Stability of the Myelin Membrane

Lipid molecules may impart stability to the myelin membrane through intermolecular cohesion.

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Myelin is a structure of vital importance. This fact is clearly demonstrated by the devastating consequences of its breakdown or dysgenesis in disease. Myelin is normally one of the most stable of anatomical components. In order to determine why myelin is unstable in disease it is important to understand why it is normally such a stable structure. In this article I present some information which has led to a partial understanding, at the molecular level, of the normal stability of myelin and of its instability in disease.

The Anatomy of Myelin

Myelin is a sheathlike structure which invests the nerve axon circumferentially, somewhat like insulation around a wire. It is formed by a wrapping of the external membrane of the Schwann cell (*1*) or the oligodendrogliocyte (*2*) around the nerve axon (Fig. 1). The result is a structure comprised of a series of tightly packed membranes of uniform thickness layered in concentric fashion (Fig. 2, *A* and *B*). A single membrane constituent of this structure, when examined under high-resolution electron microscopy, is seen to be bounded by two dense lines —(i) a thick line, the major dense line, and (ii) a thinner line, the intraperiod line; there is a clear zone between them (Fig. 2*C*).

Myelin contains lipids, proteins, polysaccharides, salts, and water. The spatial arrangement of these molecules in the myelin membrane has been determined by different procedures. X-ray diffraction analysis of myelin reveals a characteristic pattern for each individual membrane unit (*3–6*) (Fig. 3*b*), in which two high-density regions (peaks) are separated by a low-density region (trough). The most reasonable interpretation of these curves is that the peripheral regions are occupied by electron-dense polar groups of lipid molecules and adjacent proteins (or polysaccharides) while the central region is occupied by the less dense hydrocarbon tails of the lipid molecules (Fig. 3*c*). Hydration studies of myelin, in combination with x-ray diffraction analysis (*5, 6*) and electron microscopy (*7*), substantiate this interpretation by demonstrating that the peripheral regions of the membrane unit are hydrophilic and associated with the presence of polar groups while the central region is hydrophobic and associated with the presence of nonpolar hydrocarbon chains. In addition, the center of the myelin membrane is lightly stained with osmium, indicating that hydrocarbon chains are present here, since lipid hydrocarbon chains do not bind osmium as avidly as their polar groups do (*8*). The central region in each membrane unit is approximately 51 angstroms wide (*4–6*). There is room for two lipid molecules packed tail-to-tail in this region, since the average length of lipid molecules, from phosphate group to hydrocarbon tail, is approximately 26 to 28 angstroms. Since this central region fails to expand or shrink when myelin is hydrated or dehydrated (*5–7*), this region must be water-free and must be occupied entirely by hydrophobic groups—that is, the hydrocarbon tails of lipid molecules. These studies indicate that the molecular arrangement in myelin is that of a bimolecular lipid layer bounded by two protein monolayers, with the polar groups of the lipid molecules adjacent to the protein layers, and with the hydrocarbon tails of the molecules extending into the center of the lipid membrane (Fig. 3*c*).

Metabolic Stability of Myelin

Myelin exhibits a remarkable degree of metabolic inertness. Twenty-five year ago Waelsch *et al.* (*9*) demonstrated that cholesterol in the brain of adult rats given deuterium did not become labeled, whereas under the same conditions there was considerable labeling of cholesterol in the brains of young rats (*9*). These results indicated that cholesterol in the adult brain is not synthesized and therefore does not turn over. Davison and his co-workers extended these observations (*10*), studying both the incorporation of radioactive cholesterol into gray matter and white matter and the turnover of this lipid in each locale. They found that labeled cholesterol was actively incorporated into both gray and white matter in the brains of immature experimental animals. However, radioactive cholesterol, once incorporated in white matter, did not turn over; no evidence of turnover was found even as long as 1 year later. In gray matter some turnover occurred during the first few months, but after this time there was very little loss of label. In contrast, radioactive cholesterol in the heart, liver, and kidneys disappeared within a few weeks after administration. Related investigations (*11, 12*) were made, in which other major lipids were labeled and studies were made of their turnover. It was found that cerebroside, cephalins, and sphingomyelin, like cholesterol, underwent very little metabolic turnover in white matter, whereas in gray matter there was a much higher turnover of these lipids. Since these lipids were thought to be constituents of myelin, Davison *et al.* concluded (*12*): "The uniform persistence of these three myelin lipids over this long period is consistent with the belief that when once deposited in the myelin sheath at the time of its formation, they undergo little subsequent 'turnover'; the myelin sheath must therefore be regarded as one of the more permanent tissue elements."

Lipid Composition of Myelin

It is not known why lipids in myelin are "metabolically inert" whereas lipids in gray matter turn over readily. One possible explanation is that white matter

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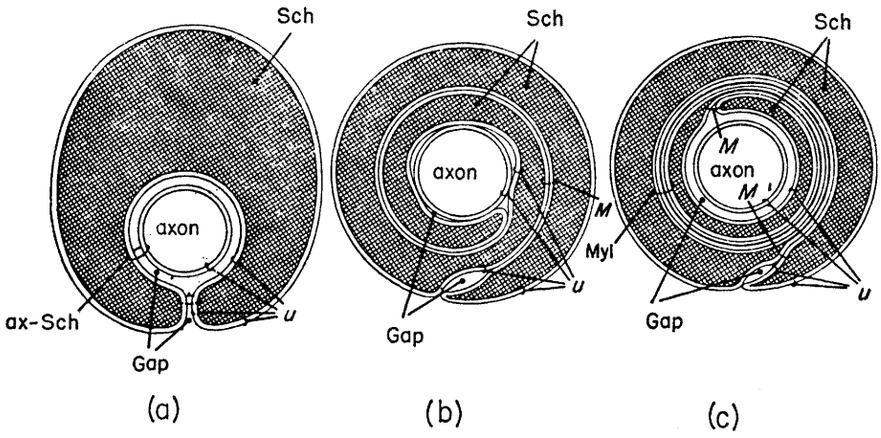

Fig. 1. Schematic drawing of myelin formation in peripheral nerve. (*a*) Schwann cell (Sch) envelops the axon. A double membrane layer, the mesaxon (*M*) is formed from two apposed external membrane layers of the Schwann cell. The mesaxon elongates (*b*), and myelin (*Myl*) is formed by the concentric layering of this double-membrane structure (*c*). [From Robertson (*34*), courtesy *Progr. Biophys. Biophys. Chem.*]

and gray matter are anatomically different, white matter being a "membranous," myelin-rich tissue, gray matter being a "cytoplasmic" tissue containing a large proportion of cytoplasmic organelles. In myelin the lipid molecules are sandwiched between protein layers out of contact with the metabolic pool, and thus they exhibit a low rate of metabolic turnover. However, as far as is known, lipids in gray matter are localized in membranes as well, and the membranes are arranged so that the lipid molecules are sandwiched between two layers of protein, either perpendicular to the long axis of the protein layer (as in the case of plasma membranes and nuclear membranes) or parallel to it [as in mitochondrial membranes (*13*)]. Thus the lipid constituents of membranes in the two types of tissues should show similar degrees of metabolic inertness, being isolated from the metabolic pool because of their location between protein monolayers. The obvious objection is that not all membranes are identical, since variations occur both in the composition and in the structure of the molecules they contain. The arrangement of lipids in a membrane could be such that these molecules are loosely held and readily exchangeable in one membrane and tightly held and immobilized in another. It is logical, then, to ask, (i) whether myelin lipids possess characteristics which lead to a tightly organized, closely packed, highly stable membrane structure; (ii) whether lipids in adjacent glial cells and neurons possess characteristics which lead to a more loosely organized, less stable membrane structure; (iii) whether such differences could account for the greater degree of metabolic inertness of myelin lipids; (iv) whether the stability of myelin itself may be largely due to the packing of its lipid molecules, since the lipid content of myelin is extraordinarily high; and (v) whether diseases involving myelin might result from changes in lipid structure and composition which lead to a less cohesive, more easily disrupted, structure.

The most straightforward approach to such questions is to isolate the major lipids from myelin and from gray matter, analyze the fatty acid compositions of each, and decide whether the composition and structure of myelin lipids *could* result in a highly compact structure. Although various studies have been made from time to time on the lipids of the brain, no studies have been reported in which the composition of the major lipids and their fatty acids have been determined in gray matter, white matter, and myelin. My associates and I therefore set about performing this task in normal humans at various ages (10 months, 6 years, 9 years, and 55 years). Gray matter and white matter were separated by careful dissection from the frontal lobes obtained at autopsy from patients free of cerebral pathology. Myelin was isolated from a portion of the separated white matter by ultracentrifugation, by means of the elegant technique of Autilio, Norton, and Terry (*14*). Each individual lipid class was isolated by the column-chromatography techniques reported previously (*15, 16*). The fatty

Table 1. Lipid content of normal frontal lobes.

Tissue	Total lipid (% of dry wt)	Individual lipids (molar percentage of total lipid)								
		Cholesterol	Ethanolamine glycerophosphatides*	Serine glycerophosphatides*	Choline glycerophosphatides	Sphingomyelin	Cerebroside	Cerebroside sulfate	Ceramide	Uncharacterized†
					Subject, 10 months					
Gray matter	36.4	35.7	14.9	5.7	24.7	4.3	4.1	1.5	2.5	6.6
White matter	49.0	37.7	16.2	3.6	14.4	3.6	13.6	3.7	2.5	4.7
Myelin	78.0	38.8	15.3	5.1	12.7	4.9	13.8	4.7	1.7	3.0
					Subject, 6 years					
Gray matter	35.8	31.1	25.1	7.9	19.6	3.1	2.4	1.4	2.7	6.7
White matter	58.4	37.7	12.3	5.0	11.8	3.8	17.2	3.3	1.6	7.3
Myelin	80.9	42.4	11.4	4.1	9.1	4.3	18.1	3.3	1.3	5.9
					Subject, 9 years					
Gray matter	37.6	31.6	22.1	5.8	20.1	6.4	4.2	0.8	1.5	7.5
White matter	66.3	33.5	15.9	6.2	11.2	6.3	12.8	4.3	0.8	9.0
Myelin	78.0	38.7	15.3	5.5	12.8	4.8	13.8	4.6	1.8	2.7
					Subject, 55 years					
Gray matter	39.6	31.3	19.6	5.7	19.4	4.2	4.7	1.5	1.6	12.0
White matter	64.6	38.5	11.9	5.2	10.3	6.3	15.3	3.3	1.3	7.9
Myelin	78.0	40.4	11.8	5.3	8.4	4.4	15.7	3.5	1.5	9.0

* Includes both diester and plasmalogen forms of these lipids. † Includes inositol glycerophosphatides as principal components, and free fatty acids, gangliosides, phosphatidic acid, and polyglycerophosphatides as minor components. An average molecular weight of 800 was assumed for this fraction.

acid and fatty aldehyde compositions of pure lipids of each class were determined by gas-liquid chromatography (*16–18*).

The lipid compositions of each of the three tissues are given in Table 1 (*19*). The lipid content of myelin was found to be very high, ranging between 78 and 81 percent of the dry weight; it was much higher than the lipid content of gray matter or white matter. In fact, the lipid content of myelin is the highest reported for any body tissue, with the exception of adipose tissue. With regard to the individual lipids, each value is given as a molar percentage, so that the lipid composition in one tissue can be compared directly with that in another. It was possible to accurately determine the molecular weight of each lipid, since its fatty acid composition had been determined. The pioneering studies of Koch *et al.* (*20*), Brante (*21*), and Folch-Pi (*22*) had established that cholesterol, cerebroside, plasmalogens (aldehyde-containing lipids), and proteolipids are predominantly constituents of myelin. In accordance with these results we found that myelin contained the highest proportions of cholesterol, cerebroside, plasmalogens, and cerebroside sulfate. The proportions of sphingomyelin or serine glycerophosphatides were about equal for the three tissues. Myelin contained the lowest proportions of ceramide, ethanolamine glycerophosphatides, and choline glycerophosphatides. Thus the lipid compositions of gray matter and myelin differ in that myelin has an extremely high lipid content and contains much higher molar proportions of cerebroside, cerebroside sulfate, and plasmalogens, slightly higher molar proportions of cholesterol, and much lower molar proportions of ethanolamine and choline glycerophosphatides than gray matter.

The fatty acid compositions were very different for myelin lipids and for gray matter lipids (Table 2). Gray matter glycerophosphatides contained very high proportions of highly unsaturated fatty acids, while myelin glycerophosphatides contained much lower proportions of these acids. Brain glycerophosphatides (ethanolamine glycerophosphatides, serine glycerophosphatides, and choline glycerophosphatides) contain saturated, monounsaturated, and polyunsaturated fatty acids, the latter molecules being from 20 to 22 carbon atoms long and containing four, five, or six double bonds (*16*). The glycerophosphatides from gray matter contained the most highly unsaturated fatty acid, docosahexaenoic acid (22 carbon atoms, 6 double bonds), in amounts proportionately about 10 times the amounts present in the glycerophosphatides from myelin, while the total content of polyunsaturated acids was 3 times the total in the glycerophosphatides from gray matter. On the other hand, myelin sphingolipids (cerebroside, cerebroside sulfate, ceramide, and sphingomyelin) from the three younger subjects contained fatty acids of longer chain length than the fatty acids present in gray matter sphingolipids from these subjects. Brain sphin-

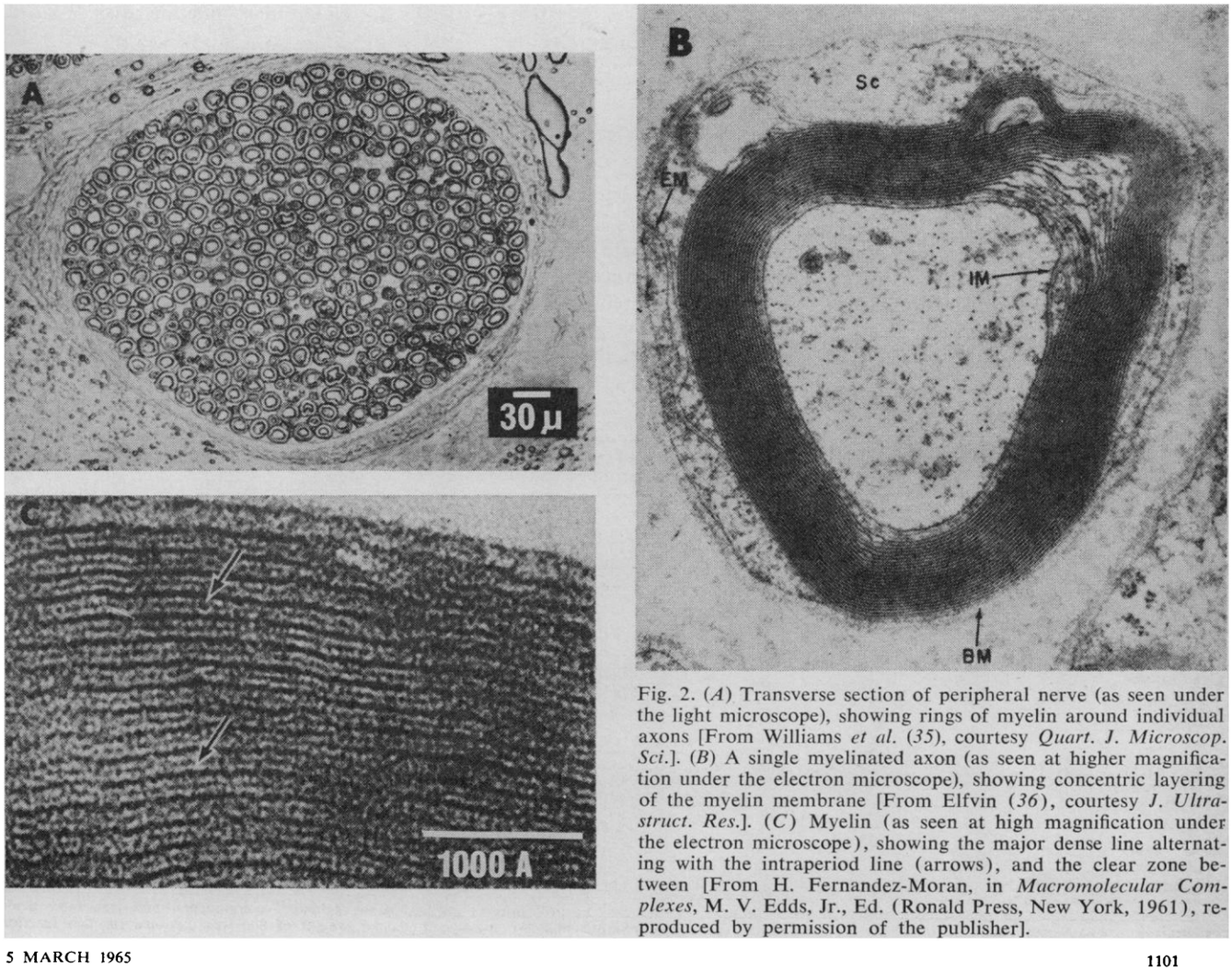

Fig. 2. (*A*) Transverse section of peripheral nerve (as seen under the light microscope), showing rings of myelin around individual axons [From Williams *et al.* (*35*), courtesy *Quart. J. Microscop. Sci.*]. (*B*) A single myelinated axon (as seen at higher magnification under the electron microscope), showing concentric layering of the myelin membrane [From Elfvin (*36*), courtesy *J. Ultrastruct. Res.*]. (*C*) Myelin (as seen at high magnification under the electron microscope), showing the major dense line alternating with the intraperiod line (arrows), and the clear zone between [From H. Fernandez-Moran, in *Macromolecular Complexes*, M. V. Edds, Jr., Ed. (Ronald Press, New York, 1961), reproduced by permission of the publisher].

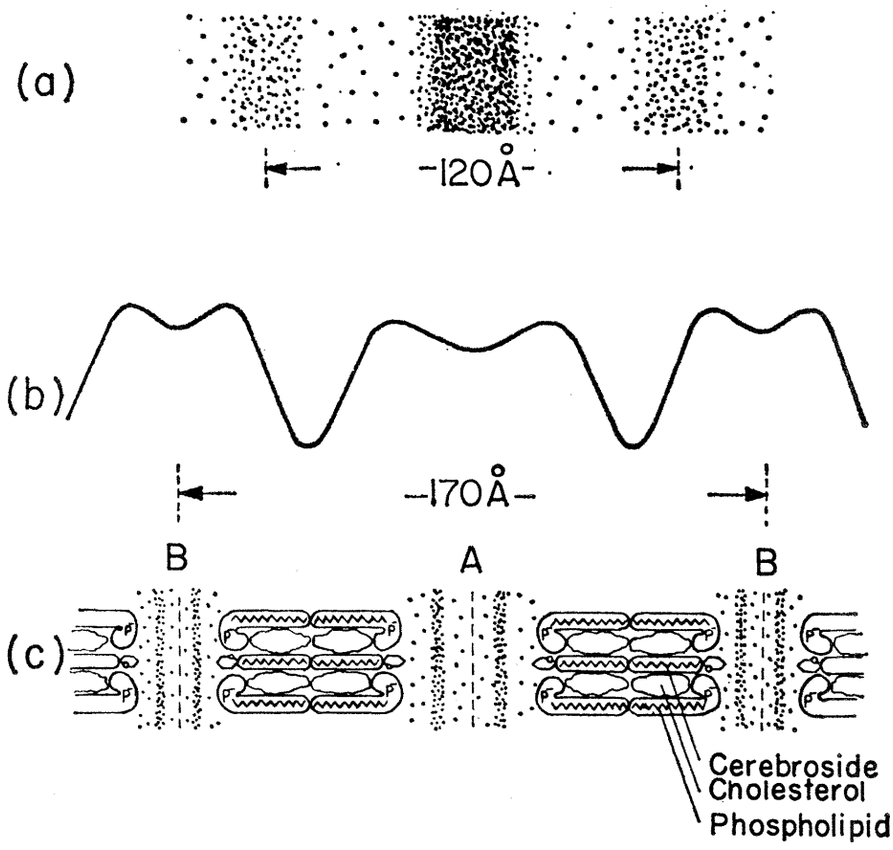

Fig. 3. Schematic drawing of (*a*) the appearance of myelin under the electron microscope; (*b*) x-ray diffraction pattern of myelin; and (*c*) Finean's molecular model of the constituents of myelin (for more recent x-ray diffraction analysis, see *6*). [From Finean (*5*), courtesy Elsevier]

golipids contain saturated or monounsaturated fatty acids which range in length from 14 to 27 carbon atoms (*18*). In general, two groups of fatty acids are present—the shorter-chain fatty acids (14 to 18 carbon atoms) and the longer-chain fatty acids (19 to 26 carbon atoms). Cerebroside and cerebroside sulfate from myelin contained longer-chain fatty acids in amounts proportionately 3 to 9 times the amounts in cerebroside and cerebroside sulfate from gray matter, while for sphingomyelin the amount differed by factors of 5 to 9. Even the myelin sphingolipids from the baby (10 months old, an age when myelination is at a very early stage) contained nearly the same proportions of longer-chain fatty acids as myelin sphingolipids from the older subjects. Unlike the glycerophosphatides, the sphingolipids from the three tissues did not differ appreciably in degree of unsaturation.

Table 2. Nature of lipid fatty acids in brain.

Tissue	Percentage of polyunsaturated fatty acids* in:			Percentage of fatty acids with chains longer than 18 carbon atoms in:		
	Ethanolamine glycerophosphatides	Serine glycerophosphatides	Choline glycerophosphatides	Sphingomyelin	Cerebroside	Cerebroside sulfate
			Subject, 10 months			
Gray matter	46.9	53.0	9.1	2.7	9.4	16.1
White matter	13.9	23.5	3.5	20.3	70.1	71.2
Myelin	12.9	11.5	1.0	23.6	80.1	
			Subject, 6 years			
Gray matter	41.2	36.0	9.5	5.8	15.0	34.0
White matter	24.9	9.0	2.6	50.4	65.7	88.3
Myelin	5.7	2.4	Trace	54.2	86.5	90.0
			Subject, 9 years			
Gray matter	28.4	25.1	7.9	7.9	34.7	32.5
White matter	23.7	12.5	4.3	42.8	85.1	85.6
Myelin	17.4	9.0	3.7	46.0	87.1	89.4
			Subject, 55 years			
Gray matter	41.0	48.5	7.6	25.5	90.2	90.0
White matter	15.5	14.9	2.1	63.2	86.4	82.1
Myelin	4.1	4.4	0.4	59.8	87.5	86.0

* Fatty acids containing from 18 to 22 carbon atoms and from 2 to 6 double bonds.

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The overall effect of these differences in lipid composition and structure is as follows. Myelin contains one-fifth the molar proportion of lipids containing polyunsaturated fatty acids that gray matter does. On the other hand, the molar proportions, in myelin, of lipids containing very-long-chain fatty acids (19 to 26 carbon atoms) are 10 times the molar proportions in gray matter. In the myelin group of lipids, 1 in 17 fatty acids is polyunsaturated; in the gray-matter group the value is 1 in 5. In the myelin group of lipids, 1 in 5 fatty acids has a chain longer than 18 carbon atoms; for gray matter the value is 1 in 100.

Intermolecular Cohesion of Lipid Constituents of Myelin

The intermolecular forces acting to hold lipid molecules in the myelin membrane can best be understood from a consideration of molecular models. Myelin membranes assembled with molecular models by Finean (*23*) (Fig. 3*c*) and Vandenheuvel (*24*) provide a framework for evaluating the importance of molecular size, shape, and composition on lipid packing. The recent model by Vandenheuvel (*24*) was made by constructing Dreiding stereomodels of individual lipid molecules and assembling them in bimolecular leaflet form (Fig. 4). This model illustrates the following points.

Lipids in myelin are not covalently bound, and the three major forces holding these molecules within the membrane result from (i) electrostatic interactions between polar groups of lipids and oppositely charged groups in adjacent proteins, (ii) hydrogen bonding between oxygen and nitrogen atoms in lipids and adjacent proteins, and (iii) London-van der Waals dispersion forces between CH_2 pairs in hydrocarbon tails of adjacent lipid molecules (*24, 25*). The major force holding such molecules together may be that resulting from interactions between CH_2 pairs in adjacent hydrocarbon chains. The total force due to CH_2

pair interaction is sizable, since such interactions are additive and the number of CH_2 pairs is large. For example, the maximum London-van der Waals dispersion force arising between a lecithin (choline glycerophosphatide) molecule containing saturated 18-carbon fatty acids and two identical molecules on either side of it in closest possible contact is approximately −18 kilocalories (*26*). On the other hand, the total electrostatic interaction between the ionically charged phosphorylcholine group of this lecithin molecule and the oppositely charged groups of an adjacent protein in closest contact is approximately −10 kilocalories (*25, 26*). It is apparent that the hydrocarbon tail of a lipid molecule has a large influence on its binding to adjacent lipid molecules.

The introduction of unsaturated fatty acids into a bimolecular leaflet will lead to a more loosely packed, less stable structure. The introduction of a single *cis* double bond into a hydrocarbon chain results in a kinking of the chain, since the bond angle between double-bonded carbons is 125°27′ (*27*) while that between single-bonded carbons is 109°28′. The inclusion of additional *cis* double bonds results in a further kinking of the chain; for example, docosahexaenoic acid, the major polyunsaturated fatty acid in the brain, is depicted as a C-shaped molecule by Vandenheuvel (*24*) (Fig. 5). Lipids containing such unsaturated fatty acids are hooklike structures which cannot approach neighboring molecules as closely as saturated lipids can, since the latter are rodlike structures (Fig 6). Because of steric hindrance it can be predicted that lipids containing polyunsaturated acids will not be held as rigidly in a bimolecular leaflet as those containing saturated fatty acids. The total CH_2 pair interaction between an unsaturated lipid and a neighbor molecule will be much less than that between a saturated lipid and a neighbor molecule since these forces are very sensitive to changes in interatomic distances; that is, the interaction for the unsaturated lipid is inversely proportional to the *fifth* power of the intermolecular distance between interacting CH_2 pairs (*25*). The total attractive force between three adjacent lecithin molecules containing saturated 18-carbon acids in closest possible contact can be diminished by as much as 20 to 30 percent if the central lecithin molecule is replaced by one containing a single highly unsaturated acid, such as arachidonic acid (*26*). Not considered in this cal-

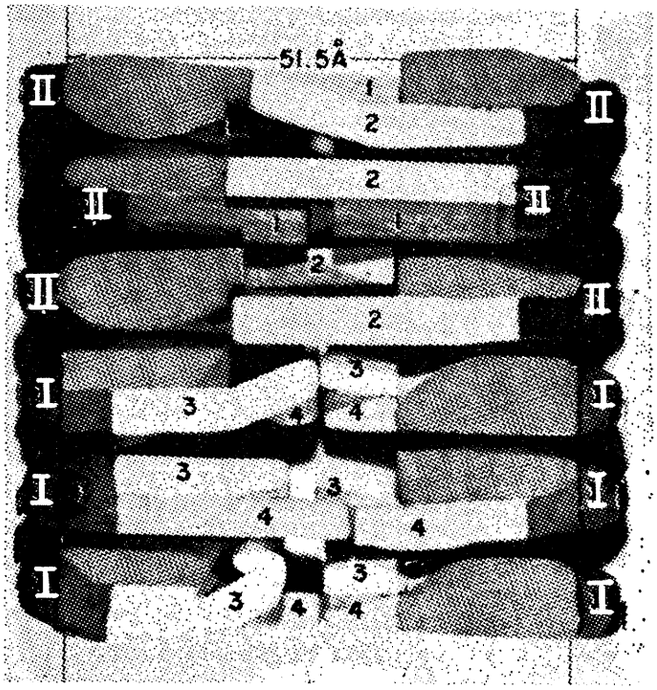

Fig. 4 (above). Vandenheuvel's molecular model of the myelin lipid bimolecular leaflet. (I) Phosphatidyl; (II) sphingo; (unnumbered dark hatching) cholesterol; (1) sphingosine; (2) fatty acid in sphingolipid; (3) fatty acid in phosphatidyl lipid (unsaturated); (4) fatty acid in phosphatidyl lipid (saturated). 1, 2, 3, and 4 are chains. The polar groups of the lipid molecules are on the outside of the leaflet, the fatty acids are inside. Sphingolipid fatty acids (2) interdigitate, but phosphatide fatty acids (3 and 4) are not long enough to do so. Unsaturated fatty acids (3) are depicted as bent or hooklike, depending on the degree of unsaturation. [From Vandenheuvel (*24*), courtesy *J. Am. Oil Chemists' Soc.*]

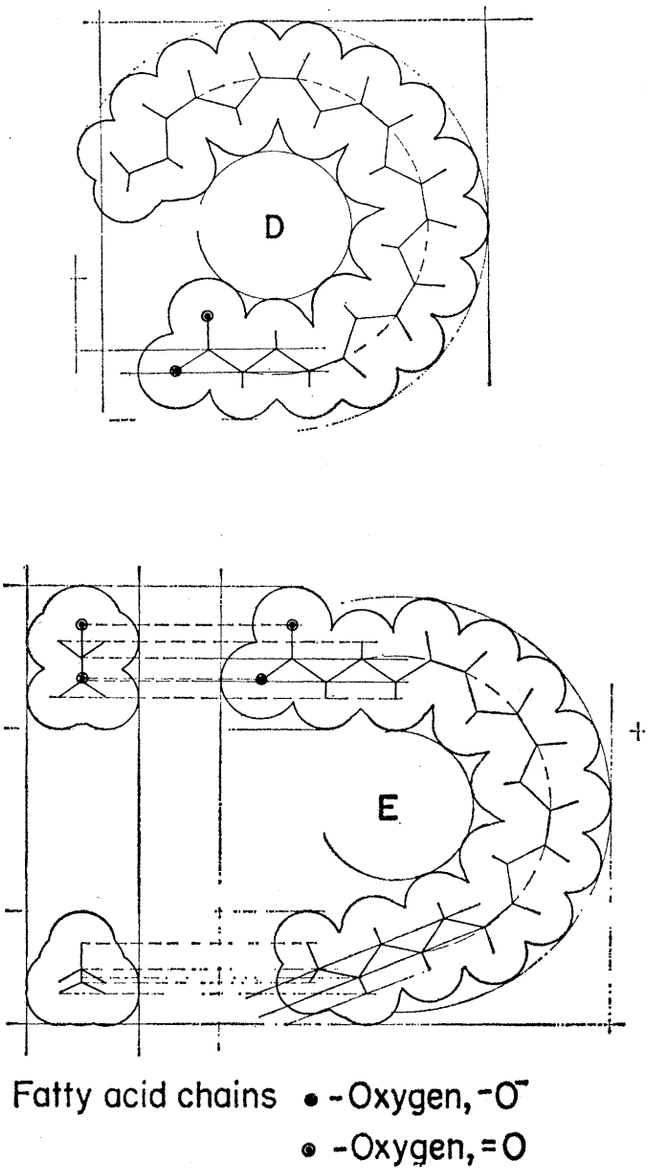

Fig. 5 (right). Effect of unsaturation on fatty acid structure. (*D*) Methyl docosahexaenoate (methyl Δ 4,7,10,13,16,19-docosahexaenoate); (*E*) methyl arachidonate (methyl Δ 5,8,11,14-eicosatetraenoate), illustrating the pronounced curvature induced by *cis* unsaturation. [From Vandenheuvel (*24*), courtesy *J. Am. Oil Chemists' Soc.*]

culation is the fact that, in myelin, lecithin probably occurs in a complex with cholesterol (*24*) (Fig. 6*A*). Such a complex, if actually present in vivo, would result in additional CH_2 pair interactions between lecithin and adjacent lipids (*24*). Polyunsaturated fatty acids such as those present in brain, which contain four, five, and six double bonds, if present in glycerophosphatides, would prevent the formation of such a complex because of their configuration (Fig. 6*B*) and would result in a further loss in potential binding force between adjacent lipid molecules.

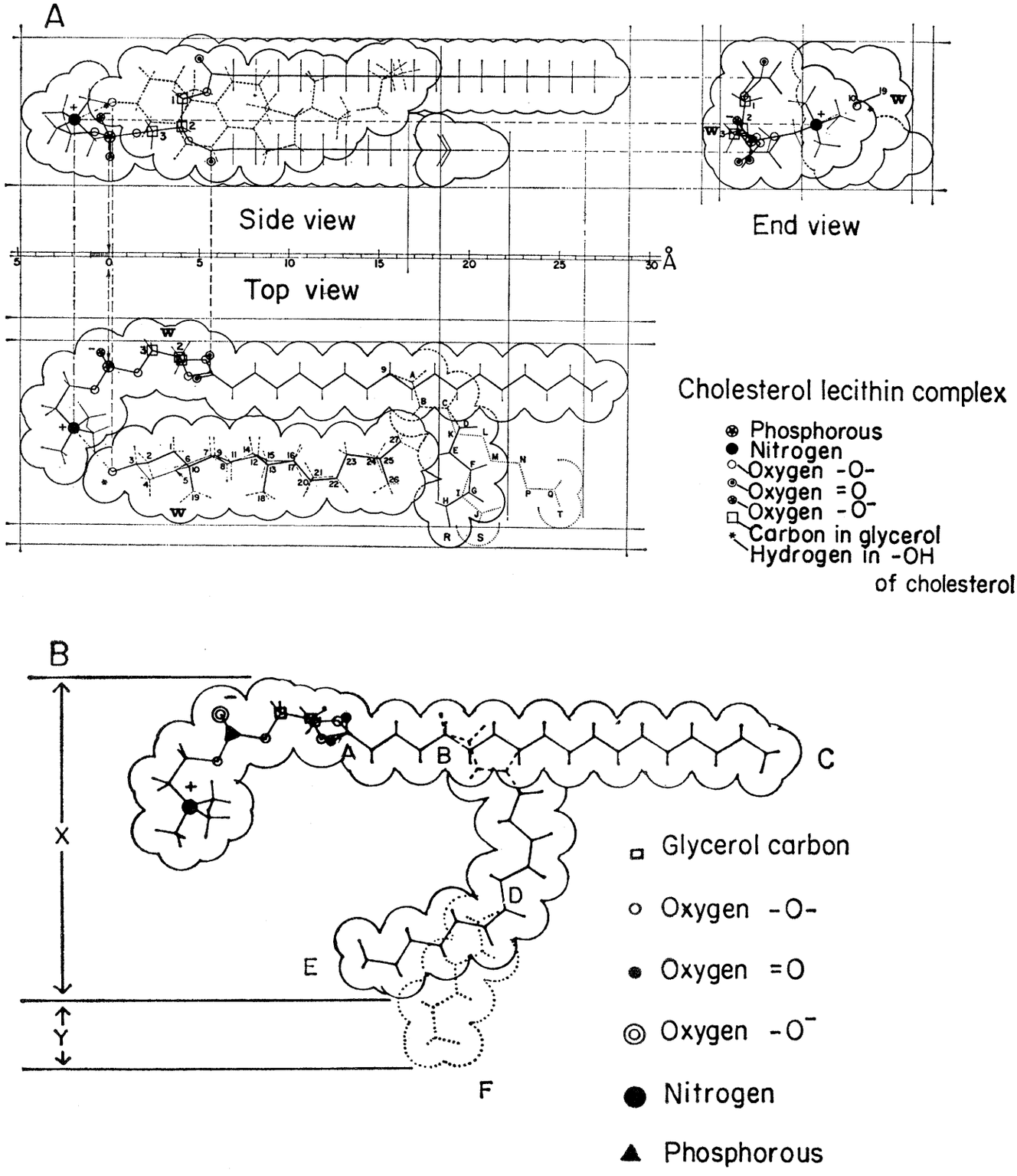

Fig. 6. Effect of unsaturated fatty acids on lipid structure. (*A*) Lecithin, containing linolenic acid (9-A-B-C-D-E-F-G-H-R), linoleic acid (9-A-B-C-D-E-F-I-J-S), and oleic acid (9-A-B-C-K-L-M-P-Q-T) [From Vandenheuvel (*24*)]. (*B*) Lecithin containing stearic acid (A-B-C) and arachidonic acid (A-B-D-E), or 5,8,11-eicosatrienoic acid (A-B-D-F) [From Walker and Kummerow (*29*), courtesy *Proc. Soc. Exptl. Biol. Med.*]. These models illustrate the hooklike structure of lipids containing polyunsaturated fatty acids and the rodlike structure of lipids containing saturated fatty acids.

Since the three major brain glycerophosphatides have similar molecular structures, as do the major polyenoic acids (*24*), the effects of introducing polyunsaturated fatty acids into various members of this group of lipids should be similar; that is, a glycerophosphatide containing a polyunsaturated fatty acid will be held more loosely in a bimolecular leaflet membrane, and hence be more easily dissociated and metabolized, than one containing saturated fatty acids. The fact that gray-matter glycerophosphatides have a polyunsaturated-acid content 3 times that of myelin glycerophosphatides may be the basis for the higher rate of metabolic turnover of these lipids in gray matter. Even more important is the effect of these structural differences on the stability of the membranes which contain them. Myelin will have greater stability than membrane structures in gray matter since the lipid molecules in myelin can be more closely packed.

Finally, lipids containing longer-chain fatty acids will be more tightly held in a membrane than those containing shorter-chain acids, since the longer the hydrocarbon chain is, the greater is the number of potential CH_2 pair interactions. Since cerebroside and cerebroside sulfate in myelin contain fatty acids whose chains are 25 percent longer than the chains of fatty acids contained in cerebroside and cerebroside sulfate in gray matter (for sphingomyelin the corresponding value is 10 percent), the binding force holding these lipids in the myelin membrane will be greater than that holding them in gray matter membranes. In addition, Vandenheuvel (*24*) has proposed that sphingolipids containing fatty acids with chains longer than 18 carbon atoms can form interdigitated complexes, while those containing fatty acids with chains shorter than 18 carbon atoms are not capable of doing so (Fig. 7). Such a complex is formed by interdigitation of the hydrocarbon tail of a sphingolipid on one side of the bimolecular leaflet with the hydrocarbon tail of a lipid on the opposite side of the leaflet. This complex, if actually present in vivo, would be highly stable and would further tend to immobilize sphingo-

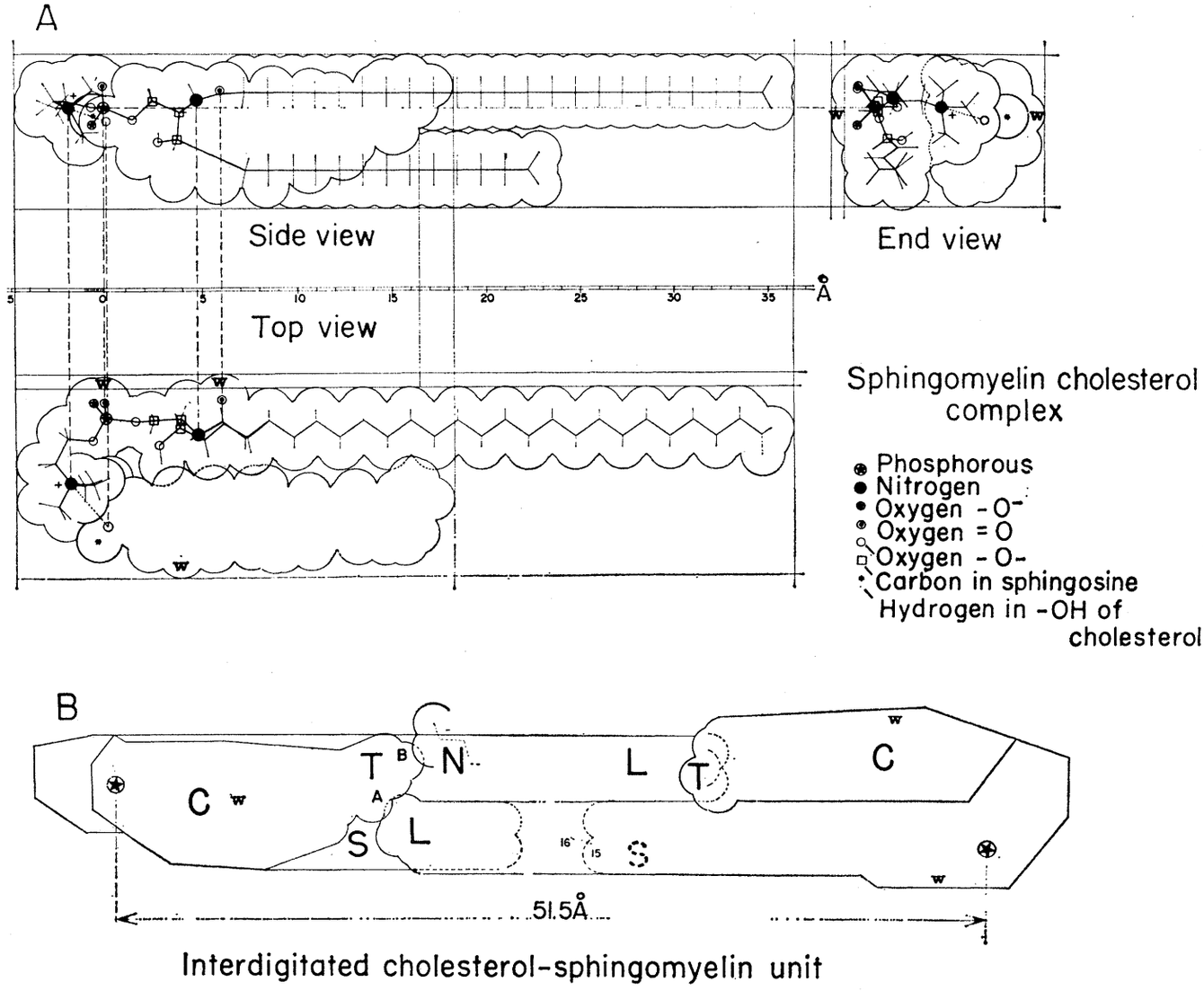

Fig. 7. Sphingolipids and the interdigitated cholesterol-sphingolipid unit. (*A*) Sphingomyelin-cholesterol complex. Sphingomyelin in this case contains lignoceric acid (24 carbon atoms), and the chain is 8 angstroms longer than that of the corresponding cholesterol-lecithin complex (Fig. 6*A*). (*B*) Interdigitated sphingomyelin-cholesterol unit. This complex was made by overlapping of two sphingomyelin-cholesterol units so that closest contact of all atoms was made without steric hindrance. *C*, cholesterol; *S*, sphingosine chain; *T*, cholesterol tail; *L*, lignoceric acid chain; *N*, tail end of nervonic acid chain when present. Stars indicate location of phosphate groups. [From Vandenheuvel (*24*), courtesy *J. Am. Oil Chemists' Soc.*]

lipids in the myelin membrane. Thus, the much lower rate of metabolic turnover of sphingolipids in myelin than in gray matter may result from the tighter binding of these molecules in myelin, due to the longer average chain length of the contained fatty acids. Once again, the overall effect of a preferential localization to myelin of sphingolipids containing long-chain fatty acids is to impart a high degree of stability to this membrane structure.

Role of Lipids in Stabilization of Myelin

It is logical to ask whether there are any physiological data which support the contention that the degree of saturation or the chain length of the fatty acids of membrane lipids can influence the stability of membranes in vivo. Kogl *et al.* (*28*) and Walker and Kummerow (*29*) have shown that an increase in the unsaturation of fatty acids in the erythrocyte membrane leads to a more permeable structure (*30*). Furthermore, Kogl *et al.* demonstrated (*28*) that a correlation exists between the stability of the erythrocyte membrane and its content of unsaturated fatty acids: the higher the content of unsaturated fatty acids in the membrane the more readily the membrane ruptured. These experiments with erythrocytes support the prediction that an increase in the proportion of lipids containing unsaturated fatty acids leads to a more permeable and less stable membrane. It is logical to apply this rule to myelin as well, since, like the erythrocyte membrane, it is an external cell membrane structure, and it is thought that the lipids in both membranes are arranged in bimolecular leaflet form.

Regarding the stabilization of a membrane by lipids containing long-chain fatty acids, the following indirect evidence is given. First of all, myelin is the most stable membrane known (lasting the lifetime of the animal), and its content of sphingolipids containing longer-chain fatty acids (19 to 26 carbon atoms) is, proportionately, 10 times that of any other membrane structure analyzed. It is tempting to suggest that these facts are related causally—that long-chain sphingolipids act as myelin stabilizers. Secondly, in two inborn errors of metabolism in which myelination is faulty (metachromatic leukodystrophy and Niemann-Pick disease) I have demonstrated, in white matter, a seven- to tenfold deficiency of sphingolipids containing long-chain fatty acids (*31*). The defect was specific; sphingolipids containing shorter-chain fatty acids were present in normal or higher-than-normal proportions, while the amounts of the remaining lipids were diminished, relative to normal, to a much smaller extent. Other workers have recently found similar deficiencies of sphingolipids containing long-chain fatty acids in a variety of diseases in which myelin is unstable, including multiple sclerosis (*32*), globoid cell leukodystrophy, and infantile Gaucher's disease (*33*). These observations prompt the suggestion that a deficiency of these long-chain sphingolipid molecules leads either to cessation of myelination or to the formation of unstable myelin, or to both. Finally, myelin in the baby is "chemically mature"—that is, it closely approximates myelin in the adult, both in total lipid content and in composition. When one examines the brain of a baby one finds that the major gross difference between it and the adult brain is the presence of a thin ribbon of white matter in the baby and of a thick mass of white matter in the adult. These observations suggest that myelination does not begin until a specific chemical composition is reached, one in which saturated glycerophosphatides and long-chain sphingolipids are present in high proportions.

Conclusions

The hypothetical questions which were asked earlier may now be answered as follows. Myelin lipids do possess characteristics which could lead to a tightly organized, closely packed, highly stable membrane structure, while lipids in adjacent neurons and glial cells do possess characteristics which could give rise to a more loosely organized, less stable membrane structure. The greater degree of metabolic inertness of myelin lipids may be explained on this basis. The stability of myelin itself may also be based largely on the intermolecular cohesion between lipid molecules, since the lipid content of myelin is extraordinarily high. Physiological and clinical studies tend to support the concept that a surfeit of polyunsaturated lipids or a deficiency of long-chain sphingolipids can result in a more easily disrupted membrane. The most important prediction to be drawn from these speculations is that myelin may be rendered unstable in disease if its lipid composition is altered so that higher proportions of lipids containing polyunsaturated fatty acids and lower proportions of lipids containing very-long-chain fatty acids are present.

I do not mean to imply that this is the only explanation for the stability of myelin. Very little is known about the structure and composition of some of the other constituents of myelin, which include proteins, polysaccharides, salts, and water. The molecular models which have been used as a framework of reference here are simplifications of a highly complex structure, of which the precise organization and molecular sequence are unknown. Also, I have drawn comparisons between myelin membranes and membranes of glial cells and neurons on the assumption that lipids occur predominantly in membranes in each locale, and that in these membranes they are arranged in parallel array with their hydrocarbon tails adjacent to one another. This assumption is an incautious one, since there is so little information on the molecular arrangement of the lipid molecules in the membranes of these cells. Furthermore, the multilayered arrangement of myelin should, by itself, restrict turnover of its constituents, and this undoubtedly accounts, in part, for the different turnover rates of gray-matter and white-matter lipids. Despite these uncertainties, I am prompted by the urgency of the clinical situation to make these speculations, in the hope that they will lead to further knowledge about demyelinating diseases. The primary purpose of presenting these speculations, then, is to provide a framework for fruitful experiments which will disprove or substantiate them.

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Quaternary Correlations across Bering Strait

Recent Soviet and American studies cast new light on the history of the Bering land bridge.

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The broad continental shelf extending beneath the Bering and Chukchi seas from Siberia to Alaska holds great interest for biogeographers. It has been the site, repeatedly, of a land connection, and thus of a migration route for land biota between Asia and North America; and when this land connection has been interrupted, as it is at present, the resulting seaway has been an avenue of migration for marine biota between the North Pacific and the North Atlantic oceans (*1*). Knowledge of the history and past environments of land bridges and seaways in this area must be sought largely through the study of the late Cenozoic sediments exposed along the Alaskan and Siberian shores of the Bering and Chukchi seas and through attempts to establish the synchroneity of individual sets of late Cenozoic sediments on opposite sides of Bering Strait.

The presence of marine sediments of late Cenozoic age in scattered places on the Siberian and Alaskan shores of the Bering and Chukchi seas (Fig. 1) has been known for many years, but, until recently, the more exact dating of these deposits has been very uncertain. Through stratigraphic and paleontologic investigations conducted during the last few years we are beginning to have a better idea of the probable ages of the late Cenozoic marine sediments of Chukotka and Alaska. In this article, and in a companion paper recently published in the *Izvestia* of the Academy of Sciences of the U.S.S.R. (*2*), we compare and attempt to correlate these sequences (Fig. 2). However, the comparison is complicated by several factors.

1) There are strong contrasts at present between the temperatures of coastal waters on the eastern and the western sides of Bering Sea, Bering Strait, and Chukchi Sea, because a strong current of relatively warm water from the North Pacific Ocean flows north along the Alaskan coast and a much weaker and colder return current flows south and west along the coast of Chukotka (*3*). If the correlations proposed in this article are correct, then the temperature contrast, the resulting faunal contrast, and presumably the circulation pattern that causes both, existed whenever Bering Strait was open during Quaternary time and perhaps during late Tertiary time as well.

2) American and Soviet paleontologists have somewhat different concepts of some of the difficult and variable species complexes that characterize arctic and boreal molluscan faunas. Because of this, published faunal lists for Siberian and Alaskan deposits are not strictly comparable. In some cases, different taxa are given the same species name, and in other cases the same taxa are given different names on opposite sides of Bering Strait. These taxonomic problems probably cannot be resolved until an opportunity arises for American and Soviet paleontologists to examine their respective collections together.

3) The late Cenozoic marine sequences have been at least slightly deformed in many areas in both western Alaska and Chukotka. Therefore cor-

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