REVIEWS OF MODERN PHYSICS VOLUME 31, NUMBER 2 APRIL, 1959

49

Molecular Organization of the Nerve Fiber*

FRANCIS O. SCHMITT

Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

IN the context of these studies in the biophysical sciences, it is the purpose of this contribution to identify some of the basic problems of nerve that may be effectively studied at the molecular level by the methods of biophysics and biophysical chemistry, and to describe certain nerve structures, such as the myelin sheath, which provide great scope for the application of such methods and which have important application also to such general biological problems as the nature of cell membranes.

The primary function of neurons is the transfer of information from one region to another, the message being propagated along the fiber from cell body to axon terminals by means of a local disturbance or propagated bioelectric impulse. To many, particularly to those studying the activity of the larger nerve units in the processing of information by the central nervous system or in the behavior of the organism as a whole, the individual neuron is but the structural and functional unit in a highly complex network whose basic function involves the all-or-nothing, on-or-off responses of the units. Thus, in neurophysiology, as well as in bioelectrical studies generally, the most elementary problem which could be posed to the molecular biologist or biophysicist would doubtless be the elucidation of the mechanism by which the impulse is propagated along nerve fibers—i.e., the nature of the changes in the properties of the surface film of the axon that underlie excitation and propagation of the action wave.

If this were in fact the only problem to be dealt with, the present contribution would be very short indeed. Little is known about the molecular composition of the axon surface membrane and even less is known about changes that occur in it during propagation of the impulse. However, for several reasons, I do not wish to take such a limited point of view. In the first place, the axon surface membrane does not exist as a separate entity. It is but part of the neuron which, as a complex reacting system, contains many constituents. Even if one were interested only in bioelectric processes, it would be impossible to understand the phenomena going on in the surface membrane without reference to the structural and biochemical properties of the fiber as a whole. Moreover, nerve performs many functions other than that of propagating impulses. Some of these functions are known, some can be guessed, while others are completely unknown.

For example, somewhere in the neuron there are synthesized certain highly active substances, such as acetyl choline and adrenergic substances, which have transmitter functions; i.e., they transmit the impulse across the junction from the nerve fiber to the muscle fiber or other end organ. The site of synthesis of this material in the neuron, its mode of transfer from this site to the nerve endings (possibly enclosed there in vesicles) and its transmission to the innervated tissue are at present very poorly understood. Certain neurons also produce neurosecretions that can be identified cytologically and histochemically and can be demonstrated to pass from the nerve cell body down the axon to the axon terminals. Possibly other hormonal substances are similarly produced and transmitted, but have thus far escaped detection.

Neurons also manifest certain properties, especially during embryogenesis or regeneration, that are highly specific. Thus, each motor fiber grows out and eventually innervates its appropriate muscle. This innervation is not only highly specific with respect to the muscle fiber which is "sought out" and innervated, but also it is necessary for the stability and maintenance of the normal properties of the muscle fiber. Cutting the nerve fiber to a muscle may cause the latter to change its physiological and pharmacological properties markedly, or even to degenerate.

Finally, it is probable that certain aspects of nerve function may involve chiefly the nerve cell or axoplasm without obvious relationship to the axon surface film. Such processes would probably not lend themselves to study or detection by bioelectric methods. For example, as is discussed in more detail below, certain constituents, such as the fibrous protein, seem to be present in all neurons and to represent one of the characteristic features of neurons generally. However, there is still not the foggiest notion as to the biochemical and physiological function of this protein or protein complex.

It is desirable, therefore, that the biophysical and biochemical investigation of the neuron be not limited to bioelectric processes, but be systematic and exhaustive so as to reveal "what is there," particularly at the molecular and macromolecular level. One has only to consider the history of other fundamental biological problems, such as that of muscle contraction, to realize the importance of such a systematic biochemical and biophysical approach. Only when significant advances in knowledge of the composition and molecular organi-

* These studies were aided by a research grant (B-24) from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare; by a contract between the Office of Naval Research, U. S. Department of the Navy, and the Massachusetts Institute of Technology (NR 101-100); and by grants from the Trustees under the wills of Charles A. King and Marjorie King, and from Lou and Genie Marron.

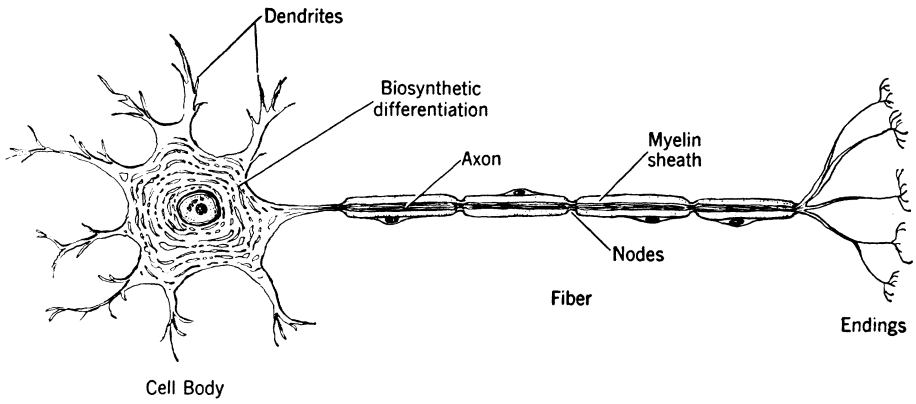

FIG. 1. Diagrammatic representation of a myelinated nerve fiber. Note cell body with dendrites and cytoplasmic organelles, axon, myelin sheath with Schwann cells and nodes of Ranvier, and terminal twigs making synaptic connection with peripheral tissue.

zation of muscle and its fibrous proteins were obtained, could the fundamental problem of the molecular mechanism of contractility be fruitfully attacked. By comparison with muscle, despite much research extending over a century, knowledge of the composition and molecular organization of nerve is still primitive. Moreover, impulse propagation in the nerve fiber is a subtle phenomenon which, unlike contractility in muscle, is not revealed in structural alterations thus far demonstrable even in the electron microscope.

The investigation of the molecular biology of nerve is, therefore, very challenging, particularly to young biophysical scientists not yet steeped in traditional concepts of neurophysiology. This article, which is necessarily limited in space, is meant to be provocative rather than exhaustive in its treatment of basic matter. For more extensive information, the reader is referred to reviews by Schmitt.[1–3]

I. NEURON

The unit of the nervous system, the neuron, consists of a nerve cell body with its dendritic arborization and synaptic connections with the terminal twigs of other neurons; of an axon which is the thin, cylindrical outgrowth of the nerve cell; and of the endings or terminal twigs which make apposition with the innervated cell or tissue through the synapse (see Fig. 1). The neuron has physiological as well as morphological polarization. The impulse passes from presynaptic fiber to nerve cell, down the axon and across the terminal synaptic membrane to the innervated tissue, but not in the reverse direction. For present purposes, it is convenient to consider the cell body, axon, and endings separately.

(A) The Cell Body

The nerve cell body has the task of spinning out the axon in development and of regenerating it in the event of injury. Since the volume of axoplasm to be biosynthesized in a long fiber may be hundreds and, in some cases, thousands of times that of the cell body, it is not unexpected that the metabolism of the nerve cell is very high. To accomplish its impressive task of biosynthesis, the cell is equipped with the cytological differentiation found by electron microscopists characteristically in highly active, secreting gland cells. Particularly prominent are the membrane-limited structures (endoplasmic reticulum, ergastoplasm) richly studded with RNA-rich ribosomes (see Palay[4]). These basophilic structures are identical with the Nissl substance, which has long been studied by cytological methods and by microabsorption spectroscopy and which is known to reflect the synthetic activity of the nerve cell. With improved spectrophotometric methods, such as those developed by Hydén, it has been possible to follow quantitatively the changes in RNA, protein, lipid, and dry weight of regenerating neurons and to correlate these changes with the peripheral growth of the axon (see Brattgård *et al.*[5]). Despite the increase in protein, lipids, and water during the axon outgrowth, the amount of RNA appears to remain constant. However, changes occur in the state of aggregation of RNA; small, finely dispersed particles are formed in the process of "chromatolysis," long known to occur during regeneration. When contact is re-established between the outgrowing axon and the peripheral innervated cell, there is characteristically an increase of RNA and of cell volume until the original values are restored. According to these authors, the only type of cell that can compete with the nerve cell in biosynthetic activity and with the degree of cytological differentiation for such processes, particularly that involving the RNA system, is the exocrine cell of the pancreas of small animals. The neuron is visualized as "an enormous gland cell structure whose lively protein metabolism serves the specific nerve function."[5]

The high metabolic activity of nerve cells (as distinguished from nerve fibers), long known to be characteristic also of normal, unregenerating cells (as in the brain), may be owing in part to the constant synthesis of axoplasm which—according to Weiss and Hiscoe[6] and supported recently by Waelsch[7] and by Ochs and Burger[8]—passes continuously, during the life of the neuron, as a plastic, gelled mass down the axon at a slow rate (*ca* 1 mm/day). The manner in which axoplasm disappears peripherally, e.g., by oxidation or

other metabolic process, is not known. If nerve cells are in fact constantly synthesizing axoplasm, it becomes important to establish the purpose served by such a metabolically expensive process. It is possible that one such purpose may be the maintenance of the turgor of the axon over its long extent to the endings. One constituent that would also be constantly synthesized in such a process is the fibrous protein of the axon. When the function of this protein is discovered, light may be shed also on the apparent necessity for its constant synthesis.

The metabolism and the cytological differentiation of the nerve cell are responsive not only to the need for active biosynthesis, as in the regeneration of a severed axon, but also to physiological activity of the neuron. This apparent energy dissipation is in contrast to the highly conservative nature of the process of impulse propagation in the peripheral fiber.

The evidence for the highly developed membrane-limited structures in the cytoplasm of living nerve cells and for the presence of fibrous protein oriented with long axes parallel to the distinguishing directions of the nerve cell is not merely cytological and electron microscopical, but derives also from polarization-optical studies of fresh nerve cells.[9]

(B) Axoplasm and the Axon

As a differentiated type of nerve-cell cytoplasm, axoplasm is a highly complex system. Noteworthy is the low absorption at about 2600 A which characterizes the region peripheral to the axon hillock, roughly at the junction of the nerve-cell cytoplasm with the axon. This is in agreement with the relative scarcity in the axoplasm of ribosomes, as seen in the electron microscope, and of basophilic material, as seen cytologically. Membranous material, possibly consisting chiefly of fragments of the membrane-rich cytoplasmic system, have been observed in squid axoplasm in this laboratory by Maxfield[10] during the process of protein fractionation and physicochemical analysis of axon proteins. Peripheral axoplasm is low in materials absorbing at 2600 A (see von Muralt[11]), consistent with the apparent lack of substantial biosynthesis in this region of the neuron.

Among the particulates commonly present in cytoplasm, only the mitochondria of axoplasm have been subjected to special study. These have properties similar to those isolated from other tissues (Foster[12]). In thin sections of lobster fibers, they have been observed to occur preferentially near the Schwann-cell surface, suggesting the possibility that metabolic energy utilization may occur in this region (Geren and Schmitt[13]).

Although axoplasm is a highly complex, specially differentiated form of nerve-cell cytoplasm, it is highly desirable that the main features of its composition and macromolecular organization be established, not merely for the light it might throw upon the energy coupling involved in active ion transport and in other processes related to impulse propagation, but also as clues to the discovery of other processes carried out by nerves, possibly unrelated to, and not predictable from, those which may be studied by electrical methods.

For such studies of axoplasm, the squid giant fiber is uniquely suited. Axoplasm, relatively uncontaminated by sheath constitutents and other nonaxonic material, can be readily obtained from these fibers. From axoplasm obtained from *Loligo pealii*, the New England squid, analyses have demonstrated (Koéchlin[14]) the presence of previously unknown substances, particularly isethionic acid, which turns out to be the major anion in the acid-base balance of the squid nerve fiber. The fibrous protein of axoplasm has also been studied in this material (Maxfield,[10] Maxfield and Hartley,[15] Schmitt[16]).

More recently, the large squid, *Dosidicus gigas*, that abound in the waters of the Humboldt current off the western coast of South America, have been utilized for chemical investigations. Deffner and Hafter[17] have demonstrated the presence in this axoplasm of some fifteen free amino acids, as well as cysteic amide (a sulfonate closely related to isethionic acid and taurine, which are also present in relative abundance) and other low molecular weight constituents (see Table I), including some five peptides whose composition is now being investigated and whose function is not known. Using high-capacity methods of electrophoretic fractionation and large amounts (10 to 20 g) of freeze-dried axoplasm, these substances are being isolated in quantities sufficient to permit determination of their composition and biological properties.

Among the macromolecular constituents of axoplasm, the fibrous protein is of special interest. Polarization-optical analysis (Bear *et al.*[18]) demonstrated (1) that the fibrous protein exists in the normal fresh axon where it is oriented with long axes parallel to the fiber axis, (2) that the intrinsic birefringence of the protein macromolecules is similar in magnitude to that of other fibrous proteins, and (3) that the partial volume occupied by the oriented fibrous protein is probably relatively small. Electron-microscopic observations[19,20] revealed that the fibrous protein occurs characteristically as thin (*ca* 100 to 200 A) filaments of indefinite length and with no demonstrable axial discontinuities or periodic structure. P. F. Davison and E. W. Taylor in this laboratory have made recent physicochemical studies (unpublished) on fresh axoplasm of *Dosidicus* shipped iced from Chile to Cambridge. From these, it appears that the fibrous protein may have a molecular weight in the order of millions and may be very long and thin. However, the possibility cannot be excluded that the protein as it occurs in the axoplasm so far available (i.e., suspended in cold saline and studied some days after removal from the squid) may in fact be a high polymer, of which the monomer may have a length of the order of 2500 A. In addition, the material which has so far been isolated may represent a complex of two or more proteins. Only when this protein or protein complex has been isolated and characterized chemically and structurally will it be

TABLE I. Dialyzable substances in squid axoplasm. Expressed as micromoles per gram dry weight.

Substance	Reaction at physiological pH (~6.7) Acidic Loligo	Acidic Dosidicus	Neutral Loligo	Neutral Dosidicus	Basic Loligo	Basic Dosidicus
Aspartic acid	73.0	74.8				
Glutamic acid	19.6	26.2				
Glycine			10.7	10.4		
Alanine			7.8	9.3		
Serine			3.7	1.0		
Leucine and isoleucine			2.7	0.2		
Valine			2.2	0.5		
Threonine			1.9	0.3		
Proline			1.0	0.1		
Tyrosine			0.7	0.3		
Phenylalanine			0.6	0.15		
Methionine			0.4	0.15		
Arginine					3.2	4.1
Lysine					2.4	0.2
Ornithine					1.8	0.3
Isethionic acid	220.0[a]					
Taurine			98.0	31.0		
Cysteic acid amide			4.4	0.4		
Glycocol, betaine			+	+		
Glycerol			+	+		
Homarine			17.1	18.6		
Peptides			+	+		
Fumaric acid						
Succinic acid	15.0[a]					
K					344.0[a]	+
Na					65.0[a]	+
Ca					7.0[a]	+
Mg					20.0[a]	+
PO_4	16.6[a]	16.1				
SO_4	+	+				
Cl	140.0[a]	+				

[a] Values taken from Koéchlin.[14]

possible to determine its biological role. Meanwhile, as it is purified and fractionated, it will be subjected to screening tests intended to provide clues regarding its biological activity.

(C) The Axon Surface Membrane

Electron microscopy permits one to visualize directly the axon surface membrane, though admittedly this is possible only in fibers which have been fixed, embedded, and cut into ultrathin sections. This membrane, or some component of it, presumably corresponds to the irritable membrane whose physical properties before, during, and after the passage of the nerve impulse have been studied by electrophysiologists. Some of the functions that must be subserved by membrane constituents are shown schematically in Fig. 2.

At high resolution, the membrane appears as a double-edged structure with two dark edges separated by a less dense area (see Robertson[21,22]). The total thickness is about 60 to 70 A, the dark and light lines having about equal thickness. The apparent thickness of the dense regions in the membrane may be reduced as methods are improved. A satisfactory interpretation of this structure, in terms of the lipid and protein components of the membrane, must await a detailed study of model systems of lipids and lipid-protein systems. The consensus at present seems to be that the dark regions represent the aqueous, protein-containing interfaces. The less dense regions represent the lipid phase with its preponderant aliphatic hydrocarbon chains. This point of view is not shared by all. Stoeckenius[23,24] believes that the osmium-tetroxide deposits electron-dense material in the region of the unsaturated double bonds of the lipids rather than at the protein, aqueous interface.

Whatever may be the eventual interpretation of the structure of the axon surface membrane, it is perhaps safe to say that the membrane includes a bimolecular

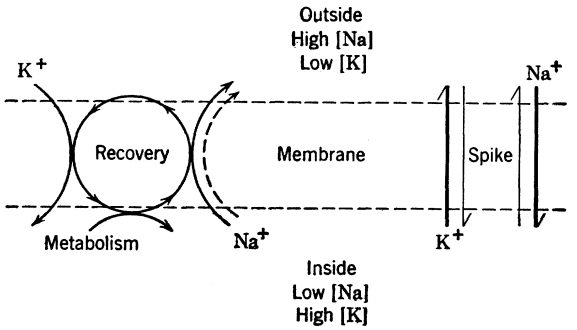

FIG. 2. Diagrammatic illustration of ion movements through the nerve membrane [after A. L. Hodgkin and R. D. Keynes, J. Physiol. 128, 28 (1955)]. Ion movements occurring during the action wave are shown at the right; active transport, involving coupling of metabolic energy, is shown on the left. The excitable membrane pictured is presumably identical with the limiting surface membrane of the axon, or some portion thereof.

leaflet of mixed lipids and at least a monolayer of protein or other hydrophilic, osmiophilic material on either side of the lipid layer. This represents the "unit" membrane structure, thought by many competent electron microscopists to be characteristic of biological membranes generally. In addition to this structure, which might be thought of as the "floor space" of the cellular factory, one supposes that there are mounted upon or within this membrane the enzymes responsible for the special molecular and biochemical processes which underlie the rapid change in cation permeability at the time of impulse propagation and the "pumping" of sodium ions from axoplasm against an activity gradient into extracellular space. Obviously, much research is required before the details of the molecular organization of the membrane can be established. Meanwhile, in describing the membrane structure, it is perhaps desirable to avoid terms such as "triple" membrane (so-called because of the two dense and one less dense regions); this might cause investigators of the nature of the true "physiological" membrane (i.e., that deduced from indirect, chiefly electrical, methods) to identify one or another component of the fixation artifact seen in the electron microscope as a self-sufficient entity, ignoring the fact that the membrane must almost certainly be considered as a complex system of interacting, interdependent components. Precisely in such situations are the methods of molecular biology different from those of descriptive morphology.

(D) Nerve Endings and the Synapse

At axon endings, electron microscopy reveals an accumulation of particulates, particularly mitochondria. Also characteristic is the presence of small (*ca* 200 to 500 A) vesicles, appearing sometimes in large quantity. It has been suggested that these vesicles contain acetyl choline, adrenalin-like materials, and other physiologically and pharmacologically active substances such as histamine and serotonin. If transmitter substances occur in packets and if such packets were presented at the end organ, perhaps as a secretion across the membrane, certain electrophysiological studies of synaptic potentials might thus find explanation (Katz, p. 524). At present, the origin of the vesicles is still unknown, as are the details of the mechanism by which they or their contents find their way into the endings.

Of considerable theoretical significance is the problem of the nature of the relationship between the limiting membranes of the nerve terminals and of the muscle or other innervated cell. If, as current consensus indicates, there is a space of at least a few hundred Ångström units between these membranes, the problem of the mechanism whereby transmitter substances pass from neuron to innervated tissue is a different one than if the two membranes were strictly in molecular contact. This is still a matter for discussion. The factors involved in determining membrane separation by electron microscopy are considered below in more detail.

II. SATELLITE-CELL DIFFERENTIATIONS AND RELATIONSHIP TO THE AXON

Extending to the farthest reaches of the body, portions of the nerve axon may be very remote from the metabolic center near the nucleus of the cell body. This would constitute an inherently very unstable system. However, the axon is probably seldom actually remote from metabolic centers because it is everywhere surrounded by thin satellite cells, the Schwann cells, which are themselves metabolically active. These cells may well have intimate biochemical relationship with the axon. Such cell-to-cell biochemical relationship and interdependence have in fact been demonstrated *in vitro*. Puck,[25] for example, has shown that a monolayer of cells of a certain type, which are themselves unable to obtain all of their nutritional requirements from the medium, can be stabilized by depositing upon them a layer of cells which can provide or utilize the required factor. These act as "feeder" cells to the underlying cells and constitute a stable system. It seems possible that the evolutionary advance to the higher organisms, with their extensive nervous system, depended upon the development of the process whereby satellite cells come to envelop outgrowing axons, thus permitting the fibers to extend to the extremities of the body as stable neurons.

The satellite cells may function in relation to the axon in other ways also. In any homogeneous group of nerve fibers, the velocity of impulse propagation is a direct function of the diameter of the fiber. In invertebrate fibers, to achieve relatively high velocity of propagation (30 to 40 m/sec), the axon diameter becomes relatively enormous (0.5 to 1.0 mm in the squid). However, in myelinated fibers in which the axon is covered to some 99% by myelin, impulse-propagation velocity may be high (50 to 100 m/sec) in fibers of modest diameter (10 to 20 μ). In these cases, impulse propagation is believed to be saltatory in nature rather than continuous; i.e., the membrane changes responsible for excitation occur in very restricted regions (at the nodes of Ranvier), and internodal segments respond as entire units. The investigation of the molecular organization of the myelin, which acts as internodal insulation, and of its mode of formation by the Schwann cell, present a most rewarding opportunity for the methods in the molecular biology of nerve.

(A) Myelin

From polarization-optical analyses, it was shown by Schmidt[26] that the lipid molecules in the myelin sheath are oriented with their paraffin chains extending radially, whereas the protein components are oriented with their distinguishing directions perpendicular to this direction, i.e., tangential. The lipid-protein layers must be thin with respect to the wavelength of light, constituting a Wiener mixed body.[27]

X-ray diffraction studies supported this view and

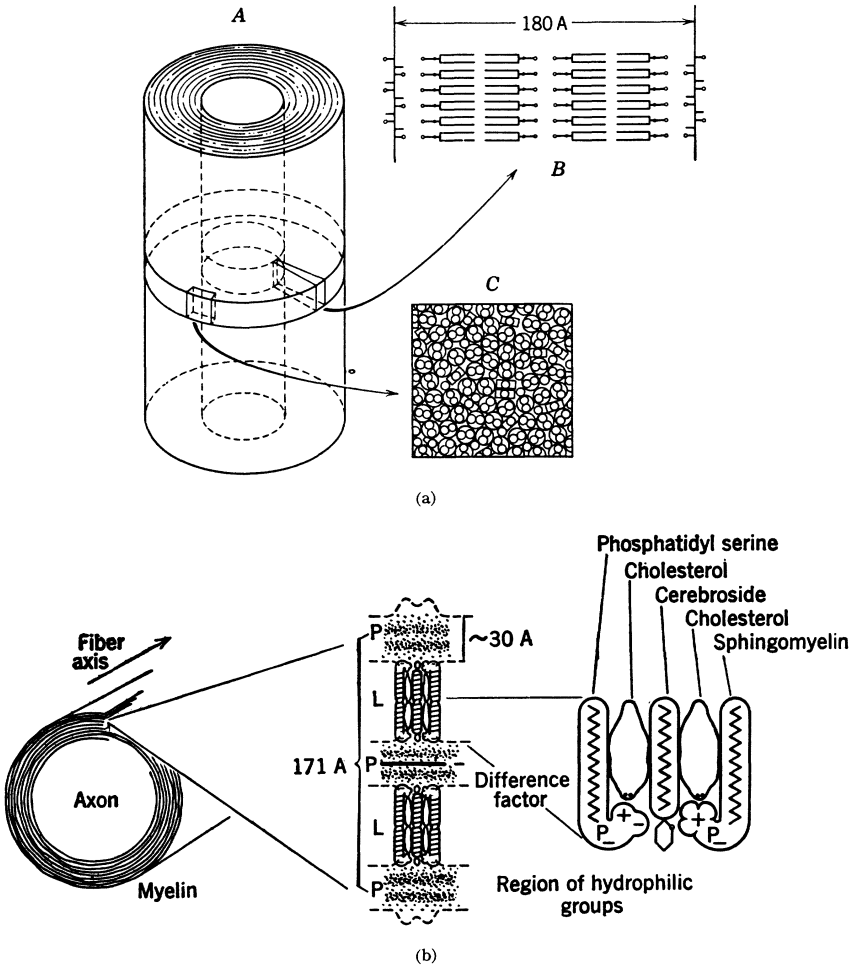

FIG. 3. (a) Schematic representation of molecular organization of the nerve myelin sheath (after Schmitt, Bear, and Palmer[28]). *A*, concentric lipid-protein layered structure deduced from polarization optics and from small-angle x-ray diffraction; *B*, unit lipid-protein structure in the radial direction (perpendicular to the planes of the layers); *C*, section through the molecular chains in the planes of the layers. (b) Diagram summarizing molecular organization of nerve myelin from x-ray diffraction data, as suggested by Finean.[34]

small-angle diffraction showed that the lipid-protein layers have a characteristic thickness of about 171 A in cold-blooded animals and about 185 to 190 A in warm-blooded animals (Schmitt *et al.*[28]). On the basis of extensive studies of the individual lipid types both dry and in aqueous systems, these authors showed that the identity period in the radial direction must include two bimolecular leaflets of mixed lipids, and that the protein and water components must occupy approximately 25 A each. Several alternative models involving bimolecular leaflets flanked by monolayers of protein were proposed (see Fig. 3). It was pointed out[29] that nerve myelin, being susceptible to x-ray diffraction analysis and having a lipid-protein architecture similar to that of cell membranes, might provide a model for the study of such membranes which, because of their thin paucimolecular nature, could not be similarly studied.

The concentric-layered structure of myelin was confirmed by direct visualization in the electron microscope (see Fernández-Morán[30] and Sjöstrand[31]) after development of the technique of thin sectioning for electron microscopy. The thickness of the layers was somewhat less than that demonstrated by x-ray diffraction for the fresh fibers. This difference was shown to be owing to the fixation, dehydration, and embedding required in the electron-optical technique; nerves were subjected to diffraction analysis after each step in the preparative technique, and the thickness of the layers was determined by electron-microscopic examination of thin sections of the same specimens.[32–34]

After the main features of myelin architecture had been determined, the question arose as to how such a structure is produced by the Schwann cell, in collaboration with the axon. A clear-cut answer to this question was provided by Geren,[35,36] at least in the case of peripheral fibers. From observations of embryonic sciatic nerves in chick embryos,[37] Geren proposed her membrane theory which is schematically illustrated in Fig. 4. According to this ingenious theory, now verified by the observations of many investigators (e.g., Robertson[38]), the Schwann cells, after enveloping the outgrowing axons and causing their respective surface membranes to adhere firmly, wrap themselves many times around the axon by a continuous infolding of the outer Schwann-cell surface membrane. The mechanism of myelination in the central nervous system is less clear. Although

various authors have seen the myelin layers as a spiral (in transverse section), as in peripheral fibers, others (Luse[39] and DeRobertis *et al.*[40]) have obtained evidence which they interpret as indicating that lipid-protein layers are formed in the cytoplasm of the oligodendrocyte, perhaps by the fusion of vesicles 200 to 800 A in diameter, which condense into the compact myelin layers. The myelin layers are hence not concentric but are spirally wrapped about the axon. After many wrappings (or few, depending upon the fiber type) the Schwann-cell cytoplasm is pressed from among the layers which become condensed into the smectic, paracrystalline material of the finished myelin. This process requires a prolific biosynthesis of lipid-protein material. Where this is synthesized in the cell and how it comes to be incorporated in the cell membrane is not yet known. The myelination process would seem to provide an ideal system in which to study the molecular mechanism of lipid-protein synthesis and of membrane formation in general.

From Geren's membrane theory, it immediately becomes obvious why there are *two*, rather than one, bimolecular layers of lipids in the x-ray identity period in the radial direction (see Schmitt, Bear, and Palmer[28]). As the outer Schwann-cell membrane infolds, the outer surfaces of the membrane remain in contact. As the layers condense, with the expulsion of Schwann-cell cytoplasm, the membrane surfaces which faced the Schwann-cell cytoplasm also adhere, fuse, and form the darkly staining line seen in the electron micrographs (see Fig. 5). The alternate less-dense line represents the fusion of membrane surfaces which originally faced outward to extracellular space. Finean,[34] who has continued the x-ray analysis in a systematic fashion over the last decade, has proposed a molecular model of myelin in which the cholesterol molecules fit in between the phospholipid and cerebroside molecules in characteristic fashion (see Fig. 3). Further refinements in the x-ray and electron-microscopic techniques, together with the study of various lipid-protein models, may provide important new information about membrane structure which will be of vital importance in the rapidly expanding field of molecular biology.

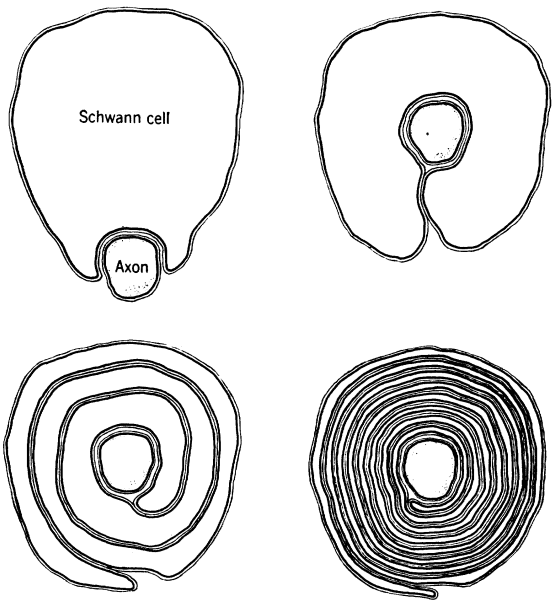

FIG. 4. Schematic representation of the membrane theory of the origin of nerve myelin, (after Geren[35]). Four stages in the engulfment of the axon by the Schwann cell and the wrapping of many double layers which, after condensation, form the compact myelin.

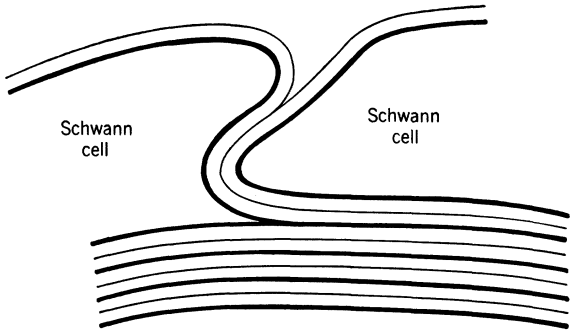

FIG. 5. Illustrating enfolding of Schwann-cell surface membranes to form compact myelin, after the theory of Geren.[35] Note differentiation between inner and outer surfaces of enfolding membrane; in the double membrane, this gives rise to Finean's "difference factor" and explains why the full repeat distance in the radial direction involves two bimolecular leaflets of lipo-protein (two membranes).

As Schwann or satellite cells envelop the outgrowing axon and start to wrap their surfaces in multiple folds about it, adjacent Schwann cells come into contact and form the nodes of Ranvier. This nodal region of the finished, myelinated fiber has not yet been exhaustively studied in the electron microscope. In view of the critical nature of this region, suggested by the theory of saltatory conduction, its ultrastructure merits careful study. According to a preliminary report by Robertson,[41] the Schwann cells on either side of the node form finger-like processes which interdigitate closely over the node. The region between the processes, presumably containing extracellular fluid, is of the order of several hundred Ångström units thick. To this extent, the axon surface membrane is exposed to extracellular fluid at the node, according to Robertson.

The incisures of Schmidt-Lantermann presented a problem because, if they are continuous channels between the axon and the exterior through the myelin, they would offer conducting paths for current. However, electron microscopy has demonstrated that this is not the case.[42,43] The incisures are produced by a systematic shift in the relationship of all of the helically wrapped membranes, but each membrane is continuous across the incisural gap. There are thus as many layers traversing the incisures as occur in the compact region of the myelin, and the incisures are therefore not regions

through which ions and currents can freely flow into the axon.

III. SATELLITE-CELL-AXON RELATIONSHIPS IN "UNMYELINATED" FIBERS

It has long been known to neurohistologists, particularly to those of the Spanish school, that nerve fibers are everywhere covered by satellite cells. The fact that these cells and their possible functional role—in the mature as well as in developing and regenerating fibers—have not greatly interested physiologists, or even histologists, is to some extent owing to their small size (*ca* 0.1 to 1 in most invertebrate fibers) or to the fact that myelin (which has been much studied by histologists and physiologists) was not recognized as satellite-cell substance (i.e., a compact helical layering of double surface membranes). The fact that, early in the development of histology, a sharp distinction was made between "myelinated" and "unmyelinated" fibers (primarily on the basis of staining reactions), led some physiologists to the mistaken notion that a substantial fraction of the animals' nerve fibers are in effect naked axons. From this, it is obvious why the cellular covering of the axon attracted little interest except to those studying nerve development or regeneration, where the satellite-cell covering is unquestionably of functional significance.

Polarization-optical studies (see Schmitt and Bear[29]), based largely on a reinterpretation of the mechanism of the so-called metatropic reaction discovered by Göthlin,[44] strongly suggested that at the surface of all nerve fibers, vertebrate or invertebrate, myelinated or unmyelinated, oriented lipid-protein material is present which has qualitative similarity of molecular organization though quite variable quantitatively from one fiber type to the next. This led to the notion that "all nerve fibers are to some extent myelinated."[29]

This matter could be clarified only when the region at the surface of nerve fibers—i.e., the monolayer of satellite cells—could be observed at high resolution by the electron microscope. Details of the discoveries thus brought to light are beyond the scope of this paper, but a few of the more significant aspects at the molecular level, particularly insofar as they bear on basic physiological concepts, are considered.

Investigation with the electron microscope of the detailed structure of the satellite cells and their relation to the axon is not yet a decade old, and it is, therefore, not unexpected that knowledge of the subject is still very primitive. No doubt, special methods of fixation and preparation will have to be developed to permit investigation of the most fundamental aspects of the problem, such as the possible variation of intercellular relationship, as manifested morphologically by the apparent spaces or channels between axon surface and satellite cells and between satellite cells themselves, with function.

The typical invertebrate, unmyelinated (but not unsatellited!) nerve fibers, such as those of the lobster leg or claw nerve, are surrounded by satellite cells which are very thin (*ca* 0.1 μ). Except at the region of the nuclei, there is relatively little space between the two parallel surfaces of the cells (i.e., facing the axon surface membrane and that facing the basement membrane, connective tissue investment), although mitochondria and some of the typical equipment of the cytoplasm of active cells may be seen. The axon surface is characteristically highly contorted, and axoplasmic mitochondria are seen with the long axes of their ellipsoids oriented into the folds of these contortions, suggestive of energy utilization at this interface (see Fig. 6). The outpocketings of the satellite cells into the axon may manifest all of the stages to be expected if particulates (mitochondria?) were actually being formed and extruded into the axon by filling such outpocketing with satellite-cell cytoplasm (including its particulates) and pinching them off into the axoplasm (see Geren and Schmitt[13,45]).

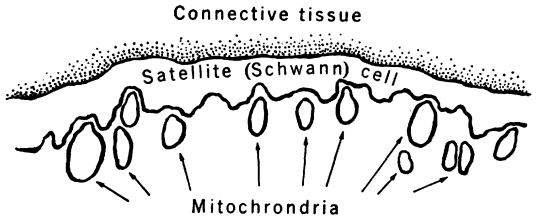

FIG. 6. Diagrammatic illustration of thinness of satellite cell in lobster fiber and of intimate relationship between mitochondria and axon surface membrane (after Geren and Schmitt[44]).

Such very thin cells would be expected to show only a very weak birefringence and metatropic effect, since only two lipid-protein surface membranes are involved. Nevertheless, this optical effect was observed and correctly interpreted, except that the layers were simply assigned to the region at the surface of the axon, rather than to the membranes of the satellite cells.[46]

The satellite cells of squid giant fibers are somewhat thicker (*ca* 1 μ) than those of lobster, though the ratio of thickness to axon diameter may actually be less. In any histological section of the giant fibers, as many as two or three nuclei may be present. If one were to chart the boundaries and domains of the satellite cells upon the axon surface membrane (e.g., by slitting the fiber and flattening out the membrane), one would see that many cells are involved in the formation of the cellular monolayer that surrounds the axon.

The most distinguishing feature of the cytoplasm of these cells is the presence of many (a half-dozen or more) osmiophilic, dense membranous layers lying in planes predominantly parallel with the cell surface. Whether these layers are related to those present in typical, metabolically active cells ("endoplasmic reticulum"), or whether they have a special structure and function in satellite cells remains to be established by more-detailed examination. However, if one assumes that the layers have the composition and ultrastructure typical

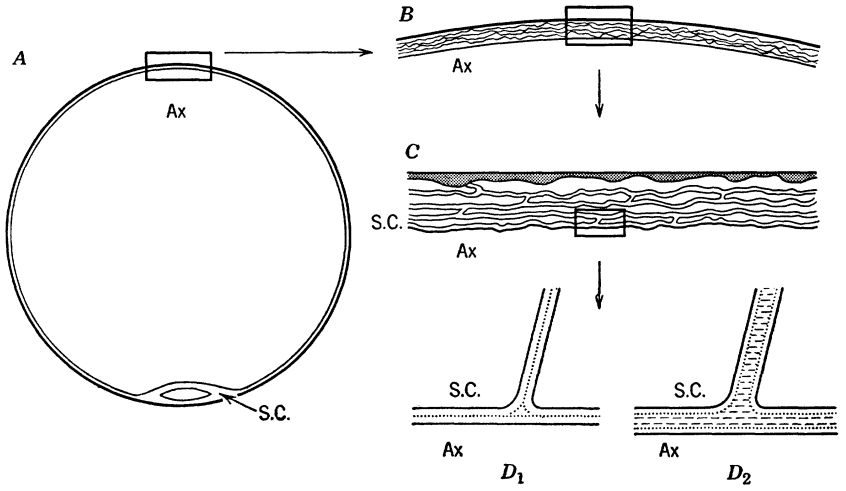

FIG. 7. Relationship of axon (Ax) and Schwann cell (S.C.) in the squid giant fiber (after Schmitt and Geschwind[2]). *A*, cross section of giant fiber showing very thin Schwann cell surrounding axon. ×125. *B*, enlarged segment portion of Schwann cell, ×3000; *C*, same as *B*, showing intracytoplasmic layers, ×9000; and D_1 and D_2, possible relationships at the molecular level of axon surface membrane and Schwann-cell membrane. D_1 presumes layers are in molecular contact. (Water layer does not exceed about 15 A in thickness.) D_2, showing thicker water layers (100 to 200 A) between adjacent membranes. ×150 000.

of lipid-protein membranes generally (hence, also of myelin), it is possible to account for the polarization-optical properties described by Bear *et al.*[47] and ascribed by them to a metatropic layer at the outer surface of the satellite cells—a kind of myelin-like layer. Geren and Schmitt[45] have shown that the optical effects can satisfactorily be accounted for on the basis of the intracytoplasmic dense layers present in the satellite cells. There is no differentiated myelin-like layer at the outside of these cells, only the amorphous basement membrane.

One of the structural details that must await further investigation for clarification, but that may be of great ultrastructural and perhaps functional significance, is the nature of the boundaries between satellite cells and the axon surface membrane, between adjacent satellite cells and between intracytoplasmic membranes. Of particular importance is the thickness of the aqueous regions between these membranes, because ions passing from extracellular space to axoplasm and in the reverse direction must traverse such channels.

The situation is illustrated schematically in Figs. 7 and 8. If the adhesion between the surface membranes were as close as in the myelin sheath, there would be no substantial channels for ion diffusion. The aqueous phase would have a thickness of the order of 12 A according to the x-ray diffraction analysis of myelin struc-

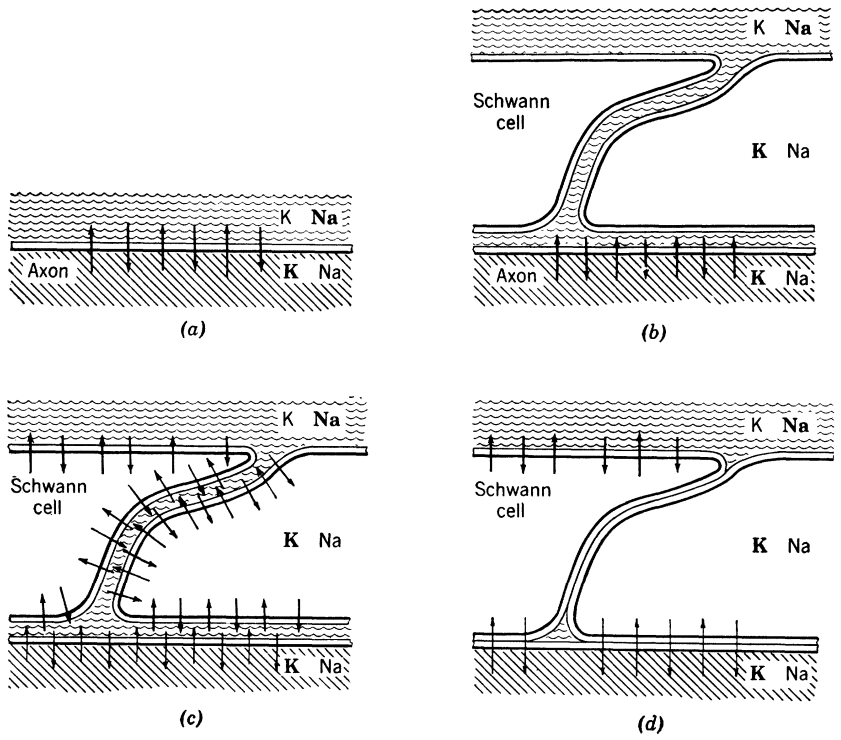

FIG. 8. Possible diffusion pathway for solutes passing between axoplasm and extracellular fluid (after Schmitt[3]). (*a*) bare-axon membrane is exposed to extracellular fluid (*b*) diffusion channel is 150 to 200 A thick, filled with extracellular fluid. Active interchange occurs only between axon surface membrane and fluid in channels; passive diffusion occurs in other channels. (*c*) same as (*b*) except that active interchange of metabolites and solutes occurs across all membrane surfaces; satellite cells participate actively in processes involving interchange between axon and extracellular fluid. (*d*) satellite cells and axon membranes are in molecular contact; water channel not more than 10 to 15 A thick with structure comparable to that of compact myelin.

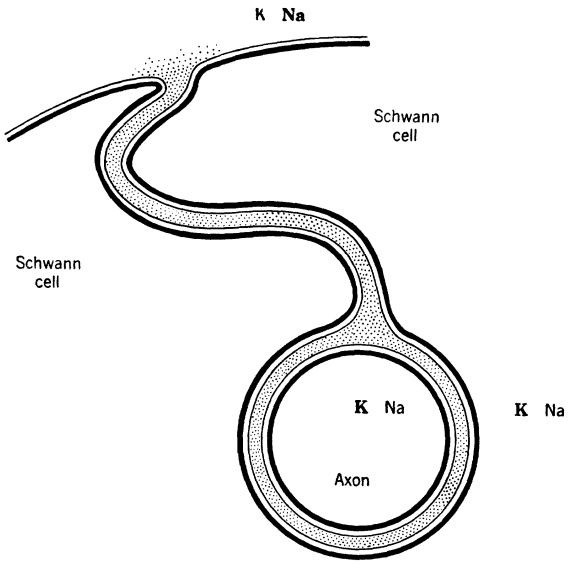

FIG. 9. Diagrammatic representation of relationship of very thin *C* fibers to Schwann cell (after Robertson[48]).

ture.[28] On the other hand, if these intercellular relationships are similar to those found by Robertson[47] and by electron microscopists to obtain between cells generally, the aqueous channels, communicating with the frank extracellular fluid of the ground substance of the surrounding connective tissue, would have a thickness of about 150 A.

Robertson[21,22,48] has found such a membrane relationship in the case of the vertebrate "*C*" fibers. In these nerves, the very thin (*ca* 0.1 μ) axons pass through the satellite (Schwann) cell tube enclosed in a fold of the surface membrane of the satellite cell in a manner analogous to that assumed by Geren's membrane theory to explain the origin of myelin (see also the earlier works of Gasser[49–52]).

In analyzing the functional significance of the intermembrane relationship and the thickness of the aqueous regions between limiting membranes *in life*, several factors must be considered. Indeed, this situation illustrates certain indeterminacies that characterize the electron microscope approach to molecular biology generally.

From the properties of the constituent membranes, including the charge on the membrane molecules, a separation of the order of 100 to 200 A between the membranes might be expected.[53–56] This equilibrium distance will vary with the ionic strength (a statistical physicochemical concept that is difficult to apply to such thin capillary spaces). If the equilibrium distance between surface membranes is responsive to the ionic strength of the environment, it is obvious that a factor of importance is the ionic strength of the fluid used to fix the nerves. Another factor is the osmotic pressure normally characterizing the intercellular fluid. If this is not identical with that of the intracellular fluid, then when the system is placed in the fixative (which presumably quickly destroys membrane semipermeability and such vital equipment as "ion pumps"), an osmotic transfer of water may be expected, and this might importantly influence the distance between surface membranes as observed in the electron microscope after fixation and sectioning.

It has become evident from electron-microscopical studies of metabolically active cells not only that the metabolic activity involves the presence of membranes separated by small distances, but also that the products of the metabolism, presumably including ions, must find their way into the aqueous space between the membranes. The presence of these metabolites in turn must affect the equilibrium separation between the membranes and the properties of the intercellular fluid within these thin capillary spaces. Such factors must be taken into consideration in dealing realistically with these spaces which are the channels of communication between the axon surface membrane (presumably the seat of the rapid alterations responsible for the propagation of the impulse in the nerve fibers) and frank extracellular space. According to Frankenhaeuser and Hodgkin,[57] if such intercellular channels are the route of diffusion of potassium ions from axoplasm to the exterior, the electrical data would require a channel thickness of about 300 A in a structure similar to that shown by Geren and Schmitt[13] assuming a process of passive diffusion calculable on the basis of properties characteristic of macroscopic bulk phases. Whether or not additional properties, deriving from the microscopic dimensions of the intermembrane system and from the presence of solutes, particularly metabolic products of the satellite cells, require modification of this view remains for further analysis to determine. That the satellite cells of the squid giant fiber are indeed metabolically active has been shown by the measurements of oxygen consumption of the sheath cells after removal of the axoplasm,[58] and from an assay of enzyme activities of sheath cells and axoplasm.[59]

BIBLIOGRAPHY

[1] F. O. Schmitt in *Metabolism of the Nervous System*, D. Richter, editor (Pergamon Press, London, 1957), p. 35.

[2] F. O. Schmitt and N. Geschwind, Progr. in Biophys. and Biophys. Chem. **8**, 166 (1957).

[3] F. O. Schmitt, Exptl. Cell Research **5**, Suppl., 33 (1958).

[4] S. L. Palay in *Ultrastructure and Cellular Chemistry of Neural Tissue*, H. Waelsch, editor (Paul B. Hoeber, Inc., and Harper and Brothers, New York, 1957), p. 31.

[5] S. O. Brattgård, J. E. Edström, and H. Hydén, Exptl. Cell Research **5**, Suppl., 185 (1958).

[6] P. A. Weiss and H. P. Hiscoe, J. Exptl. Zool. **107**, 315 (1948).

[7] H. Waelsch, J. Nervous and Mental Diseases **126**, 33 (1958).

[8] S. Ochs and E. Burger, Am. J. Physiol. **194**, 499 (1958).

[9] P. Chinn, J. Cellular Comp. Physiol. **12**, 1 (1938).

[10] M. Maxfield, J. Gen. Physiol. **37**, 201 (1953).

[11] A. von Muralt, *Die Signalübermittlung im Nerven* (Birkhäuser, Basel, 1946).

[12] J. M. Foster, J. Neurochem. **1**, 84 (1956).

[13] B. B. Geren and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. **40**, 863 (1954).

[14] B. A. Koéchlin, J. Biophys. Biochem. Cytol. **1**, 511 (1955).
[15] M. Maxfield and R. W. Hartley, Biochim. et Biophys. Acta **24**, 83 (1957).
[16] F. O. Schmitt, J. Cellular Comp. Physiol. **49**, Suppl. **1**, 165 (1957).
[17] G. Deffner and R. Hafter, Biochim. et Biophys. Acta (to be published).
[18] R. S. Bear, F. O. Schmitt, and J. Z. Young, Proc. Roy. Soc. (London) **B123**, 505 (1937).
[19] F. O. Schmitt, J. Exptl. Zool. **113**, 499 (1950).
[20] F. O. Schmitt and B. B. Geren, J. Exptl. Med. **91**, 499 (1950).
[21] J. D. Robertson in *Ultrastructure and Cellular Chemistry of Neural Tissue*, H. Waelsch, editor (Paul B. Hoeber, Inc., and Harper and Brothers, New York, 1957), p. 1.
[22] J. D. Robertson, J. Biophys. Biochem. Cytol. **4**, 349 (1958).
[23] W. Stoeckenius, Exptl. Cell Research. **13**, 410 (1957).
[24] W. Stoeckenius in *Proceedings of the Fourth International Congress on Electron Microscopy* (Springer-Verlag, Berlin, to be published).
[25] H. W. Fisher and T. T. Puck, Proc. Natl. Acad. Sci. U. S. **42**, 900 (1956).
[26] W. J. Schmidt, *Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma* (Gebrüder Borntraeger, Berlin, 1937).
[27] O. Wiener, Abhandl. sächs. Ges. Wiss. math. phys. Kl. **32**, 509 (1912).
[28] F. O. Schmitt, R. S. Bear, and K. J. Palmer, J. Cellular Comp. Physiol. **18**, 31 (1941).
[29] F. O. Schmitt and R. S. Bear, Biol. Revs. **14**, 27 (1939).
[30] H. Fernández-Morán, Exptl. Cell Research **1**, 309 (1950).
[31] F. S. Sjöstrand, Experientia **9**, 68 (1953).
[32] J. B. Finean, F. S. Sjöstrand, and E. Steinmann, Exptl. Cell Research **5**, 557 (1953).
[33] H. Fernández-Morán and J. B. Finean, J. Biophys. Biochem. Cytol. **3**, 119 (1957).
[34] J. B. Finean, Exptl. Cell Research **5**, Suppl., 18 (1958).
[35] B. B. Geren, Exptl. Cell Research **7**, 558 (1954).
[36] B. B. Geren in *Cellular Mechanisms in Differentiation and Growth*, D. Rudnick, editor (Princeton University Press, Princeton, 1956), p. 213.
[37] B. B. Geren and J. Raskind, Proc. Natl. Acad. Sci. U. S. **39**, 880 (1953).
[38] J. D. Robertson, J. Biophys. Biochem. Cytol. **1**, 271 (1955).
[39] S. A. Luse, J. Biophys. Biochem. Cytol. **2**, 777 (1956).
[40] E. DeRobertis, H. M. Gerschenfeld, and F. Wald, J. Biophys. Biochem. Cytol. **4**, 651 (1958).
[41] J. D. Robertson, J. Physiol. **135**, 56*P* (1957).
[42] M. Luxoro, Proc. Natl. Acad. Sci. U. S. **44**, 152 (1958).
[43] J. D. Robertson, J. Biophys. Biochem. Cytol. **4**, 39 (1958).
[44] G. F. Göthlin, Kgl. Svenska Vetenskapsakad. Handl. **51**, 1 (1913).
[45] B. B. Geren and F. O. Schmitt in *Symposium on Fine Structure of Cells* (Interscience Publishers, Inc., New York, 1955), p. 251.
[46] R. S. Bear and F. O. Schmitt, J. Cellular Comp. Physiol. **9**, 275 (1937).
[47] R. S. Bear, F. O. Schmitt, and J. Z. Young, Proc. Roy. Soc. (London) **B123**, 496 (1937).
[48] J. D. Robertson, J. Biophys. Biochem. Cytol. **3**, 1043 (1957).
[49] H. S. Gasser, Cold Spring Harbor Symposia Quant. Biol. **17**, 32 (1952).
[50] H. S. Gasser, J. Gen. Physiol. **38**, 709 (1955).
[51] *Ibid.*, **39**, 473 (1956).
[52] H. S. Gasser, Exptl. Cell Research **5**, Suppl., 3 (1958).
[53] K. J. Palmer and F. O. Schmitt, J. Cellular Comp. Physiol. **17**, 385 (1941).
[54] K. Hess and H. Kiessig, Chem. Ber. **81**, 327 (1948).
[55] H. L. Booij and D. Bungenberg-de Jong in *Protoplasmatologia Handbuch der Protoplasmaforschung* (Springer-Verlag, Vienna, 1956).
[56] J. Th. G. Overbeek, unpublished lecture, "Physical chemical properties of layered systems," given before the Electron Microscope Society of America (10 September 1957).
[57] B. Frankenhaeuser and A. L. Hodgkin, J. Physiol. **131**, 341 (1956).
[58] R. R. Coelho, J. W. Goodman, and M. B. Bowers (manuscript in preparation).
[59] N. R. Roberts, O. Lowry, E. J. Crawford, and R. Coelho, J. Neurochem. **3**, 109 (1958).

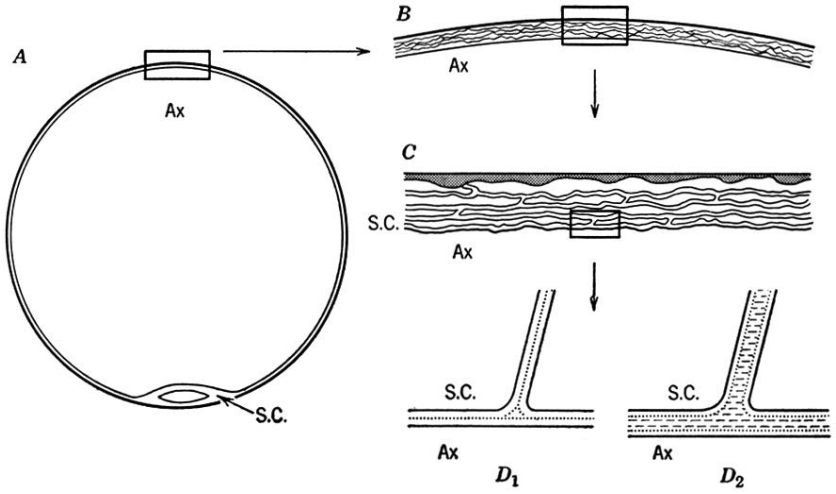

FIG. 7. Relationship of axon (Ax) and Schwann cell (S.C.) in the squid giant fiber (after Schmitt and Geschwind[2]). *A*, cross section of giant fiber showing very thin Schwann cell surrounding axon. ×125. *B*, enlarged segment portion of Schwann cell, ×3000; *C*, same as *B*, showing intracytoplasmic layers, ×9000; and D_1 and D_2, possible relationships at the molecular level of axon surface membrane and Schwann-cell membrane. D_1 presumes layers are in molecular contact. (Water layer does not exceed about 15 A in thickness.) D_2, showing thicker water layers (100 to 200 A) between adjacent membranes. ×150 000.

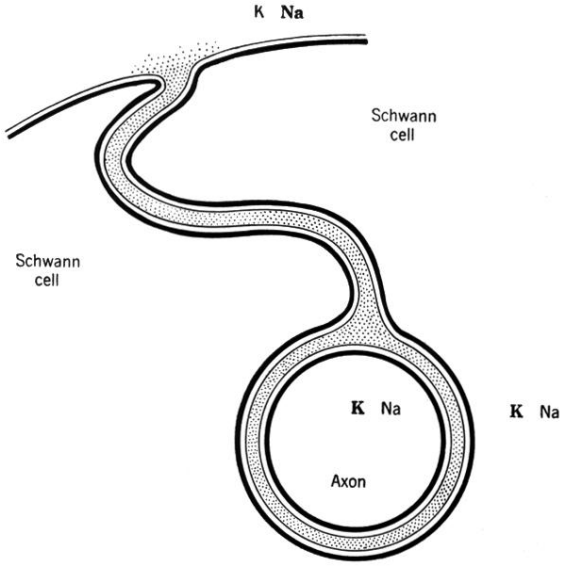

FIG. 9. Diagrammatic representation of relationship of very thin *C* fibers to Schwann cell (after Robertson[48]).