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ARTICLE

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Malnutrition and myelin structure: an X-ray scattering study of rat sciatic and optic nerves

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Abstract Taking advantage of the fast and accurate X-ray scattering techniques recently developed in our laboratory, we tackled the study of the structural alterations induced in myelin by malnutrition. Our work was performed on sciatic and optic nerves dissected from rats fed with either a normal or a low-protein caloric diet, as a function of age (from birth to 60 days). By way of electrophysiological controls we also measured (on the sciatic nerves) the height and velocity of the compound action potential. Malnutrition was found to decrease the amount of myelin and to impair the packing order of the membranes in the sheaths.

Key words Malnutrition · Myelin · X-ray scattering · Sciatic nerves · Optic nerves

Introduction

Malnutrition, a shameful scourge of our times, and its neurological effects have been actively studied in biomedical laboratories. In humans and experimental animals, malnutrition has been shown to impair brain growth: brains of rats malnourished since birth and of malnourished infants are smaller and contain less cholesterol, proteolipid protein and cerebrosides than brains from age-matched well-fed controls (Dobbing 1964; Wiggins 1982 and references therein). The changes are particularly severe if malnutrition occurs early in life, at a time when myelin synthesis is particularly active (Winick and Rosso 1969; Winick 1970). From the physiological viewpoint, malnutrition has been shown to lower conduction velocity in both the peripheral nervous system (PNS) (Osuntokun 1971; Engsner and Woldemariam 1974; Cornblath and Brown 1988 and references therein) and central nervous system (CNS) nerves (Quirk et al. 1995). In severely malnourished children, the conduction velocity may be reduced by as much as 50% (Chopra et al. 1986), an effect that is sometimes ascribed to a demyelinating neuropathy (Gilliatt 1966; Thomas 1971; Chopra et al. 1986), although the evidence pointing to a segmentally demyelinating process is scarce.

Over the past few years we have been interested in the structure of myelin, and more particularly in its order-disorder aspects, since we suspect that the structural alterations of physiological and pathological interest are more likely related to the structural disorder than to the ideal average structure of the elementary membrane pair. This work was carried out mainly on rat and mice sciatic and optic nerves, studying the action of a variety of physiologically and pathologically relevant conditions and agents: native structure (Mateu et al. 1990), swelling agents (Mateu et al. 1990), myelinogenesis (Mateu et al. 1990, 1991), local anesthetics (Mateu et al. 1992), temperature (Mateu et al. 1995), and mutations (Mateu et al. 1996). More recently (E. Vonasek et al., submitted), we have embarked on the study of human myelin, in a search for correlations between physical structure and neurological disorders. This project hinges heavily upon newly designed X-ray scattering techniques and robust algoritms for the analysis of the spectra (Luzzati and Mateu 1990).

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The results obtained so far, and the lack of information regarding the effects of malnutrition on the physical structure of myelin, prompted us to tackle the X-ray scattering study described in this work. We used sciatic and optic nerves from normally fed and experimentally undernourished rats. For assessing controls we performed conduction velocity and amplitude measurements of compound action potentials and carried out body and brain weight measurements.

Materials and methods

Nutritional procedures

The rats belong to the Sprague-Dawley strain that has been kept at the IVIC animal house for more than 100 generations. Among the different procedures for inducing experimental malnutrition, we adopted the protein calorie malnutrition (PCM) model, widely used in experimental and clinical investigations of the neurological effects of malnutrition (Chow and Lee 1964; Roy et al. 1972; Chopra et al. 1986).

The rats were distributed in control and experimental groups using 41 litters adjusted to 8 animals per dam at birth. In the control group (NF for normally fed animals) the dam during gestation and suckling and the pups after weaning were fed ad libitum with a chow containing 16% protein (Purina). In the malnourished group (MN), poor nutrition (50% of NF) was imposed first on the dams from one week before conception through the gestation and suckling periods (note that the PCM diet is thought to reduce the quantity of milk without impairing its quality) and then on the puppies after weaning (21 days after birth). Water was available ad libitum to both groups of animals.

Nerve specimens

At different ages (from 1 to 60 days) the rats were weighed, sacrificed and the brains were removed, carefully dried with filter paper and weighed. The optic nerves were dissected under a microscope and soaked in normal 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). The crural segments of the sciatic nerves were also dissected and kept in normal Ringer solution until use. The total number of nerves used for the electrophysiology and X-ray measurements exceeded 500 specimens.

Electrophysiological measurements

Sciatic nerves segments between 12 and 30 mm long, depending on the age of the animal, were mounted on a chamber provided with a pair of stimulating electrodes, a ground electrode and a pair of recording electrodes, and kept under continuous superfusion with Ringer solution. The compound action potentials were generated with a stimulation pulse of 1 ms at five times the excitation threshold intensity using a Grass SD9 stimulator isolated from the ground. Conduction velocity (CV) was calculated from the time elapsed from the stimulation artifact and the compound action potential (CAP) peak. All the measurements were carried out at room temperature, within 10 min of dissection.

X-ray scattering experiments

The X-ray scattering source was an Elliott GX6 rotating-anode generator operated at 25 kV and 30 mA. Cu K_α monochromatization ($\lambda = 1.54$ Å) and linear focusing were achieved using a nickel-coated bent glass mirror. K_β radiation was attenuated by a nickel filter intercalated between the mirror and the specimen. The X-ray diffraction patterns were recorded using a linear position-sensitive detector, 80 mm long and 10 mm wide (Gabriel 1979). The specimen-detector distance was 350 mm. A total of 2048 channels patterns were accumulated for 60 min using a Nucleus MCA interface. The patterns were stored in a PC for further analysis.

Analysis of the X-ray diffraction patterns

The patterns were analyzed via the algorithms described elsewhere (Luzzati and Mateu 1990; Mateu et al. 1990, 1991, 1992, 1995, 1996). The notation is the same as in those papers. The more usual symbols and abbreviations are: r is the position in real space (in Å); $s = (2\sin\theta/\lambda)$ is the position in scattering space (in $Å^{-1}$), where 2θ is the scattering angle; h is a positive integer that identifies the order of the reflection ($h = 0$ refers to the incident beam); $C(j)$ is the raw scattering pattern (in counts per channel) that contains the scattering produced by compact myelin, loose myelin, non-myelin components and the blank scattering from the equipment; α_{loose} is the fraction of loose lamellae (namely, of membrane pairs that are not packed in a crystallite); α_{myel} is the fraction of myelin in the sample; D and σ_D^2 (in Å and $Å^2$) are the mean and the variance of the repeat distance of a single nerve; and $< N >$ is the average number of double membranes per coherent crystallite in the same nerve.

Results

Body and brain weights

Figure 1a shows the body weight of rats of the two groups, sacrificed at various ages. At 54 days the body weight of NF animals is approximately six times the weight at weaning. At the same age, the weight of the MN animals is approximately one third that of the controls (NF). As age increases, body growth steadily increases in the two groups, whereas brain growth slows down (Fig. 1b). This phenomenon is amplified in the plots (Fig. 1c) of brain/body weight ratio versus age that display a maximum at 9 days for the NF and at 13 days for the MN animals. Note also that at all ages the brain/body weight ratio is higher in the MN than in the NF animals. In other words, malnutrition affects body weight more profoundly than brain weight (Wiggins et al. 1984).

Electrophysiological parameters

Figure 2 displays the CV of sciatic nerves as a function of age. In the two groups of animals, the CV steadily increases with age for approximately 30 days and then it reaches a plateau; no attempt was made to identify slow unmyelinated fibers. At all ages until weaning (21 days), the CV takes similar values in the two groups of animals. After weaning, the CV increases more rapidly in the NF than in the MN animals. In close agreement with the results of Cornblath and Brown (1988), at the end of the experiment (60 days) the CV is approximately 47.5 ± 2.3 m/s for the NF and 29.7 ± 6.0 m/s for the MN animals. ΔV displays a similar trend (not shown in the figure), although the experimental hazards (position of the electrodes on the nerve, contact resistance, etc.)

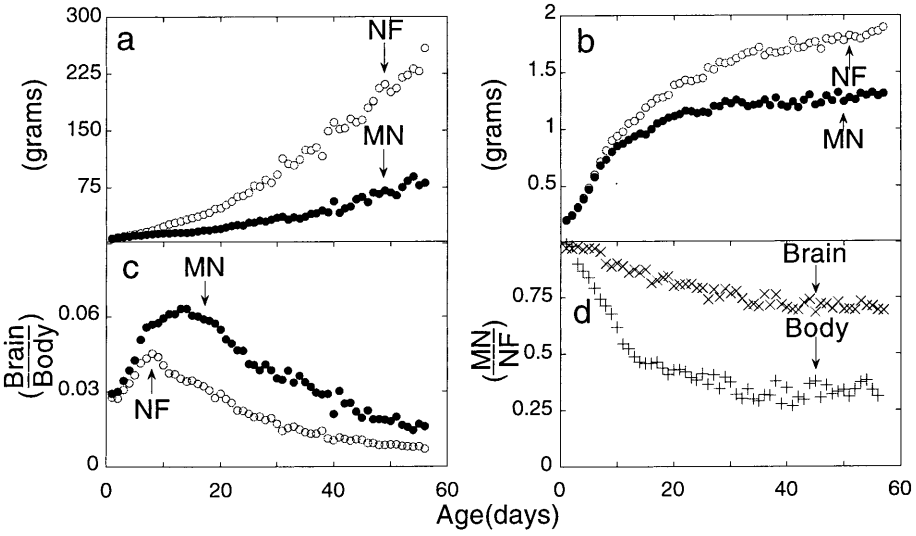

Fig. 1 Age-dependent evolution of body (**a**) and brain (**b**) weight of normally fed controls (NF, *open dots*) and malnourished animals (MN, *black dots*). **c** Brain/body weight ratio for NF and MN animals. **d** MN/NF brain (×) and body (+) ratios. Each point is the mean of several measurements. The number of measurements vary from one day to another depending on the number of available animals. The standard errors of the mean (SEM) are within the size of the dots

make the points more widely dispersed than in the case of the CV. Similar results have been reported by Quirk et al. (1995) in the CNS of malnourished rats. Lack of appropriate equipment prevented us performing electrophysiological experiments on rat optic nerves.

Myelinogenesis

A sample of raw X-ray diffraction patterns from the sciatic and optic nerves of NF and MN animals are displayed in Fig. 3 as a function of age. The patterns consist of series of equally spaced sharp reflections and are characteristic of PNS and CNS myelinated nerves (Mateu et al. 1990, 1991, 1992, 1995, 1996). At all ages, and within the same group of animals, the patterns are similar to each other.

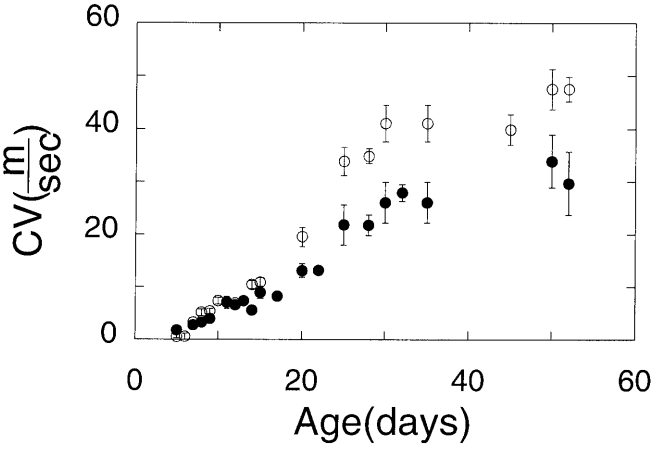

Fig. 2 Conduction velocity (CV) measured in sciatic nerves from NF (*open dots*) and MN (*black dots*) animals as a function of age. Each data point is the mean of several measurements (usually more than five). The *vertical bars* show the standard errors of the mean

Sciatic nerves

Both in NF and in MN animals, well-developed myelin is detected since day 5 (Mateu et al. 1990). After that age the spectra display several reflections, all similarly sharp and of the same relative intensity. This observation indicates that whatever the age, the amount and the structure of ordered myelin are similar in the two groups of animals. After day 10 the general aspect of the patterns remains the same, but the intensity of all the reflections increases with age, faster in the NF than in the MN animals.

Optic nerves

Well-formed myelin is detected since day 10 (Mateu et al. 1991) in NF and after 12 days in MN animals (not shown in the figure). As in the case of sciatic nerves, in optic nerves myelin develops more rapidly in NF than in MN animals.

Order-disorder parameters

The values of the structural parameters were determined using the algorithms mentioned under Material and methods. The results are plotted in Fig. 4.

Sciatic nerves

At day 5 the repeat distance D is shorter in the MN than in the NF animals (174.96 ± 0.27 and 177.05 ± 0.62 Å, respectively); with increasing age, D increases in the two groups of animals, reaching respectively 178.29 ± 0.38 Å (MN) and 179.64 ± 0.53 Å (NF). The variance σ_D remains almost constant (3.16 ± 0.34 Å) for the first 20 days in the two groups of animals; it progressively decreases after weaning and attains 2.05 ± 0.42 Å at

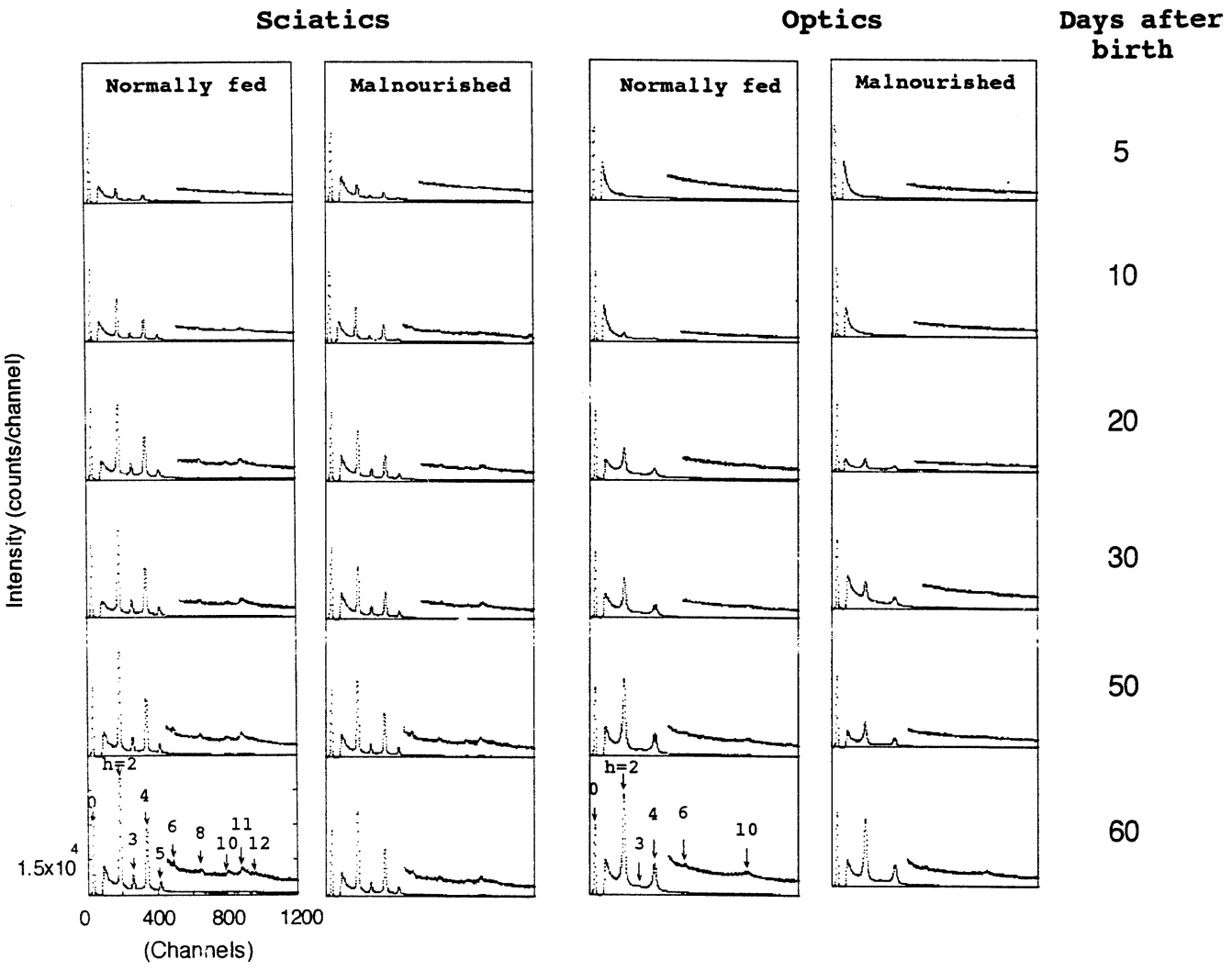

Fig. 3 The myelination process for NF and MN animals. The figure shows samples of the experimental spectra as a function of age. Each row corresponds to the same age (reported beside the right column). *Abcissae*: channel number *j*. *Ordinates*: counts per channel *j*. The spectra are normalized to the intensity of the incident beam. Note that in both sciatic and optic nerves the intensity of all the reflections increases with age and that at the same age the intensity is higher for NF than for MN animals. The position of the Bragg reflections is indicated by *arrows* in the spectra of the 60-day NF animals. Reflection $h=0$ marks the origin of the spectra

60 days. The average number $< N >$ of myelin turns per axon steadily increases with age in the two groups, somewhat faster in the NF than in the MN animals: at 5, 21 (weaning) and 60 days, $< N >$ is equal to 21.59 ± 6.8, 74.07 ± 6.22 and 86.29 ± 7.68 in the NF and 6.41 ± 1.35, 26.6 ± 6.23 and 49.23 ± 7.68 in the MN animals. The parameter α_{loose} displays a similar age dependence in the two groups of animals: high at birth ($\alpha_{loose} \approx 0.53 \pm 0.06$), it progressively decreases ($\alpha_{loose} \approx 0.24 \pm 0.03$ at 60 days). The degree of myelination (α_{myel}) steadily increases with age in the two groups of animals, and reaches a lower plateau in the MN than in the NF group (approximately 0.45 ± 0.03 and 0.57 ± 0.02).

Optic nerves

The repeat distance D and its variance σ_D are, at all ages, almost identical in the two groups of animals; moreover, D is independent of age whereas σ_D steadily decreases (from 5.75 ± 0.28 Å at day 10 to 3.26 ± 0.26 Å at day 60). $< N >$ is very small in the two groups of animals and slowly increases with age (from 2.11 ± 0.43 to 2.91 ± 0.42). The age-dependent trend of α_{loose} is also similar in the NF and the MN animals, with the exception of a small increase observed in the MN animals over the age range 10–30 days. In keeping with sciatic nerves, in the two groups of animals the degree of myelination of optic nerves increases with time. Since the age at which Bragg reflections are observed (12 days), α_{myel} is lower in MN than in NF animals. The final values at the end of the experimental period (60 days) are 0.41 ± 0.05 in MN and 0.59 ± 0.04 in NF animals.

Average structure of the elementary membrane pairs

In all the experiments relevant to the same type of nerve (sciatic or optic), the relative intensity of the reflections

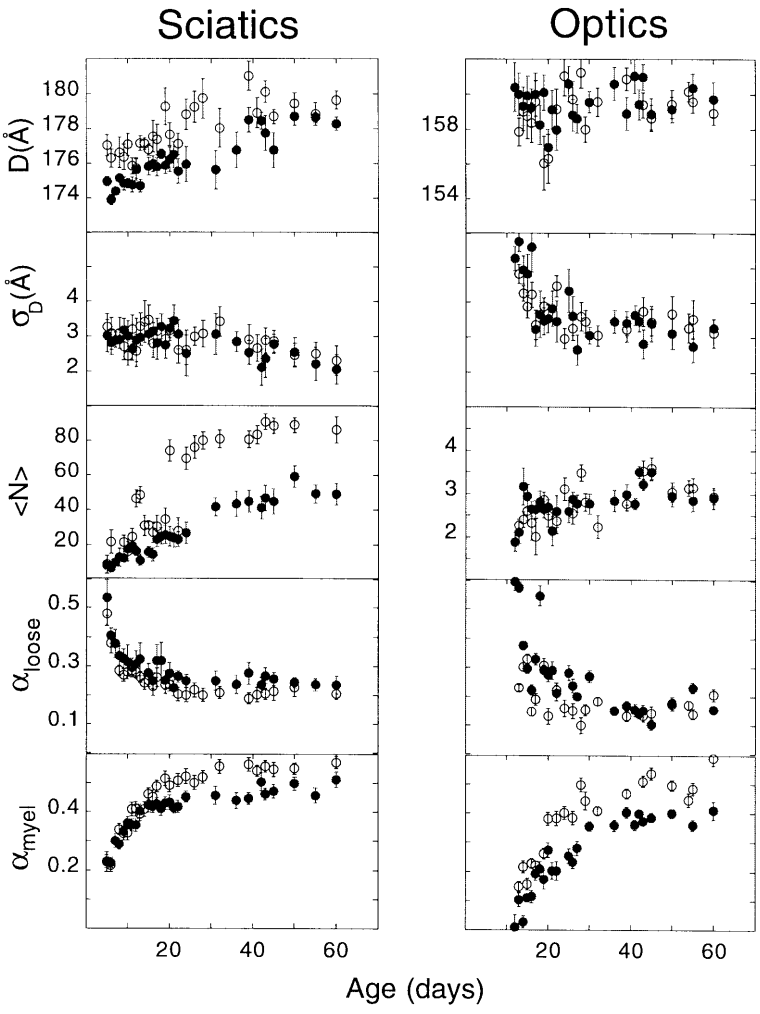

Fig. 4 Age dependence of the repeat distance D and its variance σ_D, the average number of double membrane turns per axon $< N >$, the fraction of membrane pairs not packed in a crystallite α_{loose} and the fraction of well-packed myelin in the nerve α_{myel} (see also text). Each point is the average of several measurements (from 5 to 15) depending on the availability of the animals at different ages

is found to be independent of age and of feeding history (results not shown). It can thus be concluded that neither age (Mateu et al. 1990) nor malnutrition have any effect on the average structure of myelin, at least within experimental accuracy and resolution (Luzzati et al. 1999).

Discussion and conclusions

It is timely to look into some of the technical aspects of this work. The number of experiments is large (some 300 spectra were recorded and analyzed) and all the spectra were treated in exactly the same way. As a consequence, the statistical significance of the final parameters is high. As an additional precaution, all brain extractions and weighing were carried out by one and the same person. The analysis of the X-ray scattering spectra is equivalent to fitting the experimental data (counts per channel over 2048 channels) to a model defined by 30 parameters: D and the intensity $\{I(h)\}$ of 14 reflections that define the average ideal structure; the intensity $\{I_{diffuse}\ (h)\}$ of the 14 parameters that define the diffuse scattering; σ_D, $< N >$, α_{loose} and α_{myel}, that define the disorder (Luzzati and Mateu 1990; Mateu et al. 1990, 1991, 1992, 1995, 1996). Whatever the precise physical significance of these parameters, one feels confident that the age- and nutrition-related changes documented in Fig. 4 mirror genuine structural alterations of the myelin sheaths.

Our weight determinations confirm and extend previous observations (Dobbing 1964; Osuntokun 1971; Engsner and Woldemariam 1974; Wiggins 1982 and references therein; Cornblath and Brown 1988 and references therein). For example, we find (see Fig. 1) that the body weight ratio (adult)/(pup at weaning) decreases by 55% from NF to MN animals, as compared to 0.44 and 0.39 reported respectively by Wiggins et al. (1984) and Delaney et al. (1981). Furthermore, these observations provide a rewarding confirmation that the malnourished animals whose nerves we have studied did indeed display the well-established symptoms of malnutrition.

Our results show, in keeping with Webster (1971), that well-packed myelin is detectable in both malnourished and normally fed animals since the 5th day after birth (see Fig. 3). We also find that the overall amount of myelin (α_{myel}) as well as the thickness of the sheaths ($< N >$) are still increasing several weeks after birth

(Fig. 3), at variance with Wiggins and Morell (1980) who claim that myelination is complete by 10–12 days. We also find that in sciatic nerve, and at all ages, the number $< N >$ of lamellae per sheath is significantly smaller in MN that in NF animals (Fig. 3).

Our observation that in rat sciatic nerves the effect of malnutrition is to decrease the conduction velocity confirms previous electrophysiological observations on human and rat nerves (Osuntokun 1971; Chopra et al. 1986; Quirk et al. 1995).

Regarding the nutritional model, we assume, in keeping with most workers in the field, that all the essential nutrients are present in the PCM diet, although in smaller amounts than in a normal diet. Yet the possibility exists that some particular nutrients could play a critical role. Another important question open to future investigation is the extent to which the structural perturbations observed in the myelin of MN animals can be reversed by an appropriate diet provided at some time after the onset of malnutrition.

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