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Lipid Metabolism in Peripheral Nerve Cell Culture (Rich in Schwann Cells) from Normal and Trembler Mice

*J. M. Bourre, *O. Morand, *O. Dumont, †J. M. Boutry, and †J. J. Hauw

Laboratoire de Neurochimie, INSERM U 134, and †Laboratoire de Neuropathologie Charles Foix; Hôpital de la Salpêtrière, Paris, France

Abstract: A culture of peripheral nerve cells, very rich in Schwann cells, was developed from sciatic nerve. In both normal and Trembler, typical spindle-shaped cells were seen; most of the cells were surrounded by basement membrane-like material (predominantly in-between adjacent cells). In Trembler cells, cultivated in the presence of labelled acetate, the fatty acids were slightly altered; phosphatidylcholine was slightly reduced and phosphatidylethanolamine increased. Sulfatides were increased four times. **Key Words:** Lipids—Peripheral nerve—Schwann cells—Trembler mice. **Bourre J. M. et al.** Lipid metabolism in peripheral nerve cell culture (rich in Schwann cells) from normal and Trembler mice. *J. Neurochem.* **37**, 272–275 (1981).

Peripheral nerve provides an ideal model in which to study problems related to myelination, because of its accessibility and its relatively simple organisation; the elucidation of cellular relationships in nerve may prove relevant to those present in the brain.

The long-term culture of fragments of nerve tissue provides an approach to many problems in neurobiology, and the interactions between Schwann cells and nerve fibres have been documented, especially in cultures of foetal ganglia (Bunge et al., 1967; 1978; Bunge and Bunge, 1978). Nevertheless, despite the great simplification offered by these tissue culture systems as compared with *in vivo* situations, nerve tissue in culture produces different cell types whose identity and interaction with each other cannot be easily ascertained in the complex population which is developing. Continuous cell lines derived from nervous tissue and capable of cell division are used to study some of the properties of the differentiated cells from which they are derived. Thus, Schwann cells can be harvested (Wood, 1976; Brokes et al., 1979; Brokes and Raff, 1979). The Trembler neuropathy in mice is primarily due to defect of Schwann cells, as shown by grafting experiments (Aguayo et al., 1977). The few biochemical studies (Darriet et al., 1978; Larrouquère-Regnier et al., 1979) and physicochemical studies (Viret et al., 1979) so far performed on the Trembler mutant document a drastic reduction of myelin in this mutant; thus, it is important to examine the Schwann cell lipid metabolism of these animals.

The purpose of this work was to study lipid synthesis in peripheral nerve cells (rich in Schwann cells) of normal mice and Trembler mutant.

MATERIALS AND METHODS

Peripheral Nerve Cell Culture

Sciatic nerves from 15-day-old animals (Trembler and control (B6-CBA strain) were dissected out and cut into small fragments in Eagle's Minimal Essential Medium complemented with 50% foetal calf serum (Hauw et al., unpublished). The fragments were explanted into plastic flasks (or to Leighton tubes containing cover slips for morphological examination). Specimens were allowed to culture for 90 min at 37°C with a drop of medium. Then 2 ml of this medium was added to each flask (1.2 for tubes). When confluency was reached, subcultures were obtained by trypsinization (on the average after 5–7 days).

For biochemical studies, cells from the second, third and fourth subcultures were used when confluency was reached, both for Trembler and for control mice.

Received December 12, 1980; accepted January 14, 1981.
Address correspondence and reprint requests to J. M. Bourre, Laboratoire de Neurochimie, INSERM U 134, Hôpital de la Salpêtrière, 75651 Paris Cedex 13.

Abbreviations used: N, normal mouse; Tr, Trembler mouse; PBS, Phosphate buffer solution. [8 g NaCl, 0.2 g KCl, 0.91 g Na_2HPO_4, 0.2 g KH_2PO_4 → 1 litre]; PC, Phosphatidyl choline; PE, Phosphatidyl ethanolamine; PI, Phosphatidyl inositol.

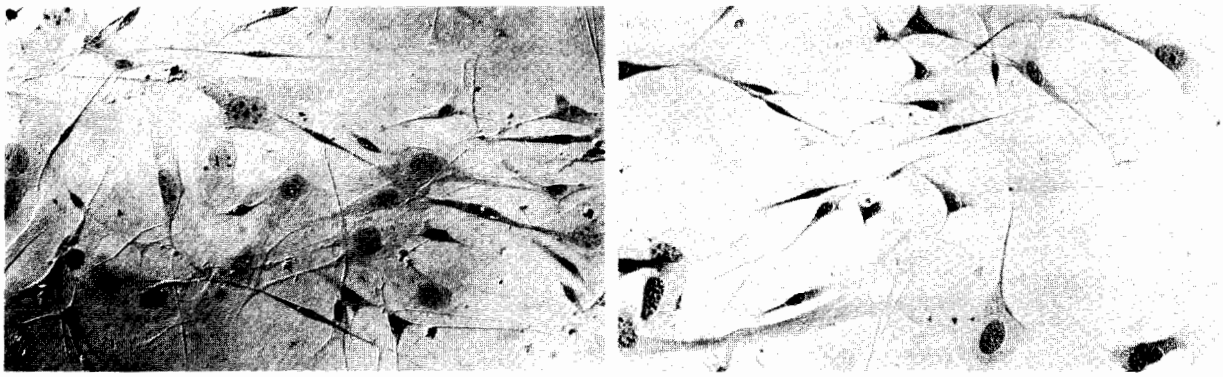

FIG. 1. Peripheral nerve cells from normal **(left)** and Trembler **(right).** Magnification: ×146.25. Hemateine-eosine staining, Nomarski's interferential contrast.

Cells were cultivated for 17 h in the presence of sodium [1-^{14}C]acetate (20 μCi in 250-ml flasks) (incorporation was linear between 5 h and 2 days).

Lipid Analysis

Cells were washed twice with phosphate buffer solution (PBS) directly in the flasks. After releasing from the flasks with a rubber policeman, a pellet of cells was obtained by a 5-min centrifugation at 200 *g* (rotor H 54 in Sorvall RC.3). The cells were further washed twice with saline and centrifuged under the same conditions and lyophilised. The lyophilised pellet was extracted with chloroform-methanol and sonicated (Pollet et al., 1978). Separatin of lipids was performed on bidimentional TLC (60 F 254 glass plates, Merck) (Bourre et al., 1977; Pollet et al., 1978).

The ratio of cholesterol to cholesterol esters was determined by another system of thin-layer chromatography (Igarashi et al., 1976). Lipids were visualised by iodine, the spots were scraped and radioactivity was determined by scintillation counting in the presence of Biofluor (New England Nuclear). Autoradiography was performed, using Kodirex film.

Fatty acids from total lipid extracts were methylated with 14% BF_3 (boron trifluoride) in methanol; unsubstituted fatty acids were separated from hydroxylated fatty acids by thin-layer chromatography (Bourre et al., 1976*b*). On emergence from GLC, the labelled fatty acids were collected in anthracene and the radioactivity was determined by liquid scintillation counting.

The fatty acid profile from unlabelled cells was determined by GLC using a glass open tubular column (50 m, Carbowax 20 M). Each experiment was performed three times.

RESULTS AND DISCUSSION

Conditions of Culture and Morphology of Cells

Typical spindle-shaped cells (Fig. 1) were regularly seen in the outgrowth zone surrounding the explant in both Trembler and normal mice. After trypsinization, the same cell type was still obtained in the first subcultures. Most cells in both cultures were surrounded by basement membrane-like mate-

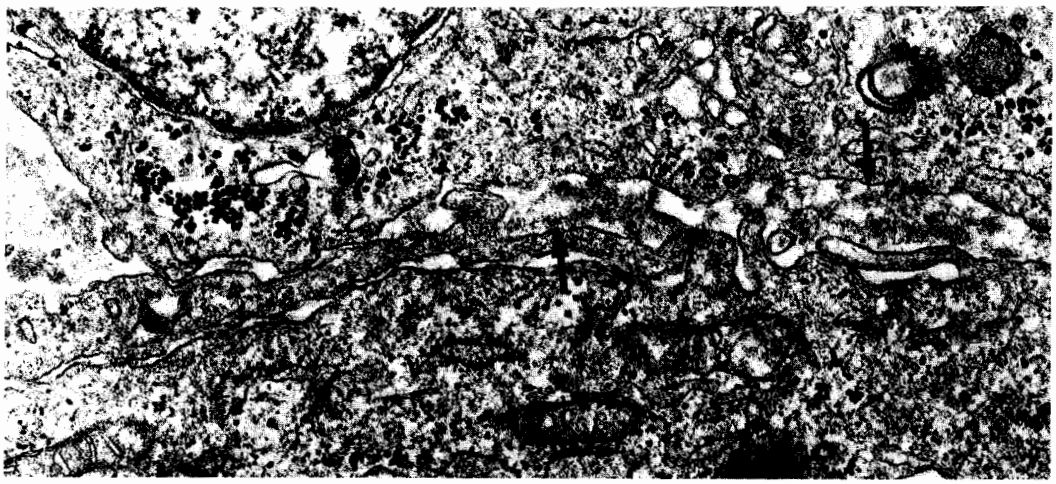

FIG. 2. Peripheral nerve cells under electron microscopy. Magnification: ×28600. The same aspect was obtained with both control and Trembler Schwann cells. Arrow: basement membrane-like material.

TABLE 1. *Lipid synthesis in normal and Trembler peripheral nerve cell culture*

Parameter	Normal	Trembler
mg lipid extract/mg dry weight	0.290	0.240[a]
c.p.m. incorporated/mg lipid extract	$1.3 \cdot 10^6$	$0.6 \cdot 10^{6}$[b]
α-OH fatty acids/unsubstituted fatty acids	0.014	0.009[b]
% c.p.m. in total lipids		
PC	59.20	52.00[a]
PI	2.40	4.00[a]
PS	1.40	1.90
PE	6.40	8.70
Sph	8.00	9.40
Cholesterol	18.50	19.50
Cholesterol esters	0.30	0.25
Cerebrosides	0.40	0.56
Sulphatides	0.16	0.65[b]
Unidentified	3.20	3.00

Significant difference between normal and Trembler: [a] $p < 0.05$; [b] $p < 0.01$.

rial predominantly in-between adjacent cells (Fig. 2). This aspect was not seen with skin fibroblast culture.

Lipid Analysis

As shown in Table 1, Trembler nerve cells contained much less lipid; this result may be partly related to a lower utilisation of labelled acetate to synthesise lipids. When considering the fatty acids, hydroxylated compounds were reduced (Table 1), but the profile of saturated fatty acids was close to normal (Table 2). The only significant alterations were found in the C_{16} and C_{18} series. As expected from the lower total lipid content, the absolute amount of fatty acids was reduced. When the results were not expressed as percentages, but instead related to milligrams of lipid extract, the unsubstituted fatty acids, though qualitatively normal, were quantitatively reduced by approximately 50%. However, the specific activities of the individual fatty acids were not identical: the specific activity of $C_{16:0}$ was about four times higher as compared with $C_{18:0}$ in both Trembler and control nerve cells. These differences could be due to the short period of incubation of cells with labelled acetate, combined with the existence of several fatty acid synthesising systems in microsomes: a *de novo* mechanism producing C_{16}, a first elongating system providing C_{18} and a second elongating system synthesising very long chains. These systems have been described in brain (Bourre et al., 1976*a*); they are probably quite similar in sciatic nerve (Cassagne et al., 1978). In terms of relative radioactivity, the lipids in nerve cells from Trembler were altered (Table 1). PC was slightly reduced, while PI was increased ($p < 0.05$), and the other phospholipids and cholesterol were not affected. Unexpected was the fourfold enhancement of sulphatide biosynthesis ($p < 0.01$), in contrast to nearly normal cerebroside synthesis (nonsignificant). Thus trembler nerve cell lipid metabolism was altered in cell culture, the greatly enhanced sulfatide biosynthesis being in contrast to the depressed myelination *in vivo*.

Although glycolipid metabolism in nerve cell culture is low, this model could be useful in analysing myelinogenesis and axonal regulation as it has been recently established that the myelinating be-

TABLE 2. *Fatty acid profile from normal and Trembler mouse peripheral nerve cell culture*

Acid	% c.p.m. after incubation with sodium [1-^{14}C]acetate[a]		Profile[b]	
	Normal	Trembler	Normal	Trembler
14:0	2.10	1.70	—	—
16:0	69.70	59.70	26.80	23.80
16:1	2.60	5.90	3.80	5.50
18:0	12.20	15.20	18.90	16.40
18:1			23.70	25.40
18:2ω6	4.60	8.20	2.10	2.60
18:2ω3			—	—
20:0	0.56	0.41	—	—
20:1	0.90	0.76	tr	0.20
20:3ω6			0.70	0.80
20:4ω6	1.80	1.80	14.40	11.70
20:5ω3			2.00	3.00
22:0	0.43	0.48	—	0.30
22:1	0.40	0.46	—	—
22:4ω6			0.70	0.90
22:5ω6	1.50	1.30	—	—
22:5ω3			—	—
22:6ω3			5.20	5.70
23:0	0.40	0.26	—	—
24:0	1.00	0.96	1.60	1.70
24:1	1.10	1.00	1.70	2.00

[a] Cells were incubated as described in Materials and Methods; the fatty acids were collected on anthracene and the radioactivities were determined. Results refer to the percentage radioactivity from [1-^{14}C]acetate found in each fatty acid.

[b] Percentage by weight of the various fatty acids, in Schwann cell culture.

havior of Schwann cells is regulated by the associated axon (Aguayo et al., 1976; Weinberg et al., 1976).

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