*Journal of Neurochemistry*
Raven Press, New York


# Recovery of Altered Fatty Acid Composition Induced by a Diet Devoid of n-3 Fatty Acids in Myelin, Synaptosomes, Mitochondria, and Microsomes of Developing Rat Brain

A. Youyou, *G. Durand, *G. Pascal, M. Piciotti, O. Dumont, and J. M. Bourre

*Unité de Neurotoxicologie—INSERM U-26, Hôpital Fernand Widal, Paris, and *I.N.R.A.-C.N.R.Z., Jouy-en-Josas, France*

**Abstract:** Rats were fed a semisynthetic diet containing either sunflower oil or soya oil. Half the litter fed with sunflower oil diet was changed to a soya oil diet when the pups were 15 days old (during active myelination). Fatty acid analysis was then performed on subcellular fractions of the animals fed (a) soya oil, (b) sunflower oil, and (c) soya oil replacing sunflower oil from the 15th day, to determine the speed of the recovery. All material from animals fed sunflower oil showed an important reduction in docosahexaenoic acid (22:6 n-3), compensated by an increase in docosapentaenoic acid (22:5 n-6), whereas arachidonic acid (20:4 n-6) was not affected. In all fractions examined, when sunflower oil was replaced by soya oil in 15-day-old pups the recovery started from the very first day but lasted more than 2 months (this recovery was determined by the increase of 22:6 n-3 up to the normal value and decrease of the 22:5 n-6). In addition a delay was found for myelin recovery, starting only from the 25th day. **Key Words:** Mitochondria—Microsomes—Myelin—Synaptosomes—n-3 Fatty acids—Diet—Polyunsaturated fatty acids. Youyou A. et al. Recovery of altered fatty acid composition induced by a diet devoid of n-3 fatty acids in myelin, synaptosomes, mitochondria, and microsomes of developing rat brain. *J. Neurochem.* **46**, 224–228 (1986).

Polyunsaturated fatty acids are essential in the mammal, and thus are derived from the diet (Holman, 1968). Accumulation of arachidonic acid (20:4 n-6) and cervonic acid (22:6 n-3) is important in the growing brain (Sun, 1972; Alling et al., 1974; McKenna and Campagnogni, 1979; Samulski and Walker, 1982; Marilla et al., 1982) and essential fatty acid requirements during pregnancy and lactation are very important as far as brain development is concerned (Crawford et al., 1981). Alteration in the linoleic (n-6) or linolenic (n-3) series in the diet can trigger dramatic alterations in brain (Menon and Dhopeshwarkar, 1981). These alterations are sometimes paralleled with changes in physical properties of membranes, alteration in activities of enzymes, receptors, carrier-mediated transport, and even alterations in cellular interactions (for a review see Stubbs and Smith, 1984). The ratio of linoleic acid to linolenic acid greatly affects the pattern of tissue lipids (Svennerholm et al., 1972). Reduced amount of linolenic acid in the diet triggered a dramatic decrease of n-3 fatty acids, mainly 22:6 n-3, compensated by an increase in 22:5 n-6 in phosphatidylethanolamines (Nouvelot et al., 1983*a*,*b*); in a comparison of 60-day-old animals fed soya oil or sunflower oil, the n-3/n-6 fatty acid ratio was reduced by 16-fold in oligodendrocytes and 12-fold in myelin, two-fold in neurons, and six-fold in synaptosomes, and three-fold in astrocytes (Bourre et al., 1984). Recently, an n-3 essential fatty acid deficiency syndrome was proposed as a dominant disease of modernized societies (Rudin, 1982) and a specific n-3 deficiency was described in humans (Holman et al., 1982).

## MATERIALS AND METHODS

Rats were fed a semisynthetic diet containing either sunflower oil (poor in linolenic acid) or soya oil (rich in linolenic acid) as previously described (Bourre et al., 1984) with slight modifications: soya and sunflower oils were used at 1.87% and 1.5% respectively. Three days after delivery, the litters were adjusted to 10 animals. At 15 days, half the animals fed sunflower oil were fed soya oil from then on. In these animals, the n-3-deficient diet

Received June 12, 1985; accepted July 11, 1985.
Address correspondence and reprint requests to Dr. J. M. Bourre at Unité de Neurotoxicologie—INSERM U-26, Hôpital Fernand Widal, 200, rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France.

was thus substituted by an n-3 normal diet. After weaning (21 days), the young rats received the same diet as their mother. Three groups of four rats were used for each subcellular preparation.

Myelin was prepared according to Norton and Poduslo (1973), synaptosomes according to Hajos (1975) with slight modifications (Morand et al., 1982), microsomes according to Eichberg et al. (1964) with slight modifications (Pollet et al., 1973), and mitochondria according to Eichberg et al. (1964) with slight modifications (Paturneau-Jouas et al., 1976). The purity of the fractions as determined by electron microscopy, marker enzymes, radioimmunoassay of specific proteins, rocket electrophoresis, electrophoresis, and lipid analysis has been published in our preceding papers (Bourre et al., 1973, 1984; Paturneau-Jouas et al., 1976).

Lipids were extracted by sonication in chloroform/methanol 2:1 (vol/vol) (Folch et al., 1957; Pollet et al., 1978) and transmethylated (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).

## RESULTS

### Myelin

Myelin from sunflower oil-fed rats presented a dramatic reduction in 22:6 n-3 (25% and 34% of the control in 15- and 60-day-old animals); this reduction was compensated by an increase in 22:5 n-6 (380% and 800% in 15- and 60-day-old animals). When sunflower oil was replaced by soya oil in the diet of 15-day-old pups, the recovery started only when the animals were 25 days old and reached the control value at the 90th day. The recovery in 22:6 n-3 occurred exclusively between the 21st and 28th days; after 28 days, the level of 22:6 n-3 was constant. However on the 28th day, the amount of 22:6 n-3 was only 50% of the control (soya oil-fed animals). The total recovery obtained at 90 days was not due to an increase of 22:6 n-3 in experimental animals but to a normal and physiological decrease in control (soya oil-fed) animals. Thus recovery was passive, in contrast with microsomes and synaptosomes (see below). Thus, although animals were actively myelinating between the 15th and 30th days, this newly formed myelin is still poor in n-3 fatty acids, confirming that there is a delay between lipid synthesis and myelin assembly.

The decrease in 22:5 n-6 was delayed to the 21st day and was rapid from then on until the 35th day. It took 2.5 months to reach the control value. Thus in myelin developmental evolutions of 22:6 n-3 and 22:5 n-6 were not symmetrical.

### Synaptosomes

The amount of 22:6 n-3 was drastically reduced (34% and 30% of the control in 15-and 60-day-old animals) in sunflower oil-fed rats. The reduction in n-3 fatty acids was compensated for by an increase in n-6 fatty acids, mainly 22:5 n-6 (480 and 600% of the control in 15- and 60-day-old animals).

In agreement with previous studies (Bourre et al., 1984; Morand et al., 1982), the polyunsaturated fatty acid content varied between 15 days and adult: 22:6 n-3 increased and arachidonic acid decreased. When sunflower oil was replaced by soya oil in the diet of 15-day-old pups, the recovery started rapidly after changing the diet (increasing amount of 22:6 n-3 and decreasing amount of 22:5 n-6) but it took $2^1/_2$ months to reach the normal value. Such a delay was unexpected, as synaptosomes are supposed to have a high metabolic activity, thus a rapid turnover of membrane components. Moreover the recovery was an active process as in soya oil-fed animals the relative amount of 22:6 n-3 increased during development, in contrast with myelin.

### Microsomes

The amount of 22:6 n-3 was reduced (17% and 27% of the controls in 15- and 60-day-old animals, respectively) and conversely 22:5 n-6 was increased (440% and 800% at the 15th and 60th days, respectively). The ratio n-3/n-6 was reduced seven- and fivefold at the 15th and 60th days. When sunflower oil was replaced by soya oil in the diet of 15-day-old animals, the recovery started from the very first day, but it took more than 2 months for 22:6 n-3 to reach the control value. As for synaptosomes, the process was active, as the amount of 22:6 n-3 increased during development in control animals (soya oil-fed). The decrease in 22:5 n-6 was delayed up to the 21st day. After the change in the diet, there is a difference between 22:6 n-3 which increases immediately, and 22:5 n-6, which decreases after 5 days.

### Mitochondria

As for the other subcellular fractions, the most significant difference between mitochondrial fatty acids obtained from animals fed soya oil or sunflower oil was found in the polyunsaturated fatty acids. 22:6 n-3 was reduced (approximately 26% of the control at any age) and 22:5 n-6 was increased (420% and 950% of the control in 15- and 60-day-old animals). The ratio n-3/n-6 was reduced approximately by sixfold in mitochondria from sunflower oil-fed rats. When sunflower oil was replaced by soya oil in the diet of 15-day-old pups, the recovery started from the very first day of changing the diet (increasing amount of 22:6 n-3 and decreasing amount of 22:5 n-6), but for mitochondria it took more than 2 months to reach the control value. As for microsomes, 22:6 n-3 increased immediately after changing the diet but the decrease in 22:5 n-6 was delayed for a few days.

## DISCUSSION

When the diet is deficient in n-3 fatty acid (sunflower oil) phospholipids (Nouvelot et al., 1983 *a,b*), brain cells (Bourre et al., 1984) and brain sub-

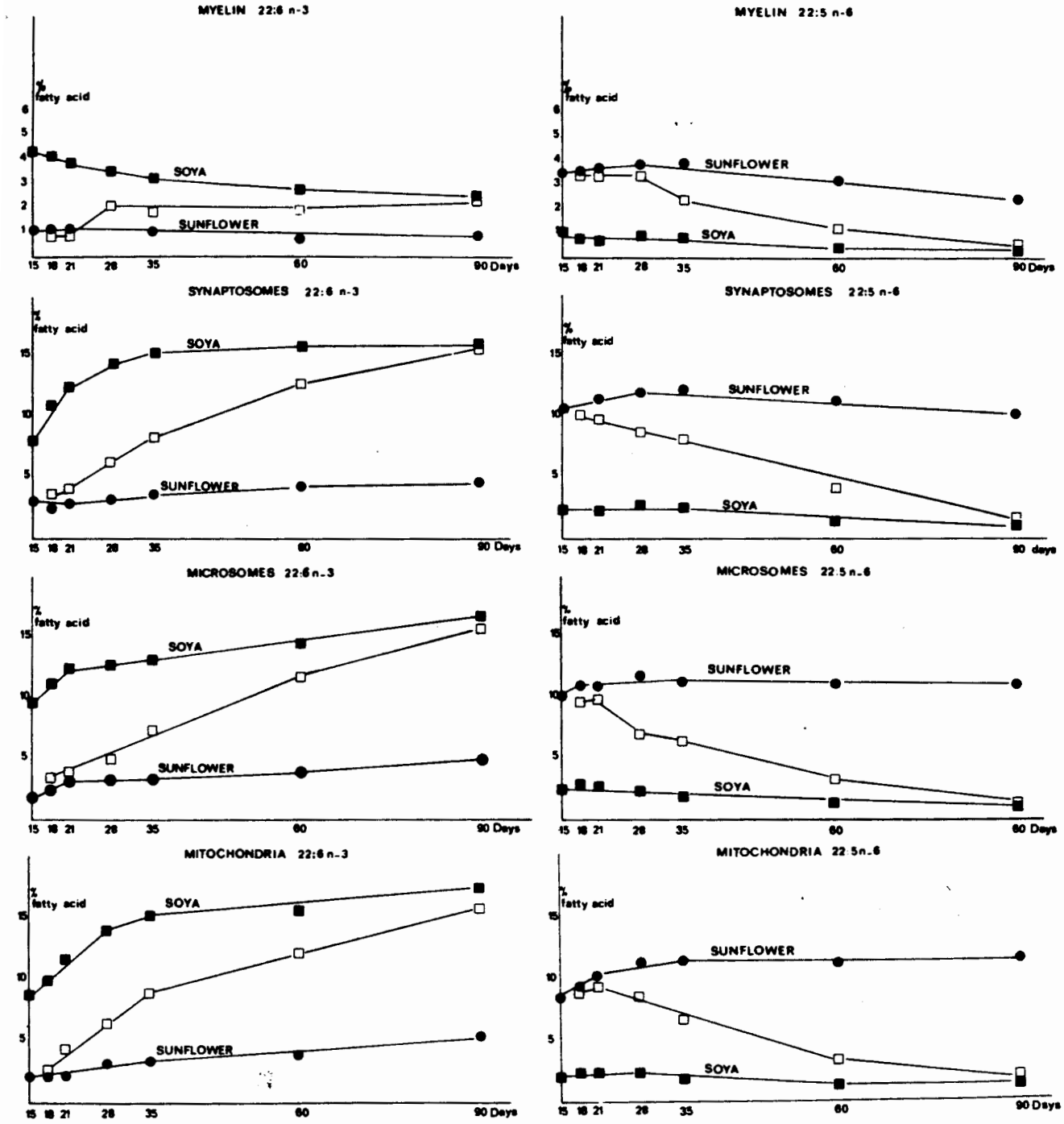


**FIG. 1.** Levels of 22:6 n-3 and 22:5 n-6 in myelin, synaptosomes, microsomes, and mitochondria of sunflower oil (●), soya oil (■) fed rats, and soya oil replacing sunflower oil from 15-day-old rats (□). See Results for details.

cellular fractions (Bourre et al., 1984 and this study for microsomes and mitochondria) contained very reduced amounts of cervonic acid (22:6 n-3) and increased amounts of 22:5 n-6. Adrenic 22:4 n-6 and arachidonic 20:4 n-6 were poorly affected. Similar results have been obtained with animals that were maintained on a fat-free diet supplemented with linoleic acid (Trapp and Bernsohn, 1977).

As arachidonic content and the total percent of polyunsaturated fatty acid are not altered in linolenic acid deficiency, it could be suggested that phospholipids are normally esterified with arachidonic acid, and that only 22:6 n-3 acid was replaced by 22:5 n-6. Moreover fatty acid changes in different phospholipids could be different as it is expected that polyunsaturated fatty acids in different types of phospholipids may exhibit different rates of metabolism (Sun et al., 1975).

The finding that the speed of recovery was very slow, whatever the subcellular fraction, was unexpected. For myelin, synaptosomes, microsomes, and mitochondria, it took more than 2.5 months to obtain the normal content of 22:6 n-3. As it is generally accepted that myelin has a very slow turnover and that other subfractions have a much faster turnover these results could mean that there are phospholipids in membranes of all subcellular fractions that contain 22:6 and that exhibit a very slow turnover. Another explanation could be that the limiting factor is the transfer of 22:6 through the blood–brain barrier, the amount that is transported during recovery being insufficient. Uptake and transport of linolenic (Dhopeshwarkar and Mead, 1973; Cohen and Bernsohn, 1973) and docosahexaenoic (Sinclair, 1975) acids through the blood–brain barrier system have been demonstrated. Cultures of brain cells in chemically defined media have suggested that brain uses 22:6 preferably to its precursor linolenic acid (Bourre et al., 1983). A third explanation could be that the rate-limiting factor is the in situ synthesis of n-3 fatty acids fom linolenic acid, due to low desaturase activities.

**Acknowledgment:** This work as supported by INSERM, INRA, and ASTRA CALVE. The authors are most grateful to Mrs. M. Bonneil for typing this manuscript.

## REFERENCES

Alling C., Bruce A., Karlsson I., and Svennerholm L. (1974) The effect of different dietary levels of essential fatty acids on lipid of rat cerebrum during maturation. *J. Neurochem.* **23**, 1263–1270.

Bourre J. M., Pollet S., Daudu O., and Baumann N. (1973) Evolution in brain mice microsomes of lipids and their constituents during myelination. *Brain Res.* **51**, 225–239.

Bourre J. M., Faivre A., Dumont O., Nouvelot A., Loudes C., Puymirat J., and Tixier-Vidal A. (1983) Effect of polyunsaturated fatty acids on foetal mouse brain cells in culture in a chemically defined medium. *J. Neurochem.* **41**, 1234–1242.

Bourre J. M., Pascal G., Durand G., Masson M., Dumont O., and Piciotti M. (1984) 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.

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.

Crawford M. A., Hassam A. G., and Stevens P. A. (1981), Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. *Prog. Lipid Res.* **20**, 31–40.

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.

Eichberg J., Whittaker J. P., and Dawson R. M. C. (1964) Distribution of lipids in subcellular particles of guinea-pig. *Biochem. J.* **92**, 91–100.

Folch J., Lees 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.

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.

Holman R. T. (1968) Essential fatty acid deficiency, in *Progress in the Chemistry of Fats and Other Lipids, Vol. IX*, pp. 275–348; Pergamon Press. Oxford.

Holman R. T., Johnson S. B., and Hatch T. F. (1982)A case of human linolenic acid deficiency involving neurological abnormalities. *Am. J. Clin. Nutr.* **35**, 617–623.

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.

Menon N. K. and Dhopeshwarkar G. A. (1981) Essential fatty acid deficiency and lipid metabolism of the developing brain, in *Progress in Lipid Research Vol. 20* (Holman R. T., ed), pp. 129–134 Pergamon. Oxford.

Morand O., Chanez C., Masson M., Dumont O., Flexor M. A., Baumann N., and Bourre J. M. (1982) Alteration in fatty acid compostion of neurons, astrocytes, oligodendrocytes, myelin and synaptosomes 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. (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*a*) 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.

Nouvelot A., Dedonder-Decoopman E., Sezille G., Paturneau-Jouas M., Dumont O., Masson M. and Bourre J. M. (1983 *b*) Influence de la teneur en acide linolénique du régime maternel sur la composition en acides gras polyinsaturés des fractions subcellulaires au cours du développement cérébral chez le rat. *Ann. Nutr. Metab.* **27**, 233–241.

Paturneau-Jouas M., Baumann N., and Bourre J. M. (1976) Biosynthèse des acides gras dans les mitochondries de cerveau de souris en présence de malonyl-CoA ou d'acétyl-CoA. *Biochimie* **58**; 341–349.

Pollet S., Bourre J. M., Daudu O., and Baumann N. (1973) Biosynthèse des acides gras dans les microsomes de cerveau de souris. *Biochimie* **55**, 333–341.

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

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. and Walker B. (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.

Stubbs C. D. and Smith A. D. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **779**, 89–137.

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.

Sun G., Winniczek H., Go J., Sheng S. L. (1975) Essential fatty acid deficiency: metabolism of 20:3 w9 and 22:3 w9 of major phosphoglycerides in subcellular fractions of developing and mature mouse brain. *Lipids* **7**, 365–373.

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.

Trapp B. D. and Bernsohn J. (1977) Changes in phosphoglyceride fatty acids of rat brain induced by linoleic and linolenic acids after pre and post-natal fat deprivation. *J. Neurochem.* **28**, 1009–1013.