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Is There a Blood-Brain Relationship for Saturated Fatty Acids during Development? [1]

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Abstract. Transport of subcutaneously injected [1-^{14}C]-stearic acid through the blood-brain barrier is compared with endogenous biosynthesis (within the brain) during postnatal brain development in mice. The uptake is very important during glial cell multiplication and myelination; endogenous microsomal synthesis is most active during myelination, soluble *de novo* mechanism is prominent during cell multiplication (mitochondrial systems are not directly related to these events). A parallel is drawn between myelin fatty acids, microsomal synthesis and uptake from the blood.

Animal experiments provide the only available method of experimental investigation for studying the biochemistry of brain development. As myelination occurs after birth in mice, this animal is a very good model.

Brain saturated and mono-unsaturated fatty acids are derived in two ways: by synthesis within the brain and transport into the brain of fatty acids synthesized elsewhere or obtained in the diet. Indeed, the biosynthesis is operative in three compartments (1–7) (microsomes, mitochondria and cytosol); myelin lipids are synthesized in endoplasmic reticulum. However, at least during myelination, it is clear that the developing brain is dependent on exogenous saturated fatty acids (8, 9): palmitic and oleic acid in cerebral lipids are derived in part from the uptake of these fatty acids from the circulation (10) and subcutaneously injected stearic acid is incorporated into brain membrane lipids (11) including myelin (12) and nerve endings (13). The purpose of this work is to make an approach of the blood-brain relationship versus *in situ* biosynthesis during brain development at the period of myelin deposition in mice. Comparison of transport versus biosynthesis was investigated for stearic acid, this acid being necessary to the synthesis of very long chain fatty acids of myelin.

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Techniques

Subcutaneous injection of labelled [1-^{14}C]-stearic acid was performed as previously described (11). Animals were injected in relation to the body weight (10 μCi/g) and sacrified 17 h later. At all ages, the incorporation of label was linear under these conditions. The lipids were analyzed by thin-layer chromatography (13) and the fatty acids (from either blood or brain) were quantified by gas-liquid chromatography with automatic counting of the eluate.

For enzymatic analysis, subcellular fractionation was achieved for cytosol (2) and microsomes (14), and fatty acids biosynthesis was performed in the usual way (9) using acetyl-CoA (25 μM), malonyl-CoA (50 μM), NADPH (500 μM) for the *de novo* system (from either cytosol or microsomes), palmityl-CoA or stearyl-CoA (25 μM), malonyl-CoA (50 μM) and NADPH (500 μM) for the two microsomal elongating complexes.

Myelin was isolated by density gradients with some modifications for young animals (15); lipids were extracted according to *Folch et al.* (16).

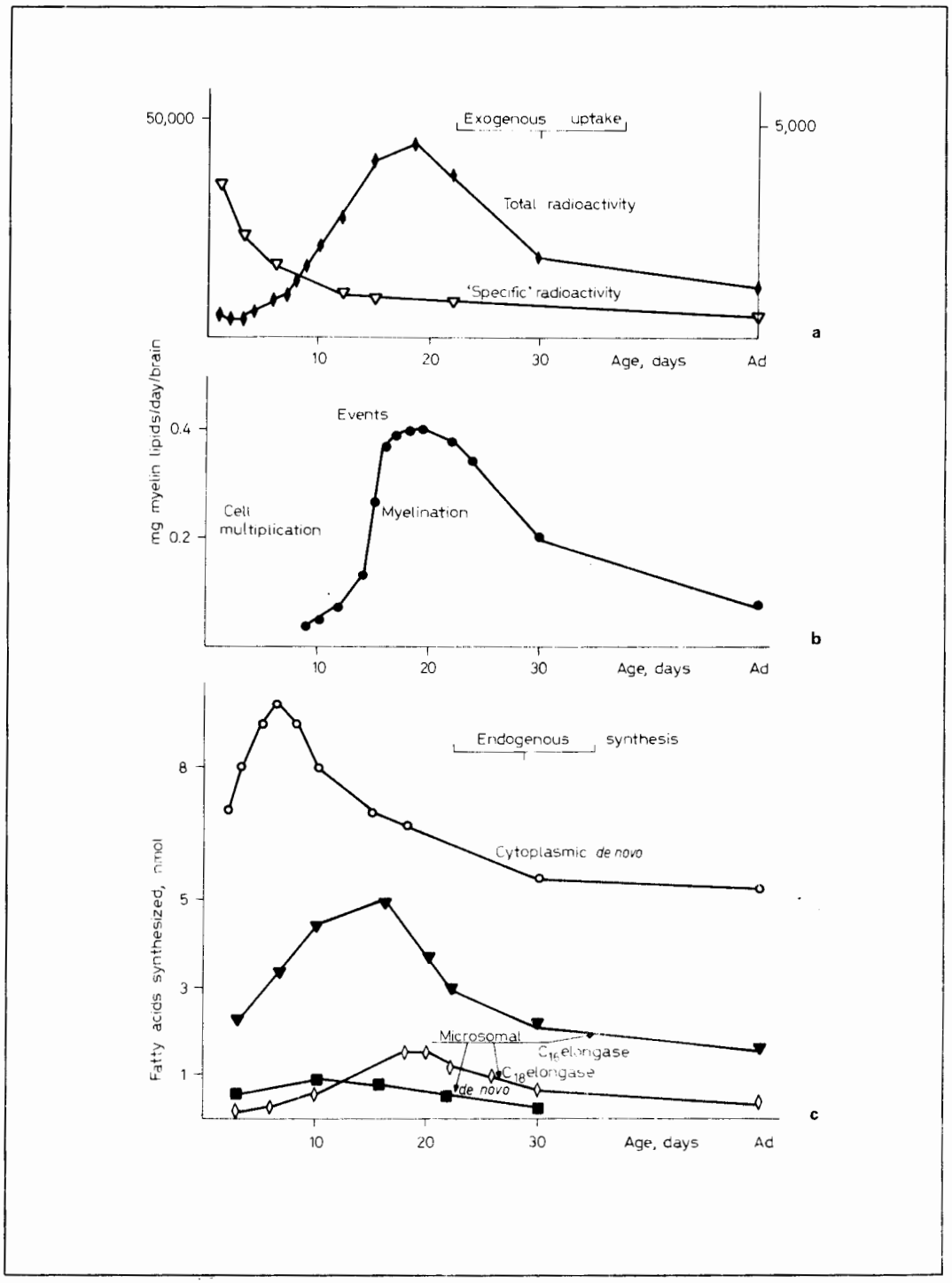
Results and Discussion

Figure 1b shows that the velocity of myelin lipids deposition increases drastically between 10 and 15 days, is nearly stable in the 16- to 24-day period and slowly decreases afterwards. The myelin synthesis has fallen off in the adult but there is not a steady state: low but measurable quantity of new myelin is synthesized throughout life. This curve is close to the one obtained in the rat (17).

Figure 1a shows that stearic acid is transported from blood into brain at varying rates during myelinogenesis. Total radioactivity (cpm/total brain lipids, i.e., mole fatty acids/total brain lipids) increases in parallel to myelination and decreases thereafter. As stearic acid contains most of the radioactivity in brain and as no other labelled molecule is found in blood, the role of an exogenous saturated fatty acid on brain lipid biosynthesis is clearly demonstrated and these results indicate a variation in the blood-brain relationship during development. However, when stearic acid incorporation is expressed in relation to the amount of brain lipids, there is a decrease as a function of age (as expressed in specific radioactivity: cpm/mg lipids, i.e., mole fatty acids per milligram lipids). Although this curve takes into account the amount of lipids deposited before injection, it may indicate the importance of exogenous fatty acids at the period of glial cell multiplication which occurs at the first week after birth (18). Thus exogenous stearic acid seems to be relatively more important during cell multiplication that during myelination. These curves are not related to an increased catabolism in situ as β-oxydation is very low in the brain.

Figure 1c shows that the very long chain fatty acid biosynthesis during development is related to myelination. The microsomal elongating system parallels myelin deposition; but the mitochondrial system is not involved in plasma membrane biosynthesis (9). We have previously shown that microsomes, at variance with mitochondria (7), contain a *de novo* system and two elongating complexes, the primers of the two latter being, respectively, palmityl-CoA and stearyl-CoA. The curve here presented shows that the synthesis of stearic acid by the first elongating complex (C_{16}-elongase) occurs somewhat before the biosynthesis of very long chain (C_{18}-elongase). This is in agreement with the composition of young myelin which is richer in medium chain than the adult one (19). As previously shown (20), the cytosolic *de novo* system is optimum at 6 days after birth. We suppose that it is not directly involved in myelination but it is related to cell multiplication.

Indeed the notion of essential fatty acids is familiar and, as established in the rat (22),


50,000
Exogenous uptake
5,000
Total radioactivity
'Specific' radioactivity
10
20
30
Age, days
Ad
a
mg myelin lipids/day/brain
0.4
0.2
Events
Cell multiplication
Myelination
10
20
30
Age, days
Ad
b
Endogenous synthesis
8
Cytoplasmic de novo
5
3
1
Fatty acids synthesized, nmol
Microsomal
C_{16} elongase
C_{18} elongase
de novo
10
20
30
Age, days
Ad
c

poly-unsaturated fatty acids from milk may provide a source for the developing brain. The nutritional need during development for saturated fatty acids demonstrated in our experiments has not been previously described. Thus, it is fundamental to determine the origin of other brain fatty acids during development. It would also be important to see if brain structure and function could be modified by alteration in its fatty acid incorporation due to abnormal diet.

As far as we know, the critical period for human babies (when the brain is more sensitive or vulnerable) begins with the second half of the fetal life and ends at about 18 months after birth. During this 'growth spurt' (18) brain vulnerability is critical and the fatty acids nutrition of both pregnant mother and the newborn afterwards will contribute to a normal brain development.

As catabolism of fatty acids within the brain is very restricted, if a long chain gets in, it will be likely to stay and if complex lipids are degraded (by physiological turnover), the fatty acid moiety is reutilized for synthesis of new complex molecules. Thus, introduction of non-physiological fatty acids (such as branched chain or trans-isomers) may have a long-term effect. This is possibly the case in multiple sclerosis and diabetes. Data have been presented (23) showing that the predisposing factor in multiple sclerosis may reflect dietary differences, this factor being directly related to milk consumption (and possibly fatty acids). A production of defective myelin will increase the susceptibility to the etiological agent. In diabetes the level of free fatty acids is increased in blood and the composition of these free fatty acids is abnormal; as a consequence the brain fatty acids could be changed in diabetes as has been shown for other organs.

Fig. 1. **a** Exogenous uptake for stearic acid. △ = Specific radioactivity expressed as cpm/mg lipid extract (moles fatty acid/mg lipid extract); ♦ = total radioactivity in cpm/total brain (moles fatty acid/one brain lipid extract). After subcutaneous injection of [1-^{14}C]-stearic acid most of the radioactivity is found in brain lipids as labelled [1-^{14}C]-stearic acid. Animals are killed 17 h after injection: for all ages the incorporation into brain is linear with time. **b** Myelin lipid deposition. Myelin was isolated from animals at different ages (with slight modifications for young animals (15). Lyophilized myelin weight was determined, proteins dried content measured according to *Lowry* and lipids extracted and weighed. The curve here presented is the velocity of myelin lipid deposition (mg myelin lipids/day in one brain). **c** Endogenous fatty acids biosynthesis expressed in nanomoles of fatty acids synthesized per mg protein (lower part f the figure). *De novo* system was studied in the presence of [1-^{14}C]-acetyl-CoA (25 μM), malonyl-CoA (50 μM) and NADPH (500 μM). For C_{16}-elongase, the medium contained palmityl-CoA (30 μM) [1,3-^{14}C]-malonyl-CoA (50 μM) and NADPH (500 μM). For C_{18}-elongase, palmityl-CoA was replaced by srearyl-CoA.

References

1 Brady, R.O.: Biosynthesis of fatty acids. II. Studies with enzymes obtained from brain. J. biol. Chem. *23:* 3099–3103 (1960).

2 Pollet, S.; Bourre, J.M. et Baumann, N.A.: Etude de la biosynthèse *de novo* des acides gras dans le cerveau de souris normale, Quaking et hétérozygote pour le caractère Quaking. C.r. hebd. Séanc. Acad. Sci., Paris *268:* 2146–2149 (1969).

3 Bourre, J.M.; Pollet, S.; Dubois, G. et Baumann, N.: Biosynthèse des acides gras à longues chaînes dans les microsomes de cerveau de souris. C.r. hebd. Séanc. Acad. Sci., Paris *271:* 798–801 (1970).

4 Bourre, J.M.; Pollet, S.; Chaix, G.; Daudu, O. et Baumann, N.: Etude *'in vitro'* des acides gras synthétisés dans les microsomes de cerveau de souris normales et Quaking. Biochimie *55:* 1473–1479 (1973).