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ARTICLE

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A reversible abnormal form of myelin: an X-ray scattering study of human sural and rat sciatic nerves

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Abstract A sural nerve dissected from a recently dead patient displayed an unusual X-ray diffraction pattern, suggesting that in situ and at the time of the patient's death the myelin sheaths were in a swollen state. Diffraction patterns of the swollen type were also recorded from: (1) a sural nerve from the corpse of a neurologically healthy person after soaking the nerve with Ringer solution at pH 5.5; (2) sciatic nerves dissected from rat cadavers at increasing time after death. In all the cases the swollen patterns reversed to the native type upon superfusion with Ringer solution at pH 7.3. The postmortem effect is to decrease the pH of the fluids surrounding the nerves in the cadavers. Our experiments show that the early postmortem processes have the effect of acidifying PNS nerves and that as a consequence of acidification the myelin sheaths swell.

Key words Peripheral neuropathies · Structural disorder · Acidosis · Myelin swelling

Introduction

Myelin structure has been studied extensively by X-ray scattering techniques (reviewed by Kirschner and Blaurock 1992). Most studies, however, are focused on the average structure of the elementary pair of membranes (reviewed by Mateu et al. 1990, 1991, 1992, 1995, 1996). In our opinion, though, the physiological (and the pathological) properties of myelin have to be more likely related to the order-disorder phenomena that underlie the packing and wrapping of the elementary double membranes than to the average structure of the membrane pair. A few years ago this hypothesis prompted us to undertake an X-ray scattering study of myelin specifically focused on the order-disorder phenomena (Mateu et al. 1991).

Our approach is based, on the one hand, on an experimental technique that yields fast and accurate X-ray diffraction patterns of nerve fibers kept under continuous superfusion. The exposure times are of the order of minutes, the temperature of the nerve and the chemical composition of the superfusing solutions are kept under control and can be easily changed. On the other hand, we have developed robust and fast algorithms to perform the data reduction of the spectra and to carry out the mathematical analyses. As a result, we are now equipped to routinely perform and analyze X-ray diffraction patterns and to determine the average structure of the elementary pair of membranes and the disorder parameters that characterize the spiral packing and wrapping (Mateu et al. 1990, 1991, 1992, 1995, 1996).

Initially, we applied these techniques to sciatic and optic nerves of rats and mice and tackled a variety of problems: (1) the ''native'' structure of nerves from adult animals (Mateu et al. 1990); (2) the effects of swelling agents (Mateu et al. 1990); (3) the structural phenomena occurring in the course of myelinogenesis (Mateu et al. 1990, 1991); (4) the effects of local anesthetics (Mateu et al. 1992); (5) the effects of temperature (Mateu et al. 1995); (6) the effects of some mutations (jimpy, shiverer, quaking) (Mateu et al. 1996).

More recently, we engaged the new experimental and analytical techniques in the study of human myelin, in a search for possible correlations between physical structure and neurological disorders. For this purpose we used sural nerves (external saphenous) routinely

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removed for biopsies performed for diagnostic purposes from patients suffering from peripheral neuropathies and control autopsies from individuals who had died accidentally or after an acute illness.

Materials and methods

Biopsies and autopsies were carried out in the Neurology Service of the Department of Neurology of the Hospital Universitario de Caracas and in the Autopsy Service of the Instituto de Anatomía Patológica, both at the Universidad Central de Venezuela, Caracas. The nerves were obtained according to the rules set by the Human Protection Committee.

The patient nerve

JRO (patient from the Hospital Universitario de Caracas) was a 36-year-old female who suffered from high blood pressure (under treatment for 10 years). Eight months before her death she underwent a hysterectomy because of metastatic cervix cancer. Seven months later she was admitted to the hospital with a diagnosis of left cerebral apoplexy. Her hands, feet, and legs were in a critical state, showing several gangrenous lesions which suggested arterial occlusions. During her last days at the hospital we tried several times to register electrophysiologically her peripheral functions, but we never succeeded in obtaining any response from these routine measurements. While in the hospital her neurological and respiratory functions progressively deteriorated until she died a few days later. At that time (because of edema) her weight was 113 kg, her height 1.66 m. The autopsy was performed 4 h after death. The results of the autopsy revealed a huge tumoral mass, invading the pelvis bones, the rectum, sigmoid and ascendent colon sections of the intestine, the urinary bladder, and the peritoneal and aortic ganglia. Upon histological examination the cancer was found to be a moderately differentiated adenocarcinoma, with metastases in the colon, rectum, bladder, vagina, ureters, and lymph nodes. The heart morphology was consistent with a hypertensive cardiomyopathy in the expanded phase, with lesions of the aortic and mitral valves, suggesting an infectious endocarditis, which in turn may explain the presence of the gangrenous lesions of hands, feet, and legs. Kidney and brain atherosclerosis was detected, probably related to the long-lasting hypertension which, in conjunction with obesity and the hemodynamic disorders caused by endocarditis, may have led to ventricular overload and hypertrophy. The lung metastases and the tromboemboli seem to be the sources of hypoxia and of brain edema. The left sural nerve was dissected during autopsy.

The reference nerve

The left sural nerve was dissected 11 h after death from a 71-year-old female who had died of respiratory infection.

Rat nerves

Sciatic nerves from Sprague-Dawley rats were used for postmortem studies. The animals were anesthetized with sodium thiopental and then sacrificed by an overdose of anesthetic. One of the sciatic nerves was dissected immediately (rat native); the other was kept within the animal body at room temperature. The second nerve was dissected and the pH of the surrounding fluid measured at variable time intervals after death with an Orion model 4104 pH meter provided with an Orion sure flow electrode.

X-ray scattering experiments

The dissected human nerves were transferred to the laboratory under controlled humidity, mounted in the scattering camera, and kept under continuous superfusion in a Ringer solution (137 mM NaCl, 5 mM KCl, 1.1 mM $MgCl_2$, 1.1 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, 12.5 mM $NaHCO_3$, 1.25 mM $CaCl_2$, 10 mM glucose) at pH 7.3. With the patient nerve a sequence of 15-min X-ray diffraction patterns was registered under superfusion in Ringer solution at pH 7.3. Recording of the first spectrum started 5 h after death (time $= t_0$). For the reference nerve, an initial 2-h X-ray diffraction pattern (initiated 12 h after death) was recorded under superfusion with Ringer solution at pH 7.3. The reference nerve was then transferred from the X-ray camera to a Petri dish where it was soaked overnight in Ringer solution at pH 5.5, mounted again in the X-ray camera (time $= t_0$), superfused with Ringer solution at pH 7.3, and a series of diffraction patterns recorded from time t_0 onwards.

Rats were sacrificed as indicated above, their sciatic nerves dissected at variable times after death, and the epineural sheaths carefully removed under a microscope. The naked nerves were mounted in the X-ray camera and their diffraction patterns recorded. Immediately after dissection the pH was measured at the place were the nerve had been originally located.

Diffraction patterns were obtained with an Elliott GX6 rotating anode X-ray generator operated at 30 kV and 30 mA. Linear focusing was achieved using a nickel-coated bent glass mirror. K_β radiation was attenuated by placing a nickel filter between the mirror and the nerve. The patterns were recorded at room temperature using a position-sensitive detector 80 mm in length and with a 10 mm aperture width (Gabriel 1979) and stored in a PC for later analysis. Nerve to detector distance was 254 mm. The analysis of the patterns is equivalent to fit the experimental data (counts per channels over 2048 channels) to a model defined by 30-odd parameters: the mean of the repeat distance D and the intensity $\{I(h)\}$ of the reflections that define the average structure of the membrane, the intensity $\{I_{\text{diffuse}}(h)\}$ of the parameters that define the diffuse scattering, the standard deviation of the repeat distance σ_D, the average number of elementary membrane pairs $\langle N \rangle$, the fraction of loose membrane pairs not packed in a crystallite α_{loose}, and the fraction of myelin in the nerve α_{myel} that define the disorder (Luzzati and Mateu 1990; Mateu et al. 1990, 1991, 1992, 1995, 1996).

This analysis yielded the values of the 30 parameters plus the continuous function $i_{\text{motif}}(s)$, which is proportional to the intensity scattered by the elementary pair of membranes. It is worthwhile to stress that $i_{\text{motif}}(s)$ is an empirical function, fully determined by the data, that suffices to specify the electron density profile $\rho(r)$ (Mateu et al. 1991, 1992, 1995, 1996).

Results

Some of the patterns from the patient nerve, the reference nerve, and the rat nerve are shown in Fig. 1. The values of some of the structural parameters are displayed in Table 1.

The patient nerve

The initial pattern from the patient nerve (Fig. 1, a1) is strikingly different from the patterns of the native myelin of the reference (b6) and of the native rat nerve (c6). Upon superfusion with normal Ringer at pH 7.3, the patient nerve pattern changes with time (a2–a5) and after 195 min it takes a stable form similar to that of the reference native nerve (compare a5 and b6). Besides, the repeat distance D in the initial patient nerve is much larger than in the native reference nerve (see Table 1). These observations indicate that the patient nerve was initially swollen and that under superfusion with Ringer

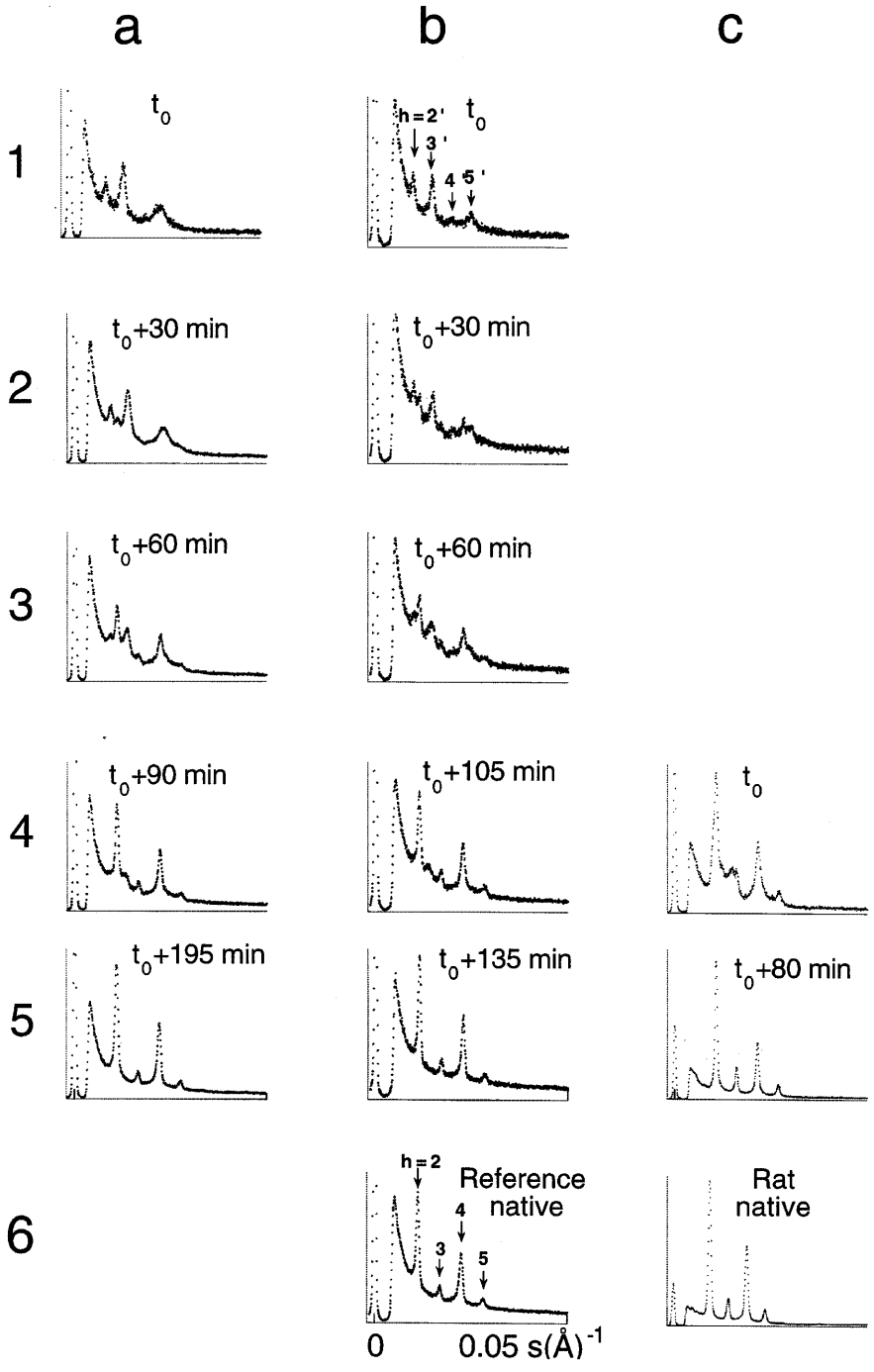

Fig. 1 Raw X-ray diffraction patterns in counts per channel versus channel number. *s* (*b6*) is equal to 2sin ϕ/λ, where 2ϕ is the scattering angle and λ the wavelength ($\lambda = 1.54$ Å in this work). The exposure times are 15 min, with the exception of the "reference native" (*b6*, 120 min) and the "reference, $t_0 + 135$" (*b5*, 60 min). The label specifies the time at which the recording of each pattern begins. Column *a*: patient nerve, superfused with normal Ringer at pH 7.3 from the initial time t_0 onwards. Column *b*: reference nerve. *b6*: native nerve superfused with Ringer solution at pH 7.3 immediately after dissection; the sharp reflections are indicated by *arrows*. *b1*: spectrum at $t = t_0$ after soaking the nerve for 12 h in Ringer solution solution at pH 5.5. The reflections of the swollen lattice are indicated by *arrows*. *b2–b5*: nerve initially swollen and then superfused with Ringer solution at pH 7.3 from time t_0 onwards. Column *c*: rat sciatic nerve. *c6*: native nerve immediately after dissection. *c4*: nerve left for 30 h after death within the cadaver. *c5*: same nerve after superfusion with Ringer solution for 80 min. Note that all the spectra of the same row are similar to each other and that the spectra of row 5 of the three columns are similar to those of the native nerves (*row* 6)

solution at pH 7.3 it shrank back to a state similar to that of native myelin.

The reference nerve

Figure 1 (b6) shows the reference nerve pattern registered shortly after dissection (see Materials and methods). The pattern is similar to those of nerves from the peripheral nervous system (PNS) of other vertebrates (Kirschner et al. 1989). Since it is known that low pH induces the myelin lattice to swell (Inouye and Kirschner 1988), we then soaked the reference nerve in Ringer solution overnight at pH 5.5 and recorded its diffraction pattern. As expected, it was of the swollen type (b1) (Mateu et al. 1990). Furthermore, and in keeping with the experiment performed with the patient nerve (see above), we reversed the swelling by superfusion in Ringer solution at pH 7.3. The time course of the phenomenon is documented by the sequence of spectra b2–b5. Note the close similarity of patterns with those of the patient nerve (sequence a2–a5).

Table 1 Parameters characterizing the disorder. The nature of the nerve samples and the experimental conditions are specified in the text. Note that the D value is typical of native PNS myelin (approximately 180 Å) in the reference native nerve, in the patient nerve after 195 min superfusion, and in the rat nerve recovered after swelling (80 min superfusion). Note also that D is much larger in the patient initial and overnight-swollen reference samples. The swollen samples are more disordered – namely σ_D is larger and $\langle N \rangle$ is smaller – than the non-swollen ones (see text)

Sample	Frame in Fig. 1	Time (min)	D (Å)	σ_D (Å)	$\langle N \rangle$	α_{loose}
Patient initial	a1	t_0	211.6	14.2	2.8	0.10
Patient recovered	a5	t_0+195	181.7	1.6	12.0	0.23
Reference native	b6	–	176.2	4.5	34.0	0.22
Reference swollen	b1	t_0	204.6	6.4	1.8	0.32
Reference recovered	b5	t_0+135	179.4	4.2	> 100	0.18
Rat native	c6	–	180.2	3.5	52.0	0.16
Rat recovered	c5	t_0+80	182.3	4.1	39.0	0.19

Figure 2 shows the functions $si_{motif}(s)$, where $i_{motif}(s)$ is the intensity scattered by the elementary pair of membranes, determined according to Mateu et al. (1995). The functions of the reference native nerve and the final spectrum from the patient nerve (patient t_0+195 in Fig. 2) are similar to each other and are typical of PNS myelin with the packing disorder preferentially (or entirely) located at the cytoplasmic space (Mateu et al. 1995). In contrast, the functions $si_{motif}(s)$ of the initial spectrum from the patient nerve and from the reference nerves soaked at pH 5.5 are characteristic of swollen myelin with the packing disorder preferentially (or entirely) located at the external space (Mateu et al. 1991, 1996). We thus conclude that the patient nerve in its original state, as well as the reference nerve incubated at pH 5.5, are swollen in their external space, and that the swelling can be reversed by superfusion in normal Ringer at pH 7.3.

The swelling in the external space is further documented by the electron density profiles from the patient and the reference nerves in their initial states (Fig. 3).

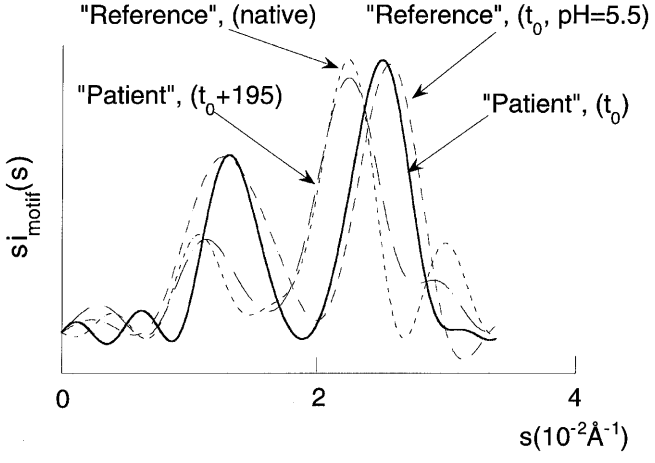

Fig. 2 Functions $si_{motif}(s)$ of the patient and of the reference nerve calculated from the spectra a1 (patient, t_0), b1 (reference, t_0 at pH 5.5), a5 (patient, t_0+195), and b6 (reference native). Note that the functions of the (reference native) and the (patient, t_0+195) samples are similar to each other and typical of native PNS myelin with packing disorder at the cytoplasmic space (see text), whereas those of the (reference, t_0 at pH 5.5) and of the (patient, t_0), that are also similar to each other, are typical of swollen myelin with packing disorder at the external space

We have discussed elsewhere (Mateu et al. 1995, 1996) the connection between these profiles and the functions $i_{motif}(s)$.

The rat nerves

Figure 1 (c6) shows the X-ray diffraction patterns from a freshly dissected rat sciatic nerve (rat native). Notice that the relative intensity and the spacing of the reflections

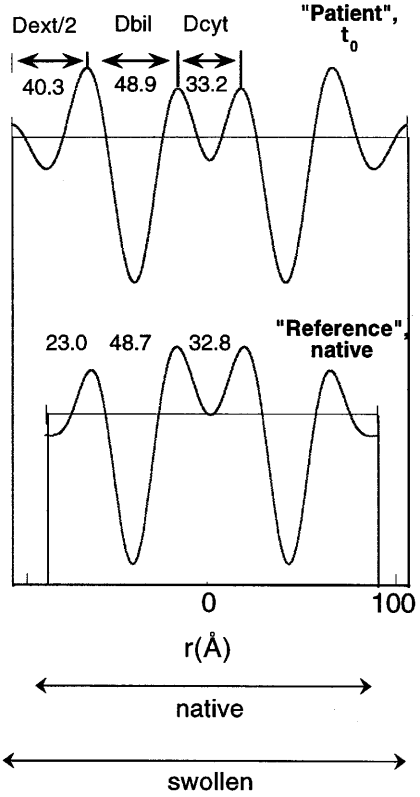

Fig. 3 Electron density maps $\rho(x)$ of the patient nerve and of the reference native nerve in their initial state (t_0). The two profiles are centered at the cytoplasmic space. Note that the thickness of the cytoplasmic space (D_{cyt}) is almost identical in the two profiles (respectively 33.2 and 32.8 Å), whereas the thickness of the external space (D_{ext}) in the patient nerve (80.6 Å) almost doubles that of the reference myelin (46.0 Å)

are similar to those of the reference native spectrum (b6), indicating that peripheral myelin has a similar structure in rats and humans. The pattern from the companion nerve of the same rat kept within the body of the death animal for 30 h is shown in c4; this spectrum is similar to the partially swollen spectra from the patient and reference nerves (a4 and b4). Incubation in Ringer solution at pH 7.3 leads to a recovery of myelin native state; see, for example, in c5 the effect of 80 min incubation on a swollen rat nerve (c4). Note also that the recovery time is different in the three nerves (compare rows 4 and 5 of columns a, b, and c), a phenomenon that may be ascribed to differences in chemical composition.

Figure 4 shows the pH of the fluid surrounding sciatic nerves kept within the rat cadaver, as a function of time after death. The pH steadily decreases with time; its value, moreover, is closely correlated with the progressive swelling observed in the patterns of nerves dissected at the same time.

Discussion

The main result of this work is to show that (in human sural and rat sciatic nerves) at the onset of necrotic decay the myelin sheaths adopt a swollen form. A variety of observations suggest that this native → abnormal swollen transformation is mediated by acidic pH:

1. The patterns from rat sciatic nerves dissected at increasing time after death from cadavers kept at room temperature progressively transform from the native to the swollen type.
2. At the same time, the fluid surrounding the nerve progressively acidifies.

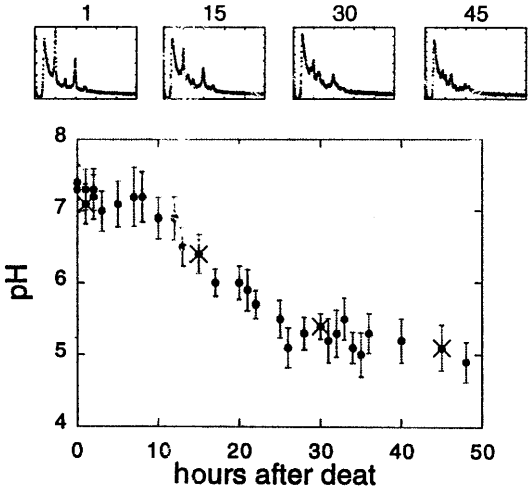

Fig. 4 pH measured in the vicinity of the sciatic nerve within rat cadavers at increasing time after death. Each data point is the mean of five measurements. The vertical bars show the standard errors of the mean. *Inserts*: X-ray diffraction patterns recorded from sciatic nerves dissected at variable time after death (*crosses* in the main plot). Note the correlation between pH value and myelin structure

3. Native myelin from both human sural and rat sciatic nerves reversibly transforms to the swollen type upon exposure to acidic pH.
4. The pattern of the swollen type originally observed in the sural nerve from the corpse of a patient with a complex clinical history also reversed to the native type by exposure of the nerve to physiological pH.

This abnormal swollen form of myelin seems to be similar to the swollen myelin previously observed in rat sciatic nerves exposed to acidic pH (unpublished), with a substantial increase of the thickness of the external space (Fig. 3).

Note that acidosis is often associated with cell injury and low pH is known to have structural and functional effects on myelin (Balentine and Green 1987 and references therein) and also that myelin swelling is a fairly common event, observed by microscopy techniques in a variety of cases: Waldestrom's macroglobulinemia (Vital et al. 1997); triethyltin-induced myelin injury (Mehta et al. 1998); absence of myelin membrane proteolipids (Griffiths et al. 1998); hereditary motor and sensor neuropathies (Santangelo et al. 1994); dysglobulinemic neuropathy (Obnishi and Hirano 1981); presence of ciguatoxin (Benoit et al. 1996).

Since X-ray scattering studies on human myelin are rare in the literature (Chandross et al. 1978a, 1978b), it is timely to stress that the initial pattern of the reference sural nerve (b6 in Fig. 1) is of the same type as 150-odd spectra that we have recorded under similar conditions from human sural nerves either dissected from corpses within a few hours of death or from biopsies performed for diagnostic purposes (manuscript in preparation). In spite of minor, but significant, differences related to the nature of the neuropathologies (in preparation), in no other case did we observe a diffraction pattern nearly as different from the reference (b6) as the initial one from the patient nerve (a1). In keeping with the gangrenous lesions in the patient's feet and legs it thus seems that her sural nerve was undergoing a necrotic decay, probably associated with cell injury, with the subsequent acidification and myelin swelling, and that at the end of her life the myelin was in the swollen state in situ. It must be stressed that in normal physiological conditions the myelin sheath is a highly structured assembly of membranes, in which the elementary double membrane is spirally and tightly wrapped around the nerve fiber. The separation between the membranes cannot be increased (i.e. myelin cannot swell) without the membrane pairs slipping past each other and the number of the spiral turns decreasing. It is surprising that myelin should undergo such conspicuous structural alteration within the patient's body that, no matter how seriously ill, was still alive. It is unfortunate (see Materials and methods) that in the last days of the patient's life it was impossible to obtain any functional register from the routine electrophysiological evaluation of her peripheral functions. In fact, in none of the trials could we detect any

functional response from this patient, indicating that the peripheral nerves of the JRO had lost their functional properties to generate and propagate action potentials. This could be accounted for by the fact that the myelin sheaths were being transformed from a 180 Å-repeat, well-packed, native structure into a 200 Å, loose, swollen array. We hope to be able in the future to look more carefully into this problem and to complete our study with some functional tests of the rat nerves, both in vivo and in vitro.

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