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Alterations in the Fatty Acid Composition of Rat Brain Cells (Neurons, Astrocytes, and Oligodendrocytes) and of Subcellular Fractions (Myelin and Synaptosomes) Induced by a Diet Devoid of n-3 Fatty Acids

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Abstract: Rats were fed through four generations with a semisynthetic diet containing 1.0% sunflower oil (6.7 mg/g n-6 fatty acids, 0.04 mg/g n-3 fatty acids). Ten days before mating, half of the animals received a diet in which sunflower was replaced by soya oil (6.6 mg/g n-6 fatty acids, 0.8 mg/g n-3 fatty acids) and analyses were performed on their pups. Fatty acid analysis in isolated cellular and subcellular material from sunflower-fed animals showed that the total amount of unsaturated fatty acids was not reduced in any cellular or subcellular fraction (except in 60-day-old rat neurons). All material from animals fed with sunflower oil showed an important reduction in the docosahexaenoic acid content, compensated (except in 60-day-old rat neurons) by an increase in the n-6 fatty acids (mainly C22:5 n-6). When comparing 60-day-old animals fed with soya oil or sunflower oil, the n-3/n-6 fatty acid ratio was reduced 16-fold in oligodendrocytes, 12-fold in myelin, twofold in neurons, sixfold in synaptosomes, and threefold in astrocytes. No trienes were detected. Saturated and monounsaturated fatty acids were hardly affected. This study provides data on the fatty acid composition of isolated brain cells. **Key Words:** Neurons—Astrocytes—Oligodendrocytes—Myelin—Synaptosomes—n-3 Fatty acids—Diet. **Bourre J. M. et al.** Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43,** 342–348 (1984).

Deficiency in essential fatty acids (EFA), initiated before birth and continued until 1 year, induces extensive modifications in rat brain. For instance, brain weight is decreased, total lipid and phospholipid concentrations are diminished, and the fatty acid composition of phospholipids, especially ethanolamine phosphoglyceride, a major phospholipid in membranes, is considerably modified (White et al., 1971). A number of other parameters are modified by EFA deficiency (McKenna and Campagnoni, 1979; Samulski and Walker, 1982). EFA deficiency induces alterations in the fatty acid compositions of various subcellular fractions (Paoletti and Galli, 1971; Sun, 1972; Svennerholm et al., 1972), and intrauterine growth retardation due to malnutrition caused by vascular ligation induces modification in the polyunsaturated fatty acid compositions of neurons and oligodendrocytes; astrocytes are affected to a much lower extent (Bourre et al., 1981; Morand et al., 1981*b*, 1982). Nevertheless it is difficult to assess the specific roles of dietary linoleic (18:2 n-6) and linolenic (18:3 n-3) acids, as most studies were performed with a reduction of both acids. Actually, the ratio of linoleic acid to linolenic acid greatly affects the pattern of tissue lipid distribution (Svennerholm et al., 1972) and an increase of the linolenic acid level in the diet produces a decrease in n-6 fatty acids and an increase in n-3 fatty acids (Lamptey and Walker, 1976). Similar observations were made when long-chain fatty

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Abbreviations used: EFA, Essential fatty acid; GFA protein, Glial fibrillary acid protein.

TABLE 1. *Diet composition*

	Sunflower oil diet	Soya oil diet
Casein	225	225
DL-Methionine	1.6	1.6
Vitamins	10	10
Minerals	40	40
Cellulose	20	20
Corn starch	464.4	461.9
Saccharose	229	229
Sunflower oil	10	—
Soya oil	—	12.5

Values are expressed in g/kg. Vitamins are the "vitamin diet fortification mixture" (United States Biochemical Corporation, Cleveland, OH). Mineral mixture is $CaHPO_4 \cdot 2H_2O$ (38%); K_2HPO_4 (24%); $CaCO_3$ (18.1%); NaCl (7%); MgO (2%); $MgSO_4 \cdot 7H_2O$ (9%); $FeSO_4 \cdot 7H_2O$ (0.7%); $ZnSO_4 \cdot H_2O$ (0.5%); $MnSO_4 \cdot H_2O$ (0.5%); $CuSO_4 \cdot 5H_2O$ (0.1%); NaF (0.1%); KI (0.08%); $CoCO_3$ (0.08%); $Al_2(SO_4)_3$ $K_2SO_4 \cdot 24H_2O$ (0.02%); $Na_2SeO_3 \cdot 5H_2O$ (0.001%).

TABLE 2. *Fatty acid composition of oils and diets*

	Soya		Sunflower	
Fatty acid	Oil	Diet	Oil	Diet
C14:0	tr	0.2	tr	0.2
C16:0	10.1	10.3	5.9	6.4
C17:0	0.1	0.1	tr	0.1
C18:0	3.8	3.8	4.9	5.0
C18:1 n-9	22.6	22.7	17.6	17.8
C18:2 n-6	55.4	54.7	70.0	68.2
C18:3 n-3	6.7	6.5	0.1	0.2
C20:0	0.4	0.4	0.3	0.4
C20:1 n-9	0.3	0.2	0.2	0.2
C22:0	0.4	0.4	0.8	1.0
C22:5 n-3	tr	0.3	tr	0.2
C24:0	0.2	0.4	0.2	0.3

Values in percent of identified fatty acids. Each value is the average of a duplicate analysis on three separate samples. Tr, Trace amount.

acids, instead of their precursors, were added to the diet (Galli et al., 1971). On the other hand, when the amount of EFA in the diet is changed the amount of polyunsaturated fatty acid remains nearly constant. Recent studies using two diets differing only in the level of 18:3 have determined the respective effects of linolenic and linoleic acids in the diet on rat brain phospholipids during development (Nouvelot et al., 1983).

The specific role of 18:3 n-3 has hardly been examined. Deprivation of this fatty acid during pregnancy and during the life span of the progeny caused learning impairment in the rat (Lamptey and Walker, 1978) and biochemical abnormalities were found even in the fetus (Samulski and Walker, 1982). Recently, it was proposed that the dominant disease of modernized societies consisted of an n-3 essential fatty acid deficiency syndrome (Rudin, 1982). Since manipulation of maternal dietary fat during gestation modifies the fatty acid profile in the newborn and the learning capacity in the older progeny, the aim of the present investigation was to determine the changes occurring in brain cells and major subcellular elements in rats fed a diet deficient in 18:3 n-3 only, but having a normal intake of 18:2 n-6.

MATERIALS AND METHODS

Animals

Wistar rats were fed through four generations with a semisynthetic diet containing only 1% lipid (sunflower oil, poor in linolenic acid). Ten days before mating, one group (half of the animals) was fed the same diet, and another group was fed a diet in which sunflower oil was replaced by 1.25% soya oil, rich in linolenic acid (Table 2). Taking into account the composition of the diet (Table 1) and the occurrence of minute amounts of lipids in the protein source, the two diets contained 1.1% and 1.35% lipids, respectively. The sunflower oil diet contained approximately 675 mg n-6 fatty acids and 4 mg n-3 fatty acids/100 g; the soya oil diet contained 665 mg n-6 fatty acids and 83 mg n-3 fatty acids/100 g. Since animals fed either diet ate similar amounts of food, they ate the same amount of n-6 fatty acids. Soya oil-fed rats received approximately 21 times more n-3 fatty acids than animals that were fed sunflower oil. Both groups of rats received similar amounts of n-6 fatty acids. Pregnancy was normal in all animals, but delivery was difficult in animals fed with sunflower oil; a number of pups and mothers died (Francois et al., 1980). Three days after delivery the litters were adjusted to eight animals. After weaning, the young rats received the same diet as their mothers. Animals were killed at 15 or 60 days. Three groups of four animals were used for each cellular or subcellular preparation.

Previous investigations (White et al., 1971) showed that substitution of a control diet for the EFA-deficient diet, after different periods of deficiency, induced an increase in the content of polyunsaturated fatty acids in the brain. Thus, when sunflower oil was replaced by soya oil 10 days before mating, the pups were considered as offspring of nondeficient animals.

Cellular and subcellular fractionation

Neurons, astrocytes (Norton and Poduslo, 1971), and oligodendrocytes (Chao and Rumsby, 1977) were prepared by sieving and sucrose gradient with minor modifications (Morand et al., 1979). Purity of the cell preparations was assessed by morphological criteria after examination of the cells under a phase-contrast microscope (Morand et al., 1979). Moreover, basic protein was measured by radioimmunoassay (Delasalle et al., 1980) and glial fibrillary acid protein (GFA) was quantitated by the use of a rocket electrophoresis technique (Jacque et al., 1978).

Myelin was prepared (Norton and Poduslo, 1973) and its purity was checked by electron microscopy (Baumann et al., 1973), marker enzymes [2',3'-cyclic nucleotides 3'-phosphodiesterase; Bourre et al. (1982)], and protein analysis (Bourre et al., 1978). Synaptosomes were prepared (Hajos, 1975) by a slight modification of the Hajos technique (Morand et al., 1982). Purity was checked by electron microscopy and lipid analysis (Y. Maurin, unpublished results; Morand et al., 1982). Synaptosomes

TABLE 3. *Neuronal fatty acids*

Fatty acids	15-Day-old animals		60-Day-old animals	
	Soya	Sunflower	Soya	Sunflower
C14:0	2.1	1.3	3.1	1.8
C16:0	28.8	26.2	24.3	31.5[a]
C16:1 n-9	0.6	0.7	1.0	0.4
C16:1 n-7	1.1	0.9	1.2	0.8
C18:0	18.0	18.6	18.9	24.4[a]
C18:1 n-9	12.8	12.9	15.5	15.2
C18:1 n-7	2.2	2.6	1.1	2.2
C18:2 n-6	3.1	2.5	6.9	2.1[a]
C20:0	0.4	0.2	2.4	1.0
C20:1 n-9	0.4	0.5	1.0	0.6
C20:4 n-6	15.1	16.4	10.3	7.8[a]
C22:0	0.2	0.3	tr	0.8
C22:1 n-9	0.5	0.5	tr	0.2
C22:4 n-6	2.1	2.7	1.0	1.8
C22:5 n-6	2.2	6.4[a]	2.2	4.7[a]
C22:5 n-3	2.2	2.2	1.8	1.5
C24:0	tr	0.5	1.0	0.8
C22:6 n-3	8.2	4.6[a]	8.3	2.4[a]
C24:1 n-9	tr	tr	tr	tr
n-3 + n-6	32.9	34.8	30.5	20.3[a]
n-3/n-6 Ratio				
This study	0.46	0.24	0.50	0.24
Morand et al. (1981*a*)	0.47		—	
Morand et al. (1982)	0.41		0.43	
Cohen and Bernsohn (1973)	—		0.46	

Values are expressed as percent of identified fatty acids. (n-3) + (n-6): sum of linoleic and linolenic series. Each value is the average of a duplicate analysis on three separate samples. Tr, Trace amount.

[a] $p < 0.01$.

were submitted to an osmotic shock with distilled water. Synaptosomal membranes were pelleted by centrifugation, lyophilized, and stored at −30°C.

Transmethylation and gas-liquid chromatography

Lipids were extracted by sonication in chloroform-methanol, 2:1 vol/vol (Folch et al., 1957; Pollet et al., 1978) and methylated (Morrison and Smith, 1964). Fatty acid methylesters were separated by GLC on an open tubular capillary column coated with FFAP (0.30 mm in diameter, 45 m long), using a flame-ionization detector. Identification of fatty acids was performed with commercial standards by means of relative retention times. Areas were calculated with an integrator ICAP 10 (LTT, France).

RESULTS AND DISCUSSION

Purity of preparation

The purities of cellular and subcellular preparations were published previously. In brief, the neuron preparation was 93% pure, on the basis of morphological criteria (Raine et al., 1971) that were assessed under a phase-contrast microscope (Morand et al., 1979). The remaining material consisted of membrane fragments and some unidentified cells. Astrocytes were 88% pure (contamination was essentially due to clumps of astrocytes and membrane fragments).

Oligodendrocytes were 84% pure (contamination consisted of some red blood cells; other unidentified cells possibly were small neurons and membrane fragments). As determined by radioimmunoassay, the basic protein was not detected in neurons and astrocytes, but only in oligodendrocytes. GFA protein was present only in astrocytes, as determined by rocket electrophoresis.

Synaptosomes were examined by electron microscopy. They were not contaminated by myelin. Basic protein was absent. Myelin specific complex lipids (cerebrosides and sulfatides) were not detected. Moreover, this study shows that synaptosomes were free of saturated and monounsaturated very long chain fatty acids (for instance, lignoceric and nervonic acids that are specific to myelin membrane).

In electron microscopic appearance, the myelin preparation compared favorably with that of other reports (Baumann et al., 1973), and the number of lamellae as well as the periodicity were normal. The protein profile of myelin was in agreement with previously published gel electrophoreses (Bourre et al., 1978) in its resolution of basic proteins, proteolipid protein, and Wolfgram proteins. The activity of the myelin-specific enzyme 2′,3′-cyclic nucleotide 3′-phosphohydrolase was 2,110 and 309 μmol/h/mg protein in myelin and homogenate respectively, in agreement with many reports.

TABLE 4. *Synaptosomal fatty acids*

Fatty acids	15-Day-old animals		60-Day-old animals	
	Soya	Sunflower	Soya	Sunflower
C14:0	1.0	1.8	0.7	0.4
C16:0	25.7	24.8	24.3	24.0
C16:1 n-9	1.4	1.7	tr	tr
C16:1 n-7	1.7	2.4	1.8	1.5
C18:0	19.7	18.1	24.9	24.9
C18:1 n-9	13.4	12.9	16.2	15.2
C18:1 n-7	3.4	3.6	3.7	3.9
C18:2 n-6	1.6	2.1	0.5	0.5
C20:0	—	—	—	—
C20:1 n-9	—	—	tr	tr
C20:4 n-6	18.7	18.9	12.2	13.5
C22:0	—	—	—	—
C22:1 n-9	—	—	—	—
C22:4 n-6	2.6	3.0	2.3	2.9
C22:5 n-6	2.2	8.3[a]	0.9	9.8[a]
C22:5 n-3	—	—	—	—
C24:0	—	—	—	—
C22:6 n-3	8.5	1.9[a]	12.5	3.4[a]
C24:1 n-9	—	—	—	—
n-3 + n-6	33.6	34.2	28.4	30.1
n-3/n-6 Ratio				
This study	0.34	0.06	0.78	0.13
Morand et al. (1982)	0.59	—	0.83	—

Same remarks as in footnote to Table 3. Saturated, all saturated fatty acids; monounsaturated n-9, all monounsaturated fatty acids of the oleic acid series; monounsaturated n-7, all monounsaturated fatty acids of the (n-7) series.

Neurons and synaptosomes

The most significant difference between neuronal fatty acids obtained from animals fed soya oil or sunflower oil was found in the polyunsaturated fatty acids (Table 3).

In the brains of 60-day-old rats fed sunflower oil, the total amount of unsaturated fatty acids was diminished by 33%, but was not reduced in the 15-day-old animals. The dramatic reduction in 22:6 n-3 fatty acid in the neurons of sunflower oil-fed rats was not compensated by an increase in n-6 fatty acids. Alteration was greater in 60-day-old animals than in 15-day-old animals.

In agreement with our previous studies (Morand et al., 1981*b*, 1982) the saturated/monounsaturated fatty acids ratio in control animals (soya oil) decreased during development (3.5 and 2.5 in 15- and 60-day-old animals, respectively). The n-3/n-6 ratio decreased in neurons of sunflower-fed rats by 50% in both age groups (Table 3). Neurons from soya oil-fed animals could be accepted as controls, since the values obtained were similar to previously reported results. Animals that were fed a standard commercial diet and soya oil diet showed similar fatty acid profiles.

In synaptosomes (Table 4), alterations were much more important when compared with the neuronal perikaryon. Although the total amount of polyunsaturated fatty acid was not altered in synaptosomes of sunflower oil-fed rats of both age groups, the amount of 22:6 n-3 was drastically reduced. The n-3/n-6 ratio decreased approximately sixfold in both age groups. The reduction in n-3 fatty acids was compensated by an increase in n-6 fatty acids.

Saturated and monounsaturated fatty acids were not altered in synaptosomes of rats fed sunflower oil.

The finding of more important alterations in synaptosomes than in neurons is at variance with results that we previously obtained in intrauterine growth retardation (Morand et al., 1982). In the neurons obtained following malnutrition induced by vascular ligation, the alterations in polyunsaturated fatty acids were important, but the alterations in synaptosomes were less prominent. Thus the alterations observed in intrauterine growth retardation are unlikely to be related to a deficiency in essential fatty acids only.

Oligodendrocytes and myelin

The total amount of polyunsaturated fatty acids decreased in oligodendrocytes of sunflower oil-fed rats by approximately 9% in 60-day-old animals; 22:6 n-3 fatty acid decreased drastically in oligodendrocytes of sunflower oil-fed rats; 22:5 n-6 increased (Table 5); and the n-3/n-6 ratio decreased 16-fold. Saturated and monounsaturated fatty acids were not affected. The occurrence of saturated and

TABLE 5. *Fatty acid composition of oligodendrocytes*

Fatty acids	60-Day-old animals	
	Soya	Sunflower
C14:0	3.9	6.5
C16:0	17.8	20.1
C16:1 n-9	tr	tr
C16:1 n-7	6.4	9.4
C18:0	18.4	16.6
C18:1 n-9	17.3	16.2
C18:1 n-7	5.6	4.5
C18:2 n-6	2.7	2.9
C20:0	1.0	0.8
C20:1 n-9	1.9	1.8
C20:4 n-6	9.3	7.4
C22:0	0.3	0.3
C22:1 n-9	2.4	2.2
C22:4 n-6	tr	tr
C22:5 n-6	3.5	8.4[a]
C22:5 n-3	tr	tr
C24:0	1.3	1.4
C22:6 n-3	5.1	0.1[a]
C24:1 n-9	1.6	1.5
n-3 + n-6	20.6	18.8
n-3/n-6 Ratio		
This study	0.33	0.02
Morand et al. (1981*a*)	—	—
Morand et al. (1982)	0.72	—
Cohen and Bernsohn (1973)	0.32	—

See footnote to Table 3.

monounsaturated very long chains probably reflects their location in the plasma membrane together with other components previously found in the plasma membrane of oligodendrocytes (Poduslo, 1975). In myelin, a membrane derived from oligodendrocytes, the changes were very similar, with a very important decrease in 22:6 n-3 (Table 6). The levels of saturated and monounsaturated fatty acids did not change in myelin of sunflower oil-fed rats. The saturated/monounsaturated fatty acid ratio might be an index of development: it decreased with age (2.1 and 0.9 in 15- and 60-day-old animals, respectively), in agreement with our previous studies (Morand et al., 1982). In the myelin of 60-day-old rats fed sunflower oil, the n-3/n-6 fatty acid ratio was reduced by a factor of 12.

Our results are in agreement with the very long-chain saturated and monounsaturated fatty acid concentrations calculated from Abe and Norton (1979).

Astrocytes

The total amount of polyunsaturated fatty acids was not affected in astrocytes of sunflower oil-fed rats, regardless of age. However, the amount of 22:6 n-3 was reduced, and, conversely, the n-6 fatty acids were increased (Table 7). The n-3/n-6 ratio was more reduced in young animals than in old (six- and threefold decrease, respectively). Saturated

TABLE 6. *Fatty acid composition of myelin*

Fatty acids	15-Day-old animals		60-Day-old animals	
	Soya	Sunflower	Soya	Sunflower
C14:0	1.2	1.6	0.3	0.4
C16:0	24.5	24.9	8.0	8.3
C16:1 n-9	1.6	1.8	tr	tr
C16:1 n-7	0.6	1.1	0.8	0.7
C18:0	22.0	20.3	20.2	20.1
C18:1 n-9	14.5	12.2	35.7	31.9
C18:1 n-7	3.0	3.1	4.3	4.1
C18:2 n-6	0.8	0.8	0.4	0.3
C20:0	0.8	0.9	2.3	2.6
C20:1 n-9	1.5	1.7	0.8	0.9
C20:4 n-6	11.6	12.0	8.6	8.8
C22:0	1.0	0.7	2.1	2.3
C22:1 n-9	1.1	2.3	0.4	0.5
C22:4 n-6	3.6	3.5	2.6	3.5
C22:5 n-6	2.2	7.6[a]	0.2	2.4[a]
C22:5 n-3	—	—	—	—
C24:0	2.1	0.9	6.0	6.5
C22:6 n-3	5.8	2.3[a]	1.4	0.2[a]
C24:1 n-9	2.1	2.3	6.2	6.8
n-3 + n-6	24.0	26.2	13.2	15.2
n-3/n-6 Ratio				
This study	0.32	0.09	0.12	0.01
Morand et al. (1981*a*)	0.41	—	—	—
Morand et al. (1982)	—	—	0.11	—
Cohen and Bernsohn (1973)	—	—	0.19	—

See footnote to Table 4.

TABLE 7. *Fatty acid composition of astrocytes*

Fatty acids	15-Day-old animals		60-Day-old animals	
	Soya	Sunflower	Soya	Sunflower
C14:0	1.4	1.5	0.9	2.3
C16:0	31.6	32.0	29.5	33.0
C16:1 n-9	0.4	0.5	tr	tr
C16:1 n-7	0.6	0.5	0.3	0.2
C18:0	18.6	17.8	23.8	21.0
C18:1 n-9	12.2	13.2	12.5	9.7
C18:1 n-7	1.9	2.2	1.5	1.0
C18:2 n-6	1.4	1.1	1.2	3.8[a]
C20:0	0.3	0.3	0.5	1.7
C20:1 n-9	3.4	1.1[a]	0.9	0.5
C20:4 n-6	10.1	13.5	10.3	8.3
C22:0	0.3	0.4	0.1	1.2[a]
C22:1 n-9	0.4	0.5	tr	0.3
C22:4 n-6	2.4	2.7	2.7	2.1
C22:5 n-6	2.7	9.4[a]	2.5	8.6[a]
C22:5 n-3	1.3	tr	0.7	tr
C24:0	0.4	0.2	0.5	0.6
C22:6 n-3	10.6	3.1[a]	12.1	5.7[a]
C24:1 n-9	tr	tr	tr	tr
n-3 + n-6	28.5	29.8	29.5	28.5
n-3/n-6 Ratio				
This study	0.72	0.12	0.76	0.25
Morand et al. (1981*a*)	0.75	—	0.67	—
Morand et al. (1982)	1.0	—	—	—
Cohen and Bernsohn (1973)	—	—	0.57	—

See footnote to Table 3.

and monounsaturated fatty acids were not different in neurons from animals fed either type of diet. The saturated/monounsaturated fatty acid ratio increased during development, in contrast with our previous findings (Morand et al., 1982).

In conclusion, the sunflower oil diet triggered dramatic alterations in the polyunsaturated fatty acid composition of brain cells. Oligodendrocytes were affected more than neurons and astrocytes. The important decrease in 22:6 n-3 fatty acid was largely counterbalanced by an increase in the 22:5 n-6 fatty acid except in neurons. It was expected that 20:4 n-6 would not be dramatically affected by either diet. Myelin and synaptosomes were affected via their precursors (oligodendrocytes and neurons, respectively).

EFA deficiency leads to a rise in the n-9 triene levels, resulting in an increase of the triene/tetraene ratio (Paoletti and Galli, 1972); we did not detect this effect in rats fed sunflower oil, as a normal amount of n-6 fatty acids in the diet probably provides sufficient unsaturated fatty acids to maintain the unsaturation index in the phospholipids.

The values obtained with animals fed soya oil can be considered as controls and they compare favorably with our previous studies. However, there are some differences, mainly in the levels of saturated and unsaturated fatty acids. These discrepancies might reflect differences in the diets, since these fatty acids originate from synthesis in the brain and blood stream, and from the diet (Dhopeshwarkar and Mead, 1973; Sinclair, 1975; Bourre, 1980).

Our results raise the question of the functional significance of the alterations in the fatty acid pattern in brain cells and subfractions induced by dietary deficiencies in n-3 fatty acids.

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REFERENCES

Abe T. and Norton W. (1979) The characterization of sphingolipids of oligodendroglia from calf brain. *J. Neurochem.* **32**, 823–832.

Baumann N., Bourre J. M., Jacque C., and Harpin M. L. (1973) Lipid composition of Quaking mouse myelin in the adult and during development. *J. Neurochem.* **20**, 753–759.

Bourre J. M. (1980) Origin of aliphatic chains in brain, in *Neurological Mutations Affecting Myelination* (Baumann N., ed), pp. 187–206. INSERM Symposium No. 14. Elsevier/North Holland Biomedical Press, Amsterdam.

Bourre J. M., Jacque C., Nguyen-Legros J., Bornhofen J. H., Araoz C., Daudu O., and Baumann N. A. (1978) Pelizaeus-Merzbacher disease: biochemical analysis of isolated myelin (electron microscopy; protein, lipid and unsubstituted fatty acids analysis). *Eur. Neurol.* **17**, 317–326.

Bourre J. M., Morand O., Chanez C., Dumont O., Flexor M., and Baumann N. (1981) Influence of intrauterine malnutrition on brain development: alteration of brain cell lipid composition associated with defective myelination, in *Physiological and Biochemical Basis for Perinatal Medicine* (Monset M. and Minkovski A., eds), pp. 323–333. Karger, Basel.

Bourre J. M., Chanez C., Dumont O., and Flexor M. A. (1982) Alteration of 5'-nucleotidase and Na^+,K^+-ATPase in central and peripheral nervous tissue from dysmyelinating mutants (jimpy, quaking, Trembler, shiverer and mld). Comparison with CNPase in the developing sciatic nerve from Trembler. *J. Neurochem.* **38**, 643–649.

Chao S. W. and Rumsby M. G. (1977) Preparation of astrocytes, neurons and oligodendrocytes from the same rat brain. *Brain Res.* **124**, 347–351.

Cohen S. and Bernsohn J. (1973) Incorporation of 1-^{14}C labeled fatty acid into isolated neuronal soma astroglia and oligodendroglia from calf brain. *Brain Res.* **60**, 521–525.

Delasalle A., Jacque C., Drouet J., Raoul M., Legrand J. C., and Cesselin F. (1980) Radioimmunoassay of the myelin basic protein in biological fluids, conditions improving sensitivity and specificity. *Biochimie* **62**, 159–165.

Dhopeshwarkar G. and Mead J. (1973) Uptake and transport of fatty acids into the brain and the role of the blood brain barrier system. *Adv. Lipid Res.* **11**, 109–142.

Folch J., Rees M., and Sloane-Stanley G. H. (1957) Simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–509.

Francois M., Pascal G., and Durand G. (1980) Effets de la carence alimentaire en acide linolénique chez le rat. *Ann. Nutr. Metab.* **34**, 443–450.

Galli C., Treciak H., and Paoletti R. (1971) Effects of dietary fatty acids on the fatty acid composition of brain ethanolamine-phosphoglyceride: reciprocal replacement of n-6 and n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta* **248**, 449–454.

Hajos A. (1975) An improved method for the preparation of synaptosomal fractions in high purity. *Brain Res.* **95**, 485–489.

Jacque C. M., Vinner C., Kujas M., Racadot J., and Baumann N. A. (1978) Determination of glial fibrillary acidic protein (GFAP) in human brain tumors. *J. Neurol. Sci.* **35**, 147–155.

Lamptey M. and Walker B. (1976) A possible essential role for dietary linolenic acid in the development of the young rat. *J. Nutr.* **106**, 86–93.

McKenna C. and Campagnogni A. (1979) Effect of pre and postnatal essential fatty acid deficiency on brain development and myelination. *J. Nutr.* **109**, 1195–1204.

Morand O., Baumann N., and Bourre J. M. (1979) In vivo incorporation of exogenous [1-^{14}C] stearic acid into neurons and astrocytes. *Neurosci. Lett.* **13**, 177–180.

Morand O., Masson M., Baumann N., and Bourre J. M. (1981*a*) Exogenous [1-^{14}C] lignoceric acid uptake by neurons, astrocytes and myelin, as compared to incorporation of [1-^{14}C] palmitic and stearic acids. *Neurochem. Int.* **3**, 329–334.

Morand O., Chanez C., Masson M., Dumont O., Flexor M. A., Baumann N., and Bourre J. M. (1981*b*) Intrauterine growth retardation (malnutrition by vascular ligation) induces modifications in fatty acid composition of neurons and oligodendrocytes. *J. Neurochem.* **37**, 1057–1060.

Morand O., Chanez C., Masson M., Dumont O., Flexor M. A., Baumann N., and Bourre J. M. (1982) Alteration in fatty acid composition of neurons, astrocytes, oligodendrocytes, myelin and synaptosomes in intrauterine malnutrition in rat. *Ann. Nutr. Metab.* **26**, 111–120.

Morrison W. R. and Smith L. M. (1964) Preparation of fatty acid methyl esters and dimethyl-acetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **5**, 600–608.

Norton W. T. and Poduslo S. E. (1971) Neuronal perikarya and astroglia of rat brain: chemical composition during myelination. *J. Lipid. Res.* **12**, 84–90.

Norton W. T. and Poduslo S. E. (1973) Myelination in rat brain: method of myelin isolation. *J. Neurochem.* **21**, 749–757.

Nouvelot A., Bourre J. M., Sezille G., Dewailly P., and Jaillard J. (1983) Changes in the fatty acid patterns of brain phospholipids during development of rats fed with peanut or rapeseed oil, taking into account differences between milk and maternal food. *Ann. Nutr. Metab.* **27**, 173–181.

Paoletti R. and Galli C. (1972) Effects of essential fatty acid deficiency on the central nervous system in the growing rat, in *Lipids, Malnutrition and the Developing Brain*, pp. 121–140 (Ciba Foundation Symposium). Elsevier North Holland, Amsterdam.

Poduslo S. E. (1975) The isolation and characterization of a plasma membrane and a myelin fraction derived from oligodendroglia of calf brain. *J. Neurochem.* **24**, 647–654.

Pollet S., Ermidou S., Le Saux F., Monge M., and Baumann N. (1978) Microanalysis of brain lipids: multiple two dimensional thin-layer chromatography. *J. Lipid Res.* **19**, 916–921.

Raine C. S., Poduslo S. E., and Norton W. T. (1971) The ultrastructure of purified preparations of neurons and glial cells. *Brain Res.* **27**, 11–24.

Rudin D. (1982) The dominant diseases of modernized societies as omega-3 essential fatty acid deficiency syndrome: substrate beriberi. *Med. Hypotheses* **8**, 17–47.

Samulski M. A. and Walker B. L. (1982) Maternal dietary fat and polyunsaturated fatty acids in the developing foetal rat brain. *J. Neurochem.* **39**, 1163–1168.

Sinclair A. (1975) Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rat. *Lipids* **10**, 175–184.

Sun G. (1972) Effects of a fatty acid deficiency on lipid of whole brain microsomes and myelin in the rat. *J. Lipid Res.* **13**, 56–62.

Svennerholm L., Alling C., Bruce A., Karlsson I., and Sapia O. (1972) Effects on offspring of maternal malnutrition in the rat, in *Lipids, Malnutrition and the Developing Brain* (Ciba Foundation Symposium), pp. 141–157. Elsevier North Holland, Amsterdam.

White H. B., Galli C., and Paoletti R. (1971) Brain recovery from essential fatty acid deficiency in developing rats. *J. Neurochem.* **18**, 869–882.