LETTER TO THE EDITOR

Some Comments on "Myelin Membrane Structure as Revealed by X-Ray Diffraction" by David Harker

Dear Sir:

Recently David Harker has put forward a low-resolution electron density profile for the myelin membrane (1). He analyzes the low-angle X-ray data previously reported by C. K. Akers and D. F. Parsons (2), but he derives a profile which is quite different from their own. Furthermore, both profiles are markedly different from the type of bilayer profile proposed independently by J. B. Finean and R. E. Burge (3), by C. R. Worthington and myself (4, 5), and by D. L. D. Caspar and D. A. Kirschner (6). These conflicting results must leave many nonspecialists in doubt about determining the membrane structure by X-ray diffraction. One hopes now to allay their doubts.

The Akers and Parsons X-ray data from Os-treated myelin (2) fall far short of the three-dimensional, high-resolution data needed for a conventional heavy atom analysis. Single Os atoms cannot be located, and therefore the phases (signs) needed to compute a profile cannot be derived with the usual reliability (see p. 619 ff. in reference 7). Because they cannot resolve single Os atoms, Harker (1) and Akers and Parsons (2) need to find some other starting assumption in order to locate the stain deposits and hence phase the X-ray reflections. I concur with them (1, 8) that neither of their starting assumptions is decisively better. What is more, it has not been shown that either assumption is likely to be correct. All in all, these are not firm grounds for doubting the bilayer profile established by myelin-swelling experiments.

To analyze the swelling experiments, it is assumed that the membrane structure does not change during swelling. The assumption is physically reasonable, and its correctness has been tested by comparing, first, the diffracted intensities, and, second, the computed profiles (5). In this way and assuming a bilayer profile, a probable set of signs ($- + + - -$ for an origin at the cytoplasmic interface) was proposed for the first five orders of the normal diffraction pattern by Finean and Burge (3) and M. F. Moody (9). Moody also gave an alternative set. Using simplified bilayer profiles, the same probable set of signs was found by Worthington and myself (4). Then without the need to assume a bilayer profile, I have shown (5) that two kinds of swelling, which occur independently under different conditions, together uniquely define the signs. The set of signs given above is the only one consistent with all the swelling data. The same five signs have also been derived by Caspar and Kirschner (6) by comparing different myelins.

On page 1292 of his report (1) Harker considers two sets of signs, the above set and its negative. He then invites the reader to ignore the above set because it requires an assumption about the Os deposits which "seems to me much less probable"; there is no further comment. Harker thereby rules out the set of signs indicated by the myelin-swelling experiments. However, it is this set, and not the set preferred by Harker, which is confirmed by swelling myelin in different solutions, as follows.

In my report (5), Fig. 4 *a* compares profiles of myelin swollen in water and in 0.24 M sucrose. The bilayer profile of the membrane in water superimposes well on the profile of the

membrane in the sucrose solution. Outside the bilayer are broad fluid spaces. The level of electron density for the sucrose solution is higher than the level for water. This is the expected result since the electron density is known to increase with the concentration of sucrose. However, if one accepts Harker's profile, then my Fig. 4 *a* needs to be turned upside down, and consequently the sucrose solution appears less electron dense than water. Thus Harker's starting assumption, that Os is deposited in a narrow layer in the membrane, leads to a contradiction. But one finds no difficulty in rejecting his assumption: Harker does not claim it is unique, nor is it compelling.

Instead of attempting to deduce signs from the Akers and Parsons data, I have assumed the above set of signs in order to compute a profile for the Os-stained membrane. Subtracting the normal profile then gives a profile for the Os alone. This profile is, of course, Harker's Fig. 4 *a* turned upside down. Consequently the membrane can be said to stain least in a region near the center. It also follows that if one had assumed this distribution of stain, then the derived signs would be just those found by swelling. One may observe that the electron microscope image of the Os-fixed membrane shows a light band at the center.

In passing, I note that my analysis (5) does not assume that "the average electron density of the double layer itself was less than that of the interstitial liquid medium" during swelling (reference 1, p. 1291). Rather, I have concluded that the average electron density of the pair of membranes is greater than that of water but less than that of 0.8 M and, a fortiori, 1.8 M sucrose solutions. Thus one finds no grounds for Harker to claim that his profile is consistent with the swelling experiments.

The bilayer profile (5, 6, 10) is amply supported by diffraction data (5). It is also physically plausible, for it closely resembles the profile (11) computed for a mixture of phospholipid and cholesterol; these together are the predominant solids in myelin. Happily, one finds no sound evidence against the bilayer profile.

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