(*6*), we were able to see the outer wall layer. This is well illustrated in a dividing cell of marine *Nitrosomonas* sp. (cover photo). In this organism, the outer wall layer is composed of repeating subunits arranged in a regular array and measuring approximately 150 Å. The only break in the pattern is at the point of division between the two cells. With the same technique, it was shown that another marine ammonia-oxidizer, *Nitrosocystis oceanus* (Barbados strain), displays a similar yet different outer cell wall surface (Fig. 1a). This surface is composed of small 20-Å particles forming a grid of diamond shaped units. This pattern is also clearly seen when cell wall fragments are negatively stained with phosphotungstic acid (Fig. 1b).

The sculptured, outer wall layer of these marine ammonia-oxidizing bacteria is believed to be composed primarily of structural protein, with the protein units linked together through metal-oxygen bonds. This theory is based on the fact that the outer layer is unaffected by hydrogen-bond breakers, such as urea, urethane, acetamide, formamide, and glycolic acid, but is broken down when treated with formic acid, which is known to break the metal-oxygen bond. In addition, this outer layer of repeating subunits is almost completely removed from the cell wall when treated with ethylenediaminetetraacetate which, of course, chelates metal ions. How the metal is linked and what metals are involved are, at present, not known; however, Ca^{++} or Mg^{++} would probably be likely candidates since they are known constituents of the cell wall.

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Fast Transport System of Materials in Mammalian Nerve Fibers

Abstract. *A fast transport system of materials is shown in cat sensory fibers of sciatic nerve. After injection of tritiated leucine into the lumbar seventh ganglia, the distribution of activity was measured in the sciatic nerves from 2 to 8 hours afterward. The distribution showed a crest of activity advancing down the fibers at a rate of approximately 410 millimeters per day. An intraaxonic locus of the activity was indicated by a block of the downflow induced by local freezing which causes the fibers to close off. Some of the activity in the nerve was due to free tritiated leucine, with most of it incorporated into polypeptide, soluble protein, and subcellular particulates.*

Both a fast and a slow rate of axoplasmic flow has been found in cat motoneuron axons after injection of small volumes of the precursor ^{3}H-leucine into the cat lumbosacral spinal cord in the vicinity of the somas of the large motoneurons (*1*, *2*). After uptake and incorporation into polypeptide, protein, and particulates (*3*) by the somas, the transport of labeled material was shown by the distribution of activity found in 3-mm segments taken along the length of the ventral roots. A rate of approximately 930 mm/day was determined for the fast axoplasmic flow component; the furthest extent of activity found in an hour was (*1*, *2*) used as an estimate of rate.

Although desirable from the point of view of their homogeneity of fiber composition, the ventral roots are too short to show the full extent of such rapid transports. Also, some part of this spread of activity could be due to diffusion. We therefore used the longer lengths of sensory nerve fibers in the sciatic nerve after injecting the ^{3}H-leucine locally into the lumbar seventh (L7) ganglia. Lasek (*4*) used this system and, at times varying from 14 hours to 60 days after injection, found changes expected of an axoplasmic flow with a break present in the distribution of activity in the nerve at 1 day which also suggested a fast flow component.

In order to see the moving front of activity at the times expected of a fast axoplasmic flow, it was necessary to take the nerves at earlier times. The L7 ganglia were injected with ^{3}H-leucine (7 μl; 5 mc/ml) (*5*), and the roots and sciatic nerves were removed at times from 2 to 8 hours afterward. They were cut into 3-mm sections, and each section was placed into a vial to which 0.5 ml of Hyamine 10-X was added; they were heated and shaken to dissolve the tissue. Scintillator solution was added to each vial, and radioactivity was counted in a Packard liquid scintillation spectrometer (*3*).

The distribution of activity in the sciatic nerves and dorsal roots is shown in Fig. 1. As expected, the highest amount of activity was found in the ganglia. At 2 hours, a fast flow was indicated by the slope of activity into the dorsal root and sciatic nerve. However, at 3 hours and thereafter, the activity in the nerves showed a peak, with a wave front being moved down the nerve. The activity distal to the

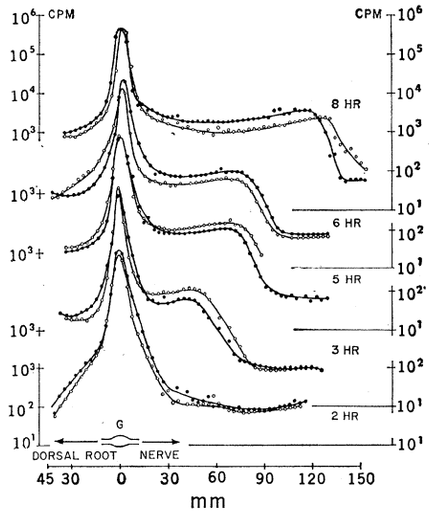

Fig. 1. Distribution of activity in the dorsal roots and sciatic nerves of five cats at different times from 2 to 8 hours after injection of ^{3}H-leucine into the L7 ganglia (*G*). The symbols (●, ○) represent the activity in roots and fibers of each side. The ordinate scales are shown only in part. That for the 2-hour roots and nerves is given at the bottom left, showing divisions from 10 to 1000 counts per minute (*CPM*). At bottom right, only the lowest amount (10 counts per minute) is shown. Above it on the right is the scale for the 3-hour nerves. Other scales are indicated only by the scale from 10 to 1000 on the right and 1000 counts per minute on the left. At the top on the right, a full scale is given for the 8-hour nerves and roots.

ganglia had a plateau rising to a crest at the front of the advancing wave. When additional time was allowed between injection and removal, the crest had an increasingly distal position along the nerve, with its displacement indicating a rate of movement of approximately 410 mm/day. Just ahead of the crest, the activity is only several times the background, probably representing a diffuse leakage of precursor into the circulation.

That the activity found in the nerve is in fact intraaxonal was shown by interrupting the fibers in the sciatic nerve without gross disruption of other nerve elements. This is accomplished by a brief period of freezing with a metal bar cooled in dry ice, which causes the nerve fibers to bead and then, in several hours, to close off and in effect form blind-ended cylinders (*2*, *3*, *6*). Sixteen hours after freeze-block of the sciatic nerve on one side, ^{3}H-leucine was injected into the L7 ganglia of both sides, the opposite side serving as a control. Seven hours was allowed for downflow of activity, and the distribution showed an increase due to damming in the freeze-blocked nerve just proximal to the freeze zone, with a fall toward the background activity distally. Some of the activity distal to the freeze zone may represent some remaining unblocked fibers. This result is similar to our previous work with this technique and it indicates an intraaxonal location of the activity (*3*, *6*).

The composition of labeled substance in the nerves was determined by subcellular centrifugation and Sephadex gel filtration. Activity was found in all the subcellular fractions, with a somewhat higher amount of specific activity present in the small particulate and soluble fraction (100,000*g* supernatant) compared to that in the nuclear and mitochondrial fractions (Table 1). The soluble fraction was further analyzed by passing it through Sephadex G-100 columns.

Labeling was present in two peaks of the effluent. The first peak contains soluble protein; the second peak contains free leucine and polypeptides with lower molecular weights (*1*, *3*). The relative proportion of activity in the two peaks changes with time; at the beginning, there is more activity in the second peak, and then after 14 hours, much more activity is present in the first peak (Table 2). This is in line with our evidence that the bulk of the activity in the soluble protein present in the first peak is associated with the slow phase of axoplasmic flow (*2*, *3*). Our work with Sephadex G-200 columns shows that most of the soluble protein has a molecular weight of approximately 450,000 with a component having a molecular weight of approximately 68,000.

The rate of approximately 410 mm/day found for the fast transport in the sensory fibers is lower than that determined in the motor fibers (*1*, *2*). This may reflect either differences in the way the rates were measured (crest movement versus the furthest extent of spread of activity), differences in the rate of transport in the proximal in contrast to the distal portion of the nerve fibers, or differences between different kinds of nerve fibers. Similar possibilities are involved in comparisons with the rates of fast axoplasmic flows reported for adrenergic granules (*7*) and glutamate in snail and frog nerve (*8*). It is, however, apparent that such fast rates of axoplasmic flow are present in various kinds of neurons, rates much faster than the 1 to 2 mm/day or the "fast" flows of 30 to 60 mm/day reported earlier (*9*).

Neither the mechanism responsible for fast transport at these high rates nor the mechanism underlying the slow rate of axoplasmic transport are known (*10*). The crest of activity at the advancing front excludes diffusion or a propulsion from the soma; therefore, the underlying mechanism of fast transport must be considered present locally all along the axon, possibly related to the neurofilaments or the neurotubules of the axoplasm (or both) (*1*, *11*).

Table 1. Subcellular fractionation of sciatic nerve homogenates gives the following fractions: nuclear (N); centrifuged at 800*g* for 20 minutes; "mitochondrial" (M), centrifuged at 20,000*g* for 30 minutes; small particulate (P) and supernatant (S) fractions separated at 100,000*g* for 90 minutes. Specific activity (SA) is given in counts per minute per milligram of protein. Percentage of activity is given with respect to the activity of the homogenate. Time is given in hours after injection of ganglia.

Time (hr)	N		M		P		S	
	SA	Percent	SA	Percent	SA	Percent	SA	Percent
3	330	5.1	1,115	29.2	3,341	39.2	921	26.4
5	667	7.1	1,500	31.6	5,056	33.9	1,518	27.3
7	943	3.6	2,209	51.2	4,320	18.9	2,293	26.3
14	2,250	6.8	4,510	29.9	12,647	21.1	11,175	42.1

Table 2. Activities in the peaks of effluents from Sephadex G-100 gel-filtration columns. At early times, there was more activity in the second peak, which contained free leucine and lower molecular-weight polypeptide. Later, particularly at 14 hours, there was more activity in the first peak which contained higher molecular-weight soluble protein. The shift in activity is further indicated by the ratios of activity present in the peaks as shown in the last column.

Time (hr)	Peak 1	Peak 2	Free leucine in peak 2 (%)	Ratio of peak 1 to peak 2
3	480	760	21	0.63
5	2990	1104	30	2.7
7	1420	586	39	2.4
14	3950	306	30	12.9

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