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A DYNAMIC X-RAY DIFFRACTION STUDY OF ANAESTHESIA ACTION

CHANGES IN MYELIN STRUCTURE AND ELECTRICAL ACTIVITY RECORDED SIMULTANEOUSLY FROM FROG SCIATIC NERVES TREATED WITH *n*-ALKANES

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Summary

Changes induced in the structure and electrical activity of myelin were recorded simultaneously from frog sciatic nerves treated with *n*-alkanes. The results suggest that the effect of *n*-alkanes seems to be two-fold: (a) there is an initial reversible phase, in which a significant modification of the X-ray diffraction patterns, concomitant with the continuous fall of the action potential, is observed; (b) there is a final phase which is irreversible. This occurs some time after the complete abolition of the electrical activity. At this stage, further changes of the X-ray diffraction patterns are detected, the most significant of them being in the *n*-pentane-treated myelin, and consist of an increase in the membrane bilayer thickness.

Introduction

Anaesthetics in suitable concentrations affect several cellular processes such as electrical excitability and membrane permeability [1]. A well known effect of anaesthetics on nerve fibres is the reversible blocking of the action potential generation. This effect is likely to be referable to the interactions between the anaesthetic molecules and the membrane and the consequent changes in some of their physical properties as a result of these interactions.

Recent discussions on current theories about anaesthesia tend to focus the nature and location of the sites of action of anaesthetics on lipids [2] or pro-

teins [3,4], or on both protein and lipid molecules [1] of the excitable membrane. Interestingly, most of the proposed models share a common feature: the site of action of these molecules is a hydrophobic region of the membrane. On the other hand, while Seeman [1] postulated that anaesthetics and other nerve-blocking drugs adsorb to the membrane causing an expansion of the volume and/or the area of its hydrophobic domains — a process which blocks the ionic channels responsible for action potential —, Haydon et al. [2] proposed that during anaesthesia by *n*-alkanes there occurs a thickening of the lipid bilayer region of the membrane, also a process which blocks the excitable ionic channels. On the other hand, Franks and Lieb [3] and Richards et al. [4] concluded that the site of action of general anaesthetics is the hydrophobic portion of a membrane-bound protein.

The use of a physical technique such as X-ray diffraction could give further insight about the mechanism by which *n*-alkanes block the electrical activity of biological membranes. Unfortunately, this is not easy to perform since excitable membranes are not naturally ordered. A reasonable alternative for obtaining useful structural information is the study of this process on a membrane system such as the nerve myelin sheath, which, inexcitable though it is, is naturally ordered. In addition, it has been suggested that myelin could be passively involved in the saltatory phenomenon of nerve conduction [5,6]. Finally, after the extensive related work that has been carried out by several groups during the last three decades, the structure of myelin is well known at present (see review in Ref. 7). Indeed, Caspar and Kirschner [8], on the basis of a high-resolution X-ray diffraction study, have proposed a molecular model for myelin membranes. More recently, these structural concepts have been exhaustively revised and enriched by Nelander and Blaurock [9].

We have recently reported [10] a dynamic X-ray diffraction study on the effect of a hydrocarbon solution on the structure of myelin (at the low resolution of 34 Å). We found that the perfusion during 24 h of frog sciatic nerves with *n*-pentane-saturated Ringer solution caused a 6% increase in the thickness of the myelin membrane lattice, although such a change was hardly visible for the first 18 h. In the present communication, a more detailed study of the effect of several *n*-alkanes on the structure of myelin is carried out. The aim of these experiments was to determine the relationship existing between the suppression of the nerve impulse and the structural changes of myelin (membrane thickening) and its reversibility, at the much improved resolution of about 15 Å. In order to do this, the nervous electrical activity and the X-ray diffraction patterns were simultaneously recorded on the same frog sciatic nerve.

Materials and Methods

Freshly dissected frog (*Rana pipiens*) sciatic nerves were deprived of the epineurium (desheathed) and mounted on a specimen holder with a continuous-perfusion system which permits frog Ringer solutions to flow around the nerve [11]. These *n*-alkane-saturated Ringer solutions were prepared by equilibrating normal frog Ringer solution with liquid alkane for 24 h with gently stirring. Great care was taken to avoid emulsions. The alkanes assayed were *n*-pentane, *n*-hexane and *n*-decane. These hydrocarbons (purest grade) were purchased

from BDH (Poole, U.K.), or Koch-Light (Colnbrook, U.K.) and used without further purification.

The specimen holder was provided with two pairs of platinum wire electrodes for nerve stimulation and for the recording of the compound action potential. The circuitry was similar to that currently used in electrophysiological practice [13]. The compound action potential height was registered in a storage oscilloscope.

The small-angle X-ray diffraction techniques were those currently used in our laboratory [10—12,14,15]. The X-ray patterns were recorded with a linear position-sensitive detector [16] from a 1 cm long sciatic segment, which was located between the two pairs of electrodes. The patterns were stored in an on-line computer. This experimental arrangement facilitated the simultaneous recording of the X-ray diffraction patterns and the electrical activity of the nerve.

For control purposes, an X-ray diffraction pattern was accumulated during 4 h from a sciatic nerve in normal Ringer solution. At the end of this data collection, the perfusion was exchanged to the alkane-containing solution and the structural modifications induced by the alkane were sequentially followed by recording a series of 5-min counting spectra. The perfusion was continued until no further change in the patterns was observed. At this point, another 4-h counting spectrum was accumulated from the alkane-perfused sciatic nerve. This register was compared with the original pattern recorded from the same nerve before the alkane treatment during an identical period of time.

The diffraction patterns were displayed on a cathode-ray tube and photographed on a 35 mm film. Periodicity and intensity measurements were determined from logarithmic plots of the computer-stored data. The error estimated for the repeat period was ±1%. The intensity of the reflections was measured from peak areas after background subtraction. Electron-density profiles were calculated as previously described [10]. Different profiles were scaled by setting $\Sigma F^2(h)/d$ = constant [17].

Results

The action of *n*-pentane, *n*-hexane and *n*-decane on the structure and electrical activity of myelin was investigated for several sciatic nerves. X-ray pattern modifications of different nerve specimens were entirely reproducible for a given alkane from one nerve preparation to the next. However, depending on nerve dimensions and the flow rate of perfusion, time-course variability was observed. In order to minimise these variations, the results reported herein will refer to sciatic nerves having similar thickness (approx. 1 mm), which were perfused at an identical flow rate (5 ml/min).

Reversible effects of n-alkanes on nerve impulse blockage and myelin structure

As stated in Materials and Methods, 5-min counting X-ray diffraction patterns were sequentially registered during the perfusion of the nerves with different alkane-containing solutions. The time courses of the action potential blockage and X-ray pattern modifications are shown in Fig. 1 for sciatic nerves treated with *n*-pentane, *n*-hexane and *n*-decane, together with the results for a

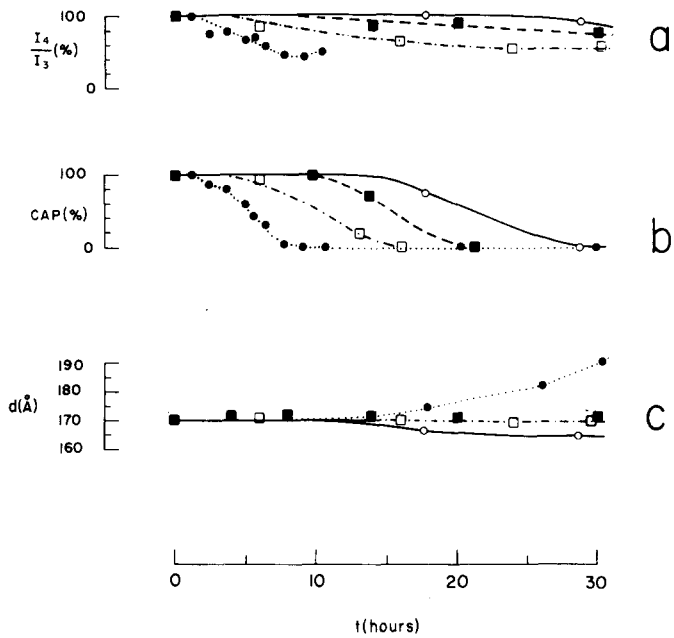

Fig. 1. Effect of the electrical activity and the X-ray diffraction pattern of the perfusion of frog sciatic nerves with Ringer solutions saturated with: (■) *n*-decane, (□) *n*-hexane and (●) *n*-pentane. (○) Control nerve in alkane-free Ringer solution. (a) Time course of the relative intensity ratio between the fourth- and third-order reflections. This ratio refers to that of the corresponding native pattern which was recorded from freshly dissected nerves in normal Ringer solution (100% at time 0 h). (b) Time course of the compound action potential (CAP) blockage as referred to the maximum amplitude (100%) at the onset of the experiment. (c) Time course of the change in the myelin lattice thickness for the different experimental conditions stated above.

control nerve perfused with normal Ringer solution during a similar time period. In agreement with other workers [2,18], it was found that the period of time taken by *n*-alkanes to block the nerve electrical activity increases with the number of carbon atoms of the hydrocarbon. Under our experimental conditions, a 50% reduction in the height of the compound action potential occurred at 5, 10 and 15 h of the perfusion for sciatic nerves treated with *n*-pentane-, *n*-hexane- and *n*-decane-containing solutions, respectively (see Fig. 1b). For the control nerve it took 21 h to develop a similar reduction in the height of the compound action potential. It is clear then, that, although the excitability will spontaneously disappear in an untreated nerve, the effect of *n*-alkanes, even *n*-decane, will occur well within a period of adequate physiological conditions as judged by the excitability properties.

As it has been previously reported [10], the alkane-induced changes of the X-ray patterns involved a progressive decrease in the relative intensity of the fourth order in comparison with the other reflections. These modifications were independent of the number of carbon atoms of the alkane used. As an example of this, the ratio of the intensities $I(4)/I(3)$ — which was arbitrarily selected as a structural parameter — is shown as a function of time in Fig. 1a for three alkane-treated nerves as well as for the control nerve. Notice that the significant changes in the ratio of the intensities (Fig. 1a) followed the time

course of the process that led to the suppression of the propagation action potential (Fig. 1b) in nerves exposed to alkane-containing Ringer solution, while the control nerve did not show such a change. Fig. 1c reveals that the myelin lattice thickness remained constant during the process in which the compound action potential was progressively reduced by alkane treatment. At this stage, neither the background nor the half-width peak intensity was significantly altered. It should be observed that prolonged exposure to alkane, specifically *n*-pentane, caused the already reported thickening of the myelin lattice [10], a process which will be shown below to be irreversible.

The reduction in the ratio of the intensities and in the compound action potential height seen through the exposure of sciatic nerves to Ringer solution-containing alkanes, was found to be reversible: nerve specimens in which the perfusion was exchanged from an alkane-containing solution (either *n*-pentane or *n*-hexane) to normal Ringer solution, recovered their electrical activity and their X-ray diffraction patterns. Fig. 2 shows a typical experiment in which the time course for the ratio of the intensities $I(4)/I(3)$ (Fig. 2a), height of the compound action potential (Fig. 2b) and myelin lattice thickness (Fig. 2c) were recorded in a sciatic nerve briefly exposed (40 min) to Ringer solution saturated with *n*-pentane. Notice that a strikingly close relationship exists between the time course for the fall of the compound action potential (functional aspect) and the reduction in the ratio of intensities (structural aspect) observed with *n*-pentane treatment, although the relationship is less conspicuous for the reversal phase.

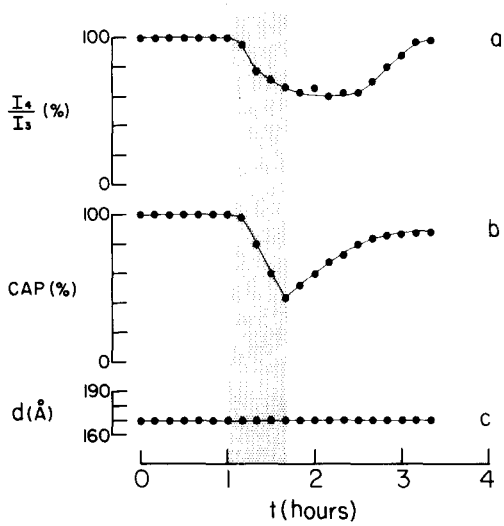

Fig. 2. Effect on the electrical activity and the X-ray diffraction pattern of the perfusion with *n*-pentane-saturated Ringer solution of a frog sciatic nerve, for a brief period of time. The sciatic nerve was mounted on the holder and the measurements of intensity of the reflections (a), compound action potential (CAP) height (b), and unit cell dimensions (c), were continuously performed as indicated in the text. These parameters remained unchanged during the first hour in normal Ringer solution. The shaded bar indicates the period in which the sciatic nerve is exposed to Ringer solution saturated with *n*-pentane. Notice that the time course of the action potential fall (b) is closely followed by the change in the ratio of intensities $I(4)/I(3)$ (a), and that after 40 min of exposure to the *n*-alkane these two parameters recovered their original levels when the perfusing solution was exchanged with normal Ringer solution. For the duration of the experiment, the myelin lattice thickness remained unchanged (c).

Irreversible structural changes occurring after the suppression of the electrical activity

After the action potential was completely abolished, perfusion of the various nerves was continued with the corresponding solutions. Besides the changes in the intensities described previously, no further modification in the X-ray diffraction patterns from nerves treated with either *n*-hexane or *n*-decane was observed: the relative intensity distribution and the repeat distance remained unchanged many hours after the electrical activity had been abolished (Fig. 1). However, all the reflections were irreversibly diminished in intensity.

The X-ray patterns of the pentane-treated nerve showed important variations both in lattice dimensions and in relative intensity distribution. The thickness of the unit cell, which had remained unchanged at 170 Å for the first 15 h (lapse in which the compound action potential disappeared), progressively started to increase and continued to do so, attaining, within 31 h, a value of about 186 Å (Fig. 1c).

Concomitant with the thickening, it was observed that the relative intensities of the odd orders decreased continuously whereas those of the even orders increased. In addition, the intensity of the sixth-order reflection also decreased steadily down to zero and then reappeared, finally adopting an intensity which was even higher than that recorded in the freshly dissected sciatic nerve (see Fig. 6). In agreement with our previous observations [10], the process of thickening was paralleled with a considerable decrease in the intensity of the pattern. The control nerve, which was perfused with normal Ringer solution, showed a detectable thinning of the lattice (from 170 to 166 Å) with a simultaneous slight decrease in the intensity of the reflections after 15 h of perfusion.

The changes observed in unit cell dimensions and intensity ratio long after the suppression of the compound action potential were completely irreversible. The nerves recovered neither their electrical activity nor their native X-ray diffraction patterns, even after extensive perfusion with alkane-free Ringer solution.

Comparison of X-ray diffraction patterns from alkane-treated and control sciatic nerve myelin at 15 Å resolution

15 Å-resolution X-ray spectra from the alkane-treated sciatic and control nerves in Ringer solution were accumulated during 4 h after about 30 h of perfusion. These high-resolution patterns are shown in Figs. 3b—5b and correspond to the sciatic nerves referred to in Fig. 1. In each figure, high-resolution patterns, recorded as controls from the same nerves before treatment with alkanes, are included for comparison (Figs. 3a—5a). The structure factors and lattice dimensions for these sciatic nerves are tabulated in Table I.

For the control condition and for the *n*-hexane- and *n*-decane-treated sciatic nerves, the quality of the spectra was adequate. Bragg reflections 2—11 were accurately recorded in a 4 h collection time. The peak half-width was not significantly altered. However, the X-ray pattern recorded from the nerve perfused for 30 h with *n*-pentane-containing Ringer solution had deteriorated: the intensities were considerably diminished as compared to a control pattern recorded from the same sciatic nerve before the treatment with pentane. This is shown in Fig. 5a and b. In additon, the spatial resolution was found to be impaired, since discrete reflections were only observed up to 28 Å spacing ($h = 6$).

TABLE I

COMPARISON OF REPEAT PERIODS AND STRUCTURE FACTORS BETWEEN FROG SCIATIC MYELINS PERFUSED WITH ALKANE-CONTAINING SOLUTIONS AND THEIR CONTROLS

F(h), structure factor of order h; t, incubation time of different sciatic nerves in the corresponding Ringer solutions; *d*, repeat distance; *h*, diffraction order; *N* is the normalisation constant for scaling the experimental structure factors at the origin peak of their Patterson functions [16]. σ is the standard deviation calculated from *I(h)* values [19]. NFR, normal frog Ringer solution.

h	*F(h)* ± σ							
	NFR		NFR + *n*-decane		NFR + *n*-hexane		NFR + *n*-pentane	
	0 h (*d* 170 Å, *N* = 1)	30 h (*d* = 166 Å, *N* = 1.33)	0 h (*d* = 170 Å, *N* = 1)	28 h (*d* = 170 Å, *N* = 1.54)	0 h (*d* = 170 Å, *N* = 1)	30 h (*d* 170 Å, *N* = 1.52)	0 h (*d* 170 Å, *N* = 1)	31 h (*d* = 187 Å, *N* = 3.23)
1	0.14 ± 0.009	0.17 ± 0.012	0.14 ± 0.003	0.11 ± 0.009	0.12 ± 0.003	0.16 ± 0.004	0.15 ± 0.002	0.20 ± 0.012
2	1.41 ± 0.004	1.41 ± 0.007	1.49 ± 0.003	1.51 ± 0.003	1.45 ± 0.001	1.53 ± 0.003	1.47 ± 0.001	1.79 ± 0.012
3	0.70 ± 0.009	0.82 ± 0.013	0.74 ± 0.003	0.82 ± 0.007	0.67 ± 0.006	0.72 ± 0.006	0.73 ± 0.003	0.53 ± 0.044
4	1.47 ± 0.009	1.42 ± 0.014	1.44 ± 0.003	1.39 ± 0.007	1.48 ± 0.003	1.40 ± 0.006	1.43 ± 0.003	1.51 ± 0.029
5	0.78 ± 0.015	0.79 ± 0.023	0.78 ± 0.005	0.79 ± 0.029	0.77 ± 0.004	0.73 ± 0.010	0.81 ± 0.005	0.48 ± 0.067
6	0.20 ± 0.047	0.15 ± 0.187	0.12 ± 0.029	0.10 ± 0.043	0.15 ± 0.063	0.00	0.13 ± 0.002	0.37 ± 0.102
7	0.06 ± 0.056	0.11 ± 0.054	0.08 ± 0.040	0.08 ± 0.057	0.00	0.00 ± 0.052	0.10 ± 0.040	0.00
8	0.24 ± 0.067	0.20 ± 0.050	0.14 ± 0.029	0.17 ± 0.125	0.23 ± 0.019	0.25	0.16 ± 0.027	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.24 ± 0.081	0.14 ± 0.045	0.14 ± 0.085	0.10 ± 0.075	0.18 ± 0.030	0.16 ± 0.065	0.17 ± 0.032	0.00
11	0.47 ± 0.060	0.32 ± 0.058	0.37 ± 0.034	0.30 ± 0.080	0.44 ± 0.019	0.46 ± 0.046	0.41 ± 0.020	0.00

Electron-density profiles

Electron-density profiles were calculated on an arbitrary scale using the normalised data of Table I. Since in the X-ray patterns from normal Ringer solution-, *n*-decane- and *n*-hexane-treated nerves, none of the reflections attained a value of zero during the perfusions, it is plausible to assume that the phases remained unchanged. Therefore, the corresponding electron densities were calculated by adopting the set of signs proposed by Caspar and Kirschner [8] for frog sciatic myelin [18]. Fig. 3c shows the electron density for freshly dissected sciatic nerve myelin and for the myelin after 30 h of perfusion with normal Ringer solution. The membrane unit profiles are relatively scaled and look very similar. The largest difference is observed at the centre-to-centre separation of the high-density peaks: this distance is 45 Å in the freshly dissected sciatic nerve, while it decreased to 44 Å in the same nerve when perfused during 30 h.

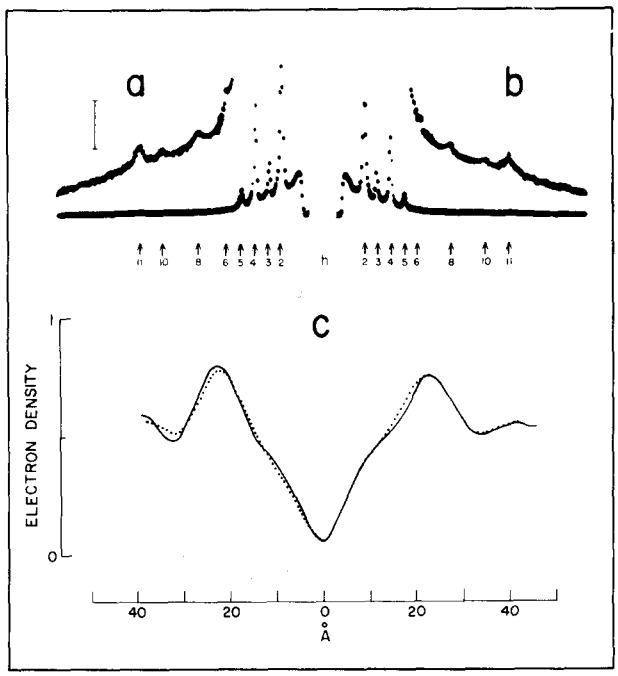

Fig. 3. Effect on the myelin structure of the perfusion during 30 h, with normal Ringer solution, of a frog sciatic nerve. (a) Diffraction pattern from a freshly dissected sciatic nerve recorded during 4 h with the position-sensitive counter [16] such as displayed in the CRT of the computer. The lower trace shows the first five orders from a 170 Å repeat period. The upper trace is at a magnification of 16 X in which the higher orders (h = 6,8,10 and 11) are clearly visible. The calibration bar refers to the lower trace and represents 10 000 counts per channel. The positions of the reflections are indicated by arrows. The counter used in this work showed a considerable increase in the non-linearity from the centre to the outer edges. This is the reason why the spacing between the reflections becomes larger at higher angles (i.e., compare the spacing between arrows 10 and 11, and 2 and 3). (b) Diffraction pattern accumulated during 4 h from the same sciatic nerve shown in a after 30 h of perfusion with normal Ringer solution. As is apparent in both the lower trace and the upper magnification thereof, the reflections have decreased in intensity (see also parameter N in Table I). The repeat period has decreased from 170 to 166 Å. (c) Comparison of electron-density profiles from the patterns shown in a (———) and b (. . . . .). The profiles have been calculated from the normalised data ($F(h)$) shown in Table I with the phasing proposed by Caspar and Kirschner [8]. The electron density scale is arbitrary. The 4-Å changes observed in the lattice dimensions are mainly due to a decrease in thickness of the intraperiod line.

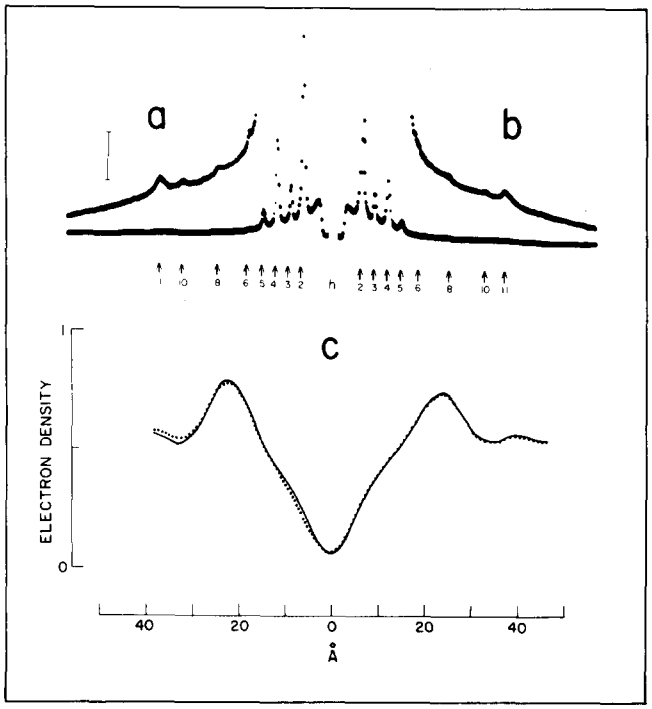

Fig. 4. Effect on the myelin structure of the perfusion with *n*-decane-saturated normal Ringer solution during 30 h, of a frog sciatic nerve. (a) 4-h counting diffraction pattern recorded from a freshly dissected sciatic nerve in normal Ringer solution. The repeat period is 170 Å. (b) Diffraction pattern accumulated during 4 h from the same sciatic nerve shown in a, after 28 h of perfusion with *n*-decane-saturated normal Ringer solution. The repeat period remains constant at 170 Å. (c) Comparison of electron densities from the patterns shown in a (———) and b (. . . . .). The profiles look almost the same. The electron-density scale is arbitrary. See legend of Fig. 2.

Membrane unit density profiles for freshly dissected and 28-h decane-treated myelins are shown on a relative scale in Fig. 4c. The profiles look almost the same in both thickness and density distribution.

A comparison of profiles from the myelin membrane unit before and after a 30-h perfusion with hexane-containing Ringer solution is shown in Fig. 5c. The bilayer thickness is the same in both the freshly dissected and in the 30-h hexane-treated myelin. The main difference between the two profiles is a slight decrease in the density of the shoulders in the low-density region of the hexane-treated myelin.

Fig. 6c shows the comparison of membrane unit density profiles involving the sciatic nerve treated with *n*-pentane. This comparison is limited to low resolution, since as previously mentioned, the diffraction pattern of the pentane-treated myelin (Fig. 6b) showed discrete reflections only up to 28 Å spacing ($h = 6$). During the perfusion it was observed that the intensity of the sixth-order reflection progressively decreased to zero and then reappeared (see above), suggesting that $F(6)$ probably changes its sign. Consequently, the profiles were calculated considering two possible sets of phases (—, +, +, —, —, ±). The centre-to-centre separation of high-density peaks in the profile of freshly dissected myelin is 45 Å, but depending on the chosen sign combination (see

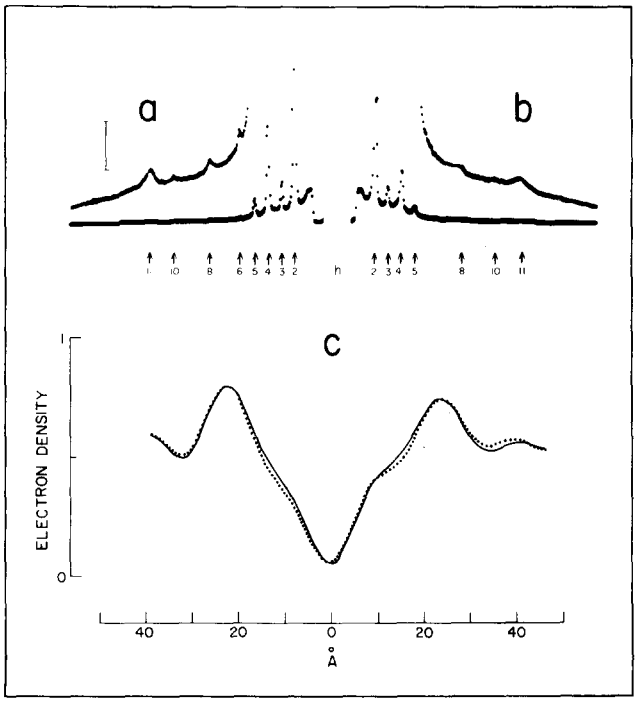

Fig. 5. Effect on the myelin structure of the perfusion with *n*-hexane-saturated normal Ringer solution, during 30 h, of a frog sciatic nerve. (a) 4-h counting diffraction pattern recorded from a freshly dissected sciatic nerve in normal Ringer solution. The repeat period is 170 Å. (b) Diffraction pattern accumulated during 4 h from the same sciatic nerve shown in a after 30 h of perfusion with *n*-hexane-saturated normal Ringer solution. The sixth order has disappeared completely and the high-angle reflections have decreased considerably in intensity. The repeat period is 170 Å. (c) Electron-density profiles from the patterns show shown in a (———) and b (. . . . .). The profiles look very similar except for the slight decrease in density of the shoulders within the bilayer as a consequence of the *n*-hexane treatment. The electron-density scale is arbitrary. See legend of Fig. 2.

above) it varies for the pentane-treated myelin. Indeed, the peak-to-peak separation was found to be 49 Å for the profile calculated with the set of phases (— + + — — +). The 5 Å increase in thickness, as compared to the control myelin, is almost completely located at the cytoplasmic side of the membrane bilayer. On the contrary, if the electron-density profile is calculated with the set of phases (— + + — — —), the peak separation is 58 Å and the 14 Å increase in thickness is located at the external side.

Discussion

Previously results from our laboratory [10] have shown that myelin — a membrane system closely associated to the nodal excitable membrane — started to increase its thickness only after many hours of exposure to *n*-pentane-containing solutions. This fact apparently supported the thickness-tension hypothesis of anaesthesia by *n*-alkanes proposed by Haydon et al [2]. It was then suggested that the long delay observed between the start of the myelin thickening (more than 18 h as recorded by X-ray diffraction [10]) and the action

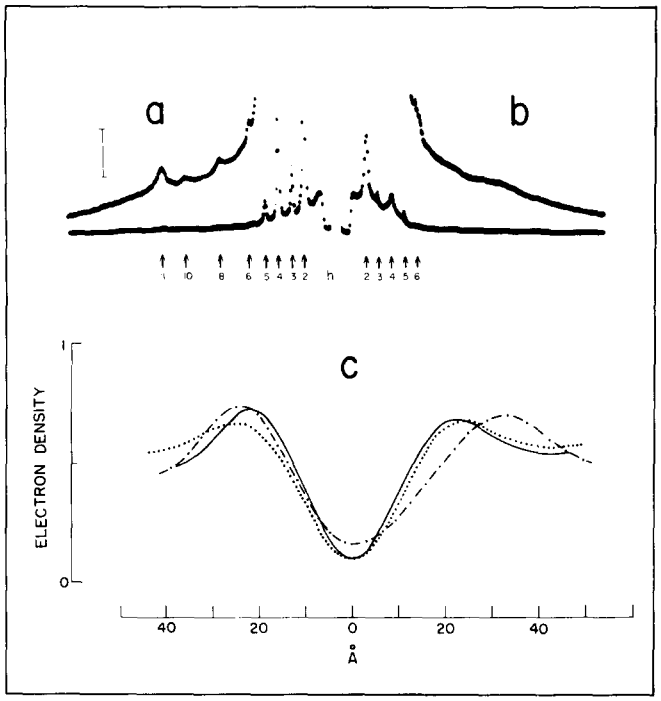

Fig. 6. Effect on the myelin structure of the perfusion with *n*-pentane-saturated normal Ringer solution, during 31 h, of a frog sciatic nerve. (a) 4-h counting diffraction pattern recorded from a freshly dissected sciatic nerve in normal Ringer solution. The repeat period is 170 Å. (b) Diffraction pattern accumulated during 4 h from the same sciatic nerve shown in a after 31 h of perfusion with *n*-pentane-saturated normal Ringer solution. Notice that, as a consequence of the alkane treatment, the pattern has been significantly modified: the reflections are very weak and the high-angle orders have dissapeared completely. Also, notice that the intensity of the sixth order in the pentane-treated myelin is higher than that in the native myelin (compare with the pattern shown in a). The increase in the background scattering is clearly observed (see text). The repeat period is 186 Å. (c) Comparison of the low-resolution (34 Å) electron densities of native and pentane-treated myelin from the diffraction data to the sixth order of patterns shown in a and b. Native myelin (———). Profiles from *n*-pentane-treated myelin calculated with the set of phases (— + + — — +) and (— + + — — —) shown in (. . . . . .) and in (· — · — ·), respectively. The electron-density scale is arbitrary.

potential blockage of the excitable membrane (less than 1 h as reported by Haydon et al [2]) could be accounted for by a difference in the accessibility of the *n*-pentane to the myellin sheath as compared to the Ranvier node.

The results reported in the present work suggest that the action of *n*-alkanes on frog sciatic nerves appears to be two-fold, as is shown in Fig. 1. Firstly, there is an initial stage, in which the changes in the electrical activity and in the myelin membrane structure occur at the same time without modifications in the lattice thickness. The changes during this phase were reversible, as shown in Fig. 2. At the end of this stage, the X-ray patterns from all the sciatic nerves showed similar changes independently of the alkane used: the intensity of the fourth-order reflection decreased while that of the third order was enhanced. The half-width peak and the background were not significantly altered.

For more prolonged exposures to alkanes, a second stage is clearly distinguished: the effects on the myelin structure were irreversible and occurred long

after the electrical activity was completely abolished. For instance, the nerve treated with *n*-hexane showed slight modifications within the bilayer structure. In agreement with other workers [2,18], it was found that the effects on the compound action potential observed during the treatment with *n*-alkanes decreased with increasing chain length (compare the diffraction patterns shown in part b of Figs. 3—5). Indeed, the density profile of the nerve specimen treated with *n*-decane was very similar to that of the freshly dissected sciatic nerve (Fig. 3).

In agreement with our previous results regarding *n*-pentane [10], a 9% increase in the myelin membrane lattice is observed after 20 h of exposure to the alkane. However, the myelin thickness is associated with the irreversible stage since, for the first 15 h, no detectable thickening was observed.

The final X-ray diffraction of pentane-treated myelin showed a considerable deterioration as compared with the native pattern: the background increased considerably and the higher order reflections disappeared completely. This is shown in Fig. 6b. This effect suggests that some of the myelin become more disordered. However, in the results presented above, neither the lattice disorder in the freshly dissected myelin membrane nor the possible changes in this disorder induced by alkane treatment have been considered as far as the analysis of the diffuse scatter is concerned [9]. This is certainly an important point which should be investigated using a two-dimensional position-sensitive detector. Unfortunately for us, though, such an instrument is not available at present.

The electron densities of Fig. 6c indicate that whichever the sign of the sixth-order reflection, the myelin membrane unit is thickened by the action of *n*-pentane. If we assume that the sign of $F(6)$ remains positive during the experiment, then the increase in thickness is mainly located at the cytoplasmic side of the membrane. On the contrary, a larger change is detected at the external side when the profile is calculated with $F(6)$ being negative. At present, the answer to this crystallographic problem is unknown. However, the fact that the sixth-order intensity continuously disappeared and reappeared during the perfusion suggests that probably $F(6)$ is negative.

In a previous report from our laboratory [10], it was suggested that the effect of *n*-pentane was faster on nerve conduction than on myelin structure. This was inferred from morphological considerations such as the fact that the Ranvier node, where an action potential is generated, is more accessible to external solutions than myelin.

The results presented here and summarised in Figs. 1 and 2 disprove the notion that alkanes interact slowly and/or in a restricted fashion with myelin. Indeed, concomitantly with the fall in the eletrical activity produced by the action of *n*-alkanes, there is a structural modification in myelin which is not an increase in the lattice dimensions.

The experiments have been performed in myelin, which although not being excitable, is a natural membrane system that may be used, in a first approximation, as a model system for studying the structural effect of anaesthetics on biological membranes. If anaesthesia by diverse neutral molecules arises from a unique mechanism common to any cell membrane, it should be emphasised that if the excitability blockage is chosen as a measure of the uptake of *n*-pen-

tane by the axolemma at the node of Ranvier, the structural parameter — ratio of the intensities $I(4)/I(3)$ — arbitrarily adopted here appears to follow equally well the interaction of *n*-pentane with myelin. The fact that *n*-alkanes do not thicken the membranes is not a direct proof that the nodal excitable membranes behave in the same way. However, the structural modification detected as a change in the intensity of Bragg reflections calls for studies at higher resolution. It is clear, though, that in order to obtain direct information about the molecular mechanisms of anaesthesia, these experiments have to performed on excitable membranes and using either general or local anaesthetic molecules (Padrón, R. and Mateu, L., unpublished data).

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