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DECREASED BIOSYNTHESIS OF SATURATED C20–C24 FATTY ACIDS BY THE TREMBLER MOUSE SCIATIC NERVE

F. BOIRON, D. DARRIET, J. M. BOURRE* and C. CASSAGNE

Institut de biochimie Cellulaire, et Neurochimie du CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux Cedex and *INSERM U26 Unité de Neurotoxicologie, Hôpital F. Vidal, 200 rue de Faubourg Saint-Denis, 75475 Paris Cedex, France

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Abstract—Excised Trembler mouse sciatic nerves synthesize, from acetate, only minute amounts of C20 and C22 saturated fatty acids (about 1/10 of the normal value) and almost no lignoceric acid. The elongation activity is localized in the microsomal fraction. The microsomes from Trembler sciatic nerves can elongate stearoyl-CoA into C20, C22 and C24 saturated fatty acids. The elongation rate is only 1/3 of the normal value, whereas the stearoyl-CoA hydrolysis is 3 times higher than in the control; the malonyl-CoA concentration remains at the same level in microsomes from normal and trembler sciatic nerves. When ATP-Mg^{2+} is added to the Trembler microsomes, the stearoyl-CoA hydrolysis is reduced, the stearoyl-CoA concentration remains nearly normal and the elongation reaches an almost normal level.

The Trembler mouse is a neurological mutant which bears a dominantly inherited mutation showing phenotypic involvement of the peripheral nervous system, and an apparently intact central nervous system (Low and McLeod, 1975).

Morphologically, the Trembler defect is correlated with a severe peripheral nervous system hypomyelination, with segmental demyelination (Ayers and McAnderson, 1973), uncompacted myelin sheaths, endoneurial and perineurial connective tissue proliferation and onion bulb formation (Ayers and McAnderson, 1976).

From the biochemical point of view, Trembler sciatic nerves exhibit a 66% decrease in their total lipid level, compared to that of normal nerves. The analysis of the lipid composition of the former shows a nearly parallel decrease of every lipid class level, except for that of the cholesterol esters, which is increased 5-fold (Larrouquère. Régnier *et al.*, 1979). In good agreement with these data, the total amount of fatty acids in Trembler mouse sciatic nerves is only 50% of the control value, but, interestingly, this decrease does not affect the various fatty acids equally (Darriet *et al.*, 1980). Whereas unsaturated fatty acids have nearly normal levels, the levels of C20, C22 and C24 saturated fatty acids, which are the most characteristic fatty acids of the myelin lipids, are drastically reduced (by 80% for C20 and by more than 90% for C22 and C24).

This decrease could reflect the hypomyelination of the Trembler mouse sciatic nerve (Darriet *et al.*, 1980); but the nature of the metabolic events inducing or resulting from this defect is unknown.

In order to obtain information on this point, we studied the ability of the Trembler mouse peripheral nervous system to synthesise fatty acids, and particularly C20, C22 and C24 saturated fatty acids, compared to that of control mice.

EXPERIMENTAL PROCEDURES

Malonyl-CoA, stearoyl-CoA, stearate, CoA, NADPH, ATP, Magnesium chloride were obtained from Sigma and [1-^{14}C]acetate (50 Ci/mol), [2-^{14}C]malonyl-CoA (59 Ci/mol), [1-^{14}C]stearoyl-CoA (49 Ci/mol), [1-^{14}C]stearate (51 Ci/mol) were from Commissariat à l'Energie Atomique (CEA, France), New-England Nuclear (NEN) or from Amersham International Ltd.

Trembler mice (Tr/+) were obtained from the B6-CBA strain (+/+). Since the neuropathy is dominantly inherited, control mice were the littermates of the mutants that presented a normal phenotype. The sciatic nerves of the Trembler and control mice (30-days old) were carefully removed and used either as such, or as membrane fractions. In the former case, about 50 mg of freshly dissected nerves were incubated for various times at 37°C, in the presence of

A preliminary report on this work was presented at the *Neurological Mutations Affecting Myelination* meeting (Seillac, France, 1980).

[1-^{14}C]acetate (7 μCi) in 50 μl of water (see the legends of figures and tables). This method of incubation of excised sciatic nerves with labelled acetate was chosen following various attempts which showed that: (1) buffering the external medium at nearly neutral pH resulted in a decreased acetate incorporation; (2) adding ATP-$MgCl_2$ or CoA-SH or NA-DPH did not modify the label found in the fatty acids; (3) the acetate incorporation into fatty acids increased linearly for at least 4 h (about 10^5 cpm/100 mg fresh weight/h). This level of acetate incorporation is equivalent to or higher than the others so far reported in the literature (Koeppen *et al.*, 1979; Natarajan *et al.*, 1982). In the case of membrane fractions, the nerves were homogenized in an all-glass tissue grinder with 10 vol 0.05 M Tris–HCl, pH 7.5 at 4°C. The homogenate was spun at 20,000 *g* for 20 min. The pellet (20,000 *g* pellet) was recovered in buffer and the supernatant centrifuged at 150,000 *g* for 90 min. The resulting membrane pellet (150,000 *g* pellet) was resuspended in 0.05 M Tris–HCl pH 7.5 and gently homogenized in a Potter homogenizer. When using subcellular fractions, the biosynthesis of the very long chain fatty acids, (C20-C24), was measured by the incorporation of [1-^{14}C]stearate, [1-^{14}C]stearoyl-CoA or [2-^{14}C]malonyl-CoA. With the [1-^{14}C]stearate as labelled substrate (from 1 to 8 μCi, 51 Ci/mol, in a final volume of 0.5 ml), the incubation mixtures contained: Tris–HCl 50 mM, pH 7.5; NADPH 0.5 μmol, malonyl-CoA 0.1 μmol, CoA 0.25 μmol, ATP 1 μmol, Mg^{2+} 1 μmol. With [1-^{14}C]stearoyl-CoA (0.2 μCi, 2 Ci/mol), for the same final volume (0.5 ml), the incubation mixtures had the same composition as above except that the CoA and the ATP-Mg^{2+} were omitted. With [2-^{14}C]malonyl-CoA (1 μCi, 50 Ci/mol), 80 nmol of stearoyl-CoA and 0.5 μmol NADPH were added to the 500 μl reaction mixture. The assays were always started by adding 50 to 150 μg of membrane proteins as the enzyme source. The incubations were carried out at 37°C for 1 h, and then stopped by adding 5 M KOH (0.5 ml). The lipids were saponified by heating this mixture for 1 h at 80°C. After extraction of the non-saponifiable matter, the fatty acid methyl esters were prepared, purified and anlayzed as previously described (Cassagne *et al.*, 1977, 1978).

Thin layer chromatography of the experimental mixtures with [2-^{14}C]malonyl-CoA as labelled substrates

After the 1 h incubation with [2-^{14}C]malonyl-CoA as labelled substrate, 600 μl chloroform-methanol (1:1, v/v) were added to 100 μl of the reaction mixture. An aliquot (50 to 75 μl) of the homogeneous phase so obtained was deposited on a 10 × 10 cm HPTLC Kieselgel 60 plate (Merck) and eluted by *n*-butanol, acetic acid, water (5:2:3, by vol.) to allow the separation of malonyl-CoA, acetyl-CoA, malonic acid and long chain acyl-CoAs in a single step. The different spots were identified by co-migration with standards. The distribution of the radioactivity in the different spots was estimated by radio-chromatography of the plates on a Packard radio-chromatograph paired with a Packard integrator, or by densitometric analysis after autoradiography.

RESULTS

Fatty acids biosynthesis by excised sciatic nerves

Excised sciatic nerves from normal and Trembler mice were allowed to incorporate labelled acetate for various times. The results of fatty acid labelling after 3 h incubation with [1-^{14}C]acetate are shown in Fig. 1. The distribution of radioactivity among the purified methyl esters was determined and expressed as cpm/mg fresh weight. It was then calculated as cpm/mg dry weight from statistical studies of the value of the ratio dry weight (DW)/fresh weight (FW), which emphasizes the hypomyelination of the mutant (see Darriet *et al.*, 1978). In the controls DW/FW = 33.2 and in Trembler DW/FW = 20.5. In the sciatic nerve of the Trembler mutant, the overall fatty acid synthesis was diminished by 80%. The C14 and the C16 biosynthesis reached about 20 to 25% of the control value, while the C18 synthesis was less than 20% of it. Some label was detected in C20 and C22 fatty acids (less than 10% of controls) but not in C24. When shorter times of incubation were used, almost no radioactive C20 and C22 fatty acids were detected. Hence, the whole metabolic pathway of the fatty acid biosynthesis is altered in the Trembler mouse sciatic nerve. However, each step is not modified in the same way: (1) The biosynthesis of C16 fatty acid usually reflects a *de novo* synthesis: it is diminished to 1/4 of the normal level ($P < 0.001$). (2) The biosynthesis of C18 fatty acids is reduced to 1/5 of the normal level ($P < 0.001$). (3) The very long chain fatty acid (VLCFA) biosynthesis reaches only

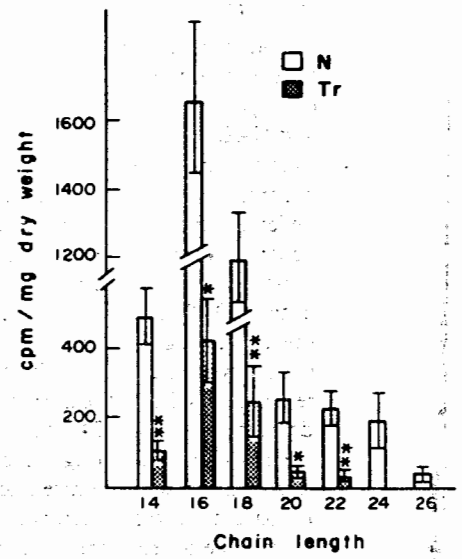

Fig. 1. Total fatty acid biosynthesis by excised sciatic nerves. 50 mg of normal or Trembler sciatic nerves; [1-^{14}C]acetate (7 μCi); 3 h of incubation at 37°C; Results are given as cpm/mg dry weight; N = normal; Tr = Trembler; Mean of 3 experiments ± SD; Significant difference between normal and Trembler: $^*P < 0.01$; $^{**}P < 0.001$.

Table 1. Stearoyl-CoA elongation by the subcellular fractions from sciatic nerves of normal and Trembler mice

	Homogenate	20,000 g pellet	150,000 g pellet	150,000 g supernatant
Normal	13 ± 5	7 ± 1	36 ± 3	1.5 ± 0.5
Trembler	4.5 ± 3*	2.7 ± 1†	13 ± 2‡	1 ± 0.5§

[1-^{14}C]stearoyl-CoA (2 μCi/mol; 0.2 μCi); NADPH 0.5 μmol; Malonyl-CoA 0.1 μmol; Tris–HCl 0.05 M pH 7.5; Final volume 500 μl; 1 h incubation at 37°C; Results are given as specific activity of the elongase (nmol incorporated into C_{20}, C_{22} and C_{24} fatty acids/mg protein/h). Mean value (3 experiments) ± SD; Significant difference between normal and Trembler *$P < 0.1$, †$P < 0.01$, ‡$P < 0.001$, §$P > 0.1$.

1/10 of the normal value in the Trembler mouse sciatic nerve ($P < 0.01$).

The latter results are in good agreement with the fatty acid analysis (Darriet *et al.*, 1980). They show that the very long chain fatty acid biosynthesis is the most altered, and thus could explain a specific deficit of these molecules in the mutant sciatic nerve. Consequently, we first studied this step of VLCFA biosynthesis, that is stearoyl-CoA elongation, after a subcellular fractionation.

Very long chain fatty acid biosynthesis by subcellular fractions

Four subfractions were prepared, and their ability to elongate the putative substrate of the elongase, [1-^{14}C]stearoyl-CoA, in the presence of malonyl-CoA and NADPH, was checked. Table 1 shows that in the Trembler mouse sciatic nerve subfractions, as in the control ones, the crude homogenate, the 20,000 *g* pellet and the 150,000 *g* pellet exhibited an elongating activity, whereas the 150,000 *g* supernatant was almost devoid of it. The maximal biosynthesis occurred in the 150,000 *g* pellet, which contained about 60% of the elongating activity; but the stearoyl-CoA elongation was markedly reduced in the mutant and reached only 1/3 of the normal level ($P < 0.001$). This decrease in C20-C24 fatty acid formation from stearoyl-CoA was in agreement with the results obtained using whole excised sciatic nerves, although, (apparently), the differences of elongation in the membrane pellets were not as marked as the C20-C24 formation from acetate in the whole nerves. This last point could be explained by the fact that, in the experiments using 150,000 *g* pellets, the membrane subfractions from normal and mutant sciatic nerves were supplied with the same amount of stearoyl-CoA; in contrast, in the assays carried out on whole nerves, using acetate as the substrate, the amount of C18 formed in the Trembler nerves is only 20% of that formed in the normal nerves.

Very long chain fatty acid biosynthesis by the 150,000 g membrane pellet

Elongation as a function of the quantity of protein. Figure 2 shows that, in the assays with the control and mutant membranes, the synthesis of C20-C24 fatty acids increased with increasing amounts of the microsomal membrane proteins (20–300 μg). With amounts of protein between 50 and 100 μg, the synthesis was about 3 times lower in the membrane fraction from the mutant sciatic nerves than in that of the control. With quantities above 100 μg and up

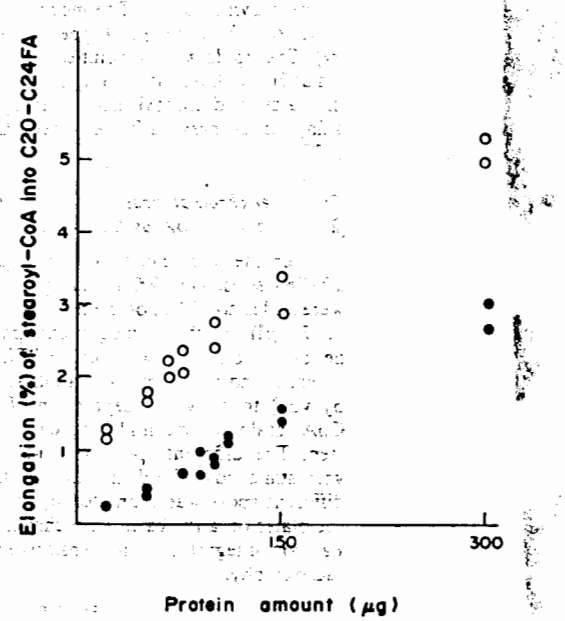

Fig. 2. Stearoyl-CoA elongation by the microsomes of sciatic nerves from normal and Trembler mice as a function of protein concentration. Experimental conditions—[1-^{14}C]stearoyl-CoA (0.2 μCi, 2 Ci/mol), NADPH 0.5 μmol, malonyl-CoA 0.1 μmol, Tris–HCl pH 7.5, 0.05 M, final volume 500 μl. Incubation time 1 h at 37°C. Results are given as percentage of stearoyl-CoA elongation (1 experiment, duplicates). ○: normal mice; ●: Trembler mice.

to 300 μg of membrane proteins, the stearoyl-CoA elongation was roughly 50% that of the control. For a quantity of membrane protein less than 50 μg, the elongation in the assays with the mutant nerves was very low and hard to detect; consequently all subsequent experiments were done with 50–150 μg of membrane protein.

Stearate and stearoyl-CoA elongation

In order to study the stearate elongation, not only malonyl-CoA and NADPH, but also ATP-Mg^{2+} and Coenzyme A were required in the experimental mixture, for the activation of stearate to stearoyl-CoA.

Table 2 shows that, whatever the stearate concentration, except for the lowest one, the specific elongating activity of the Trembler microsomes reached about 50% of the elongating activity of the controls ($P < 0.01$). In all the Trembler assays, the biosynthesis of lignoceric acid was measured and no inhibition of the elongation was observed for stearate concentrations of up to 320 μM. An increased elongation in both normal and Trembler microsomes was observed when using the [1-^{14}C]stearoyl-CoA instead of the [1-^{14}C]stearate as labelled substrate. As shown in Fig. 3 when [1-^{14}C]stearoyl-CoA was the substrate, the specific activity of biosynthesis reached 36 nmol/mg protein/h in the assays with the controls and 13 nmol/mg protein/h in those with Trembler (Fig. 3a) ($P < 0.01$), as compared to 10 nmol/mg protein/h in the assays with the controls and 5 nmol/mg protein/h in the assays with Trembler, when [1-^{14}C]stearate (Fig. 3b) was used at the same substrate concentration ($P < 0.01$).

In the mutant assays, the label distribution in the very long chain fatty acids also differed according to the labelled substrate. Thus, for an incubation of 1 h, eicosanoic acid (C20) biosynthesis was the most increased when using [1-^{14}C]stearoyl-CoA. These results demonstrated that the 150,000 *g* membrane pellet from the Trembler sciatic nerves exhibited a stearoyl-CoA elongating activity which was always decreased by about 2/3 as compared with the control. However, this cannot be taken as evidence that the elongase itself is altered in the mutant mouse since: (a) the amount of elongase in the membrane pellet is unknown; (b) it is well-known that acyl-CoAs are the substrates of many other membrane bound-enzymes, so that the amount of substrate actually available for elongation is also unknown. We have previously studied the fate of stearoyl-CoA in the membrane pellet from normal and Trembler sciatic nerves (Boiron *et al.*, 1982), but no data were available on the second substrate, malonyl-CoA, which was, therefore, further investigated.

The 150,000 *g* pellets from Trembler and normal sciatic nerves were incubated in the presence of [2-^{14}C]malonyl-CoA, stearoyl-CoA and NADPH. Table 3 shows that in both cases (normal and Trembler), [2-^{14}C]malonyl-CoA was in part hydrolyzed, in part decarboxylated, and to a lesser extent incorporated into fatty acids (as seen in the spot of long chain acyl-CoAs and in the unidentified polar lipids).

Interestingly, the label insertion into long chain acyl-CoA was 3 times lower in the mutant nerves than in the controls. But the level of the remaining malonyl-CoA was almost the same in both membrane

Table 2. Comparative study of C20-C24 fatty acid synthesis in microsomal fractions from normal and Trembler sciatic nerves as a function of stearate concentration

Stearate concentration		Chain length C20	C22	C24
40 μM	N	2.6 ± 0.4	2.0 ± 0.2	1.2 ± 0.2
	Tr*	0.3 ± 0.1	0.7 ± 0.2	0.3 ± 0.1
80 μm	N	3.9 ± 0.5	2.3 ± 0.3	1.8 ± 0.1
	Tr†	1.0 ± 0.2	1.4 ± 0.3	1.0 ± 0.2
160 μM	N	4.2 ± 0.3	3.9 ± 0.5	2.1 ± 0.1
	Tr‡	2.4 ± 0.4	1.5 ± 0.2	1.1 ± 0.2
320 μM	N	7.0 ± 0.3	3.1 ± 0.6	2.9 ± 0.1
	Tr§	4.0 ± 0.3	2.2 ± 0.3	1.5 ± 0.2

Experimental mixture: Stearate [1-^{14}C] 51 Ci/mol 20 to 150 nmol; NADPH 0.5 μmol; Malonyl-CoA 0.1 μmol; ATP 1 μmol; Mg^{2+} 1 μmol; CoA 0.25 μmol; Tris–HCl 0.05 M, pH 7.5; Final volume 500 μl, 1 h of incubation at 37°C; Results are given as nmol incorporated/mg protein/h; Mean value of 3 experiments ± SD; Significant difference between normal and Trembler: *$P = 0.001$; †$P < 0.01$; ‡$P < 0.01$; §$P < 0.01$.

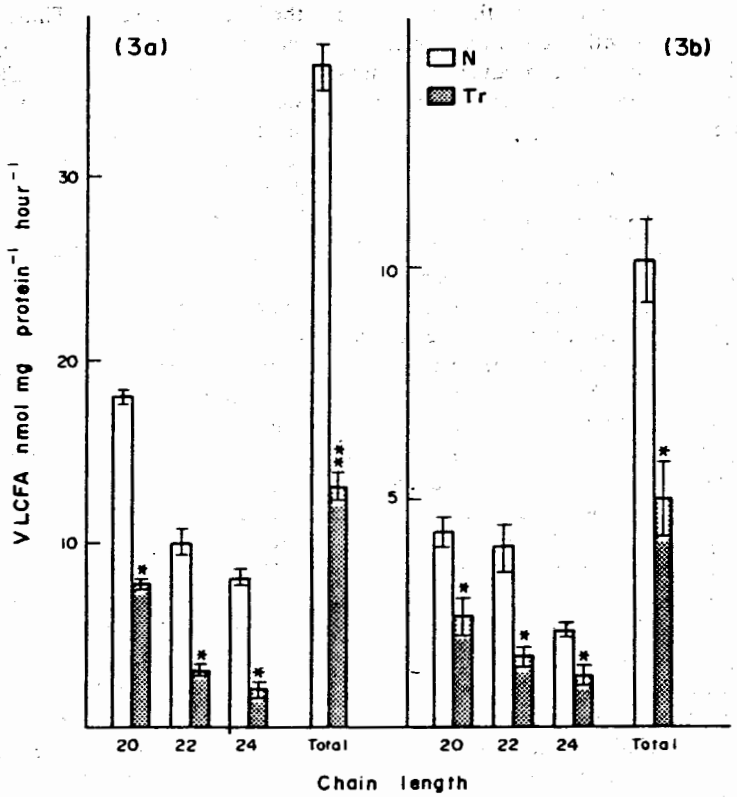

Fig. 3. (a) Stearoyl-CoA elongation. Same conditions as in Fig. 2. Each bar corresponds to the mean of ±SD of 3 experiments. *$P < 0.01$, **$P < 0.001$. (b) *Stearate elongation.* [1-^{14}C]stearate (2 μCi, 51 Ci/mol), NADPH 0.5 μmol, malonyl-CoA 0.1 μmol; CoA 0.25 μmol, ATP 1 μmol, $MgCl_2$ 1 μmol. Final volume 500 μl. Incubation time 1 h at 37°C. Each bar corresponds to the mean ±SD of 3 experiments. Significant difference between normal and Trembler: *$P < 0.01$, **$P < 0.001$.

fractions, so that a difference in VLCFA formation could not be accounted for by a specific decrease in malonyl-CoA concentration in the membrane pellet from the Trembler sciatic nerves.

Table 3. Metabolism of [2-^{14}C]malonyl-CoA in the microsomes from normal and Trembler sciatic nerves

	Normal	Trembler
Malonyl-CoA	63%	65%*
Malonic acid	18.6%	19.8%
Acetyl-CoA	10.2%	10.3%
Long chain acyl-CoA	4.7%	1.7%
PL (n.i.)	3.5%	3.2%

Experimental conditions: Stearoyl-CoA 80 μM; NADPH 0.5 μmol; Enzyme 70 μg; Tris–HCl 50 mM pH 7.5; [2-^{14}C]malonyl-CoA (50 Ci/mol, 1 μCi); 1 h of incubation at 37°C; n.i.: not identified; PL: polar lipids. Results are given as percentage of radioactivity. Mean value of 3 experiments.

*Not significantly different from control ($P > 0.1$).

Effect of an ATP-Mg^{2+} addition on stearoyl-CoA elongation

Table 4 shows that upon ATP-Mg^{2+} addition, the stearoyl-CoA elongation by the control was almost unaffected, although small changes were detected in the label distribution. This was not the case in the microsomal fraction from Trembler sciatic nerves, where the elongation was strongly increased, reaching about 80% of the control activity ($P < 0.02$). This stimulation of the biosynthesis affected all of the very long chain fatty acids equally.

DISCUSSION

In various cases of peripheral neuropathies, the fatty acid composition shows a pronounced loss of C20 to C24 fatty acids, irrespective of the etiology of the neuropathy and this modification is considered to

Table 4. Stearoyl-CoA elongation by normal and Trembler microsomes in the presence or in the absence of ATP Mg^{2+} (5 mM)

Fatty acid synthesis chain length	Control		Trembler	
	−ATP Mg^{2+}	+ATP Mg^{2+}	−ATP Mg^{2+}	+ATP Mg^{2+}
20	18 ± 0.4	14 ± 0.6	8 ± 0.2	16 ± 0.2
22	10 ± 0.6	12 ± 0.8	3 ± 0.2	6 ± 0.2
24	8 ± 0.2	8 ± 0.6	2 ± 0.4	6 ± 0.8
Total	36 ± 1.2	34 ± 2	13 ± 0.6*	28 ± 1.2†

Same experimental conditions as in Table 1; Results are given as nmol/mg prot/h ± SD (3 experiments); Significant difference between normal and Trembler.
*$P < 0.001$. †$P < 0.02$.

be associated with all forms of myelin loss (Heipertz *et al.*, 1978). Accordingly, in the sciatic nerve of the Trembler mouse, which is considered as a model of human peripheral neuropathies (Ayers and McAnderson, 1973), the amount of the saturated C20-C24 fatty acids is 3 times lower than that of any other fatty acid and reaches 20% (C20), 8% (C22) and 7% (C24) of the control values. This could reflect the hypo- and dysmyelination and/or demyelination of the Trembler sciatic nerves; in other words, a lack of synthesis and/or a degradation of these well-known myelin components. This abnormality could be directly related to the genetic defect, but it could equally well correspond to a secondary phenomenon, similar to the situation in other CNS murine mutants, in which a large number of metabolic steps related to myelination are defective, as the result of an as yet unknown fundamental genetic defect. As a first approach to this unsolved question, the role of the Schwann cells must be examined. It may be pointed out that the number of Schwann cells is greatly increased (× 10) in Trembler sciatic nerves. This increase contrasts with the 4-fold increase in the number of fibroblasts (Low, 1976). After 1 month, the Trembler Schwann cell population remains stable, in spite of the persistence of cell death among the actively dividing populations of these cells (Aguayo *et al.*, 1980). The nerve transplantations carried out by Aguayo *et al.*, 1977) and by Pollard and McLeod (1980) have shown that the Trembler defect is due to a primary Schwann cell disorder, rather than an axonal one. It was also established that the Trembler fibroblasts did not appreciably contribute to the expression of the myelin abnormality (Bunge *et al.*, 1980). On the other hand, it is assumed that Schwann cells have a higher capacity to synthesize very long chain fatty acids, while the fibroblasts will form predominantly shorter chain fatty acids. This was shown in the rabbit PNS (Darriet *et al.*, 1979), where the endoneurium, rather than the perineurium, synthesized C20 to C24 saturated fatty acids. This excluded a prominent role of mesodermal cells (fibroblasts) in this system. Thus, the drastic and specific decrease of the saturated C20–C24 fatty acid biosynthesis by the Trembler sciatic nerves in relation with the Schwann cell proliferation could be a biochemical illustration of the disorder of these cells. Short-time acetate incubation periods revealed that a decreased biosynthesis rather than an increased degradation of these acids could be involved. In the normal excised sciatic nerve, acetate is readily incorporated into fatty acids, chiefly C16 and C18, as already demonstrated in rabbit PNS (Cassagne *et al.*, 1978). In mouse PNS the label found in C20-C26 fatty acids increased from 7% of the total fatty acid label after a 60 min acetate incorporation, to about 17% after 3 h and to 30% after 5 h, in the Trembler sciatic nerve, the acetate incorporation into fatty acids was only 20% of the control and the most drastic decrease was found in C20-C24 fatty acids (5 to 7% of the control after a 3 h acetate incorporation); no label was detected in lignoceric acid. The study with the subcellular fractions from sciatic nerves showed that the C20-C24 biosynthesis is diminished by 66% in the Trembler microsomes. That decrease is not accounted for by a related decrease of the malonyl-CoA concentration, which is metabolized almost at the same rate in normal and Trembler microsomes (Table 3). However, the possibility that the stearoyl-CoA metabolism in the Trembler microsomes differs from the control should not be overlooked. Besides its role in very long chain fatty acid (VLCFA) formation, stearoyl-CoA could also be the substrate of many other membrane-bound enzymes such as stearoyl-CoA desaturase, stearoyl-CoA transacylase and stearoyl-CoA thioesterase. The analysis of the various labelled fatty acids by argentation TLC revealed that no desaturation of stearoyl-CoA occurred in our experimental conditions (Boiron *et al.*, 1982a). Although large differences concerning the

lipid acyl acceptors were observed, the yields of acyl transfer from acyl-CoA to glycolipids and phospholipids were only slightly higher in the membrane pellets from Trembler sciatic nerves than in the normal ones. The most striking difference was observed when measuring acyl-CoA hydrolysis (Boiron *et al.*, 1982b) and it was shown that the hydrolysis was about 3 times higher in the Trembler membrane pellet than in the control one. The maximal hydrolysis was about 40% of the added substrate. It follows that the stearoyl-CoA concentrations in the membrane pellets from Trembler and control sciatic nerves did not remain identical and that the direct comparison of the stearoyl-CoA elongation in both systems had to take into account the specific substrate concentration in the membrane pellet from the mutant. The addition of ATP-MG^{2+} to the reaction mixture markedly reduced the stearoyl-CoA hydrolysis (Boiron *et al.*, 1982b) and for a 5 mM ATP-Mg^{2+} addition, hydrolysis by the membrane pellet from Trembler sciatic nerves was abolished. Under these conditions, the substrate concentrations in normal and Trembler membrane pellet remained almost identical allowing the comparison in both systems of the stearoyl-CoA elongation in the presence of malonyl-CoA and NADPH. On the other hand, an addition of ATP-Mg^{2+} to the normal microsomes did not significantly modify the stearoyl-CoA elongating activity, but had a marked effect on the VLCFA biosynthesis by Trembler microsomes: the synthesis of eicosanoic acid and lignoceric acid became nearly normal and that of docosanoic acid increased 2-fold (Table 4). Thus, despite the fact that the most specific deficit of the Trembler PNS lipids is that of the saturated very long chain fatty acids, the restoration of the stearoyl-CoA elongating activity leads us to propose that the membrane bound elongase of Trembler sciatic nerves could work almost normally, provided that adequate substrate concentrations are used and proper cofactors are added. Consequently, other steps involved in the synthesis of these molecules and/or their transfer to lipids and insertion into myelin could be modified in the Trembler PNS. The study of these steps is under progress in our laboratories.

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