ELECTRON MICROSCOPE AND X-RAY DIFFRACTION STUDIES OF THE EFFECTS OF DEHYDRATION ON THE STRUCTURE OF NERVE MYELIN

II · Optic Nerve

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ABSTRACT

The dehydration of rat optic nerve has been studied by allowing specimens to become partially or fully dried before fixation and preparation for electron microscopy. A correlation is established between electron micrographs of the myelin sheath and corresponding small-angle x-ray diffraction patterns. The modifications of the optic nerve myelin layers during drying were very similar to those described in more detail for the myelin of frog sciatic nerve. The most striking difference was that the system of fine layers characteristic of the fully dried myelin was much more extensive in the case of the optic nerve, and the layer thickness was significantly greater than the corresponding layer in the frog sciatic nerve preparation. The significance of these correlations is discussed.

The small-angle x-ray diffraction characteristics of myelin of central origin have been shown to differ considerably from those of peripheral nerve myelin (1–4, 7), but the myelin lamellae revealed in electron micrographs of normal preparations of peripheral and central nerve material are virtually identical. From the diffraction data it has been suggested that different types of lipoprotein associations might be involved in the molecular organisation, but the only chemical difference so far demonstrated relates to the amount of proteolipid extracted by 2:1 chloroform:methanol solvent (3, 6).

In studying the dehydration of myelin, the comparison has been extended by doing parallel experiments with a peripheral nerve (see previous paper) and a nerve representative of the central nervous system. This second paper is concerned with the data obtained from the optic nerve myelin and with the comparison with data on peripheral nerve myelin presented in the previous paper.

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MATERIALS AND METHODS

Rat optic nerve was used in all experiments.

The experimental procedure followed was exactly analogous to that already described for frog sciatic nerve (5).

RESULTS

X-Ray Diffraction Data

X-ray diffraction results are summarised diagrammatically in Fig. 1 and illustrated in Fig. 3.

Diffraction Studies during Drying: The sequence of

changes in small-angle diffraction pattern recorded as the rat optic nerve was allowed to dry was similar to that obtained in an earlier study of bovine optic nerve by Teale (7). The changes also resembled those recorded from frog sciatic nerve in that the main features of the early stages of drying were the introduction of a new reflection at about 60 A, and the fading out of the 40 A diffraction (Fig. 3 *b*). Subsequently a decrease in the intensity of the original 80 A band also became apparent, and this was accompanied by a gradual decrease in spacing. When the 40 A diffraction was no longer detected in normal exposures the remaining pattern featured two bands of similar intensities at 70 to 75 A and 60 to 62 A (Fig. 3 *c*). This could be considered as an intermediate stage in the drying changes. As drying progressed further, the 70 to 75 A band continued to decrease both in intensity and spacing until finally it appeared to merge with the 60 A diffraction. Meanwhile, a further diffraction had appeared at about 45 A, and the diffraction pattern of the fully dried preparation featured intense bands at 63 and 45 A and a faint but clearly defined reflection at 34.5 A (Fig. 3 *d*). The 63 A band occasionally appeared to be resolved into two reflections at about 60 and 65A, and when a study was made of the effects of heating the fully dried optic nerve preparation it was noted that under conditions which would have caused the complete disappearance of the 60 A reflection (transformation to the 40 A polymorphic form) from the sciatic nerve diffraction pattern, the 63 A reflection of the dried optic nerve diffraction pattern persisted but with reduced intensity. The observations were such as to suggest that the 63 A band might represent more than one structural unit.

OsO_4-Fixed, and Fixed and Embedded Preparations: The small-angle diffraction pattern of optic nerve which had been fixed with osmium tetroxide immediately after isolation showed the first four orders of diffraction of a 140 A fundamental repeating unit. As reported in an earlier publication (4) the relative intensities of the diffraction bands differed from those in the corresponding sciatic nerve pattern in that the fourth order was more intense than the third. The diffraction pattern of the corresponding embedded preparation showed two rather diffuse bands which corresponded to orders of a $\sim$ 140 A repeat period.

The diffraction patterns of specimens fixed with osmium tetroxide after various periods of drying showed only one general type of change. An additional 55 to 58 A diffraction was introduced, and the bands of the main (120 to 140 A) periodicity showed a gradual decrease in intensity. In the diffraction patterns obtained from the corresponding embedded preparations an increasingly intense small-angle x-ray scatter eventually made it impossible to detect any discrete reflections above about 100 A, but a diffraction was observed at 60 to 70 A which could be considered as a second order diffraction from a 120 to 140 A unit, and there was a strong diffraction at about 50 A. As

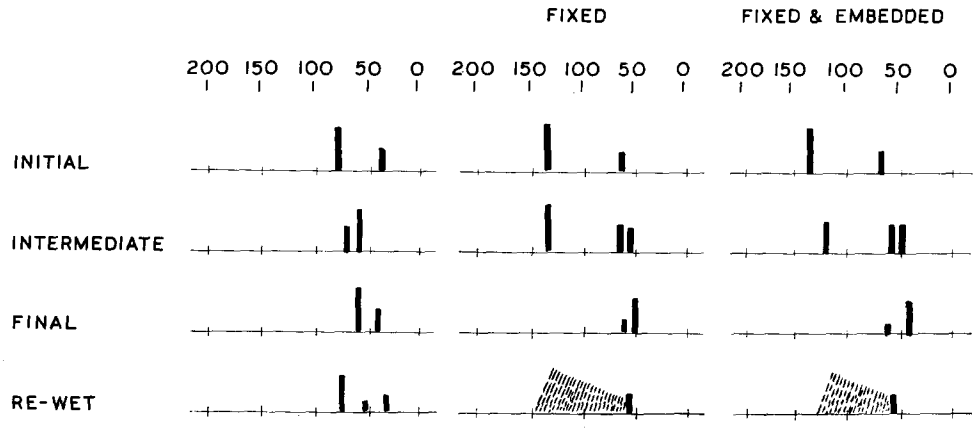


FIGURE 1

Diagrammatic summary of small-angle diffraction data. The diffraction spacings in A units, intensities, and definitions are indicated for each type of diffraction pattern recorded. Diffuse scatter is represented by intermittent line shading.

the drying period before fixation was extended, the 50 A band strengthened whilst the intensity of the 60 to 70 A band decreased. Thus, from the diffraction data derived from OsO_4-fixed, and from fixed and embedded preparations, the dehydration series appeared as a gradual change from 120 to 140 A units to 50 to 60 A units.

Rehydration: When the fully dried optic nerve was re-immersed in Ringer's solution it eventually yielded a diffraction pattern very similar to the normal, although occasionally an extra 60 A reflection was also recorded. When fixed with osmium tetroxide the preparations gave only one clear diffraction at about 60 A, and after embedding in araldite the diffraction was extremely weak, but the spacing appeared little changed.

Electron Microscopy: Electron micrographs of normal OsO_4-fixed preparations of optic nerve showed a system of close packed nerve fibres, penetrated only occasionally by cell processes (Fig. 2). In most regions there was a complete absence of connective tissue and unmyelinated fibres, and myelin sheaths of adjacent nerve fibres were closely apposed (Fig. 2). They appeared to have on the average far fewer lamellae than the peripheral nerve myelin sheaths but these lamellae were of very similar appearance. They were 110 to 120 A thick, and featured a well defined main dense line but only a very faint and somewhat variable intraperiod line.

In the electron micrographs of the partially dehydrated specimens the axons were observed to have collapsed, and at low magnification individual fibres could not readily be distinguished. Many areas of the tissue were virtually reduced to one continuous mass of myelin.

The sequence of changes in the appearance of the myelin lamellae was very similar to that previously described for the myelin of frog sciatic nerve. The intraperiod line became much more intense and better defined, and after a preliminary drying period of about 40 to 60 minutes some of the layering was seen to have collapsed to give layers 80 to 90 A thick but showing no intraperiod dense line. More prolonged drying produced specimens which again featured two types of layering (Figs. 4 and 5), one (the principal layering) consisting of 130 to 150 A thick layers with prominent intraperiod dense lines and the other a fine layering averaging about 50 A in thickness. The intraperiod dense line of the principal layering occasionally appeared to be split into two closely apposed lines (Fig. 4). In the case of the desiccator-dried optic nerve preparation the fine layering appeared to account for a greater proportion of the layered system than was the case in the corresponding peripheral nerve preparation, and the layers were significantly thicker. There was a definite continuity between the two types of layering which clearly established that two fine layers were related to each principal layer, alternate dense lines of the former being derived from the intraperiod dense line of the latter. In many cases, it was possible to see the continuity preserved at each end of the collapsed region. Another striking feature of these electron micrographs was the over-all lower density of the fine layered regions as compared with those relating to the principal layers.

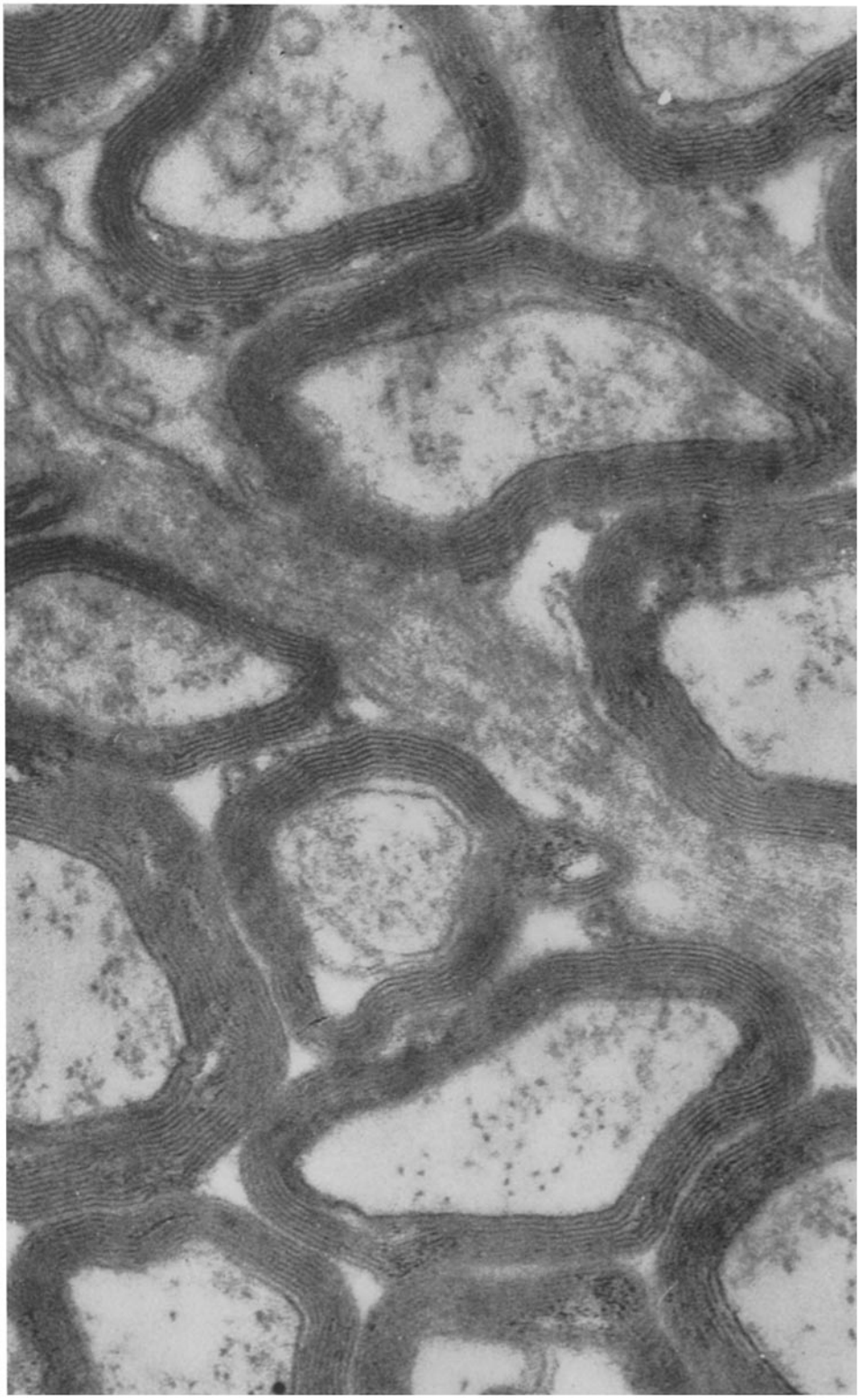
Electron micrographs of preparations which had been dried and re-wet before fixation indicated that the axon volume had been at least partially restored, but the myelin layers were on the whole very poorly defined. In the few areas in which the layer thickness could be measured, a value in the region of 60 A was obtained, although it may be that there was a small difference between alternate layers which could be related to a true periodicity of about 120 A.

DISCUSSION

From general observations on the changes in relative volumes of myelin and axons during the dehydration of optic nerve it is clear that the water content of the myelin sheath is much less than that of the axon. However, the difficulty encountered in distinguishing individual components in the low magnification electron micrographs of the partially dehydrated preparations has prevented a quantitative assessment of the water content such as was attempted in the case of peripheral nerve myelin (5).

Considering now the details of the myelin layering, the changes observed in the electron micrographs of the optic nerve preparations during dehydration tend to stress the structural similarities of the myelin in optic and sciatic nerves but also demonstrate certain differences.

The general diffraction and E.M. evidence again suggests that the fine layers seen in the myelin sheath of optic nerve are simply lipid layers, whilst the principal layers are probably lipoprotein. The striking difference in over-all densities between regions featuring these two types of layer may be related to such a chemical difference,



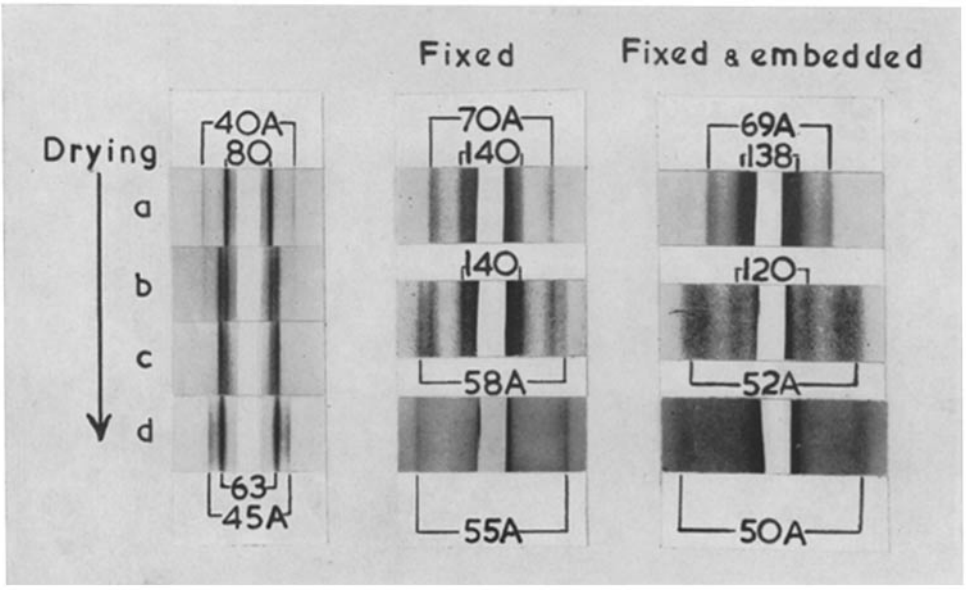


FIGURE 3

Examples of small-angle diffraction patterns recorded during the study of the dehydration of optic nerve myelin.

although, as this is now a heterogeneous system, different regions may be differently affected by the preparative and photographic procedures, and such general density variations may, therefore, not be structurally significant.

In this system there is more extensive evidence of continuity between the two types of layering, but the possibility of an independent fine layering which might be identified with the lipid phase suggested from diffraction data is not excluded. The molecular interpretation of the observations is thus subject to the same uncertainties as those already discussed for sciatic nerve preparations.

If the fine layers of both optic and sciatic dried nerve preparations are lipid layers, then the difference in dimension observed in electron micrographs might be an indication of a difference in the composition of lipid layers in the two systems. However, no comparable difference is evident in the diffraction patterns of the dried but unfixed preparations, and chemical analyses have so far revealed no appreciable differences as far as lipid types (lipid "end group" analysis) are concerned (3).

The electron microscope and x-ray diffraction data relating to the principal layering in dried optic nerve myelin indicate that this layering is almost eliminated by the drying process. However, the principal layers that are seen in the electron micrographs are very similar to those of the comparable sciatic nerve preparation.

In general the comparison of electron micrographs of the dehydrated myelin of optic and sciatic nerves indicates that in both cases there has been a partial transformation from principal ($\sim$ 140 A) layers to fine ($\sim$ 50 A) layers and that each principal layer is related to two fine ones, but the extent of transformation is appreciably greater in the optic nerve myelin and the fine layering has a higher periodicity. Such differences could be related to differences in chemical composition and type of lipoprotein association but present data does not allow more detailed speculations.

FIGURE 2

Electron micrograph of cross-section of "normal" osmium tetroxide–fixed rat optic nerve preparation. Layer spacing $\sim$ 115 A. $\times$80,000.

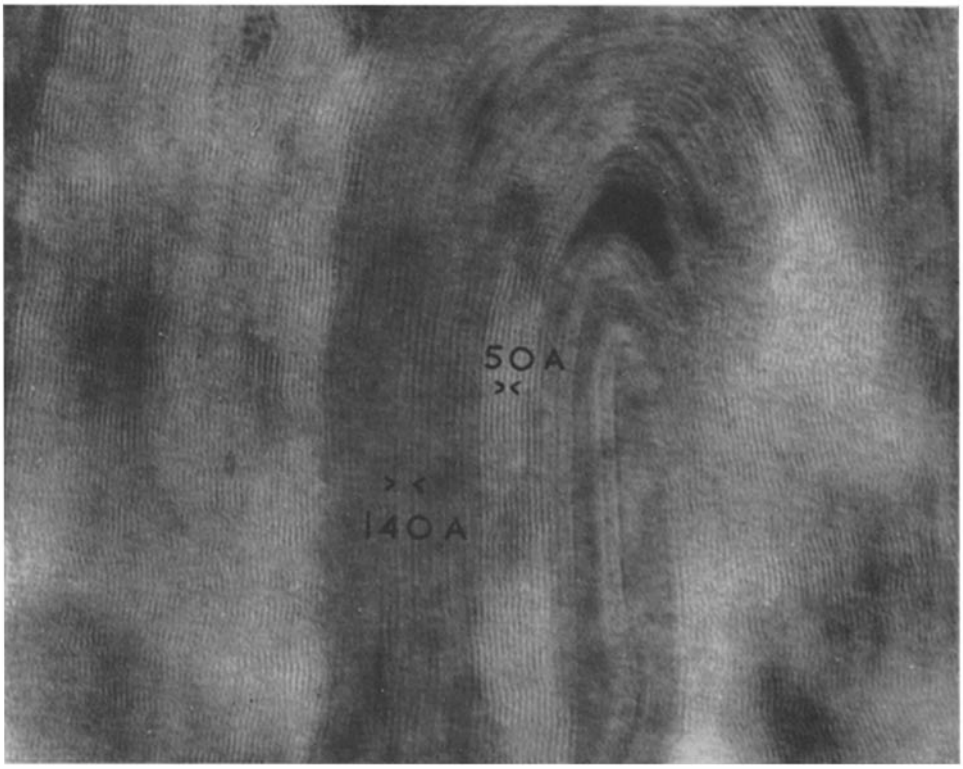


FIGURE 4

Electron micrograph of a cross-section of optic nerve preparation which had been desiccator-dried before fixation in osmium tetroxide. Principal layer spacing 130 to 150 A. Fine layer spacing ~ 50 A. × 160,000.

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FIGURE 5

Electron micrographs of cross-sections of optic nerve preparations which had been desiccator-dried before fixation in osmium tetroxide. Principal layer spacing 130 to 150 A. Fine layer spacing ~ 50 A. × 160,000.

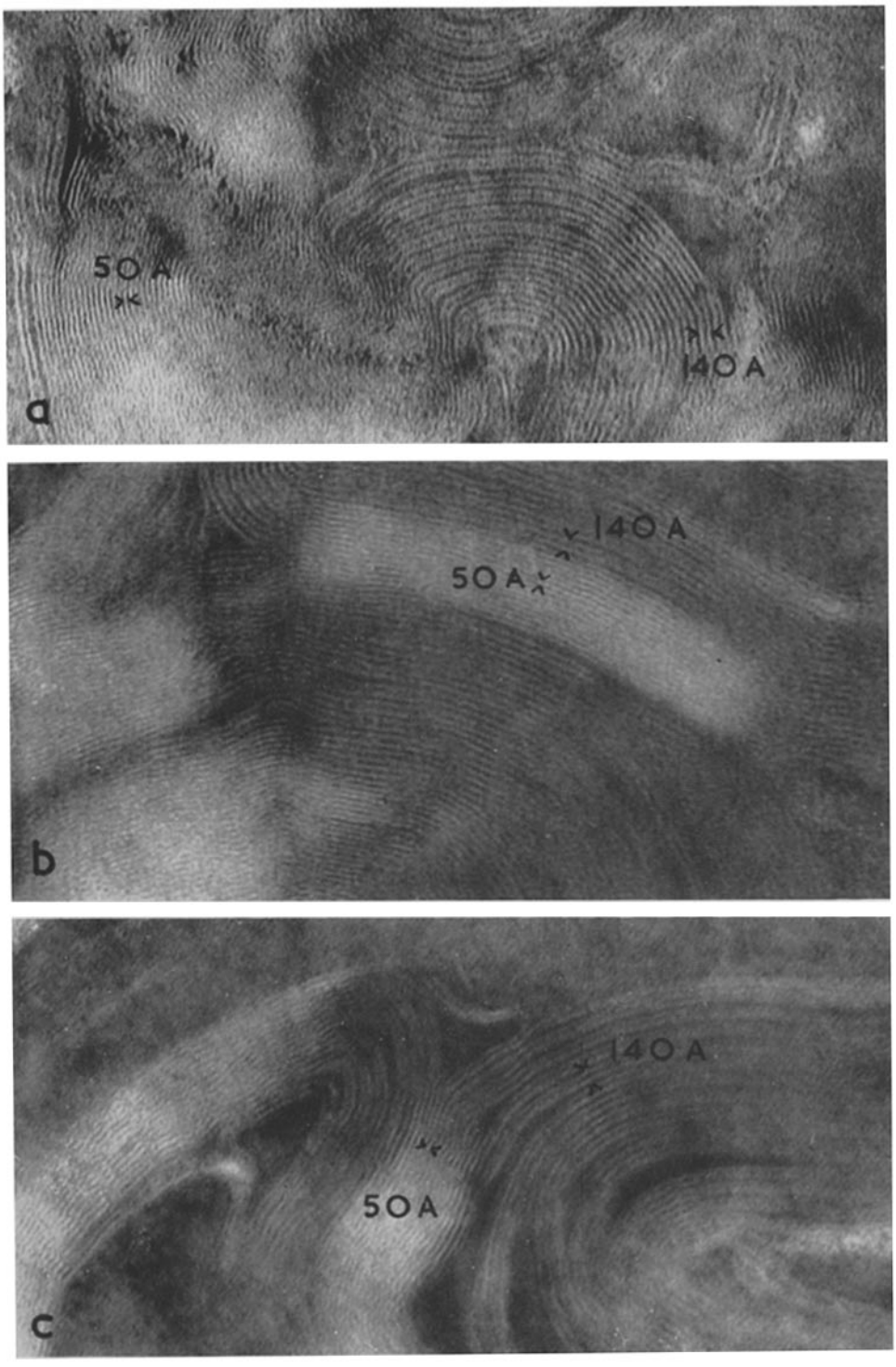

FIGURE 5