DEVELOPMENT OF THE FINE STRUCTURE OF THE MYELIN SHEATH IN SCIATIC NERVES OF CHICK EMBRYOS*

BY BETTY BEN GEREN AND JOSEPHINE RASKIND

CHILDREN'S CANCER RESEARCH FOUNDATION, THE DIVISION OF LABORATORIES AND RESEARCH OF THE CHILDREN'S MEDICAL CENTER, THE DEPARTMENT OF PATHOLOGY, HARVARD MEDICAL SCHOOL; AND THE BIOLOGY DEPARTMENT, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Polarization optical studies[1, 2] of fresh nerve fibers produced evidence of the radial orientation of lipid molecules and the tangential orientation of protein molecules in the myelin sheath. The low-angle x-ray diffraction analyses[3, 4] of fresh nerves have defined a repeat period in the radial direction of the myelin sheath of about 172 A in fresh amphibian nerve and about 185 A in fresh mammalian nerve. In 1950, Fernández-Morán[5] described concentric lamellar structures in frozen sections of osmium tetroxide fixed nerve fibers and reported the unit spacings to be about 70 A in rat sciatic and about 60 A in frog sciatic nerves. Sjöstrand[6] recently measured the unit spacing in sections of methacrylate-imbedded, osmium tetroxide fixed mouse nerve fibers and found an average spacing of 119 A. Our own measurements[7] of osmium-tetroxide fixed, methacrylate-imbedded adult frog nerve spacings average about 85–95 A.

It has been of interest here to distinguish between two possible modes of formation of the fine structure of the myelin sheath in the embryo. One possibility would be the "crystallizing out" of the characteristic fine structure in accumulated lipid and protein at some time related perhaps to cellular activity or axonal function. An alternative possibility would be the deposition of lipid and protein as oriented layers from the beginning of the myelination process. The evidence presented here gives strong support to the latter view.

Methods.—Fertile chicken eggs were incubated for 18 days at 39°C. Embryos were removed, the sciatic nerves exposed and flooded with fixative at room temperature. After preliminary blackening, the fibers were dissected and allowed to fix for a total period of 1 to $1^1/_2$ hours. The fixative used was 1% osmium tetroxide in Hank's balanced salt solution.[8] After washing, alcohol dehydration and infiltration, specimens were imbedded at 45°C. in *n*-butyl methacrylate[9] catalyzed with benzoyl peroxide. Sections were cut on the Minot-type microtome[10] with glass knives.[11] The electron microscope is an RCA type EMU fitted with an externally movable objective aperture.[12]

Results.—The concentric lamellar structure of the myelin sheath is seen in cross section of the embryonic nerve fibers. The unit spacing (the distance from the beginning of one dense band to the beginning of the

next) was measured in undisrupted areas of 22 fibers (1056 total spacings measured). Figure 1 shows a distribution curve of these measurements, each position representing the average measurement for 3 to 14 spacings. The mean is 97 A with an average deviation of 9 A. Figure 2 (*a*) illustrates a region in a cross-section of a sciatic nerve, and figures 2 (*b*) and 2 (*c*) are higher magnifications of indicated areas in the myelin sheath. The thickness of the dense lines in the spacing varies according to focus, thickness of section, orientation of the fiber in the plane of section, and degree of astigmatism caused by the objective aperture. In our best in-focus pictures

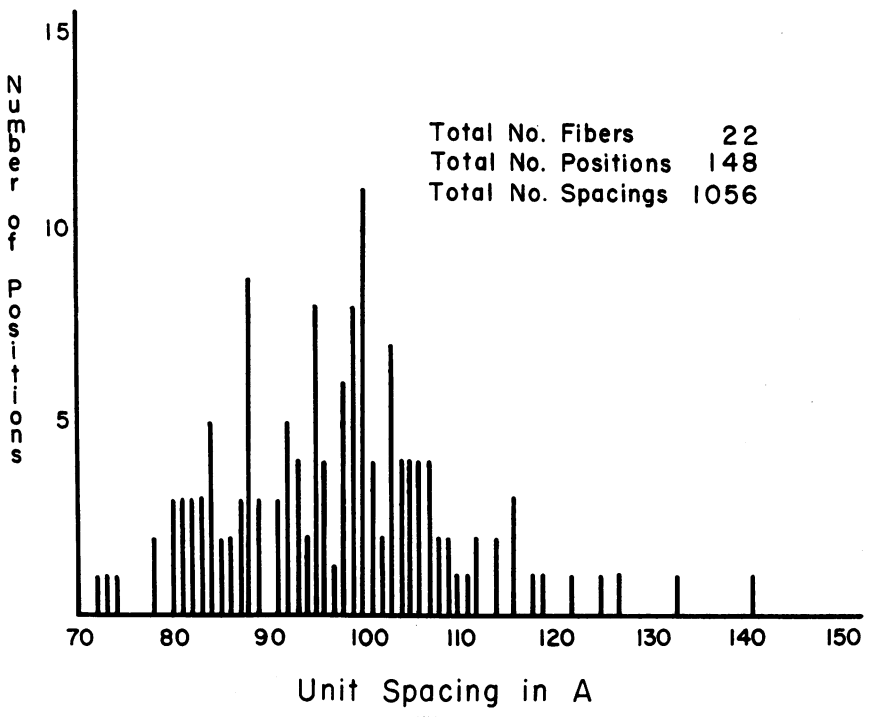

FIGURE 1

of through-focus series the width of this dense line varies between 20 and 50 A. No regularly arranged structures were observed in the less dense band of the unit repeat.

Within a single sciatic nerve are fibers with varying numbers of layers in the myelin sheath. Preliminary study of earlier ages of development (14 and 15 days) reveals many fibers with 2 to 12 concentric lamellae whereas in the 18-day material described here most fibers have 24 or more lamellae, although a few are seen with lesser numbers of layers. It is thus apparent that the increase in myelination of nerve fibers in the embryo

proceeds by the addition, in an oriented manner, of lipids and proteins to the surface of the axonal projection within the cytoplasm of the Schwann cell. The Schwann cells in the early (14-day) embryonic nerve trunk are extremely large compared to the size of the individual myelinating fibers which appear as large Schwann cell cytoplasmic inclusions. This is in marked contrast to the satellite type of appearance of the Schwann cell around the adult fiber. Detailed studies of the earlier (14- and 15-day) stages will be reported later.

Discussion.—It is apparent that the myelin sheath has well-ordered fine structure in the 18-day chick embryo. Our observations indicate that from the beginning, the deposition of lipid and protein about the axon occurs in well-ordered layers and the thickness of the myelin sheath is increased by an increase in the number of layers.

The unit spacing measures 97 ± 9 A. One might expect that a unit structure approximately one-half the large period shown by x-ray diffraction[3, 4] would be based on repeating lipid double layers arranged in some alternating fashion with the predicted tangential protein lamellae, as suggested by Schmitt, Bear, and Palmer. However, several factors make detailed studies of the period and correlation with the x-ray data still premature. Swelling and shrinking may occur during fixation with undefined changes of water content. One observes obvious areas of distortion of the myelin sheath as in figure 2 (*c*). The swelling and gross distortion of tissues imbedded in methacrylate is easily recognized and such specimens are discarded, but the evaluation of small changes in volume due to imbedding is difficult. Fernández-Morán's data on frozen sections of fixed material are of significance here, but more data are needed for detailed comparisons. Very slight distortion of the specimen from heating in the electron beam can further prevent accurate measurements. Measurements of fibers cut somewhat diagonally with respect to the fiber axis would give greater than actual values for the unit spacing in the radial direction. In our specimens, the myelin sheath and its spacings appear to be compressed in the direction of cutting.

Our lack of knowledge regarding the nature of the reactions of osmium tetroxide with tissue components further limits detailed analysis of the tissue fine structure as revealed by the present methods.

Correlation of the fine structure of developing nerve fibers with the time of appearance of conduction cannot be attempted yet, but further advances in techniques and in interpretation of results may make such correlation possible. Investigations of this type may also permit analyses of factors influencing myelin formation at levels of resolution that allow interpretation of the lipid and protein molecular arrangement.

Conclusions.—The forming myelin sheath of embryonic nerve fiber has a repeat spacing similar to that of the myelin sheath of mature fibers.

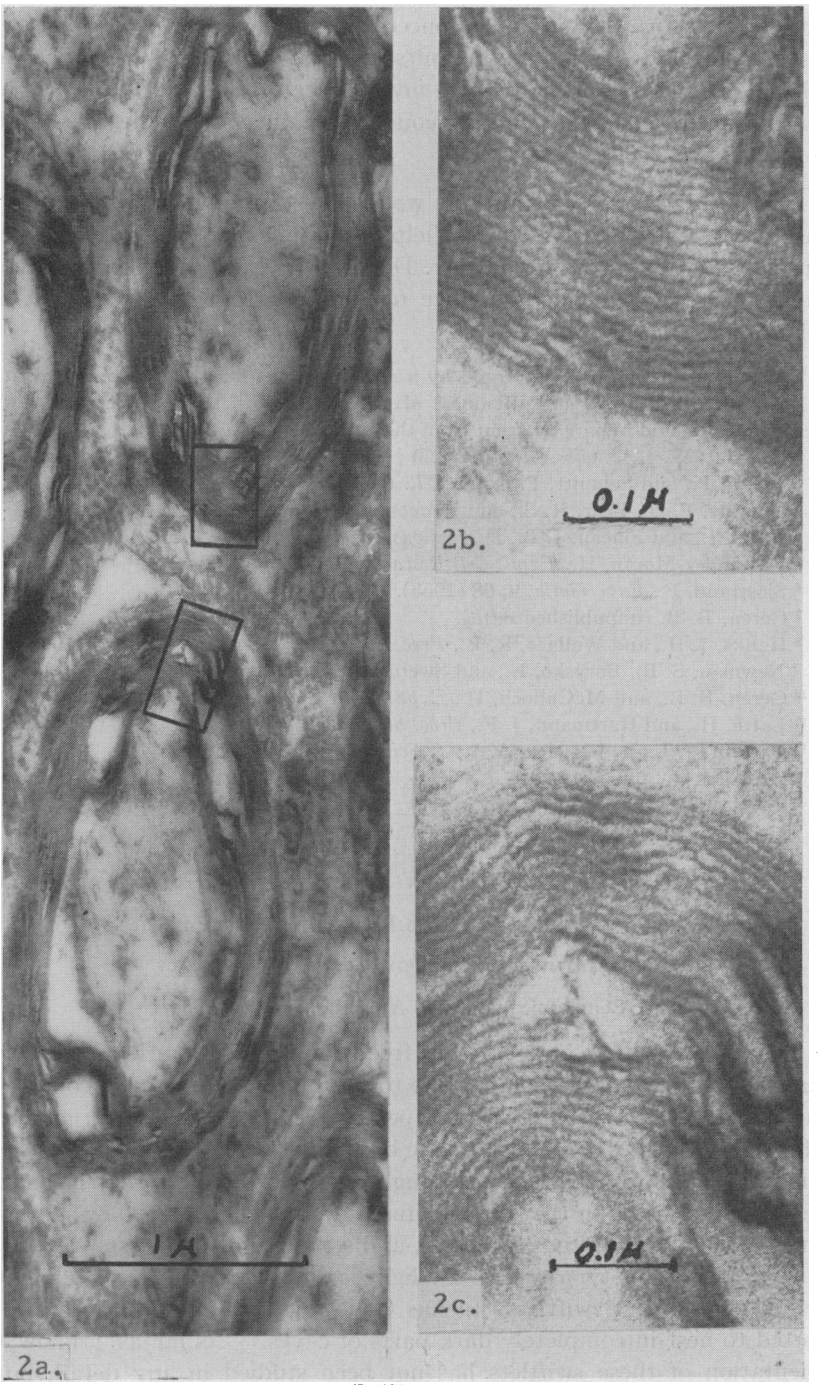

FIGURE 2

The forming myelin consists of concentric lamellar structures arranged as a sheath around the axon as it courses in the cytoplasm of the Schwann cell. The thickness of the forming myelin sheath is dependent on the number of concentric lamellae and the younger the fiber the smaller the number of unit layers.

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ACOUSTIC ORIENTATION IN THE OIL BIRD, STEATORNIS

BY DONALD R. GRIFFIN*

DEPARTMENT OF ZOOLOGY, CORNELL UNIVERSITY

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The discovery that bats use high frequency sounds to orient themselves during flight has naturally given rise to speculation and inquiry concerning the possible existence of similar types of orientation in nocturnal birds. The limited studies of owls that have been made to date indicate that these birds rely primarily on vision, although their dark-adapted thresholds may be slightly lower than that of the human eye.[1] There are a few species of birds that habitually roost in caves, and some build their nests well inside caverns of various types where the light is dim at best. For example, the "edible bird's nest swiftlets" (genus Collocalia) of southeast Asia are reported to nest in completely dark parts of caves.[2] As far as I know the orientation of these swiftlets has not been studied in any detail, but I