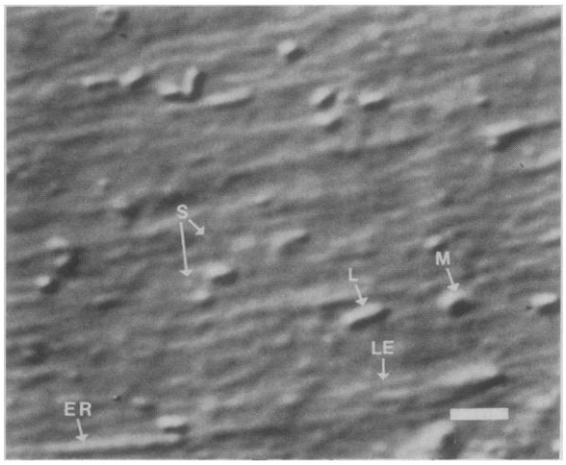

Fig. 1. A video micrograph of an optical section about 200 nm thick and 10 μm deep in the axoplasm of a squid giant axon showing examples of large (*L*), medium (*M*), and small (*S*) particles and linear elements (*LE*). Studies with the stain Fast Green suggest that structures such as that labeled *ER* may represent a segment of the smooth endoplasmic reticulum of the axon (*15*). In video records, many of these particles are moving (as described in text). Scale bar, 2 μm.

thograde movements tended to be more rapid than retrograde movements except in the cortex (Fig. 2). These observations contrast with previous reports (*8*) in which most detectable particles moved in the retrograde direction, whereas particles moving in the orthograde direction were generally large and relatively slow (presumably mitochondria).

In the squid axon, medium-sized particles (0.2 to 0.6 μm) move more rapidly in the orthograde than the retrograde direction, but at a slower mean velocity and a wider range of velocities than the smaller particles. The particles of medium size are more likely to move intermittently. For example, a particle may exhibit one velocity, stop momentarily, and then resume movement at a different velocity. Large particles (0.8 to 5.0 μm, mostly mitochondria) also exhibit intermittent movements. Their velocities tend to be greater in the retrograde direction, but more appear to be moving in the orthograde direction, confirming the observations of others.

Both medium and large particles can be seen to undergo elastic recoil-like behavior in which they stop momentarily and then resume movement at a significantly higher velocity. They recoil backward to a previous position or move forward until they have "caught up" with the position which would have been occupied had they continued moving. Recoil lateral to the main axis has also been observed. The basis of these recoil events may reveal new information on interactions of the large organelles with cytoskeletal elements. It is noteworthy that few, if any, small particles were seen to exhibit behavior suggestive of elastic recoil.

The smallest particles appear to be moving along linear elements, probably microtubules and neurofilaments (*9*), both of which can be detected but not distinguished in AVEC-DIC images. However, some particle movements orthogonal to visible linear elements can be seen. Electron micrographs (*10*) strongly suggest that the smaller particles are mostly small vesicles, dense membranous bodies, and tubulovesicular elements in the range of 30 to 50 nm in diameter. The AVEC-DIC method inflates their apparent diameters, but renders them visible as weakly contrasted structures in the living cell for the first time.

We interpret these results to mean that the fundamental process in fast axonal transport is not saltatory but continuous motion. It seems likely that this process is a form of intracellular motility that should be called cytoplasmic transport, because it is also seen in the reticulopodial networks of foraminifera (*5*, *11*) and in various tissue cells in culture (*12*).

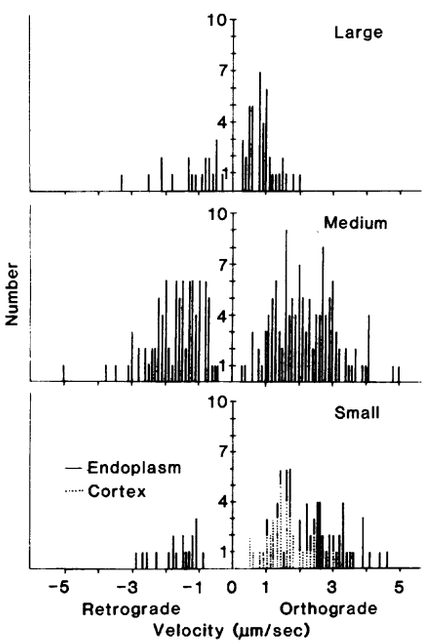

Fig. 2. A histogram of the velocities (measured in periods of 1 to 3 seconds) of large, medium, and small particles, as described in the text. Dotted lines indicate measurements in the outer 2 μm of the intact axon.

The squid giant axon is an important model system in which to study cytoplasmic transport for several reasons. First, the mass of vesicular material moved is remarkable. Second, much is known about the ultrastructure, physiology, and biochemistry of this axon. Third, extruded axoplasm can be prepared (*13*) that retains its ability to exhibit axonal transport in vitro (*14*). We believe that the visualization and demonstration of fast axonal transport in intact giant axons and extruded axoplasm from squid represent major advances in the study of intracellular transport processes.

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Fast Axonal Transport in Extruded Axoplasm from Squid Giant Axon

Abstract. *Development of video-enhanced contrast–differential interference contrast for light microscopy has permitted study of both orthograde and retrograde fast axonal tranport of membranous organelles in the squid giant axon. This process was found to continue normally for hours after the axoplasm was extruded from the giant axon and removed from the confines of the axonal plasma membrane. It is now possible to follow the movements of the full range of membranous organelles (30-nanometer vesicles to 5000-nanometer mitochondria) in a preparation that lacks a plasma membrane or other permeability barrier. This observation demonstrates that the plasma membrane is not required for fast axonal transport and suggests that action potentials are not involved in the regulation of fast transport. Furthermore, the absence of a permeability barrier surrounding the axoplasm makes this an important model for direct biochemical, pharmacological, and physical manipulations of membranous organelle transport.*

The elongate form of the neuron has made it a valuable system for the study of intracellular transport (*1, 2*). The processes of intracellular transport may take many forms, from the movements of membranous organelles to cytoskeletal translocations, and the underlying molecular mechanisms of such movements are poorly understood. Understanding of cellular phenomena at the molecular level requires model systems that permit direct analysis of the cellular elements as well as biochemical and pharmacological manipulations, and that can be maintained long enough to conduct the manipulations. No such models have been available for the study of most types of intracellular transport.

Fast axonal transport is the movement of tubulovesicular membranous organelles from the cell body to the axonal extremities at rates of 1 to 5 μm per second (*1–3*). Many of these membranous organelles are vesicles, 30 to 50 nm in diameter, which can be identified only by electron microscopy. However, the dynamics of structures are generally lost during fixation for electron microscopy and indirect methods of analysis, such as biochemical dissection of labeled materials, have not been sufficient to provide answers about the molecular mechanisms of movement. One major difficulty stems from the presence of a plasma membrane and connective tissue sheath containing glial cells which complicate manipulation and analysis of intracellular transport processes in the axon. These structures effectively insulate the axon from external influences. Thus, to study the molecular mechanisms of fast axonal transport, the minimum requirements of a model are (i) the means to detect moving 30- to 50-nm vesicles and (ii) a preparation lacking permeability barriers.

We report here on a new preparation that satisfies both of these requirements. Recent developments in video-enhanced contrast–differential interference contrast (AVEC-DIC) microscopy permit detection of structures as small as individual microtubules, 25 nm in diameter, in living cells (*4*). It has been possible to analyze the movements of the 30- to 50-nm vesicles in cultured vertebrate neurons and intact giant axons of the squid (*5, 6*). The movements of these structures were found to correspond to fast axonal transport with respect to both rate and direction of movement, but the permeability barriers of these preparations were intact. However, it is possible to separate a cylinder of axoplasm of the squid giant axon from its plasma membrane and connective tissue sheath by mechanical extrusion (*7*). This extruded axoplasm maintains its structural integrity and many metabolic activities in vitro (*8*). The elimination of permeability barriers without the use of detergents permits precise control of experimental conditions.

The axonal transport processes in extruded axoplasm and in the intact giant axon show no qualitative differences initially. If the axoplasm is extruded directly onto a cover glass, with care being taken to maintain original dimensions and orientation, the resulting image reveals more structural detail than images of the intact axon because there is no intervening tissue to scatter and depolarize light (*5*) (Fig. 1). Otherwise, the images of extruded axoplasm are indistinguishable from the images obtained with intact axons. Electron micrographs (*8, 9*) confirm that extruded axoplasm retains the morphology of axoplasm in the intact axon.

Video records of extruded axoplasm (Fig. 1, a to d) indicate that three classes of particles can be distinguished by size and characteristics of movement [see (*5*)]. The sizes and velocities of the small, medium, and large particles are comparable in intact axons and extruded axoplasm. Prominent linear elements, which appear to delineate pathways that most particles tend to follow, can be seen more clearly in extruded axoplasm than in the intact axon. Optical sections (200- to 400-nm intervals) suggest the presence of strata that differ in their complement of membranous organelles and linear elements as well as in the predominant directions of movement.

The continuation of fast axonal transport processes in the extruded axoplasm demonstrates unequivocally that the movements of membranous organelles do not require an intact plasma membrane or a membrane potential. This is in accord with previous studies (*10*) that suggested that the rate of fast transport did not change with electrical activity or alterations in the membrane potential of the nerve fibers. Thus fast axonal transport is not regulated by the number of action potentials traversing the axon. The apparent absence of regulation of fast transport by neuronal activity is consistent with the hypothesis that it operates at or near the maximal rate. This is a difference between fast transport and other forms of motility that are regulated.

The linear elements, which are thought to be microtubules and neurofilaments, are more apparent and easily visualized in the extruded axoplasm (Fig. 1a) than in intact axons. Most re-

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