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Fine Structure of Biological Lamellar Systems*

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INTRODUCTION

LAMELLAR structures represent one of the most important and widespread components of biological systems.[1,2] Ranging from the individual membranes of cells to the complex multilayered structures found in the nerve myelin sheath, photoreceptors, and chloroplasts, they embody similar patterns of molecular organization. As derivatives of cell membranes, the lamellar structures exhibit the characteristic permeability properties, electrical activity, and other functional features essential to life. Moreover, by virtue of their repetitive and orderly arrangement, the multilayered systems are uniquely endowed for performing the functions of conversion, transfer, and storage of energy in organisms.

Investigation of the morphological substrate of these processes has been handicapped by the marked lability of membranous systems when subjected to the effects of analytical techniques. However, with recent advances, a general picture of the organization of these systems at the macromolecular level is now gradually emerging. The successful application of high-resolution electron microscopy in combination with polarization-optical and x-ray diffraction studies has revealed the extraordinary degree of regularity and structural differentiation which is present throughout the various hierarchies of organization down to the molecular level.

This review describes the salient features of the fine structure of the nerve myelin sheath, photoreceptors, and other representative lamellar systems. It is becoming increasingly evident that all lamellar systems appear to have certain common structural parameters at the molecular level, possibly reflecting an underlying functional analogy. Therefore, as more information becomes available on any one of these specialized systems, new approaches are suggested in the correlation of structure and function. Thus, many of the biochemical studies and recent concepts derived from solid-state physics in investigating the mechanisms of photosynthesis,[3-7] may eventually prove to be of operational value in elucidating the primary events of nerve function and sensory reception.

FINE STRUCTURE OF THE NERVE MYELIN SHEATH

The myelin sheath of nerve fibers is one of the most highly ordered biological systems, and exhibits all of the properties of the smectic fluid-crystalline state. Despite the high water content and marked lability of the myelin sheath, its ultrastructure can be studied by combined application of different techniques under various conditions. The analysis of the fine structure of the myelin sheath can be regarded, in fact, as one of the best examples of the systematic application of complementary biophysical and biochemical methods. Since the myelin sheath derives from a multiply-folded Schwann-cell surface,[8,9] it can also be considered a model system for the study of cell-membrane structure in general.

Polarization-optical studies of the myelin sheath[10-12] indicated that it is composed of concentrically arranged protein or lipoprotein lamellae which alternate with layers of lipid molecules oriented with their long axes in the radial direction. This concept was confirmed and extended by x-ray diffraction studies of fresh nerve.[13-18] It was assumed that the thickness of the concentric lipid-protein unit layers corresponded to the fundamental small-angle x-ray diffraction spacings of 170 A recorded in amphibian, and of 180 to 185 A found in mammalian peripheral nerves.

Subsequently, the postulated layers and the concentric laminated structure of the sheath were observed directly in electron micrographs of thin nerve sections fixed with osmium tetroxide.[19-23] However, since the preparation of these specimens required fixation, dehydration and embedding in a plastic medium, followed by slicing into ultrathin sections, the possibility of artifacts had to be seriously considered. Moreover, the regular patterns of selective deposition of osmium could not be interpreted in terms of specific regions containing the lipids, lipoproteins, water, and other components of the normal myelin structure.

One is obviously dealing here with a case in which combined application of low-angle x-ray diffraction techniques and high-resolution electron microscopy can supplement each other in many ways. It is instructive to consider the merits and drawbacks of each technique in order to appreciate the type of approach required in analyzing biological systems.

The x-ray diffraction method offers the great advantage of permitting examination of the intact nerve trunk in the living animal.[13,15] The information derived from the x-ray diffraction pattern of normal active nerve can, therefore, be considered a reliable basis for all structural analyses of the myelin sheath.[13,18,24] From the low-angle x-ray diffraction pattern, the dimensions and

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approximate distribution of scattering groups in the radial direction of the sheath can be deduced. Reliable reference parameters are, therefore, provided for checking the results of electron microscopy in studies of the normal and modified myelin sheaths.[18,24]

Finally, the x-ray diffraction pattern recorded in a few hours represents an average of the main structural parameters of all of the nerve fibers contained in the exposed nerve trunk which contribute to the diffraction. The practical significance of this is immediately apparent when comparing it with the far greater time factor involved in the corresponding electron-microscope study. Examination of nearly 100 000 serial ultrathin cross sections would be required for a complete electron-microscope study of a 1-mm long segment of a few fibers only.

Conversely, electron microscopy offers the unique advantage of revealing directly the complex patterns of macromolecular organization in selected areas of the specimen.

Structure of the Normal Myelin Sheath

X-ray diffraction patterns recorded from fresh peripheral nerve in a direction perpendicular to the fiber axes feature a series of very well-defined reflections at low angles. The wide-angle reflections at about 4.7 A exhibit meridional intensifications, while the low-angle reflections are precisely oriented in the equatorial direction. The low-angle reflections can be interpreted in terms of a fundamental radial repeating unit varying from 170 A in amphibians to 184 A in mammalian peripheral nerve. The low-angle reflections also show a characteristic alternation of intensities in the even and odd orders [Fig. 1(b)]. It is assumed that the fundamental repeating unit consists of two parts having very similar distributions of x-ray scattering power; from the intensities of the odd-order reflections, the magnitude of the difference between the two parts can be estimated.[18] Finean[15–18] has determined that this "difference factor" is appreciable in peripheral nerve myelin, but appears to be negligible in the optic nerves.

High-resolution electron micrographs of ultrathin sections of osmium-fixed peripheral nerve (Fig. 1) show a series of concentrically arranged, dense lines separated by lighter spaces with an average period of 130 to 140 A. There is, thus, a correspondence of the periodic layers observed in the electron micrographs of myelin preparations and the fundamental radial unit derived from low-angle x-ray diffraction patterns.

The discrepancy of 20 to 30 A between the layer thickness and the x-ray fundamental spacing is owing to the shrinkage effects introduced by osmium fixation and other preparative procedures connected with the examination of the thin sections in the electron microscope.[24] In addition to the periodic dense bands about 30 A wide, the electron micrographs feature light intermediate bands which are much narrower (10 to 15 A). This variation in the densities of the two principal bands of osmium deposition is related to the "difference factor" which manifests itself in the low-angle pattern as an increase in the intensity of the first-order reflection.

The present lack of precise information on the chemistry and localization of the sheath proteins, the lipids, the water layers, and other components of myelin has not as yet permitted a direct correlation with the structural data.[25–27] However, by combined electron-microscope and x-ray diffraction studies of controlled physical and chemical modifications of the myelin sheath, a general picture of the molecular arrangements in the radial units can be obtained.[15,18,21,24,28] Satisfactory correlation and interpretation of structural relationships at the macromolecular level requires the preparation of a large number of ultrathin (100 to 200 A) undistorted serial sections of the same specimens utilized for the parallel x-ray diffraction studies. This has been greatly facilitated by improved thin-sectioning techniques using a diamond knife.[24,29]

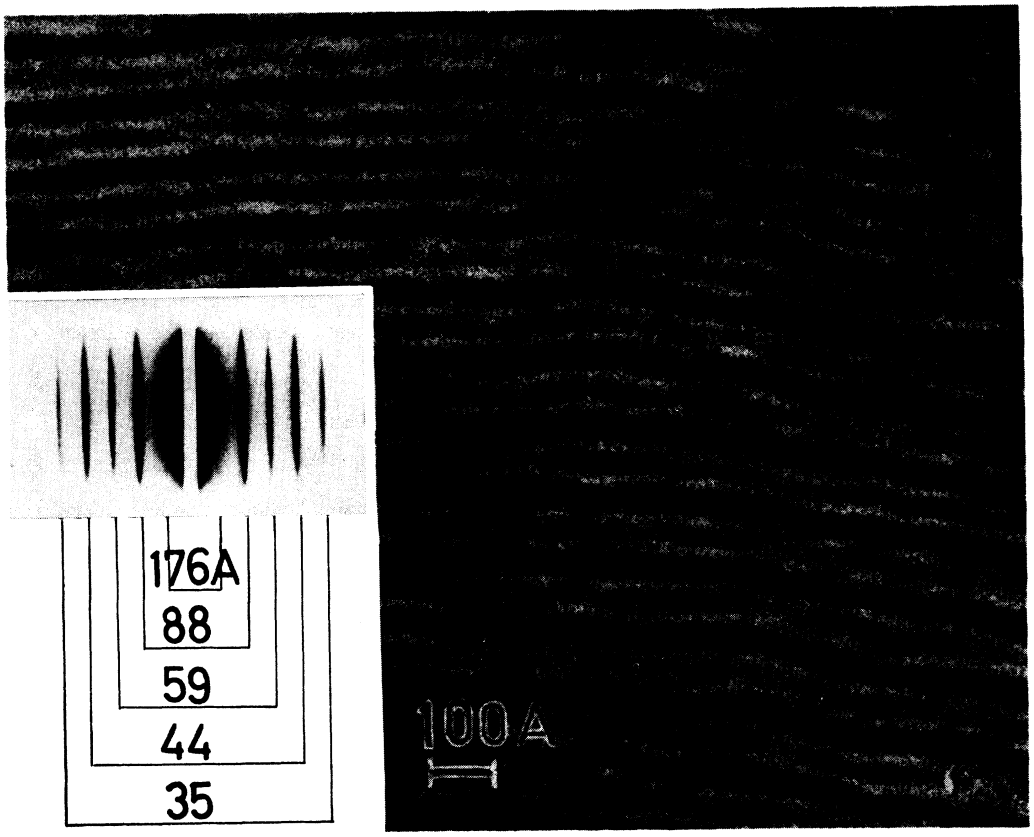
Such a combined approach has led to a detailed analysis of the preparation procedures, thus validating important findings and defining artefact sources. The extraction and enzymatic-digestion experiments performed on fresh myelin proved to be particularly revealing.

Lipid-Extraction Experiments

Extraction of fresh nerve with acetone at 0°C removes about 30% of the cholesterol,[18] leaving the other lipid components essentially intact within the framework of a still highly organized residual myelin sheath.[24] The main modifications revealed by electron microscopy and x-ray diffraction [Figs. 2(d) and 2(e)] of the residual sheath comprise expansion of the layered structure with internal rearrangements and formation of collapsed layer systems [Figs. 2(a) and 2(b)]. The dense lines are relatively resistant to lipid solvents, and their persistence in the collapsed layered structure may indicate that the heaviest osmium deposition is in the region of the protein or stable lipoprotein constituents. These modifications emphasize the importance of cholesterol in the myelin structure, and lend further support to the existence of the phospholipid-cholesterol complex postulated in the unit cell by Finean.[18]

Low-angle diffraction patterns of the lipid extract show a strong 34.2-A reflection [Fig. 2(c)] characteristic of cholesterol. When the dried lipid extract is examined by electron microscopy, thin crystalline lamellae are found embedded between condensed multilayered aggregates. These crystalline lamellae give electron-diffraction patterns which are similar to those recorded from pure cholesterol.

A more extensive breakdown of the sheath is observed after alcohol extraction. The fine-layered structures observed in certain areas may represent the refractory protein framework in addition to recrystallized lipid components. The extraction experiments suggest, therefore, that the dense bands observed in electron micro-



(b) (a)

FIG. 1. (a) High-resolution electron micrograph of myelin-sheath segment from a transverse section of an osmium-fixed rat sciatic nerve embedded in gelatin. The average layer spacing is 110 A. Note compact and well-preserved dense lines with moderate enhancement of the intermediate lines. ×850 000. (b) Low-angle x-ray diffraction pattern of rat sciatic nerve recorded with Finean camera. This pattern features a fundamental period of 176 A, with characteristic alternation of the intensities of the even and odd orders.

graphs of the normal myelin sheath represent areas of selective osmium deposition at lipoprotein interfaces, whereas the light bands are regions occupied by lipid chains and associated myelin components which do not react primarily with osmium tetroxide.

Enzymatic-Digestion Experiments

Although digestion with trypsin is not specific, its application to fresh nerve fibers produces characteristic modifications of the myelin fine structure.[19,24] In addition to slight expansion of the concentric layers, the uniform dense lines appear to dissociate into rod-shaped granules, 30 to 50 A wide and 40 to 60 A long (Fig. 3). The unit myelin lamellae isolated from the sheath after trypsin digestion likewise exhibit extensive dissociation into composite elongated granules of similar dimensions.[19,20] An analogous granular fine structure of the dense layers is commonly found after freezing and thawing of fresh nerve, $KMnO_4$ fixation, and other types of preparations.[24] Moreover, there are also numerous indications of a compact granular fine structure in the dense layers of the normal myelin sheath.

X-ray diffraction data furnish supporting evidence for the presence of a regular organization within the plane of the layers. A strong 60- to 70-A vector, which appears necessary to account for the relative intensities of the low-angle reflections, can be related to this type of organization within the planes of the lipoprotein layers.[24]

Nerve-Degeneration Studies

The breakdown of myelin known to occur during *in vitro* degeneration of nerve affords an opportunity to study the fine structure of the disintegrating sheath without introducing extraneous reagents. Comprehensive studies of nerve-fiber degeneration, which are now being carried out by the author with combined application of high-resolution electron microscopy and low-angle x-ray diffraction techniques, reveal interesting details of the layer structure.

When fresh peripheral nerve is enclosed in a glass capillary under aseptic conditions and left to degenerate at 20°C, low-angle x-ray diffraction patterns recorded at periodic intervals show characteristic changes

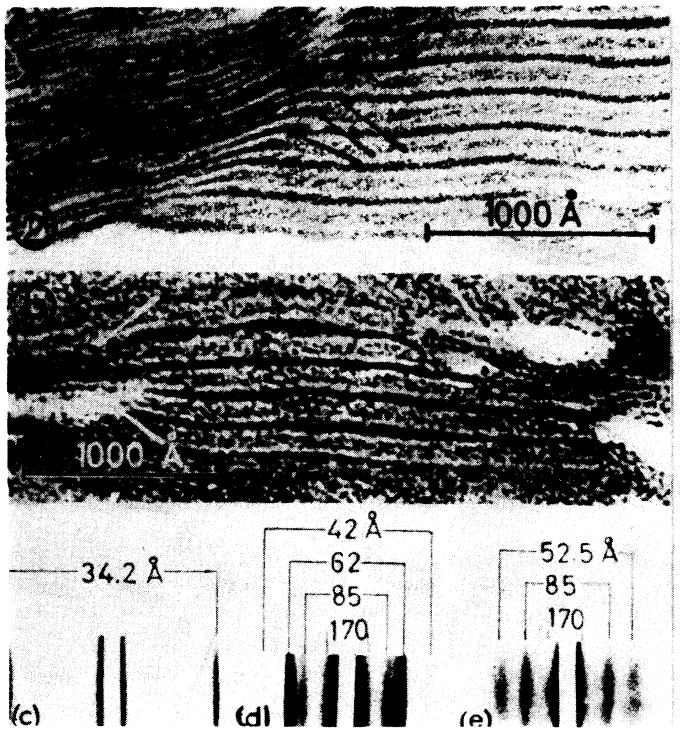


FIG. 2.

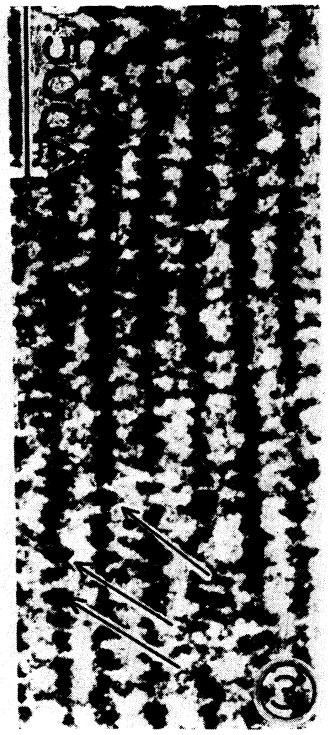


FIG. 3

FIG. 2. (a) Myelin-sheath segment from transverse section of fresh rat sciatic nerve extracted with acetone at 0°C for 12 hours prior to fixation with 2% osmium tetroxide and embedding in butyl methacrylate. Notice the expanded periods (160 A) of the modified layers at the right, and the transitions (arrows) to the collapsed period (43 A) at the left. ×300 000. (b) Myelin-sheath segment from longitudinal section of fresh rat sciatic nerve extracted with acetone at 0°C for 12 hr prior to fixation with osmium tetroxide and embedding in butyl methacrylate. Notice the transitions (arrows) from the expanded layer system to the collapsed layer system ×280 000. (c) Low-angle x-ray diffraction pattern of the lipid material extracted from fresh sciatic nerve by immersion in acetone at 0°C for 12 hours, showing the characteristic cholesterol spacing at 34.2 A. (d) Low-angle x-ray diffraction pattern of residual dried rat sciatic nerve after extraction with acetone at 0°C for 12 hours. (e) Low-angle x-ray diffraction pattern of acetone extracted nerve fixed with buffered 2% osmium tetroxide and embedded in methacrylate.

FIG. 3. Myelin-sheath segment from a rat sciatic nerve which was incubated with 1% crystalline trypsin for 12 hours prior to fixation in buffered 1% isotonic osmium-tetroxide solution and embedding in butyl methacrylate. Notice the marked dissociation of the dense lines (arrows) into elongate granules. ×520 000.

(Fig. 4). Compared with the normal pattern, marked modifications of the distribution of x-ray scattering power within the unit are noted as degeneration progresses. There is marked intensification of the second-order diffraction, followed by the appearance of a 70-A reflection, and gradual extinction of the lower orders in later stages of degeneration. The corresponding electron micrographs (Fig. 5) show various forms of granular dissociation of the dense layers, resembling the structures observed after trypsin digestion. In addition to this supporting evidence of granular structure within the layers, numerous other features of myelin organization are being uncovered as the complex dissolution process is followed at the submicroscopic level.

Suggested Molecular Organization of Myelin

Based largely on their pioneering x-ray diffraction studies, Schmitt and co-workers[12–14,30] concluded that the fundamental radial unit of the myelin sheath in the internodal portion of peripheral nerve comprised two lipoprotein layers, each of which consisted of bimolecular leaflets of 67 A sandwiched between protein layers of 25 A, with interposed water layers contributing a further 25 A. Taking into consideration the contraction of the lipid layers during drying, and the results of other experimental modifications, Finean[15–18] arrived at a more detailed picture of the molecular arrangement in the myelin unit. According to this conception, each lipoprotein layer would consist of two phospholipid-cholesterol complexes associated with a cerebroside molecule, intercalated between monolayers of protein. The two units are distinguished by a "difference factor" which has not been accurately determined, but can nevertheless be explained by the mechanism of myelin formation. As Geren[8,9] showed, the myelin sheath is formed by an infolding and multiple wrapping of the Schwann-cell membrane around the axon in embryonic fibers. Assuming that the Schwann-cell lipoprotein membrane is asymmetric, then this process of rolling

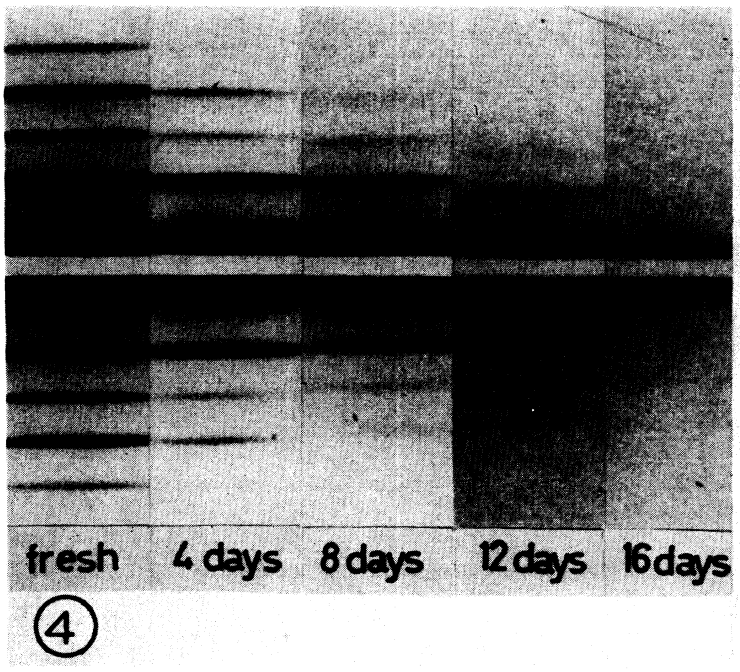


FIG. 4.

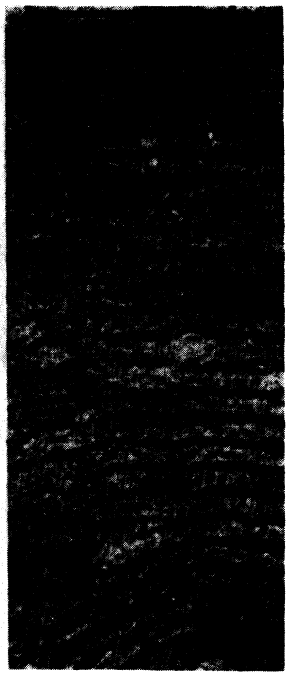


FIG. 5.

FIG. 4. Low-angle x-ray diffraction patterns of rat sciatic nerve recorded during *in vitro* degeneration at 20°C. Notice marked modifications of the distribution of x-ray scattering power within the radial unit as degeneration progresses.

FIG. 5. Electron micrograph of nerve myelin sheath during *in vitro* degeneration (4 days), showing dissociation of the dense lines into granular structures, disappearance of the intermediate line, and other modifications of the layered structure. ×280 000.

onto the axon will produce the observed symmetry difference in successive layers.[18]

The lipids are considered to be oriented with their long axes radially in the sheath. The 4.7-A, meridionally intensified ring recorded from fresh nerve has been related to the cross sections of the lipid molecules, indicating the average interchain separation of their hydrocarbon chains. However, in order to account for the permeability properties of the membranes, various possibilities including the existence of real or potential "holes" have been discussed. One of the suggested configurations envisages a continuous layer of lipids sandwiched between monolayers of protein with a fenestrated or open-network type of structure. The filtering action of the protein layer would combine with the selective solubility of the penetrating ions or molecules in the lipid layer to account for the sieve mechanism of permeability. The formation of submicroscopic pores by radial extensions of the protein layers across the lipid layers has also been considered, but no direct evidence for the existence of these discontinuities is yet available. Nevertheless, the x-ray diffraction and electron-microscope data clearly indicate that there must be a considerable degree of organization within the plane of the lipoprotein layers which remains to be investigated.

Water is one of the most important components of fresh myelin, constituting at least 35% of the myelin sheath.[12,30] Information on the precise localization of water within the myelin layers is also of great importance when considering the possible pathways of diffusion of ions and other solutes between the axon and the extracellular fluids.[28,31] The water layers at the aqueous interfaces of the fundamental repeating unit in the compact myelin are about 12 to 15 A thick.[31] Under certain experimental conditions,[17] larger amounts of water can be incorporated between the aqueous interfaces in the myelin unit. The water is presumably coordinated on the protein layer located at the aqueous surface of each bimolecular leaflet. The thickness of the water layers is largely determined by the electrical charge density at the aqueous interfaces,[31] and by the ionic strength, since water is expelled from lipid layers in the presence of ions.[14] These changes in thickness of the aqueous interfaces may be of significance in connection with ion movements in the intermembrane spaces. However, as Schmitt[31] has pointed out, the physicochemical properties, such as *p*H and ionic strength in capillary spaces of these macromolecular dimensions, would be quite different from those in bulk solutions. The effects of the electrically charged lipid groups on the structure of the hydration water in these aqueous interfaces may also induce the formation of a crystalline arrangement resembling "frozen" hydration sheaths postulated in protein molecules.[32]

Through the application of high-resolution nuclear

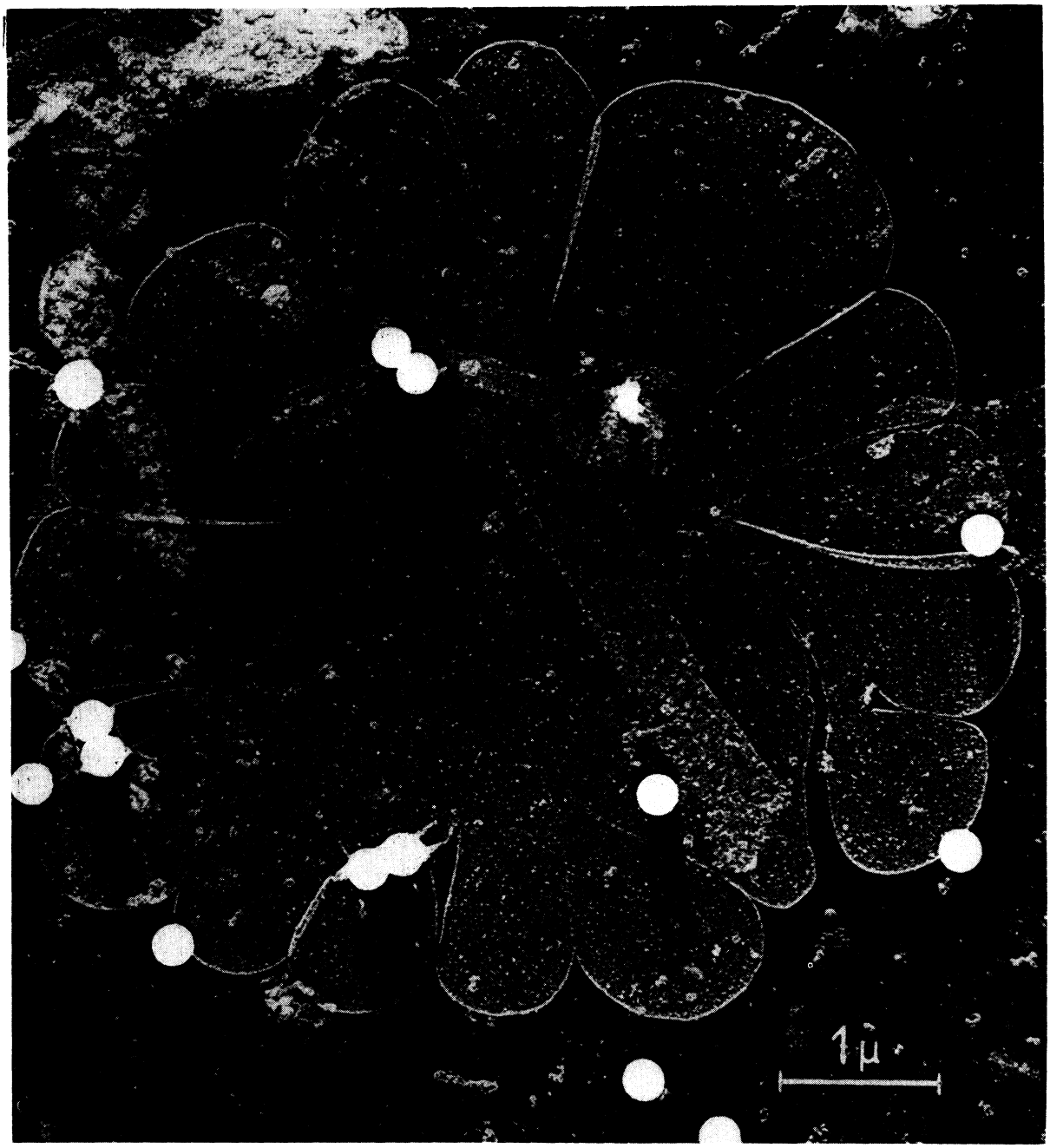


FIG. 6. Single unit disk isolated from the outer segment of frog retinal rod, showing the granular surface structure and the marginal cord. Latex particles (2800 A diam) have been added for calibration in this shadowed preparation. ×25 000.

magnetic-resonance spectrometry, it now appears possible to obtain important supplementary information on the water content of fresh whole nerve and the hydration state of its components, in a rapid and nondestructive way.[33] The size of the proton magnetic-resonance signal recorded from biological materials is proportional to the water content.[34] Since the mobility of the protons in the solid, nonaqueous constituents is much less than in the aqueous phase, the resonance spectrum consists of a narrow line owing to the water, superimposed on a broad line owing to protons in the supporting solid. After suitable calibration, the water content can be accurately determined by evaluating the line width of the proton resonance, or by measuring the amplitude of the derivative of the narrow absorption curve. Both methods have been applied in preliminary

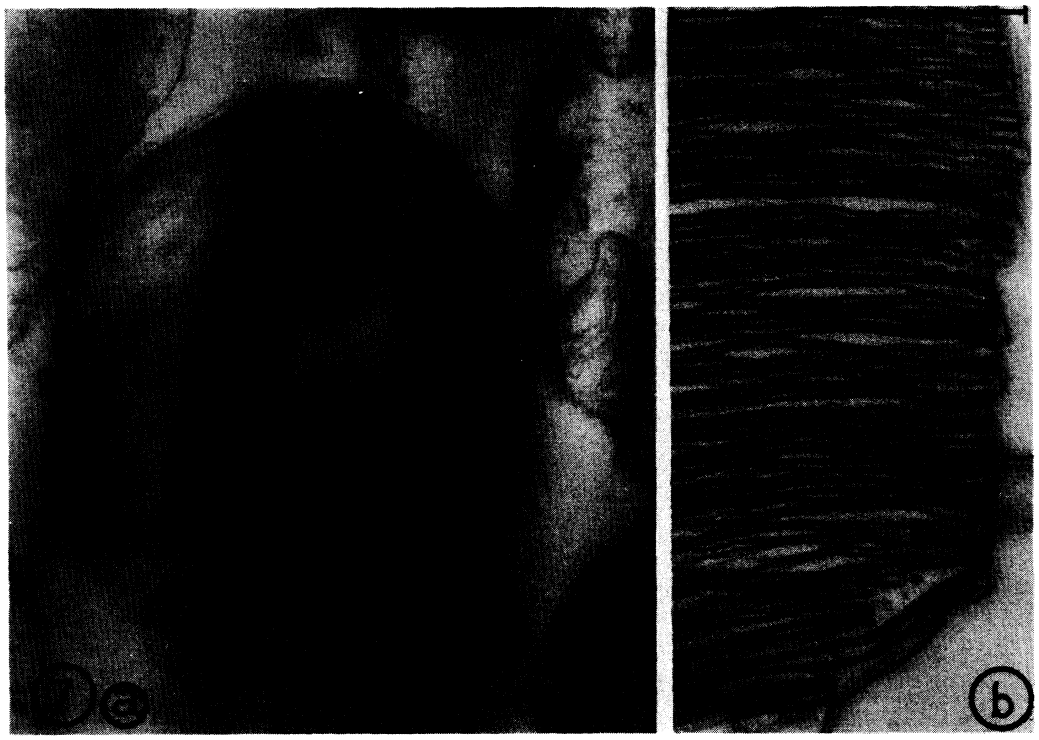


FIG. 7. (a) Transverse section through the basal region of the outer segment of a retinal rod of the guinea pig, showing the characteristic tubular structures and vesicular formations. Notice cross-sectioned filaments which establish connection with the inner rod segment. ×32 000. (b) Longitudinal ultrathin section through the basal part of the outer segment of retinal rod from guinea-pig eye showing the regular arrangement of the double membrane disks. ×48 000.

determinations of the water content of fresh nerves under various conditions, using commercially available equipment and a special transistorized NMR spectrometer.[33,35]

The protons in the hydration shells postulated around the macromolecules lack the high mobility of the water in the bulk aqueous environment, and give a broad and weak resonance line. By comparison with the strong narrow line owing to the more-mobile water protons, the proportion of water forming the hydration shells of nucleic acids has been estimated.[36] Similar experiments can be carried out on living nervous tissues, and should yield valuable information on the hydration state of the bound water in the myelin sheath.

The present concepts of the myelin's molecular architecture refer almost exclusively to the lipoprotein framework which stands up to various analytical techniques. Within this framework, no detailed localization is yet possible of the numerous enzymes, electrolytes, trace metals, and other essential components of the myelin sheath. Although deuterium and radiophosphate studies[37] indicate that myelin has a relatively low metabolic turnover, more information is required on the structural relationships with the phospholipases, phosphatases, and other enzymes involved in lipid metabolism.[38,39] Likewise, the molecular models will be incomplete until specific localizations can be assigned to the cholinesterases[40] and related enzymes which play an important role in nerve conduction. Recent investigations which link the metabolism of copper with certain demyelinating diseases[38] also focus attention on the possible relationships of trace metals with the lipoprotein layers. In view of the close association of the porphyrins[41] and related photodynamic agents with the central myelin, it may prove of interest to establish a more specific correlation with the ultrastructure of the myelin sheath.

FINE STRUCTURE OF PHOTORECEPTORS

Electron microscope studies have revealed that most of the photoreceptors in biological systems, where light is converted into chemical or electrical energy, feature a regular lamellar fine structure. In the vertebrate eye, the photoreceptor elements are composed of submicroscopic plates orderly disposed in a pile; whereas in the invertebrates, closely packed thin-walled tubules build up the light percipient constituents. The process of visual excitation is initiated by the absorption of light in the characteristic photopigments which form an integral part of all photoreceptors. In the vertebrates, the visual pigments, rhodopsin and iodopsin, are located in the outer segments of the rods and cones, respectively. Polarized-light studies[42] had already indicated that the rod outer segments consist of transversally oriented protein layers alternating with layers of longi-

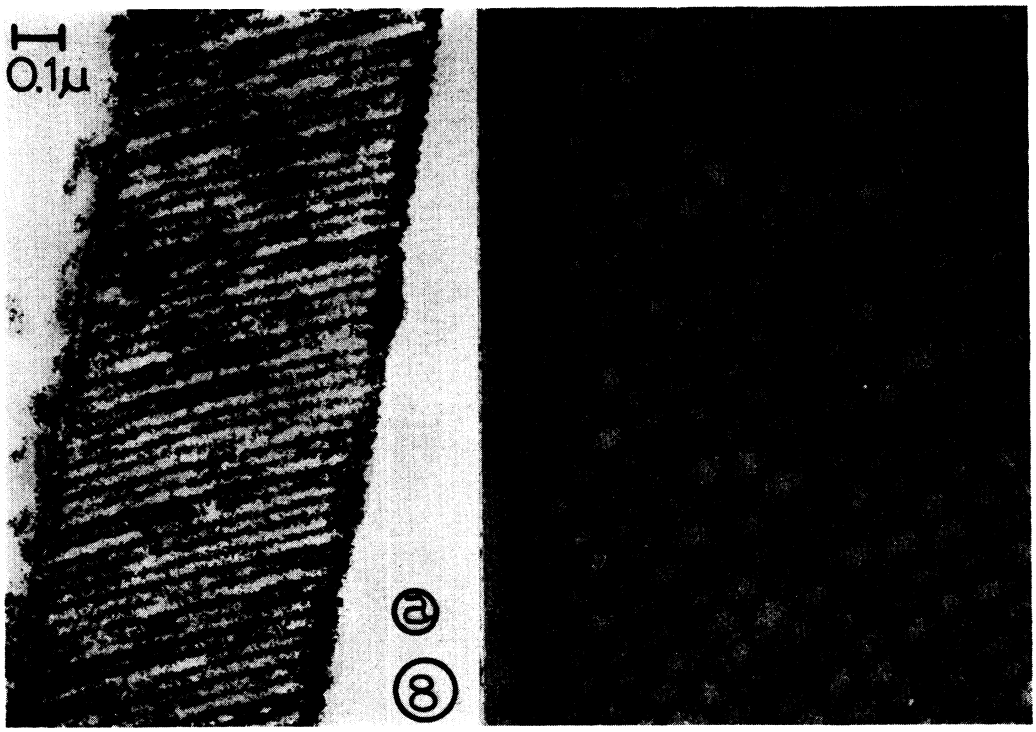


FIG. 8. (a) Oblique longitudinal section through a rhabdomere of the housefly retinula showing the lamellar type of internal structure. The highly regular submicroscopic organization is illustrated by the nearly perfect parallel array of several score dense bands occupying the entire rhabdomere. These parallel bands correspond to the dense walls of the tubular compartments sectioned longitudinally. ×60 000. (b) High-resolution electron micrograph of longitudinal ultrathin section through a rhabdomere of the housefly retinula, demonstrating the fine structure of the tubular compartments. The regular array of sharply-outlined annular profiles (400 to 500 A in diameter) bounded by a dense osmiophilic substance is interpreted as representing cross sections of the closely packed tubular compartments. This differentiation of the dense "walls" of the compartments into a thin, osmiophilic boundary line, 20 to 30 A wide, associated with a dense layer of approximately 60 A, may be of interest in relation to the postulated models of the macromolecular photoreceptor complexes. ×100 000.

tudinally oriented lipid molecules, and could, therefore, be compared with a radial cylinder of the myelin sheath. Electron microscopy confirmed that the entire rod outer segment consists of several hundred unit disks, about 150 A thick, stacked up in regular array.[21,43,44]

The unit disks of the frog retinal rods have a diameter of about 6μ and exhibit a peculiar lobulated shape with numerous incisures outlined by a dense marginal cord (Fig. 6). Thin sections disclose the remarkably regular layering of the unit disks [Fig. 7(b)], which are arranged with their planes perpendicular to the rod axis. Each disk consists of dense double membranes which stain intensely with osmium and enclose a light space of about 70 to 80 A. The repeating unit along the axis of the rod in osmium-fixed preparations corresponds closely to the spacing of about 320 A recorded in the low-angle x-ray diffraction patterns.[45] Recent studies demonstrate tubular processes at the incisures which are closely associated in the basal portion with the bundle of thin fibrils connecting the outer and inner rod segments [Fig. 7(a)]. The inner segments contain numerous densely-aggregated long mitochondria,[44,46] which are regularly encountered in all types of photoreceptors.[47,48] The retinal cones show a similar layered structure.[44]

It has been tentatively assumed that the protein membranes stain densely with osmium, while the lipid molecules may be predominantly localized in the intermembrane compartments of the disk.[44] However, knowledge of the detailed chemical composition of the photoreceptors is still too meagre, and controlled extraction and digestion studies of the type described earlier remain to be done. The exact location of the visual pigments is also unknown, but Wolken and co-workers[49] have suggested that the pigment molecules are oriented as monolayers at the aqueous protein and lipoprotein interfaces. The chloroplasts exhibit a very similar layered structure, and the chloroplastin-pigment complex appears likewise to reside in the aqueous protein-lipoprotein interfaces.

Light Receptors in Insect Compound Eyes

In the insect compound eye, each separate visual element or ommatidium consists of eight sensory retinula cells.[50] The differentiated medial part of each retinula cell, known as a rhabdomere, is built up of approximately 20 000 to 30 000 closely packed tubular

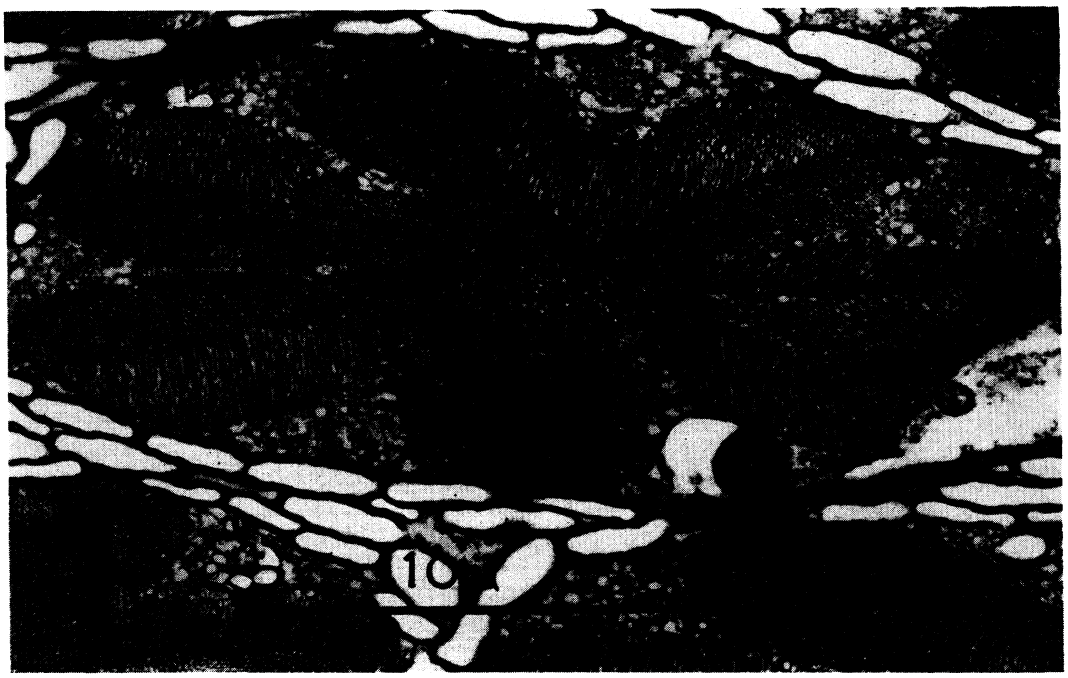


FIG. 9. Oblique cross section through a retinula from the eye of the giant tropical moth, *Erebus*, demonstrating the symmetrical arrangement of the fused rhabdomeres. Note that the general configuration and orientation of the tubular compartments of any one of the rhabdomeres is matched by the corresponding rhabdomere pattern directly or diagonally opposite to it. The equivalent rhabdomeres of contiguous ommatidia are also oriented with their main axes in the same direction, thus introducing a degree of "structural polarization" in the over-all pattern which must bear relation to the analysis of polarized light in the insect eye. ×6000.

compartments which vary from 400 to 1200 A in diameter, and are oriented in highly regular array [Fig. 8(a)] with their long axes normal to the longitudinal rhabdomere axis.[47–49,51] These tubular rhabdomere compartments are regarded as differentiated microvilli of the retinula cell membrane. The walls of the tubular compartments exhibit a dense boundary line of about 30 A in which the oriented visual pigments may possibly be located[47] [Fig. 8(b)]. Within each ommatidium, the rhabdomeres are radially arranged in a symmetrical pattern formed by matched pairs of opposite rhabdomeres displaying similar orientation of their layered structure (Fig. 9).

This differentiated submicroscopic organization of the visual elements—and their structural coupling in coordinated groups[47]—suggests a correlation with the remarkable ability of insects to recognize the regional patterns of polarization of the sky as a basis for light-compass orientation.[52–54] It is assumed that the radially arranged rhabdomere pairs containing dichroic visual pigments similarly oriented in their periodic tubular compartments may correspond to the functional units of the analyzer for polarized light postulated in the compound eye.[47–49,51,55]

The visual system of the insects and other arthropods features an intimate association with a complex three-dimensional network of air-filled tubules or tracheoles and with pigment granules, which is probably of considerable functional significance.[47]

The structural analysis of the insect compound eye is proving to be one of the most revealing examples of how the highly differentiated tubular and lamellar textures can provide a periodic array of gas-liquid-solid interfaces at the supramolecular level, which are ideally suited for selective interaction (absorption, reflection, diffraction, scattering, etc.) with incoming light signals.

RELATION OF LAMELLAR STRUCTURES TO ENERGY-TRANSFER PROCESSES

Essentially the same type of macromolecular architecture described in the unit layers of the myelin sheath and the photoreceptors is encountered with only minor modifications in the organization of the mitochondria, intracytoplasmic lamellar complexes, Golgi apparatus, and other specialized lamellar systems.[21,31,33] If this striking structural similarity is actually the expression of an underlying functional analogy, then interesting general correlations may be derived from critical collation of the available information.

Correlation of fine structure with energy-conversion processes has been most fruitful in those systems—like mitochondria and chloroplasts—which can be isolated in quantity and studied outside of the cell without appreciably impairing their functional activity. Biochemical studies[56–58] have shown that certain fragments of mitochondria are still capable of carrying out the essential functions of electron transport and oxidative phosphorylation. Based on this evidence, the mitochon-

drion has been compared with a giant polymer made up of certain repeating units incorporated in the internal and external membranes.[57] Recently, it has been possible by digitonin treatment to obtain fragments of the mitochondrial membrane which contain relatively intact "respiratory chain assemblies" including the enzymes necessary for coupling phosphorylation to electron transport.[58] These fragments contain protein, phospholipid, and a small amount of cholesterol;[58] they may possibly correspond in many respects to the double membrane components obtained by sonic fragmentation of mitochondria.[56,57] The available evidence indicates that these enzymes and related components of the electron-transfer chain are precisely arranged in specific patterns built into the matrix of the lipoprotein layers, which provide the highly ordered "floor space" where these assemblies are anchored.[57,58]

It has been suggested that the electrons are transported in this comparatively rigid structure, approximating the solid state, by being relayed through small, mobile molecules within the lipoprotein matrix.[56,57] Considering the important participation of water in all lamellar systems, it is tempting to associate the water molecules or hydrated small molecules with this transfer function.

The general problem of structural interaction between an ordered substrate and its aqueous environment appears to be of particular significance in the fluid-crystalline lamellar systems with their high water content. Many unique features of proteins in aqueous solutions suggest that the macromolecules may be surrounded by a hydration sheath of icelike character.[32,36,59] The induction of a crystalline lattice of hydration water is ascribed to the local and long-range cooperative electric-field effects of the nonpolar side chains in proteins.[32] In the case of the 10- to 15-A thick, aqueous layers intercalated between the protein-lipoprotein layers, these effects are expected to be even more pronounced in determining the formation of crystalline lamellae of "frozen" water.

On first consideration, this concept of "frozen" hydration lamellae permeating in periodic array such a multilayered complex would seem to be incompatible with the smectic fluid-crystalline character of biological systems. However, it should be borne in mind that the "icelike" hydration sheaths lodged in ultracapillary spaces of these molecular dimensions may exhibit physical and physicochemical properties which are quite different from those encountered in bulk solutions. Also, only that part of the hydration water which is in immediate contact with the protein or lipoprotein layers may be ordered in a crystalline lattice; while the remaining "liquid" aqueous strata, and the additional interstitial water channels would still allow sufficient freedom of movement of the flexible layered substrate. In this connection, it is interesting to note that the adsorbed hydration water in graphite facilitates slippage of the crystalline lamellae, and is largely responsible for the lubrication properties of this material.

It is, therefore, conceivable that the postulated crystalline hydration sheaths could provide an organized framework interconnecting the lipoprotein layers and serving primarily as a transfer medium for charged carriers like electrons, holes, protons, and ions. By virtue of the extensive hydrogen bonding in the icelike lattice, the effects of ion movements could be produced over comparatively long distances without involving bodily displacement of the ions, which would be an important factor in the relatively compact lamellar systems.

Regardless of these hypothetical assumptions, it is obvious that much more must be known about the hydration state and properties of water in lamellar systems before detailed functional correlations can be attempted. Nuclear magnetic-resonance spectrometry, supplemented by spin-echo techniques, now offers the possibility of obtaining essential quantitative data on relaxation times and correlation times of the water components in living biological systems under various physiological conditions. It is also suggested that a structural determination of the hydration shells of lattice-ordered water may eventually be possible by extending the neutron-diffraction studies already successfully carried out on ice[60] to suitable models of multilayered systems, or to liquid crystals of adequate size.

Important additional evidence that the processes of energy transfer and conversion in the chloroplasts are dependent on the highly ordered molecular arrangement in their layered structure has been furnished by comprehensive recent studies of the mechanisms of photosynthesis.[3–7,61] Calvin and co-workers[3,4,6] have strongly indicated that the initial process of energy transfer is a physical one, dependent on the specific physical organization of the chlorophyll and its associated pigments in the ordered, "quasicrystalline" layered structures. Absorption of a light quantum by chlorophyll raises an electron to a conduction band, leaving behind a "hole," both of which migrate together as an "exciton" through the oriented array of chlorophyll molecules. The conjugated carotenoid molecules act as conductors of electrons, while the lipids in the chloroplasts would function as an insulator, permitting a separation of charges when the electrons are "trapped" on one side of the layer and the "holes" on the other. This polar character is similar to that which exists at a *p-n* semiconductor junction. The electrons participate in the formation of a reducing agent leading to the reduction of carbon dioxide; the positive holes react with water to form oxygen, thus initiating the two basic reaction sequences of the photosynthetic mechanism. Direct experimental evidence has also been presented for the existence of trapped electrons and holes by recording electron spin-resonance signals from illuminated whole chloroplasts[3–6,61] at low temperatures

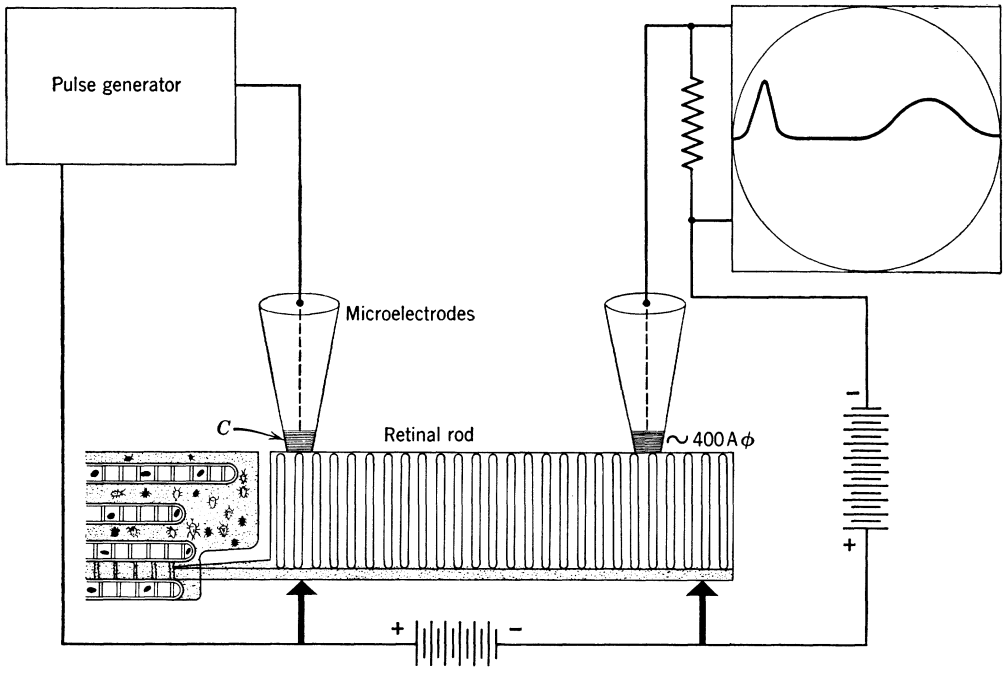


FIG. 10. Suggested experimental arrangement to investigate semiconductor phenomena associated with the mobility of electrons and holes in active biological lamellar systems. By applying microelectrodes (filled with a suitable solid free radical, "C") directly to the outer segments of retinal rods, it might be possible to determine the drift velocity and density of injected current carriers. Addition of a transverse magnetic field would then permit determination of the Hall effect and other magnetic concentration effects. Experiments of this type, carried out on photoreceptors and chloroplasts or on artificial multilayered structures containing specific photopigments, are needed to furnish direct experimental proof of the postulated semiconductor properties in biological systems.

(−150°C), and, therefore, excluding the possibility of any ordinary enzymatic process.

By tentatively extending these concepts to other lamellar systems, one arrives at a general picture of paracrystalline lipoprotein layers containing assemblies of specific enzymes associated with photopigments or specialized electron-transfer systems, all of which are organized in highly ordered patterns. These biological lamellar systems, permeated with hydration water of possible icelike character, might have many properties in common with semiconductors, although, as pointed out earlier,[21,62] the concepts of solid-state physics are not directly applicable to the fluid crystalline state. However, merely as a working hypothesis, the semiconductor analogy should prove useful as a possible basis for the physicochemical amplifying devices suggested in connection with the events leading from the primary process of sensory excitation to the complex bioelectric phenomena associated with nerve-impulse propagation.[63,64]

Although these ideas are admittedly speculative, they may, nevertheless, serve to encourage new experimental approaches. Of particular interest would be systematic investigations designed to furnish direct experimental proof in biological lamellar systems of the two processes of electronic conduction in semiconductors, corresponding to positive and negative mobile charges, which are characteristic of transistor action.[65] In the suggested experiment (Fig. 10), two capillary microelectrodes filled at the tip with a suitable solid free radical are applied directly to the outer segments of freshly isolated retinal rods, or, alternatively, to large chloroplasts. With the outlined arrangement, it might be possible to determine the drift velocity and the density of injected current carriers, in analogy to the classical experiments[65] carried out on small samples of germanium using micromanipulator techniques. By introducing a transverse magnetic field, determination of the Hall effect and other magnetic-concentration effects[65] should also be possible. Application of these techniques to simple model systems containing multilayers of organic semiconductors would probably be the first step in this type of study. The study of organic semiconductors[66,67] is just beginning now, and further developments in this field are bound to have far-reaching implications in the interpretation of biological phenomena.

Ultrastructural studies are confronted with more immediate tasks as a result of recent improvements in the preparation techniques and the useful resolving power of electron microscopy. Thus, continuation of earlier experiments based on rapid freezing of glycerine-treated tissues with liquid helium,[33] followed by special fixation and embedding procedures at low temperatures, may ultimately permit a more reliable correlation of organization at the molecular level with sequentially arrested states of activity in biological systems. Application of

moire effects[68] to directly visualize equivalent configurations of atomic dimensions may also prove to be of considerable operational value in the study of paracrystalline components.

From the foregoing cursory survey, it is evident that the correlative investigation of fine structure and function of lamellar systems poses some of the most challenging and rewarding problems in modern biology, which will require an integrated approach enlisting the best efforts of biophysicists, biochemists, and physicists.

ACKNOWLEDGMENTS

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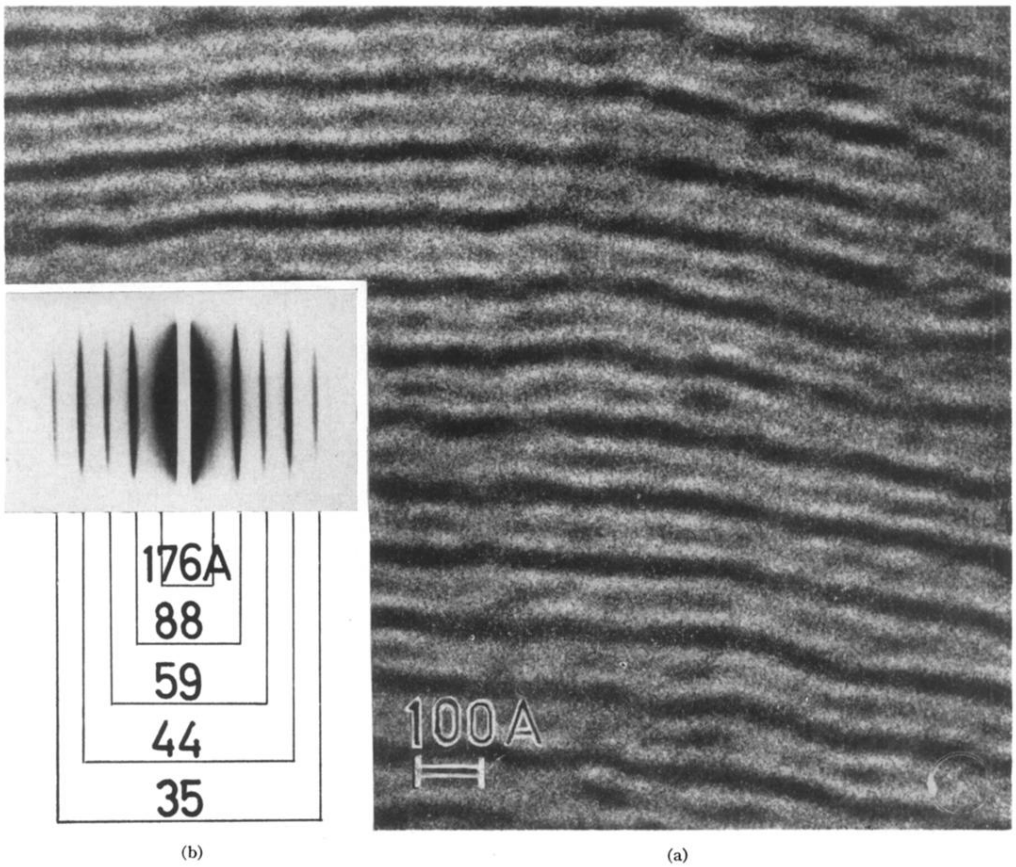

(b) (a)

FIG. 1. (a) High-resolution electron micrograph of myelin-sheath segment from a transverse section of an osmium-fixed rat sciatic nerve embedded in gelatin. The average layer spacing is 110 A. Note compact and well-preserved dense lines with moderate enhancement of the intermediate lines. ×850 000. (b) Low-angle x-ray diffraction pattern of rat sciatic nerve recorded with Finean camera. This pattern features a fundamental period of 176 A, with characteristic alternation of the intensities of the even and odd orders.

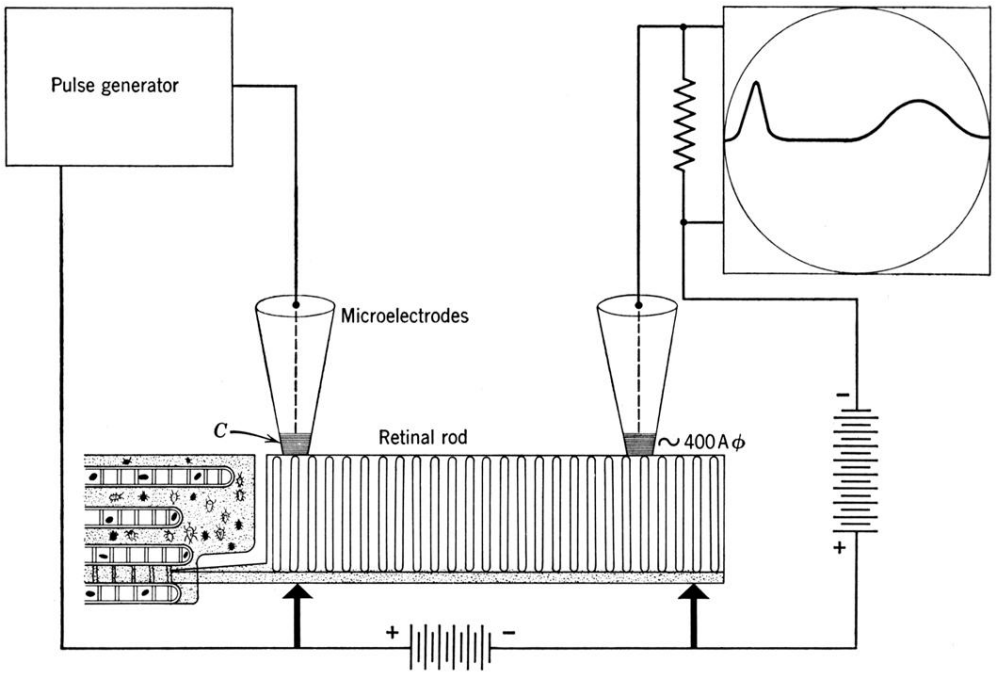

FIG. 10. Suggested experimental arrangement to investigate semiconductor phenomena associated with the mobility of electrons and holes in active biological lamellar systems. By applying microelectrodes (filled with a suitable solid free radical, "*C*") directly to the outer segments of retinal rods, it might be possible to determine the drift velocity and density of injected current carriers. Addition of a transverse magnetic field would then permit determination of the Hall effect and other magnetic concentration effects. Experiments of this type, carried out on photoreceptors and chloroplasts or on artificial multilayered structures containing specific photopigments, are needed to furnish direct experimental proof of the postulated semiconductor properties in biological systems.

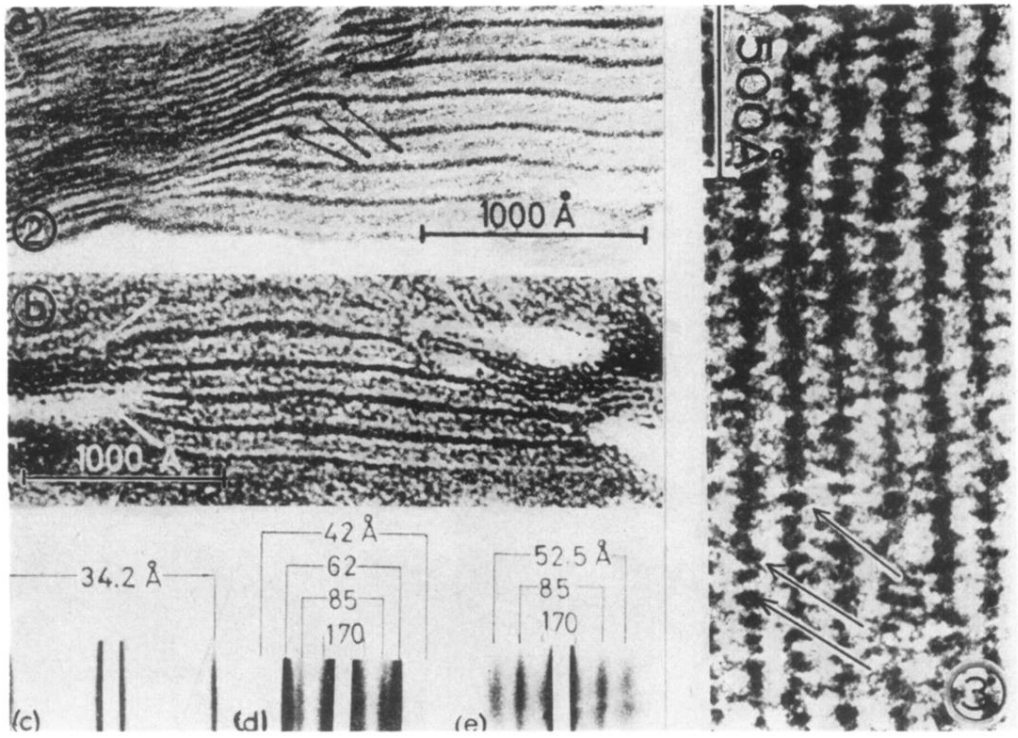

FIG. 2.

FIG. 3

FIG. 2. (a) Myelin-sheath segment from transverse section of fresh rat sciatic nerve extracted with acetone at 0°C for 12 hours prior to fixation with 2% osmium tetroxide and embedding in butyl methacrylate. Notice the expanded periods (160 A) of the modified layers at the right, and the transitions (arrows) to the collapsed period (43 A) at the left. ×300 000. (b) Myelin-sheath segment from longitudinal section of fresh rat sciatic nerve extracted with acetone at 0°C for 12 hr prior to fixation with osmium tetroxide and embedding in butyl methacrylate. Notice the transitions (arrows) from the expanded layer system to the collapsed layer system ×280 000. (c) Low-angle x-ray diffraction pattern of the lipid material extracted from fresh sciatic nerve by immersion in acetone at 0°C for 12 hours, showing the characteristic cholesterol spacing at 34.2 A. (d) Low-angle x-ray diffraction pattern of residual dried rat sciatic nerve after extraction with acetone at 0°C for 12 hours. (e) Low-angle x-ray diffraction pattern of acetone extracted nerve fixed with buffered 2% osmium tetroxide and embedded in methacrylate.

FIG. 3. Myelin-sheath segment from a rat sciatic nerve which was incubated with 1% crystalline trypsin for 12 hours prior to fixation in buffered 1% isotonic osmium-tetroxide solution and embedding in butyl methacrylate. Notice the marked dissociation of the dense lines (arrows) into elongate granules. ×520 000.

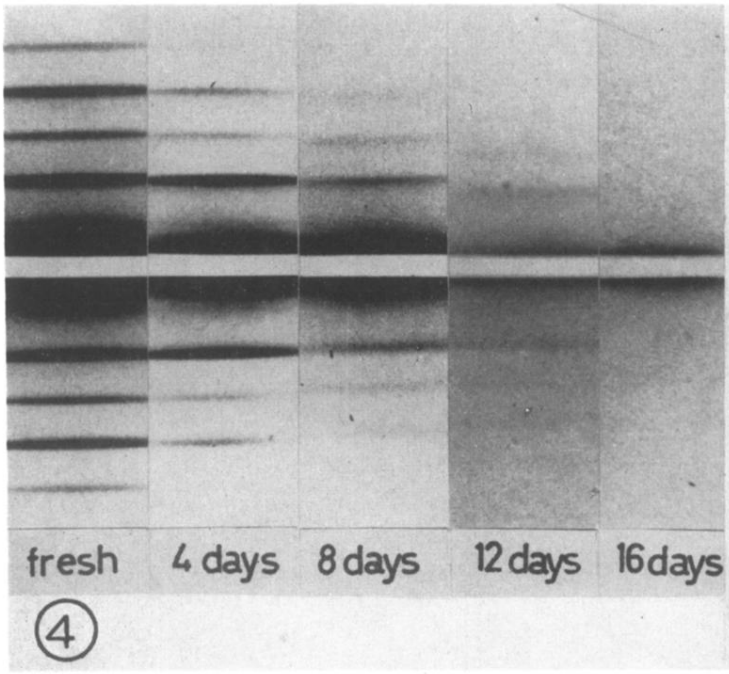

FIG. 4.

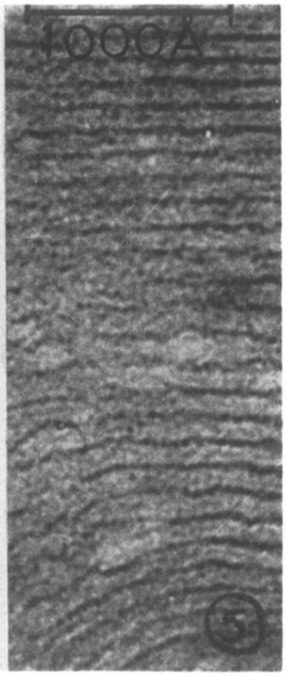

FIG. 5.

FIG. 4. Low-angle x-ray diffraction patterns of rat sciatic nerve recorded during *in vitro* degeneration at 20°C. Notice marked modifications of the distribution of x-ray scattering power within the radial unit as degeneration progresses.

FIG. 5. Electron micrograph of nerve myelin sheath during *in vitro* degeneration (4 days), showing dissociation of the dense lines into granular structures, disappearance of the intermediate line, and other modifications of the layered structure. ×280 000.

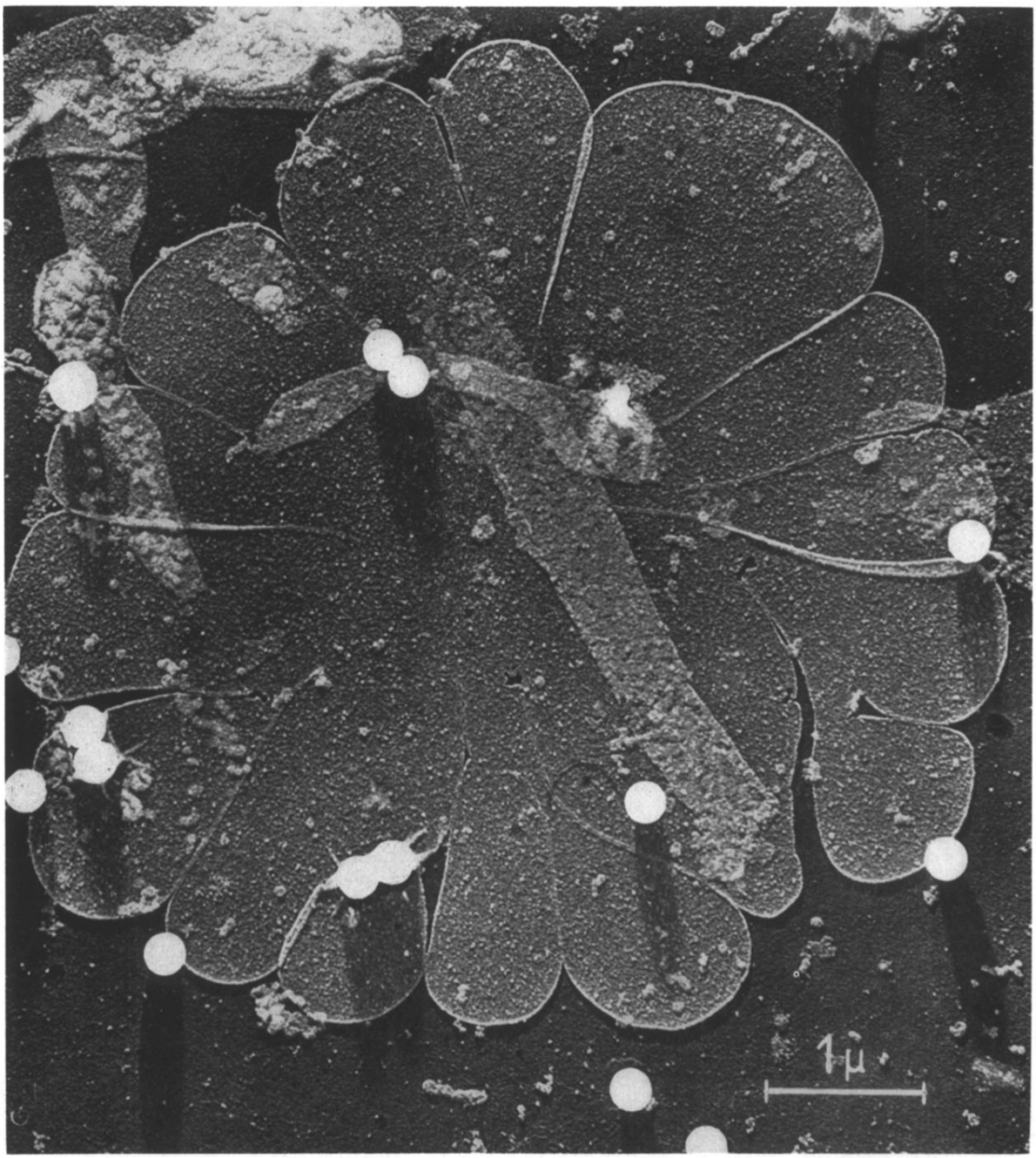

FIG. 6. Single unit disk isolated from the outer segment of frog retinal rod, showing the granular surface structure and the marginal cord. Latex particles (2800 A diam) have been added for calibration in this shadowed preparation. ×25 000.

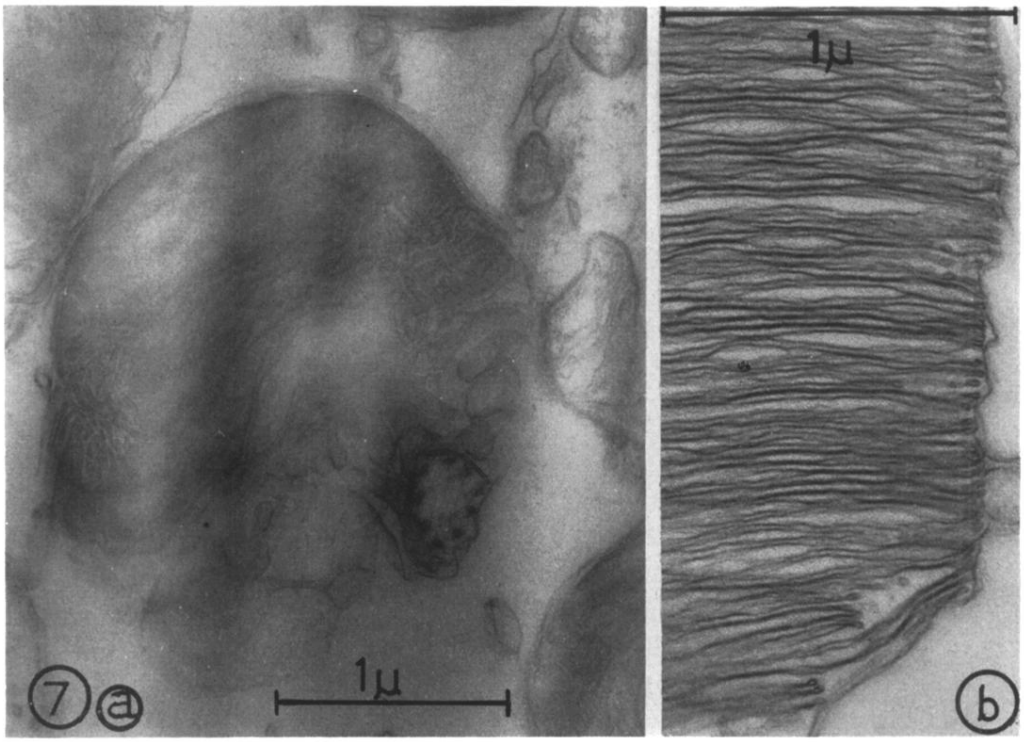

FIG. 7. (a) Transverse section through the basal region of the outer segment of a retinal rod of the guinea pig, showing the characteristic tubular structures and vesicular formations. Notice cross-sectioned filaments which establish connection with the inner rod segment. ×32 000. (b) Longitudinal ultrathin section through the basal part of the outer segment of retinal rod from guinea-pig eye showing the regular arrangement of the double membrane disks. ×48 000.

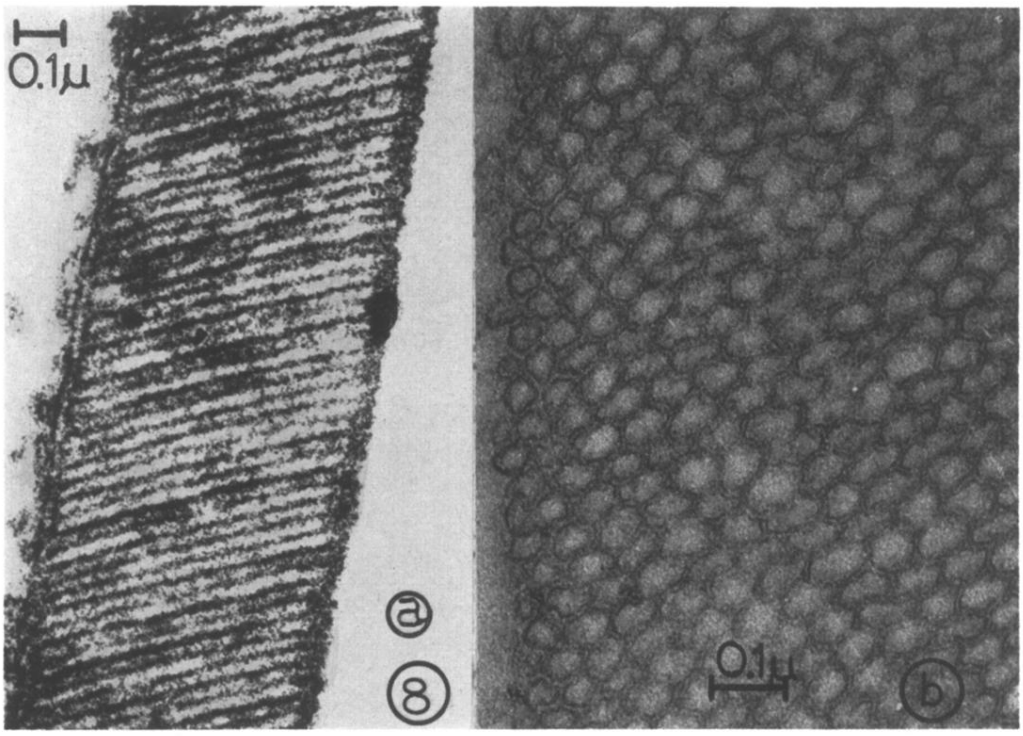

FIG. 8. (a) Oblique longitudinal section through a rhabdomere of the housefly retinula showing the lamellar type of internal structure. The highly regular submicroscopic organization is illustrated by the nearly perfect parallel array of several score dense bands occupying the entire rhabdomere. These parallel bands correspond to the dense walls of the tubular compartments sectioned longitudinally. ×60 000. (b) High-resolution electron micrograph of longitudinal ultrathin section through a rhabdomere of the housefly retinula, demonstrating the fine structure of the tubular compartments. The regular array of sharply-outlined annular profiles (400 to 500 A in diameter) bounded by a dense osmiophilic substance is interpreted as representing cross sections of the closely packed tubular compartments. This differentiation of the dense "walls" of the compartments into a thin, osmiophilic boundary line, 20 to 30 A wide, associated with a dense layer of approximately 60 A, may be of interest in relation to the postulated models of the macromolecular photoreceptor complexes. ×100 000.

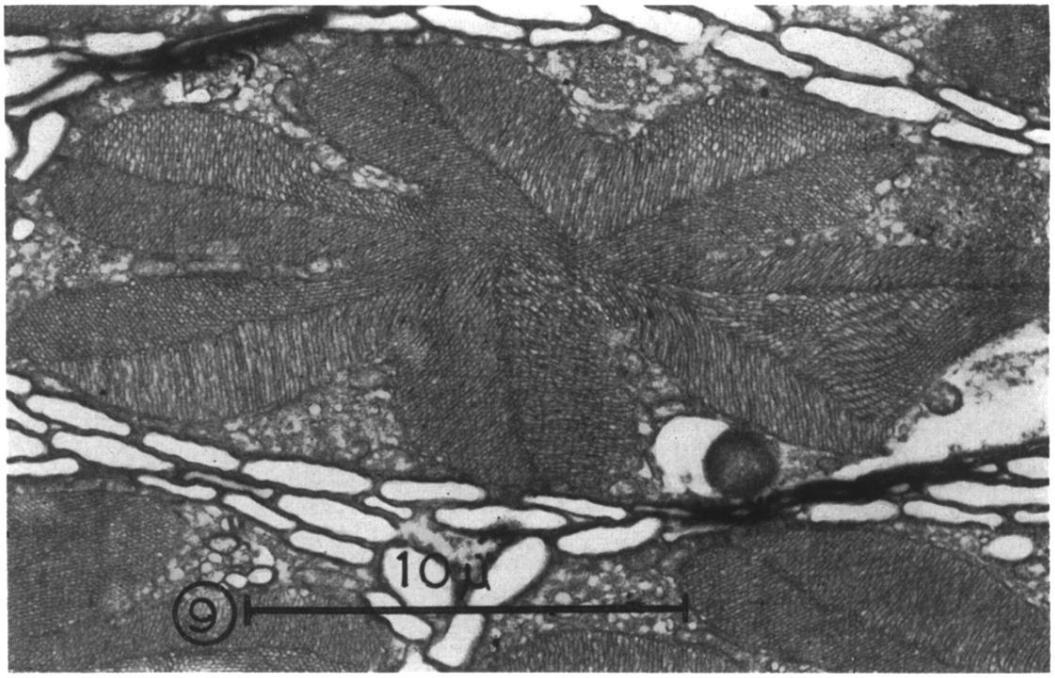

FIG. 9. Oblique cross section through a retinula from the eye of the giant tropical moth, *Erebus*, demonstrating the symmetrical arrangement of the fused rhabdomeres. Note that the general configuration and orientation of the tubular compartments of any one of the rhabdomeres is matched by the corresponding rhabdomere pattern directly or diagonally opposite to it. The equivalent rhabdomeres of contiguous ommatidia are also oriented with their main axes in the same direction, thus introducing a degree of "structural polarization" in the over-all pattern which must bear relation to the analysis of polarized light in the insect eye. ×6000.