Ten days later the fetuses were delivered, bled, and autopsied.

The rationale behind this experiment was as follows: If the fetus could be shown to reject a skin homograft in the normal way in the presence of persisting rabbit anti-sheep globulins, then the conclusion would be almost inevitable that circulating antibody does not play an obligatory role in this process. Under these conditions, fetal antibody globulins, even if produced, should be unable to reach the graft site. If, on the other hand, graft rejection were inhibited or altered by the rabbit anti-sheep globulins, then circulating anti-graft antibody must be implicated in the rejection process according to a similar line of reasoning.

In every instance, the grafts were rejected in an apparently normal fashion. In addition, each fetus showed a persisting level of circulating rabbit γ-globulin at the end of the 10-day period. Of the animals that had received rabbit anti-sheep globulins, only one showed persistence of detectable anti-sheep 7S γ-globulin and anti-sheep β_{2M}-globulin activity in its serum by tanned cell hemagglutination assay. Although the titers were only moderate (1 : 8 in each case), satisfactory negative controls and standards render the data significant. It became apparent that at this point (10 days after injection) the rabbit anti-sheep globulins were being cleared rapidly from the fetal circulation, and that we were fortunate in finding persisting anti-globulin activity even in the one fetus. In addition to the normal catabolism of rabbit protein in the lamb, some of the rabbit anti-sheep globulins were disappearing due to their binding by the continuous production of low levels of β_{2M}-globulin normally found in the fetal lamb (*5*). Perhaps the chief reason for the rapid disappearance of the rabbit anti-sheep globulins, however, was initiation of antibody production by the fetus against one or more of the rabbit protein antigens injected, since each of these animals had plasma cells in its lymph nodes and spleen. Such antibody globulin response on the part of the fetus would tend to deplete its circulation more rapidly of rabbit anti-sheep globulin antibodies. These considerations suggest that during the earlier stages of graft rejection, circulating anti-sheep globulins were probably present in all four animals. In future experiments, more highly purified rabbit globulin preparations should be employed to diminish the number of antigens to which the fetus may respond.

Despite these drawbacks, it is felt that this experimental model has provided useful data and may be capable of further improvement and broader application. Thus far, it has furnished evidence that the formation of plasma cells and immunoglobulins is not a necessary accompaniment of the homograft rejection process, and that the orthotopic skin graft in the fetal lamb is rejected normally even in the presence of circulating anti-sheep globulins. These data are offered as evidence that conventional circulating antibody may not be an *obligatory* participant in the specific rejection of solid-tissue homografts (*7*).

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X-ray Diffraction Pattern of Nerve Myelin: A Method for Determining the Phases

Abstract. *The x-ray diffraction patterns of normal myelin, and of myelin expanded by soaking in hypotonic solutions, have been compared quantitatively. Application of the "shrinkage stage" method for determining the course of a real Fourier transform allows all but 2 of the 32 possible sign combinations to be eliminated.*

The small-angle diffraction pattern of nerve myelin consists of five orders of a fundamental repeat of 150 to 180 Å (depending on the type of nerve and species). Electron microscopy shows that this repeating structure consists of two adhering plasma membranes, the surface of adhesion presumably forming a mirror plane at low resolution. Since there are two surfaces of adhesion for each repeat, there will also be two such mirror planes, respectively, between the adhering inner (cytoplasmic) and outer surfaces of the membranes. Although these mirror planes strictly apply only locally and at low resolution to the complete myelin structure, they should apply exactly to the projection of myelin's electron density onto a radial line. It is precisely this projected electron density about which the equatorial diffraction pattern provides information. From the intensity of each order h, the magnitude $|F(h)|$ of the corresponding structure factor can be calculated, but its phase is indeterminate. Because of the mirror planes, each $|F(h)|$ can have only two possible phases, which are equivalent to plus or minus sign. If these signs could be determined, the relative average electron density in successive planes of a cell membrane could be calculated, as a Fourier synthesis, to a resolution of about 30 Å.

Additional information is needed to decide between the 2^5 possible Fourier synthesis. Preliminary results, from a quantitative comparison of the diffraction patterns of normal nerves and of nerves modified by soaking in hypotonic media, are reported here. Soaking causes the myelin membranes to separate at their outer surfaces while the inner surfaces remain attached (*1*). At the same time the diffraction pattern completely changes to give successive orders of a new repeating unit of 250 to 270 Å, but this change can be reversed by reimmersion in normal Ringer solution (*2*). This reversibility suggests that the structure of the membranes is not much altered during the swelling process, and that the change in the diffraction pattern could be accounted for by the separation of the lamellae.

The expanded form should therefore allow us to apply the method of "shrinkage stages" (*3*) for determining the signs. This method depends on the fact that the structure factors of normal myelin can be regarded as values of a continuous curve (the Fourier transform of a myelin lamella) sampled at equidistant points. The structure factors of expanded myelin (appropriately scaled) are also values of the same curve, but sampled at a different set of equidistant points. Each sampled value has the same sign as the corresponding part of the transform, and (in the case of a centrosymmetric structure) the transform cannot change sign without passing through zero. With a sufficient number of sampled points we can determine where this occurs, and thence deduce how the sign of the transform changes throughout its course. The signs of the structure factors follow from this.

Diffraction patterns of normal and expanded frog sciatic nerves were photographed with a line-focus x-ray camera. The $|F(h)|^2$ values were estimated from densitometer traces of the film, and the $|F(h)|^2$ values from different photographs were scaled by making

$$\sum_h |F(h)|^2$$

constant.

Figure 1 shows $\pm |F(h)|$ for normal and expanded myelin plotted against reciprocal spacing. The reflections of expanded myelin were blurred, and in many cases there was a continuous band from about 0.021 to 0.031 Å^{-1}. This excludes the possibility that the transform changes sign in this region,

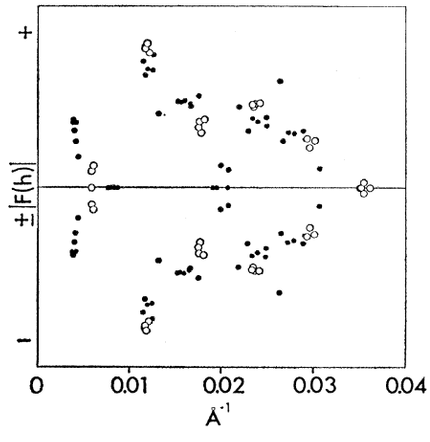

Fig. 1. Observed $\pm |F(h)|$ (arbitrary units) plotted against reciprocal spacing (2 sin θ/λ). Open circles, normal myelin; closed circles, expanded myelin.

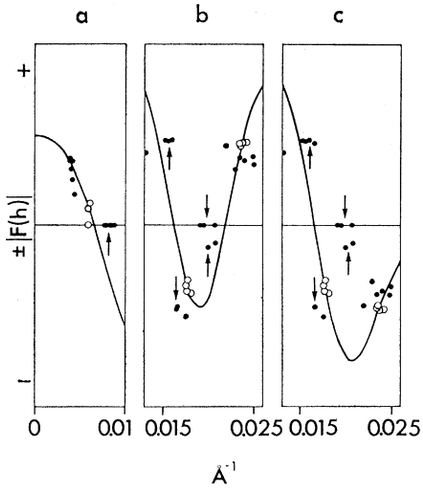

Fig. 2. Portions of transforms constructed from the averaged $\pm |F(h)|$ of normal myelin. (Different sign combinations are used in *a*, *b*, and *c*; see text for details); $\pm |F(h)|$ of expanded myelin (closed circles) which differ significantly from the transform are indicated by arrows.

in which the fourth and fifth orders of normal myelin lie: these orders therefore have the same sign. Other sign relations must be deduced from the probable course of the transform. This could be predicted from the principle of "minimum wavelength" (*4*), according to which any two successive peaks or nodes of the transform should always be separated by at least a certain minimum wavelength. However, it has been found that this principle is not always rigorously obeyed (*5*), so a more precise criterion will be used. The data from expanded myelin show considerable scatter, but the transform is sampled with relative accuracy by the $|F(h)|$ of normal myelin, and it can be rebuilt from these $|F(h)|$ by using Shannon's sampling theorem (*6*). Every sign combination gives a different rebuilt transform, which can be judged from its agreement with the expanded myelin data.

Since homogenized myelin is significantly denser than water (*7*), $F(0)$ is almost certainly positive. If $F(2)$ is negative the transform is rather large in the region 0.0076 to 0.0088 Å^{-1}, where the second order of expanded myelin should lie, but it was not visible. (The best fit transform for this region, with $F(2)$ negative, is shown in Fig. 2*a*.) So $F(2)$ is probably positive. In this case $F(1)$ is probably negative, for otherwise the transform is not sufficiently small at 0.0076 to 0.0088 Å^{-1}. Finally, $F(3)$ must be positive, for otherwise the transform seriously conflicts with expanded myelin data in the region 0.015 to 0.025 Å^{-1} [see Figs. 2*b* and 2*c*, in which the sign of $F(4)$ and $F(5)$ is respectively positive and negative]. The only remaining ambiguity concerns the sign of $F(4)$ and $F(5)$.

Thus, even with the present rough data for expanded myelin, the method allows the number of possible sign combinations to be restricted to two: $-F(1)$, $+F(2)$, $+F(3)$, $-F(4)$, $-F(5)$, and $-F(1)$, $+F(2)$, $+F(3)$, $+F(4)$, $+F(5)$. Except for the sign of the small first-order reflection, the former sign combination is the same as that preferred by Finean for other reasons (*8*). However, Finean expressed uncertainty concerning which of the two nonlipid regions of myelin coincides with the origin of his electron-density curve. This is equivalent to an ambiguity in fixing the signs, since the origin of the curve is shifted a half period (but the curve is otherwise unaltered) by reversing all the signs when h is odd. An advantage of the method presented here is that it is possible to localize the origin of the electron-density plot at the "cytoplasmic" mirror plane.

Better data should improve the certainty with which unsatisfactory transforms are eliminated, and may allow a decision to be made on the remaining ambiguity. It should also be possible to obtain an estimate of $F(0)$ by extrapolation to sin $\theta/\lambda = 0$, so that the actual density at different levels of a cell membrane could then be calculated, when the average density of myelin is known (*9*).

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