THE ROLE OF WATER IN THE STRUCTURE OF PERIPHERAL NERVE MYELIN

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Some progress has been made in deducing the detailed arrangement of lipide and protein components in the myelin sheath of nerve fibres but there have been only general comments on the role of water in this structure. Some attempt has been made to estimate the amount of water in the myelin sheath (1–3), but as myelin has not yet been isolated from the other nerve components somewhat indirect methods have had to be used with consequent uncertainty as to the reliability of the results.

From a comparison of the changes in diffraction spacings of myelin on drying with those observed for lipide emulsions (1), it was estimated that the water content of the myelin was probably of the order of 30 per cent. This was suggested to be a minimum figure because the protein component of myelin had not been considered, but as the change in diffraction spacing used in the comparison was that of the whole lipoprotein system of myelin such an argument would appear to be invalid. Further uncertainty as to the accuracy of the estimate arises from the possibility of part of the decrease in spacing being due to lipide rearrangement rather than to the simple removal of a water layer, and also from the presence of intermicellar water in the lipide emulsion used for the comparison.

Estimates from radioactive tracer studies and from x-ray microradiography have indicated a water content of 60 to 70 per cent for the myelin sheath. In the radioactive studies (3) assumptions had to be made concerning the extent of penetration of the radioactive material into the various parts of the nerve structure, and it was further assumed that in any water phase penetrated the radioactive material attained a concentration equal to that in the surrounding medium. Although this led to a reasonable over-all picture of the distribution of water in the nerve fibre, such an assumption can hardly be justified for an organised water phase such as probably exists in myelin, and the estimation of the water content of myelin on the basis of such studies can be no more than approximate. The x-ray microradiographic method, which can be applied to a single nerve fibre, should provide the most direct and reliable method for studying the water content of individual tissue com-

ponents. However, the early studies (2) on nerve fibres adopted the simplest procedure of estimating the mass of material in the dried structure, and calculated the amount of water removed during drying by assuming an original fresh tissue density of 1 and neglected any shrinkage or distortion of the structure due to the drying process. The error in the original estimations may have been considerable but the accuracy could certainly now be improved in view of the more recent refinements in the x-ray microradiographic technique.

The purpose of this paper is to present new experimental data relating to the water content of normal frog sciatic nerve, and of specimens treated with hypertonic and hypotonic Ringer's solutions, and to consider these data in relation to the detailed molecular organisation of the myelin sheath.

Methods

Only data relating to frog sciatic nerve are reported in this paper.

A study was made of the kinetics of the drying process by suspending a 10 mg. segment of nerve trunk from the arm of a torsion balance by means of a very fine stainless steel wire, the nerve being enclosed in a glass chamber over sulphuric acid (sp. gr. 1.84), and following the change in weight during drying. The specimen was initially dried superficially on filter paper, and weighings were made at intervals initially of 1 minute but at progressively longer intervals as the rate of evaporation of water decreased. The experiment was continued for 3 to 4 hours, and the drying was finally completed in vacuum over P_2O_5 at a temperature of about 60°C. Data were obtained for fresh nerve immediately after removal from the frog, and after washing briefly with Ringer's solution, and also from specimens which had been immersed in normal (N), hypertonic (10 N), and hypotonic (N/4) Ringer's solutions respectively for 20 to 24 hours. The data for fresh nerve, nerve washed briefly in Ringer's solution, and nerve left in Ringer for 24 hours showed some small variations in over-all water content, but for the purposes of this investigation the differences in the drying kinetics were not significant, and only one set of data is given.

The low-angle x-ray diffraction patterns of all types of preparation were recorded in high resolution cameras before and after drying. The detailed changes in low-angle diffraction patterns were studied by allowing the nerve specimen, slung across a wire loop, to dry freely on the open camera, and taking a series of 10 to 15 minute exposures on a single strip of film during the drying process. The camera geometry was arranged so as to give a clear resolution of diffraction spacings up to 100 A at a specimen-to-film distance of 70 mm. In order to facilitate the correlation of the changes in diffraction pattern with the characteristics of the drying kinetics, a part of the same specimen was suspended from the arm of the torsion balance so as to be within 2 to 3 cm. of the diffraction specimen, and its weight changes studied under the non-controlled conditions.

RESULTS

Drying Kinetics:

Eight drying experiments were carried out under carefully controlled conditions, and four experiments were made in which serial x-ray diffraction patterns were recorded from a freely drying specimen and parallel drying curves obtained.

The kinetics of the drying process have been expressed in terms of the variation with time of the log of the weight of water remaining in the tissue per milligram of ultimate dry weight of tissue. Such a plot should produce a straight line relationship for each "phase" from which water is being removed. The plots obtained in these experiments could be readily and consistently resolved into a small number of straight line relationships. An idea of the reliability of this method of analysis can be derived from the mean variation figures given in Table I. These figures cover a large number of curves obtained over a considerable period of time and analysed by several different workers. The results for nerve in normal (N) Ringer's solution form at least five different water "phases" in the system. Such a curve is illustrated in Fig. 1. In attempting to obtain a quantitative interpretation of the drying curves only the data from the eight carefully controlled experiments have

TABLE I

	N				10 N				N/4		
	A	B	C	D	A	B	C	D	A	BC	D
All consecutive	1.12*±0.2	1.38±0.25	0.23±0.07	0.16±0.03	0.9±0.2	0.58±0.1	0.14±0.02	0.2±0.02	1.2	2.6	0.2
All simultaneous	0.36±0.18	1.07±0.3	1.09±0.18	0.36±0.09	0.4±0.15	0.74±0.1	0.33±0.10	0.36±0.06			
B & C simultaneous	1.12±0.42	0.64±0.2	1.0±0.13	0.16±0.03	0.9±0.2	0.35±0.05	0.35±0.05	0.2±0.02	1.2	2.6	0.2

* Figures represent milligram of water per milligram of dried tissue.

been used. An average curve for these experiments is given in Fig. 2 and alongside the graph is an indication of the parallel changes in diffraction pattern. The first phase (Fig. 1) has been shown experimentally to be due to surface water, but as this is largely removed before suspending the nerve from the balance it has not been thought necessary for the purpose of calculations to distinguish it from the second phase (*A*, Fig. 2) which dominates the initial stages of drying. Furthermore, the part of the curve below 0.2 mg. per mg. is taken to be represented by one phase (*D*) though in fact there may be more but the rate of evaporation is too slow to show them. Thus for the purpose of quantitative interpretation only four phases are distinguished.

Three alternative methods of interpretation are adopted. In the first, all evaporations are considered to be consecutive; *i.e.*, all water is removed from one phase before evaporation from another commences. The amount of water in each phase is thus obtained simply from the measured amounts of water in the system at the cross-over points between phases. In the second method of interpretation, evaporation is assumed to take place from all phases simultaneously, and the water content of each phase is then obtained by extrapolating each line back to zero time and deducing the water contents from

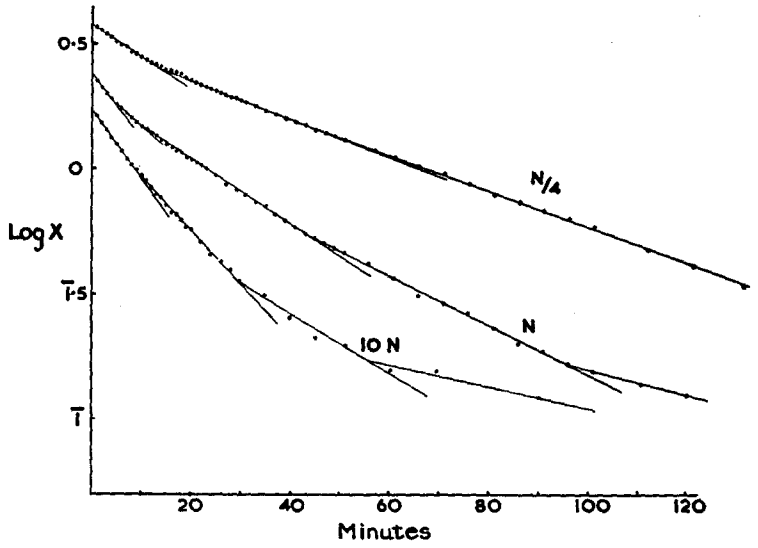

FIG. 1. Examples of curves relating $\log_{10} x$ (x is milligrams of water remaining in tissue per milligram of ultimate dry weight) and time during drying of specimens of frog sciatic nerve which had been immersed for 20 hours in Normal (N), hypertonic (10 N), and hypotonic (N/4) Ringer's solutions.

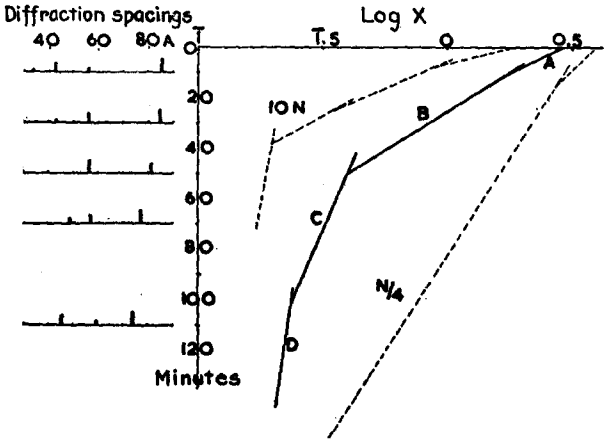

FIG. 2. Average curves relating $\log_{10} x$ and drying time and a diagrammatic representation of changes in low-angle diffraction pattern accompanying the drying of normal nerve.

the values indicated at the intersections with the ordinate. The series of diffraction patterns indicate that changes in the myelin probably do not commence until after phase *A* of the drying is completed but then continue through both *B* and *C*. It therefore seemed possible, as a third interpretation, that phase *A* of the evaporation might be independent but that *B* and *C* are

probably simultaneous. The values for phases *A* and *D* are thus as in the first method, but new *B* and *C* values are obtained by extrapolating back to the ordinate corresponding to the break between *A* and *B*. All three sets of values are given in Table I, and the mean variations from the average values are indicated. This variation is due in part to the difficulty of accurately locating the breaks between the phases, but probably also reflects some slight variations in the drying conditions and perhaps even in the water distribution in the specimen.

A similar set of results is given for nerve dried under controlled conditions after immersion for 24 hours in 10 N Ringer's solution (four experiments). Only two experiments were carried out under controlled conditions with nerve which had been immersed for 24 hours in N/4 Ringer's solution, but the results were sufficiently in agreement to quote the figures obtained. In these experiments, however, no reliable distinction could be made between phases *B* and *C*, and as the slope of *D* was also somewhat unreliable an interpretation assuming simultaneous evaporation could not be usefully considered. The curves for specimens in 10 N and in N/4 Ringer's solutions are included in Figs. 1 and 2.

X-Ray Diffraction:

Some of the intermediate diffraction patterns recorded during the drying of normal nerve are indicated schematically in Fig. 2 alongside the drying curve. Because of the time required to obtain the diffraction patterns, the relating of the pattern changes to the drying kinetics can only be approximate. Furthermore, owing to the limited resolution of the camera in this experiment, and to the lack of definition in some of the bands in the constantly changing pattern, some of the spacing changes are difficult to locate accurately. In the case of the drying of nerve which has been immersed in N Ringer (as has been reported previously (4)), there are three main characteristic changes during the drying process. The first is an increase in the intensity of the third order diffraction which seems to occur mainly during stage *B* of drying. The second is the decrease of the second order spacing which starts in stage *B* but continues in stage *C*, and the third is the appearance of an outer band in the 40 to 50 A region at the beginning of stage *C*. The specimen in 10 N and the one in N/4 Ringer's solution both have a strong band in the 60 to 70 A region (5) before drying commences, and no change in this band can be detected until the final stages of drying when its behaviour is similar to that reported for drying from N Ringer. As far as could be detected, the changes in spacing of the band in the region of 70 to 90 A in these two cases could not be confined to any particular stage of drying. The appearance of the new band in the 40 to 50 A region was fairly well defined in both cases, in the first at the beginning of stage *C*, and in the second at about the middle of stage *BC*.

DISCUSSION

The experiments reported in this paper give a direct measurement of the amount of water in the nerve tissue as a whole, but assumptions have to be made in order to allocate the water to different nerve components. The total weight of water found in the normal frog sciatic nerve is about 2.9 mg. per mg. of dry tissue; *i.e.*, about 75 per cent. Of this, a considerable amount is removed before there is a detectable change in the myelin structure, and it seems probable that this is largely surface and interstitial water which might account for the whole of phase *A* of the drying curve. Thus, the indications are that the myelin water, and probably also that of the axon, are to be found entirely in the water remaining in the tissue at the end of phase *A*. This amounts to about 1.8 mg. per mg. If equal volumes of axon and myelin are assumed (6, 7), and the water content of the axon is considered to be similar to the 90 per cent measured experimentally for the axon of the squid giant fibre (8), then the water content of the myelin sheath would be about 43 per cent (allowing $\frac{1}{10}$ of the weight of water already removed to represent the contribution of interstitial tissue to the dry weight). If the water contained in phases *B* and *C* only is considered (1.6 mg. per mg.) the figure is reduced to 38 per cent.

In the case of nerve specimens immersed in 10 N Ringer, the amount of water remaining in the tissue at the end of phase *A* is only about 0.9 mg. per mg. There are no direct observations available on the relative volumes of axon and myelin in such specimens, but the indications from x-ray diffraction data (5) are that the layer spacing of the myelin has not decreased, and the myelin might therefore be considered to be relatively unchanged in volume.

In the case of nerve specimens immersed in N/4 Ringer's solution, the x-ray diffraction data indicates a possible increase in myelin layer spacing of about 50 per cent (5). If the axon diameter remained constant then this 50 per cent increase in layer spacing would produce an increase in total myelin volume of about 50 per cent. The drying experiments indicate that the amount of water remaining in the specimen at the end of phase *A* is about 2.8 mg. per mg., and the probability is that the axon has also undergone some swelling. However, it can be shown that if the myelin layers are continuously wound in jelly-roll fashion as indicated by the studies of Geren (9) then the expansion of the axon will have little effect on the volume of the myelin sheath, and the observed increase in water content can be readily accounted for in terms of a 50 per cent increase in myelin volume and a 25 to 50 per cent increase in axon diameter.

Relating these general considerations of the drying curves and x-ray diffraction data to the attempted detailed interpretations given in Table I, it can be assumed that phase *A* probably represents surface and interstitial

water. In the consideration of evaporation of water from all phases as simultaneous the amount of water allocated to phase *A* is much lower than would be expected for surface and interstitial water, and therefore it is suggested that this method of interpretation is probably incorrect. The value obtained by assuming that evaporation from this phase takes place before "internal" water begins to emerge is reasonable. The amount of water in the final slow phase (*D*) is too small to represent the total water of any major component of nerve, and the probability is that it represents "bound" water which may be associated with strongly hydrophilic groups in any part of the nerve. If the myelin and axon water are to be distinguished, then they must be represented by phases *B* and *C*. From general considerations, the axon would be expected to contain more water than the myelin sheath, and the spatial arrangement of the two components is such as to suggest that, although the myelin water might start to come off first, much of the water from the axon would ultimately have to diffuse through the myelin during drying.

These considerations, together with the x-ray diffraction evidence, suggest that the third method of interpretation is the one most likely to be correct. This would give a value of 0.64 mg. per mg. for myelin water and 1.0 mg. per mg. for axon. Again considering equal volumes of axon and myelin and assuming the axon to be 90 per cent water, the figure for myelin would be about 58 per cent water. This figure is considerably higher than the 38 per cent deduced in the earlier considerations but agreement between the two calculations could be obtained by increasing the myelin to axon volume ratio to about 1.25, which leads to a value of about 45 per cent water in myelin. If the 0.16 mg. of water associated with phase *D* is also assigned to the myelin, agreement between the two calculations can be reached by increasing the myelin to axon volume ratio to about 1.45 when the value for the water content of myelin becomes 50 per cent. This latter figure can probably be regarded as a maximum.

In general, these considerations of the controlled drying of frog sciatic nerve and the accompanying changes in the low-angle diffraction pattern of the myelin indicate a myelin water content of 40 to 50 per cent. This figure is appreciably lower than that deduced from radioactive tracer studies and from x-ray absorption measurements, but the lower figure is more in keeping with the structural characteristics of the myelin. The small shrinkage (approximately 16 per cent) of the myelin layers on drying (1, 4) indicates only a small contribution by water to the layer spacing, and the fact that hypertonic Ringer's solution fails to produce any shrinkage in the radial direction (5) suggests that only "organised" water is present. This could be associated with the hydrophilic groups which are suggested to be localised at lipide-protein and possibly protein-protein interfaces (10), but the strength of the ionic binding at these interfaces is such as to favour only a limited hy-

dration. The strength of this binding is probably reflected in the behaviour of the structure in hypotonic solutions (5).

The general conclusion from this work is that the water content of the myelin sheath of frog sciatic nerve is probably between 40 and 50 per cent and that the information available on the molecular organisation of the myelin strongly favours a low water content.

SUMMARY

In the study of the drying kinetics of nerve fibres, at least five "phases" of water evaporation can be distinguished. A consideration of the accompanying changes in low-angle x-ray diffraction patterns permits a tentative identification of the "phases" and a quantitative interpretation of the data in terms of the water distribution in nerve fibres. These results suggest that the myelin sheath of frog sciatic nerve contains 40 to 50 per cent water, and it is suggested further that the greater part of this water is "organised" in relation to the hydrophilic groups of the lipide and protein components.

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