even h reflections never change, it is clear that they are fixed by the structure (structure invariants) and cannot be given a sign value at will. One odd h reflection can be assigned a phase arbitrarily. The phases of all of the other odd h reflections are now fixed and no further arbitrary choice can be made.

This means that if $(+++++)$ is a solution to the myelin phase problem then $(-+-+-)$ is also a solution. In terms of the Geren (1954) wrapping model for myelin, the crystallographic origin can be chosen either at the center of symmetry between the extracellular protein layers or at the center of symmetry between the cytoplasmic protein layers. One cannot a priori distinguish between them.

Akers and Parsons do not state that $(-+-+-)$ is an acceptable solution to the myelin problem, but by omission from their Table I seem to imply that it is worse than the 15 phase sets selected for inclusion. They further define an R factor which is presumably a measure of acceptability of fit in their computer-analogue studies of heavy atom labeling. They report R values for six pairs of equivalent phase sets. For example, $R(-++++) = 52$ while $R(+-+-+) = 19$ and $R(++-++) = 13$ while $R(-+++-) = 55$. These disparities cast serious doubt upon the validity of their computer-analogue procedures and their phase solution. The rule of crystallographic pair equivalences demands that if a heavy label site is found at $x = 0.0$ and, for example, $R(++-+-) = 13$, then $R(-+++-)$ must $= 13$ for the label at $x = 0.5$.

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ALBERT HYBL
Department of Biophysics
University of Maryland School of Medicine
Baltimore, Maryland 21201

Reply to "Some Symmetry Considerations in the One-Dimensional Myelin Lattice" by Albert Hybl

Dear Sir:

We reported our phase sequence, as did previous workers (Finean, 1962; Finean and Burge, 1963; Moody, 1963; Burge and Draper, 1965; Worthington and Blaurock, 1968), only in relation to one of the two possible centers of symmetry of the double membrane repeat unit. While at the present time, a center of symmetry cannot be identified with either mem-

brane surface, Hybl's point, that the equivalent set of phases (odd orders having reversed signs) should also be stated, is well taken.

It is interesting to note that in adapting the swelling-phasing technique (Perutz, 1954) to myelin (Finean and Burge, 1963; Moody, 1963), the second- and third-order reflections occur within the same swept transform loop and so must be given the same phase sign. However, the equivalent phase set indicates that it is possible for the second- and third-order reflections to have opposite phase signs.

We disagree that the computer-analogue technique for determining phase signs (Akers and Parsons, 1970) is erroneous because similar R factors were not found for several equivalent phase pairs. The program does examine all 32 phase possibilities. In the original analogue, the electron density distribution (EDD) was normalized by setting the density value at the origin to 1.0. The gaussian parameter A (A: peak height of added electron density) was then allowed to vary in steps of 0.05 from 0.00 to 1.00. Thus, unless the electron density value of the EDD was the same at $x = 0.0$ and $x = 0.5$, the normalized EDD of the equivalent phase pairs would not be the same. However, improvements in the normalization and accuracy of the calculations are now found to result in a different sequence of phase combinations with increasing R while the choice of the best phase combination remains the same as previously reported. In addition, R factors for equivalent phase combinations are now found to be identical.

In order to reduce the number of computations from 32 to 16, and to include the concept of equivalent phase pairs, the computer program was modified. The EDD was normalized by setting to 1.0, not the density value at $x = 0.0$ but the largest electron density value occurring in the EDD. This allowed the labeled EDD from both phase sequences of an equivalent pair to be equal but displaced half a unit cell. The R factors for these two EDD derived from equivalent phase pairs become equal as mentioned by Hybl (Table I). The original analogue was also updated to the "Extended Precision" mode. Computational errors were consequently improved to an accuracy of 0.001 in R.

A calculation of all the electron density distributions resulting from the 16 equivalent pairs, indicates that there are three EDD types that fulfill the general assumptions that the membrane surface be electron dense due to protein and the interior of the membrane has low electron density due to lipid. Type I ($+++++$, $-+-+-$; $-++++$, $++-+-$) shows an asymmetry in the high electron density peaks at $x = 0.0$ and $x = 0.5$ with a medium peak at $x = 0.25$ in a region of general low density. These two equivalent phase pairs produce almost identical EDD's. Type II ($++-++$, $-+++-$; $++++-$, $-+-++$) have nearly equal high electron density at $x = 0.0$ and $x = 0.5$ with an asymmetric medium peak in the low electron density center of the membrane. Type III ($++--+$, $-++--$; $-+--+$, $++--+$) corresponding approximately to a model recently proposed by Worthington and Blaurock (1969), consists of a shelf of electron density with a narrow peak of high electron density adjacent to it. The center of the membrane is a uniform trough.

Table I shows the best fitting gaussian parameters for all equivalent phase pairs that have an R factor less than 10% using the modified program. The R factor is a function of the degree of fit between the analogue and the experimental data and differs somewhat from the crystallographic R factor (Akers and Parsons, 1970). The R factor for type I combinations ($+++++$, $-+-+-$) and ($-++++$, $++-+-$) are 2.7% and 3.6%, respectively. However, they have the same R factor within the limit of error (1.5% in R). The type II phase combinations ($++-++$, $-+++-$) and ($++++-$, $-+-++$) have R factors of 7.7% and 7.5%, respectively, and have equivalent R factors within the limit of error. However, the analogue significantly favors type I combinations over type II. However, the computer analogue rejected the type III phase combinations.

TABLE I

EQUIVALENT PHASE PAIR AND THEIR BEST FITTING GAUSSIAN PARAMETERS THAT GIVE (*a*) POSITIVE INTENSITY INCREASES AND (*b*) *R* FACTORS LESS THAN 10%

Phase	*R*	Curve I*			Curve II		
		A	*B*	*C*	*A*	*B*	*C*
	%						
+++++	2.7	0.50	0.06	0.00	0.15	0.06	0.45
−+−+−	2.7	0.50	0.06	0.50	0.15	0.06	0.05
−++++	3.6	0.50	0.06	0.00	0.20	0.03	0.45
++−+−	3.6	0.50	0.06	0.50	0.20	0.03	0.05
++−++	7.7	0.50	0.03	0.00	0.50	0.06	0.45
−+++−	7.7	0.50	0.03	0.50	0.50	0.06	0.05
++++−	7.5	0.50	0.09	0.00	0.40	0.06	0.50
−+−++	7.5	0.50	0.09	0.50	0.40	0.06	0.00

* Curves I and II represent added electron density due to metal label. *A*, *B*, and *C* are parameters (fractions of the unit cell, $d = 171$ A) which describe the gaussian curve (*A*: peak height, *B*: half-width at half-height, *C*: position relative to origin)—see Akers and Parsons (1970).

The difference between equivalent phase pairs that give similar *R* factors consists in the phase sign of the first-order reflection. Taking into consideration the extremely weak intensity of the first-order reflection, the alteration of the phase sign would have a small effect upon the EDD. The analogue also indicates that the second- and fourth-order reflections must be positive. Thus, both the equivalent phase pairs (+++++, −+−+−) and (−++++, ++−+−) satisfied the modified analogue as the correct phase sequence for the myelin membrane from the labeled sciatic nerve data.

In a report in preparation, this same heavy metal labeling technique has been applied to the myelin membrane of the frog optic nerve. The problem of center of symmetry will be discussed further in that report. It should be emphasized that our metal-labeling computer analogue is a general one and promises to provide more structural information about other biological materials such as collagen, muscle, and, possibly, fiber diffraction patterns of nucleic acids.

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C. K. AKERS
D. F. PARSONS
Biophysics Department
Roswell Park Memorial Institute
Buffalo, New York 14203

Nerve Radiation and the Dipole Model

Dear Sir:

In a recent letter published in the *Biophysical Journal*, Moisescu and Mărgineanu (1) attempted to rule out the dipole model (2) for nerve excitation by comparing a rough calculation of the radiated energy when a dipole shifts its orientation by 180° to the radiated energy measured by Fraser and Frey (3) for active crab nerves. By assuming Wei's (2) values for the dipole moment and the electric field in the vicinity of the dipoles they obtain a radiated wavelength of 60 μ. Since the measured radiated band was 2–20 μ, they state that the electromagnetic emission of the active nerves cannot be explained by the dipole theory.

We are presently doing electrodiffusion calculations for the electric dipole model (4). We have so far successfully fit the potassium iso-osmotic rectification data of Gilbert and Ehrenstein (5) and the normal rectification data of Hodgkin, Huxley, and Katz (6) for the squid giant axon. The energy difference between the postulated two dipole orientations is a parameter in the fits; it assumes values between 20 and 100 mev (wavelength: 62–12 μ) depending on the ion concentration on both sides of the membrane. (Our dipole moments range from 140 to 290 Debye.) Moisescu and Mărgineanu estimated the energy difference to be 20 mev. Our calculations were done with a crude constant-electric-field assumption. We are doing the calculations without this assumption now, and the energy difference could change considerably. Our calculations are for the squid axon. It would be desirable to have radiation data for the squid.

It should be emphasized that rough calculations of physical parameters in any physical system must be, at least, one or two orders of magnitude different than the corresponding experimental quantities in order to rule out a model.

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