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A SPIN-LABEL STUDY OF SCIATIC NERVES FROM QUAKING, JIMPY AND TREMBLER MICE

JACQUES VIRET[a], FRANÇOIS LETERRIER[a] and JEAN MARIE BOURRE[b]

[a] *Division de Biophysique du Centre de Recherches du Service de Santé des Armées, 1 Bis, rue du Lieutenant Raoul Batany, F-92141 Clamart and*
[b] *Laboratoire de Neurochimie, INSERM U 134, Hôpital de la Salpetrière, 47, boulevard de l'Hôpital, F-75013 Paris (France)*

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Summary

The spin labels, 5-nitroxide stearic acid and 16-nitroxide stearic acid were incorporated into whole sciatic nerves dissected from normal, quaking, jimpy and trembler mice. With 5-nitroxide stearic acid, we have studied the thermal variation of the maximal apparent coupling constant ($T_{//}$) between 0°C and 50°C. Within this range of temperatures, we obtained identical values of 2 $T_{//}$ for nerves from normal and jimpy mice, whereas 2 $T_{//}$ was smaller for nerves from quaking and trembler mice. With 16-nitroxide stearic acid, composite spectra were recorded, particularly in the high-field range. A line characteristic of myelin was clearly observed in the spectra of nerves from normal and jimpy mice; its intensity was somewhat less in nerves from quaking mice and much less in spectra from trembler mice. A shoulder in the principal highfield line of the spectrum is modified only with nerves from jimpy mice.

The results agree well with those obtained by electron microscopy, which reveal normal myelination in nerves from jimpy mice, a slight modification of the myelin from those of quaking mice and a practically complete demyelination in peripheral nerves from trembler mice. However, the structure of the nerves of jimpy mice also seems to be modified at an, as yet, undetermined level.

The neurological mutant mice, jimpy, quaking and trembler are well-known for the presence of altered myelination in their nervous system. No

*Abbreviation: 2 $T_{//}$, maximal apparent coupling constant.

modifications have been observed by light and electron microscopy in sciatic nerves from jimpy mice [1—3], in contrast to the brain, where myelin is practically absent. For nerves from quaking mice a slight hypomyelination has been reported [4—6]. In trembler mice, the brain seems to be apparently normal, whereas an almost complete demyelination [7] and the presence of 'onion' bulb formation in the siatic nerve [8—10] were observed. Transplantation experiments have shown that the characteristic neurological alteration in tembler and quaking mice are due to a primary defect of the Schwann cells [11—12].

Spectroscopic methods have given much information about the structure of brain membranes in normal and diseased animals [13, 14]. They have not been practically applied to peripheral nerves [15] and, in this work, we present the first results obtained by the spin-label method on the sciatic nerves of quaking, jimpy and trembler mice as compared to normal mice.

The animals came from our colonies and were killed by fracture of the cervical column. The sciatic nerves were dissected from their spinal origin to the knee, at room temperature. Labeling was then performed by soaking the nerves in isotonic NaCl containing 10^{-4} M nitroxide fatty acids, for 1 min at room temperature.

Two spin labels were used: 5-nitroxide stearic acid (2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl) and 16-nitroxide stearic acid (2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl), which were purchased from SYNVAR.

Immediately before recording the spectra, sciatic nerves were washed for 30 s in isotonic saline to remove unbound label. For each determination, two nerves were introduced into a Varian quartz cell (sample volume, 100 μl) and the spectra were recorded at increasing temperatures from 5 to 45° C, with an E3 Varian ESR spectrometer. Each experiment was performed on preparations from at least three different mice.

Fig. 1 shows the spectra obtained on 5-nitroxide stearic acid-labeled sciatic nerves of normal and mutant mice. The method of labeling and washing does not allow us to eliminate the presence of lines characteristic of free spin label in solution because extensive washing quickly eliminates the labels, and the signal amplitude becomes too low for accurate measurement. As with all the biological membranes studied with this probe, the spectra are characterized by a large maximal coupling constant, 2 $T_{//}$, which shows that the amplitude of anisotropic motion of the spin label is restricted, or that the label is strongly immobilized. This constant has been measured on the different nerves as a function of temperature (Fig. 2).

In order to measure any temperature effect on the variation of 2 $T_{//}$, the slope of the mean regression line was calculated by the least square method on the three sets of experiments performed on each kind of nerve. These slopes and the coupling constant measured at 40° C are given in Table I.

No difference either in coupling constants or in temperature effects were observed between normal and jimpy mice. For quaking mice, 2 $T_{//}$ is lower and decreases slightly when the temperature is increased. These differences are much more visible with nerves from trembler mice.

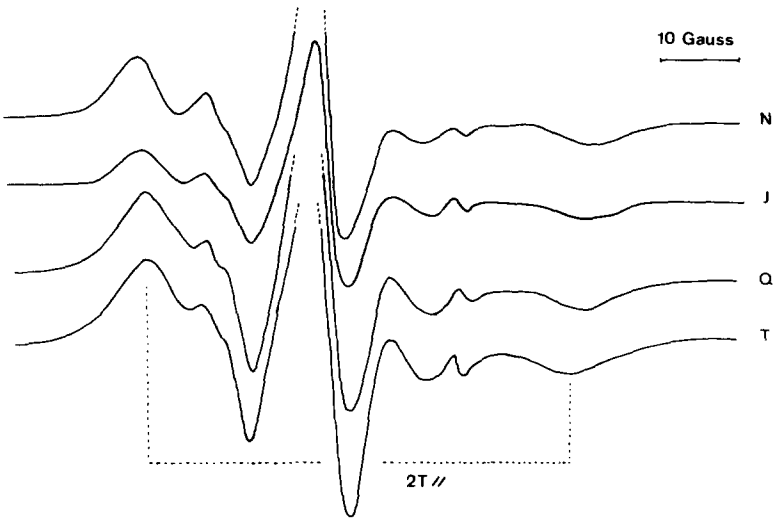

Fig. 1. Spectra obtained with 5-nitroxide stearic acid at 15°C for mice sciatic nerves. N, Normal; J, Jimpy; Q, Quaking; T, Trembler.

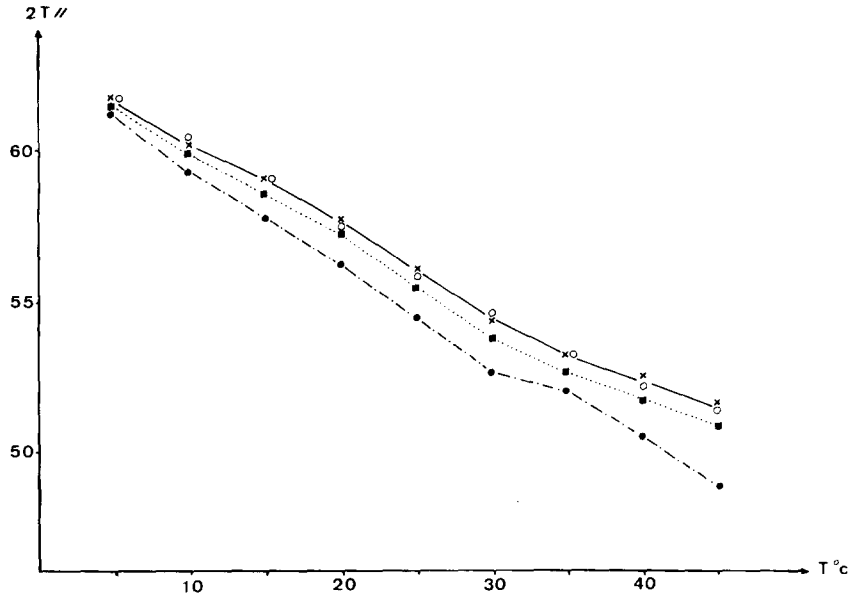

Fig. 2. Variations of hyperfine splitting constant 2 $T_{//}$ as a function of temperature on mice sciatic nerves labeled with 5-nitroxide stearic acid. X, Normal; ○, Jimpy; ■, Quaking; ●, Trembler.

TABLE I

VALUES OF 2 $T_{//}$ COUPLING CONSTANT AT 40°C, AND SLOPES OF THE 2 $T_{//}$ VERSUS TEMPERATURE VARIATION MEASURED ON EACH KIND OF NERVES

	Normal	Jimpy	Quaking	Trembler
2 $T_{//}$ (Gauss)	52.5	52.2	51.6	50.4
% Variation to Normal	0	< 1	1.71	4.00
Slope (Gauss/°C)	− 0.26	− 0.26	−0.27	− 0.30

Fig. 3a shows the spectrum obtained on 16-nitroxide stearic acid-labeled normal sciatic nerves. The high-field part of the spectrum is formed by the superimposition of different lines, which have been called A, B, C and D. Fig. 3b shows the enlargment of these parts of the spectra obtained with the different types of sciatic nerves. We observed that line D is well resolved for normal and jimpy mice. It becomes a shoulder for quaking mice and almost disappears for trembler mice. The amplitude of peak C is very low for normal mice, it increases for jimpy mice and becomes well resolved in quaking and trembler mice. It must be noted that there are differences between the samples in the region of the shoulder B.

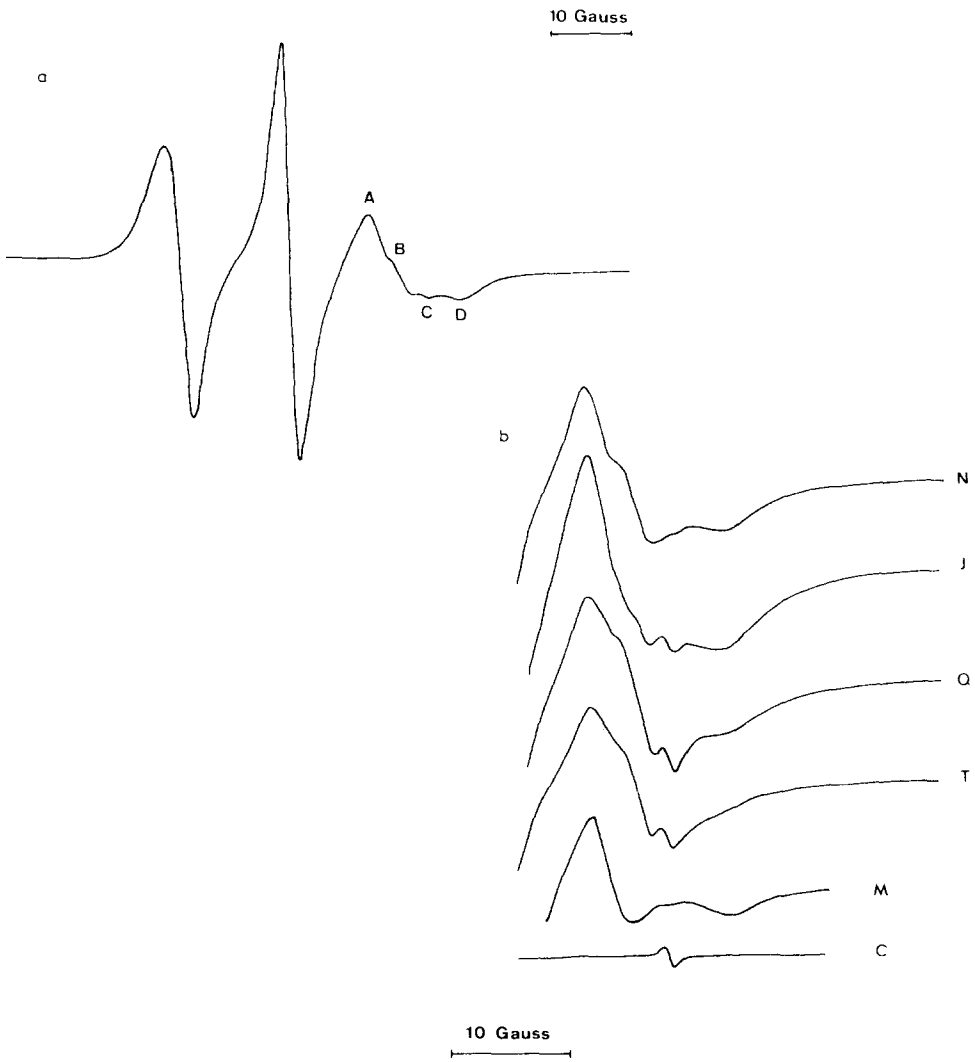

Fig. 3(a) Spectrum obtained on normal sciatic nerves labeled with 16-nitroxide stearic acid at 15°C. Definition of the lines and shoulders observed at high field. (b) High-field part of the spectra recorded at 15°C with 16-nitroxide stearic acid labeled mice sciatic nerves. N, Normal; J, Jimpy; Q, Quaking; T, Trembler. C is the control spectrum obtained with the washing isotonic saline solution, in the case of quaking mice. M is the control spectrum obtained with isolated myelin.

When peripheral nerves were incubated with fatty acid nitroxides, several different membranes were simultaneously labeled. As myelin is highly differentiated from other membranes by its very rigid organization [16], one can attempt to observe its presence on the composite spectra recorded on whole nerves. Based on this assumption, we can now discuss the results obtained with 5-nitroxide stearic acid and 16-nitroxide stearic acid.

The hyperfine splitting, 2 $T_{/\!/}$, measured on 5-nitroxide stearic acid spectra gives indications on the phospholipid organization near the polar part of the membrane and it has been shown that relative variations of 2 $T_{/\!/}$ as low as 1% indicate significant modifications of the membrane structure [14, 16—18]. The values of the 2 $T_{/\!/}$ parameter increase with the rigidity of the fatty acid chains. Our results are in good agreement with electron microscopy observations. The 2 $T_{/\!/}$ and temperature variation are the same in normal and apparently normal (ultrastructurally) nerves from jimpy mice. The main part of the spectrum is due to spin-labeled myelin, since its characteristics are very similar to pure myelin [14]. On the other hand, in the case of nerves from trembler mice, the 2 $T_{/\!/}$ values and the temperature variation are approximatively the same as in synaptic brain membranes [16]. This is not surprising, since these nerves are completely unmyelinated. With sciatic nerves from quaking mice, intermediate values were observed, in good correlation with their hypomyelination.

In the 16-nitroxide stearic acid spectra, we have to try to identify the different peaks recorded in their high-field part. Peak C is obviously due to traces of free label not entirely eliminated by washing, since its position and amplitude are identical with those measured in the spectrum recorded with the washing solution. Peak D is characteristic of the presence of myelin, since it is observed in the same position as in a reference spectrum made with pure myelin. The principal line, A, shows that the 16-nitroxide stearic acid label is weakly immobilized in all the membrane structures probed. However, the shoulder, B, reveals some differences in the fluidity of these structures.

It is not possible to measure the variation observed in the 16-nitroxide stearic acid spectra, as was done for 5-nitroxide stearic acid. However, qualitative information can be obtained, that parallels and confirms the information given by the other label. Peak D is identical in nerves from normal and jimpy mice, it decreases slightly in quaking mice and is practically non-existent in nerves from trembler mice. Since peak D is characteristic of myelin, these observations agree with the different states of myelination of the mutant mice. Furthermore, with 16-nitroxide stearic acid, differences are apparent between sciatic nerves from normal and jimpy mice with respect to the spectra observed in the region of shoulder B. The behaviour of this label within the phospholipid environment of the nerve membrane structure probably does not concern myelin. As the chemical compositions are supposed to be the same for nerves from both normal and jimpy mice [3], we suggest that the membrane molecular organization is slightly altered in the latter. It would be possible that some change in the membrane winding could modify the constraints in the hydrophobic core of the phospholipid structure.

In conclusion, the spin-label method can be proposed as a simple test to estimate the state of myelination of peripheral nerves. An alteration in the

nerve structure of jimpy mice not previously observed by electron microscopy or biochemical investigations has been detected. The nature of this alteration must be elucidated by further work. It appears possible to extend the use of this method to human nerve diseases, for example the trembler mouse is considered as a model for the human Dejerine-Sottar disease [7].

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