

# EFFECTS OF IONIC STRENGTH OF IMMERSION MEDIUM ON THE STRUCTURE OF PERIPHERAL NERVE MYELIN

BY J. B. FINEAN, PH.D., AND P. F. MILLINGTON

(From the Department of Medical Biochemistry and Pharmacology, University of Birmingham, England)

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As part of a general study of the effects of various ions on the low-angle x-ray diffraction patterns of nerve myelin, specimens were immersed in aqueous media containing ions in the proportions recommended for Ringer's solution but at hypertonic and hypotonic strengths. Observations were made which may have great significance in relation to the role of ions and of water in myelin structure.

## Materials and Methods

Only data on the frog sciatic nerve are reported in this paper. Segments of nerve trunk were immersed in ionic media for various lengths of time and then mounted in thin walled glass capillary tubes for examination by x-ray diffraction using high resolution low-angle cameras (1) and Cu  $K\alpha$  radiation. After preliminary experiments, studies were concentrated on specimens which had been immersed in normal (N), hypertonic (10 N), and hypotonic (N/4) Ringer's solutions, 0.25, 0.9, and 10 per cent saline, and Ringer's solution in which the  $Ca^{++}$  concentration alone had been increased to 10 N. After examination by x-ray diffraction, the specimens were either dried and reexamined later, or immersed in normal Ringer's solution and reexamined after a further 16 to 24 hours.

## RESULTS

The most interesting low-angle diffraction patterns were those obtained after 20 to 24 hours in 10 N and N/4 Ringer's solutions. Photometer traces of such patterns, together with a trace obtained from nerve immersed in normal strength Ringer's solution, are shown in Fig. 1. The 270 A band from the N/4 specimen can be seen to be clearly resolved in the original diffraction patterns but the photometer fails to resolve it to more than a marked shoulder in the trace.

The detailed changes of diffraction pattern resulting from immersion of the nerve specimens in hypertonic and hypotonic solutions will be described separately.

### *Hypertonic Solutions:*

In the early stage of modification of myelin structure by 10 N Ringer's solution there is an apparent strengthening of the third order x-ray reflection

of the 171 A fundamental unit. The band becomes as intense as the second order reflection, and its spacing is still such that it can be considered as a third order reflection. Subsequently the intensity of the band decreases again, and at the same time the spacing increases (to about 68 A) until it can no longer be considered as a third order reflection of the fundamental repeat, which is now increased to about 190 A. All other reflections can still be considered as orders of the fundamental repeat. This is the stage illustrated by the photometer trace in Fig. 1, and further changes take place only very slowly.

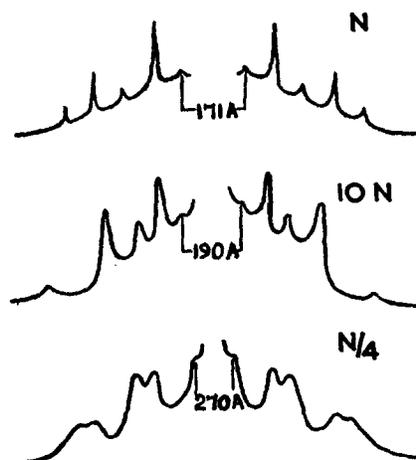


FIG. 1. Photometer traces of the low-angle diffraction patterns from specimens of frog sciatic nerve in normal strength (N), 10 N, and N/4 Ringer solutions. The probable fundamental layer spacing is indicated in each case.

Similar changes can be produced by immersion in 10 per cent saline, but Ringer's solution in which only the  $\text{Ca}^{++}$  is increased to 10 N (*i.e.*, only a small increase in over-all ionic strength) produces no appreciable change in diffraction pattern.

When the specimen is dried from this modified state, the diffraction pattern obtained is not significantly different from that of normal dried nerve (2). If the modified specimen is reimmersed in normal strength Ringer's solution for about 20 hours the pattern returns to the normal, fresh nerve pattern.

#### *Hypotonic Solutions:*

Studies of the intermediate changes in the production of the diffraction pattern N/4 of Fig. 1 show that the original fundamental repeat increases only slightly to about 180 A with little change in the relative intensities of the diffraction orders. The earliest significant change in pattern is the appearance of an extra diffraction band at about 65 A. This band is quite distinct

from the third order reflection of the 180 A repeat. It increases in intensity whilst the bands of the original fresh nerve pattern, with the exception of the second order, gradually fade out. The two intense bands can now only be related as third and fourth orders of a new fundamental repeat of 250 to 270 A. The first order diffraction at 250 to 270 A has now been resolved (Fig. 2). Furthermore, a very weak 250 to 300 A band has been recorded from specimens of nerve which have been kept in normal strength Ringer's solution for several days, though the reflections at higher angles all appear to be orders of the 171 A period. The band does not appear in the pattern of fresh nerve immersed in Ringer's solution of hypertonic strength.

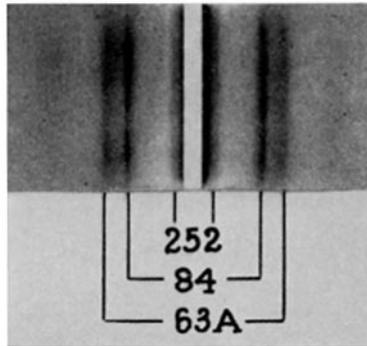


FIG. 2. Low-angle x-ray diffraction pattern from frog sciatic nerve immersed in  $N/4$  Ringer's solution.

The  $N/4$  pattern in Fig. 1 shows an additional diffuse band of diffraction at 30 to 45 A which persists until the late stages of modification. Occasionally peaks which may correspond to sixth and eighth orders of the 250 to 270 A period can be distinguished, but the definition never approaches that of the third and fourth order reflections. Prolonged immersion in  $N/4$  Ringer's solution eventually leads to an irreversible deterioration of the diffraction pattern, but if at the stage illustrated in Fig. 1 the nerve specimen is reimmersed in normal strength Ringer's solution a return to something closely resembling the fresh nerve diffraction pattern is obtained.

Drying from the modified state leads to an approximately normal dried nerve diffraction pattern.

Immersion in 0.25 per cent saline produces changes similar to those described for  $N/4$  Ringer's solution.

#### DISCUSSION

The first conclusion from these experiments is that the structural modifications revealed by the changes in diffraction pattern are due primarily to changes in the general ionic strength of the medium rather than to the specific

effect of any particular ion. The detailed effects will again be discussed separately.

*Hypertonic Solutions:*

Four of the five reflections in the diffraction diagram obtained from nerve immersed in 10 N Ringer can be accounted for as diffraction orders 1, 2, 4, and 5 of a fundamental layer spacing of 190 Å, but the reflection at 68 Å cannot be related to the other reflections in any simple way and must be considered separately. The main conclusion from the experiment is that the immersion in hypertonic solution produces a significant increase in layer spacing. This suggests that there can be little "free" water between the layers in the normal myelin structure, and the observed increase in spacing may result from a binding of additional ions (together possibly with their associated water molecules) by the layers.

The sequence of pattern changes which are featured in the development of the 68 Å reflection is similar in many ways to that observed during the drying of fresh nerve (2). The rapid intensification of a reflection in the region of the third order diffraction and then its subsequent decline in intensity and increase in spacing are featured in both series of observations. It has been suggested that this reflection may arise from a lipid phase which separates from the main lipoprotein structure (2) and produces independent reflections, but such a phase separation is not easily reconciled with the observation that the process is, to a large extent at least, reversible.

An attempt has also been made (3) to account for the reversibility of the early stages in drying by interpreting pattern changes in terms of small changes in the positions of the phosphate groups of the phospholipide molecules, but such considerations are not readily extended to the higher dimensions involved in these experiments with hypertonic solutions. One other possibility presents itself and that is that this odd reflection arises from organisation in a direction other than the radial one. If the myelin sheath is considered as a concentric layered structure, then a regular structural repeat within the plane of the layers but at right angles to the fibre direction would also give equatorial reflections when the specimen is examined perpendicular to the fibre axis.

From these and other considerations (4) it is becoming increasingly clear that in the molecular organisation of the myelin sheath there is an important 60 to 70 Å vector for which no provision has been made in the earlier suggestions (3, 5, 6) of general structural organisation in the radial direction.

*Hypotonic Solutions:*

There seems little doubt that in hypotonic solutions the 171 Å period of the fresh nerve is replaced by one of 250 to 270 Å, and, from the observed

features of the diffraction pattern changes, the transition would appear to be abrupt.

The well defined and intense reflections at about 90 Å and 67 Å are almost certainly third and fourth order reflections of the 250 to 270 Å period, and their presence signifies the continued importance of the 85 to 90 Å vector in the structure and again the rise to prominence of a 60 to 70 Å vector which has featured in so many previous experiments. The diffraction in the region of 30 to 45 Å is not at all well defined, although occasionally peaks can be distinguished at positions which correspond approximately to sixth and eighth orders.

The change of fundamental period from 171 Å to 250 to 270 Å could be accounted for in general in two ways. It could result from a structural re-arrangement of the components already present, or it could be produced by a rapid expansion of the water layers in the structure.

It has been concluded from previous studies that the 171 Å period includes two very similar lipoprotein layers (7). In these later experiments the magnitude of the increase in period (50 to 60 per cent) might suggest that a re-arrangement takes place which results in three layers forming a new repeat period. It would be expected, however, from the suggested mechanism of myelin formation from the Schwann cell membrane (8), that the radial repeating period in the sheath would consist of either one (for a symmetrical cell membrane) or two (for an asymmetrical one) membrane layers (4), and no other arrangements can be readily visualised.

On the whole, the evidence favours an explanation of the change in period in terms of a swelling of the original 171 Å unit by the inhibition of water. Certainly, as will be shown in the succeeding paper, the nerve fibre takes up a great deal of additional water when placed in hypotonic solutions. Furthermore, such a high degree of swelling with retention of crystalline regularity of layer spacings has a precedent in the swelling of pure lipid systems (9). However, the structural implications of such a high degree of swelling require more detailed consideration. The fact that the 85 to 90 Å vector remains prominent probably means that the interrelationships in at least one-half of the original unit remain unaffected by the swelling, and it may be that the entry of water into the structure is limited to one or both of the interfaces formed by the surfaces of the Schwann cell membrane in the production of myelin. In such a case the surfaces would be re-separated by at least 40 Å, and, because of the continuous nature of the layers, the surfaces would also be sheared (translated laterally with respect to each other). The observed reversibility of the process is perhaps surprising and, viewed in this way, the persistence of attractive forces between surfaces can hardly be invoked. Alongside this problem we might also place the difficulty of accounting for the limitation (for a time at least) of the expansion to 250 to 270 Å. Osmotic forces,

and perhaps also mechanical forces exerted by outer sheaths, may be involved, but further experimental data are required before detailed explanations can be put forward.

In these experiments the swelling of the structure would seem to be a prelude to the complete breakdown of the organisation and the appearance of the new reflection in the region of 250 to 300 Å may be one of the first signs of "ageing." Such results may have some significance in relation to the *in vivo* degeneration of myelin.

#### SUMMARY

A study of the effects of hypertonic solutions on the structure of peripheral nerve myelin reveals an expansion rather than a contraction of the layer spacing. This suggests the absence of "free" water between the myelin layers.

Hypotonic solutions bring about a change in radial repeat period from 171 Å to 250 to 270 Å.

These findings are of significance in relation to the structure of myelin.

#### BIBLIOGRAPHY

1. Finean, J. B., *J. Scient. Inst.*, 1956, **33**, 161.
2. Elks, J., and Finean, J. B., in Lipoproteins, *Discussions Faraday Soc.*, No. 6, 1949, 134.
3. Finean, J. B., *Nature*, 1954, **173**, 549.
4. Finean, J. B., *2nd Internat. Conf. Biochem. Properties Lipids*, Ghent, London, Butterworths, 1955, 127.
5. Schmitt, F. O., Bear, R. S., and Palmer, K. J., *J. Cell. and Comp. Physiol.*, 1941, **18**, 31.
6. Finean, J. B., *2nd Internat. Neurochem. Symp.*, Aarhus, London, Pergamon Press, 1956.
7. Finean, J. B., *Exp. Cell Research*, 1953, **5**, 202.
8. Geren, B. B., *Exp. Cell Research*, 1954, **7**, 558.
9. Bear, R. S., Palmer, K. J., and Schmitt, F. O., *J. Cell. and Comp. Physiol.*, 1941, **17**, 355.