and the nerve; this finding indicates that the properties of both F_1 and F_2 are adequate to eliminate optical signals due to changes in light scattering. (iii) With an AO-stained crab nerve, optical signals similar to those in Fig. 1 could be observed at 0°, namely, when S, F_1, N, F_2, and P were arranged in one straight line; this indicates that the fluorescent light emitted at both 0° and and 90° increases during nerve conduction. (Note that an increase in light scattering during nerve conduction tends to reduce the light intensity observed at 0°.) From these observations, we conclude that possible sources of artifact in our detection of small fluorescence changes are eliminated.

The reason for the small size of our optical signal is easy to understand. The plasma membrane, where the primary event in the process of nerve conduction takes place, is extremely thin (roughly 5 to 10 mμ in thickness). The dye molecules contributing to the optical signal during conduction must be limited to those bound to the plasma membrane and the structures in its immediate vicinity. The major portion of the background fluorescent light derives from the dye molecules bound to the connective tissue in the nerve. Therefore, the level of fluorescence change in the critical layers of the nerve is considered to be several orders of magnitude greater than those described in this report.

The fluorescence of AO is strongly influenced by changes in the microenvironment of the dye molecules (*4*, *10*). The change in the intensity of fluorescence during nerve conduction is therefore indicative of changes in the physicochemical properties of the microenvironment of the dye molecules bound to the nerve membrane. The observed change in fluorescence suggests that the macromolecules in the nerve membrane undergo drastic conformational changes during nerve conduction. This report indicates that the use of fluorescence is a powerful means of studying the process of nerve excitation and conduction.

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References and Notes

1. I. Tasaki, *Nerve Excitation* (Thomas, Springfield, Ill., 1968); D. Nachmansohn, *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York, 1959); J. P. Changeux *et al.*, *Proc. Nat. Acad. Sci. U.S.* **57**, 335 (1967); T. Hill, *ibid.* **55**, 1379 (1964); A. L. Lehninger, *ibid.* **60**, 1069 (1968).
2. L. B. Cohen and R. D. Keynes, *J. Physiol.* **194**, 85P (1968); ———, B. H. Hille, *Nature* **218**, 438 (1968); I. Tasaki, A. Watanabe, R. Sandlin, L. Carnay, *Proc. Nat. Acad. Sci. U.S.* **61**, 883 (1968); A. Watanabe, I. Tasaki, L. Carnay, *Biol. Bull.* **135**, 442 (1968).
3. O. F. Borisova, L. L. Kiselev, L. A. Tumerman, *Dokl. Akad. Nauk SSSR* **152**, 1001 (1963); G. Weill, *Biopolymers* **3**, 567 (1965).
4. R. W. Albers and G. J. Koval, *Biochim. Biophys. Acta* **60**, 359 (1962).
5. D. F. Bradley, *Trans. N.Y. Acad. Sci.* **24**, 64 (1961).
6. K. Zeiger and H. Harders, *Z. Zellforsch.* **36**, 62 (1951).
7. S. Strugger, *Fluoreszenzmikroskopie und Microbiologie* (Schaper, Hannover, 1949); M. Wolf and S. Aronson, *J. Histochem. Cytochem.* **9**, 22 (1961).
8. I. Tasaki, A. Watanabe, T. Takenaka, *Proc. Nat. Acad. Sci. U.S.* **48**, 1177 (1962).
9. I. Tasaki and M. Shimamura, *ibid.*, p. 1571; W. J. Adelman, Jr., and J. P. Senft, *J. Gen. Physiol.* **50**, 279 (1966).
10. P. Pringsheim, *Fluorescence and Phosphorescence* (Interscience, New York, 1949).
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Macromolecular Subunits in the Walls of Marine Nitrifying Bacteria

Abstract. *The outermost layer of the cell wall of all marine ammonia-oxidizing bacteria so far isolated is made up of protein subunits arranged in a regular manner and linked together through metal-oxygen bonds. This sculptured, outer wall layer appears to be unique to the marine forms and is not found in the terrestrial ammonia-oxidizing bacteria.*

The presence of macromolecular subunits in the outer cell wall of bacteria has been known for some time. These structures were first described in *Spirillum* sp. (*1*) and later in a photoorganotrophic bacterium (*2*), *Bacillus cereus* (*3*), *Halobacterium cutirubrum* (*4*), and most recently in a marine photosynthetic bacterium, *Ectothiorhodospira mobilis* (*5*). It was not surprising, therefore, to discover that marine ammonia-oxidizing bacteria also have an outer layer of regularly arranged subunits. This layer was not found in their terrestrial counterparts or in marine or terrestrial nitrite-oxidizing bacteria.

Using the freeze-etching technique

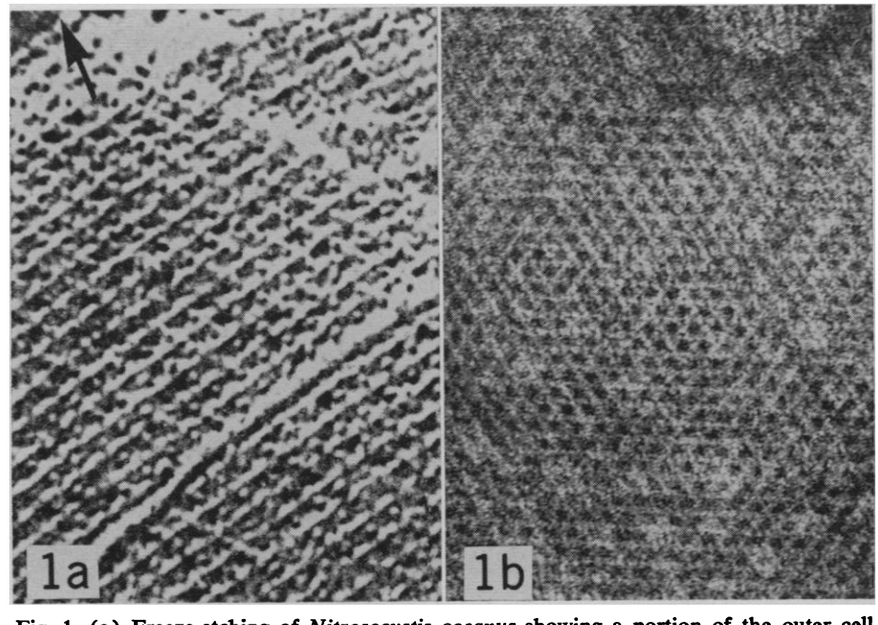

Fig. 1. (a) Freeze-etching of *Nitrosocystis oceanus* showing a portion of the outer cell wall layer consisting of small particles (about 20 Å) forming a diamond-shaped unit with side dimensions of about 110 Å. The arrow in the upper left corner indicates the direction of platinum-carbon shadow (× 750,000). (b) Negatively stained outer cell wall fragment of *N. oceanus* showing the regular pattern of subunits (× 260,000).

(*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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References and Notes

1. A. L. Houwink, *Biochim. Biophys. Acta* **10**, 360 (1953).
2. W. de Boer and B. S. Spit, *Antonie Van Leeuwenhoek J. Microbiol. Serol.* **30**, 239 (1964).
3. D. J. Ellar and D. G. Lundgren, *J. Bacteriol.* **94**, 1778 (1967).
4. D. J. Kushner, S. T. Bayley, J. Boring, M. Kates, N. E. Gibbons, *Can. J. Microbiol.* **10**, 483 (1964).
5. C. C. Remsen, S. W. Watson, J. B. Waterbury, H. G. Trüper, *J. Bacteriol.* **95**, 2374 (1968).
6. H. Moor, *Histochemie* **62**, 546 (1964); C. C. Remsen, F. W. Valois, S. W. Watson, *J. Bacteriol.* **94**, 422 (1967).
7. Supported in part by PHS grant GM 11214, and in part by AEC contract AT(30-1)-1918 (Ref. NYO-1918-174). Contribution No. 2210 from the Woods Hole Oceanographic Institution.

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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

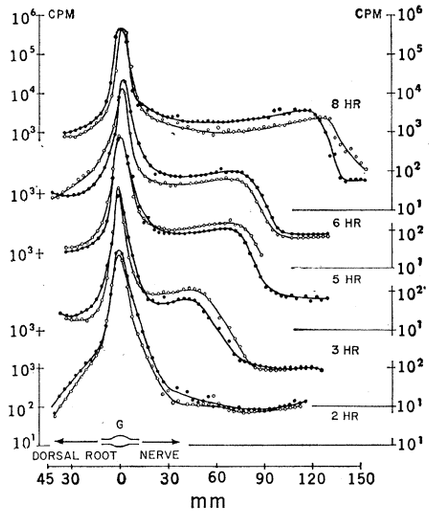

Fig. 1. Distribution of activity in the dorsal roots and sciatic nerves of five cats at different times from 2 to 8 hours after injection of ^{3}H-leucine into the L7 ganglia (*G*). The symbols (●, ○) represent the activity in roots and fibers of each side. The ordinate scales are shown only in part. 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.