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ESSENTIAL FATTY ACID DEFICIENCY AND BRAIN DEVELOPMENT

NIRMALA K. MENON and GOVIND A. DHOPESHWARKAR†

Laboratory of Biomedical and Environmental Sciences, University of California, 900 Veteran Avenue, Los Angeles, California 90024 U.S.A.

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I. INTRODUCTION

Brain growth is unique and distinct from that of other tissues because it is virtually complete in a rather brief period in early life. Although this period of rapid growth is not identical in all the species and that regions of the nervous system fail to develop at the same rate or to reach maturity at the same time, the growth of the brain is almost over long before the growth of the body has stopped. The rate of growth in the early stages of development is so phenomenal that the rat brain, for example, in the first eight days of life, gains 0.09 g/day followed by a slower gain of 0.02 g/day in the next 45 days, after which the growth rate is almost unnoticeable.[5,7]

The brain growth encompasses the anatomical, physiological, biochemical and psychological development of the brain, each occurring at its own pace with critical periods in intrauterine or early postnatal life. For this reason, the adult brain is known to tolerate severe deficiencies and other environmental abberrations. Even the reported changes in biochemical parameters in adult brain do not seem to have any functional significance.[2]

†Dr. Dhopeshwarkar passed away on April 1st 1982.

In 1973 Galli[40] concluded that dietary essential fatty acid (EFA) deficiency affected the developing central nervous system and that the nutritional stress may be of comparable gravity to those obtained by protein and/or calorie malnutrition. Since then, there has been a gradual evolution of our comprehension of the role played by EFA deficiency on brain metabolism and function. Several noteworthy advances have been made in the last few years. The main objective of the present article is to review major advances made after 1972, covering the areas of development, cellular and subcellular effects, lipid composition and metabolism, functional role of EFA, effects of maternal fatty acid imbalances on the fetal development, lipogenesis, etc., occurring in the brain. We have excluded EFA deficiency in humans entirely since this aspect was incorporated in a recent review.[119] In a few instances, we have included pertinent research monographs which appeared earlier than 1972, including important preliminary reports.

II. ANIMAL MODELS TO STUDY EFA DEFICIENCY AND BRAIN

The type of diet and the time of onset are the important aspects to be considered in developing an animal model. To study the effect of EFA deficiency on brain development during the intrauterine, suckling or post-weaning period, the maternal EFA stores need to be completely depleted as emphasized in the work of Alling *et al.*[2,3] and Sinclair and Crawford.[97] Since maternal EFA stores are only slowly released, the necessity of feeding female rats an EFA-deficient diet long before mating is necessary. One problem in this experimental design is that even though EFA deficient females conceive, this deficiency causes fetuses to be reabsorbed, animals to abort or young ones to be still-born, making it difficult to obtain viable litters.

A fat-free diet has been tried.[2,40,70,86] Such a diet cannot be imposed early in pregnancy, again due to the problem of getting viable litters. Feeding a fat-free diet has its own limitations. Withdrawal of fat from the diet may change the entire fat metabolism.[98]

Diets containing saturated fat are also used to induce EFA deficiency.[41,44] The saturated fat usually contains only traces of linoleic and no linolenic acid, thus making the unsaturation index zero, and thus in practice such a diet can be considered EFA deficient. Feeding such a diet to female rats ten days before mating, Galli *et al.*[41,44] successfully produced EFA deficiency in the brains of the progeny as indicated by the triene/tetraene ratio in the ethanolamine phosphoglyceride (EPG) component (see Fig. 1). The deficiency index, as defined by Holman, is the ratio of 20:3(n-9)/20:4(n-6). The magnitude of the ratio is by no means identical in all tissues or body fluids. In human serum which has been extensively studied, a value above 0.2 is considered to indicate EFA deficiency. Holman currently considers that the triene/tetraene ratio is less useful as an index of EFA deficiency than is the content of total ω6 acids because the ratio is affected by factors other than linoleic acid intake. In our opinion, the value should be established in all tissues. In the brain, for example, the ratio is usually very low even when by all other criteria the animal is conclusively EFA deficient. The ratio reaches deficiency index values of 0.4 only by day 60. In our experiments,[72] when saturated fat was started during the last week of gestation and continued thereafter, even though the liver and serum showed definite amounts of triene, the level in the brain EPG was negligible even after 90 days. Longer duration of feeding is thus important if saturated fat is used in the diet to produce EFA deficiency.

Our approach to produce EFA deficiency has been to keep a small amount of linoleic acid to ensure successful reproduction but to include large amounts of oleic acid that would compete for the utilization of linoleate,[22,72,74,75] which results in EFA deficiency. Under these conditions, saturated fatty acids do not compete. Using this approach, we have successfully produced fetal EFA deficiency by feeding female rats a 5% fat diet (18:1, 90–95%; 18:2, 2–2.5%) from day 1 of gestation.[74] When the same diet is started during the last week of gestation, trienoic acid starts to accumulate in the brain from postnatal day 21. There were structural changes in the fatty acid composition of myelin,

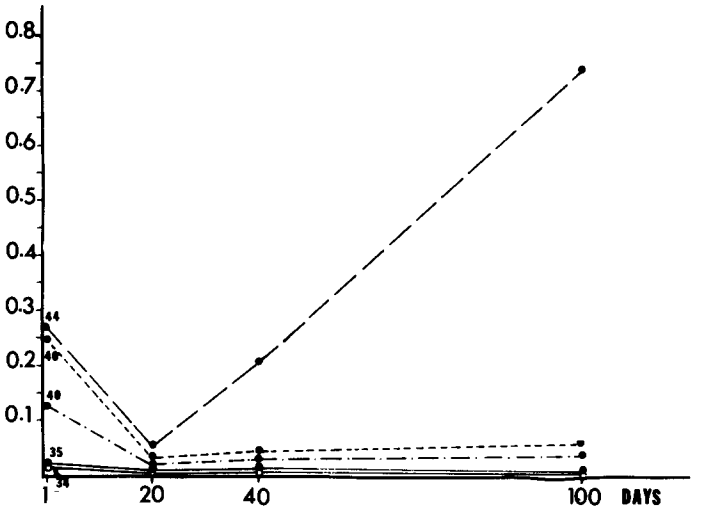

FIG. 1. Ratio of *n*-9 triene/*n*-6 tetraene in brain EGP of rats born to mothers fed the normal diet (O——O) or semisynthetic diets containing 10% w/w of either olive oil (●—·—·—●), sunflower seed oil (●——●), tallow (●– –●) or saturated fat (●— —●). The young rats were fed the same diet after weaning. Values represent the average of two determinations performed and one large pool of brains at 1 day of age on three pools of six animals, each at the subsequent age intervals. From Galli, C., Spagnuolo, C., Agradi, E. and Paoletti, R. Comparative effects of olive oil and other edible fats on brain structural lipids during development. In: *Lipids* Vol. I, p. 240 (Paoletti, R., Porcellati, G. and Jacini, G., eds.) Raven Press, New York, 1976. With kind permission.

microsomes and mitochondria.[72] The brain weight did not decrease but, in subcellular components such as microsomes, the lipid to protein ratio was decreased by 50%. This type of dietary imbalance is more prevalent in human populations than is total lack of fat or EFA.

The importance of maintaining the same ratio between linoleic and linolenic acids in the experimental and control diets is stressed by some workers.[2,49] A change in the above produces a change in the ratio of 22:5(*n*-6) to 22:6(*n*-3) in the brain phosphoglycerides. This relationship is independent of the concentration of EFA in the diet, at least when the dietary EFA concentration exceeds one calorie percent. If the dietary linoleate is less than 0.2 cal%, the absolute amount of linoleate and linolenate in the diet also becomes important in deciding the concentration of 22:6(*n*-3) and 22:5(*n*-6) fatty acids.

Essential fatty acid deficiency could also be produced secondary to other existing conditions such as disease states, zinc deficiency, excessive alcohol abuse and total parental nutrition.

III. GROWTH AND BIOCHEMICAL DEVELOPMENT OF THE BRAIN IN EFA DEFICIENCY

A. *Biochemical Parameters*

Pre- and postnatal EFA deficiency reduces body and brain weights of the developing progeny even though the mothers are asymptomatic.[50,62,69,72,74,86,104,114] There was no significant reduction in the food consumption of the dams during pregnancy or lactation in the above investigations. The reduction in body weight was more pronounced in the male than the female.[72] No morphological studies have been reported in the brain in EFA deficiency and only a few studies have evaluated the biochemical parameters of brain development. Karlsson and Svennerholm[62] found that when rats were fed 0.1 cal% of EFA for two generations the weight of the forebrains of the deficient group decreased markedly in the third generation. This difference was more noticeable after 10 and 15 days postnatally. In the deficient group, the increase in weight of the developing brain, due to growth, was smaller all through the 120 days studied. DNA and

protein content of the brains also decreased, indicating a reduction of cell number of the forebrain.

McKenna and Campagnoni[70] also found a reduction of DNA, RNA and protein in the brains of mice exposed to EFA deficiency from day 1 of gestation. The development of the brain was retarded by one week, with most striking differences at ages below 15 days. Brain DNA content of the control and deficient mice became comparable at 20–22 days but brain protein and RNA remained lower in deficient mice at all ages.

Some workers found that in EFA deficiency the concentration of total brain lipids did not vary significantly,[2,3,69,72,75] but others have reported that EFA deficiency started early enough and continued for a longer period of time reduced brain lipid concentration,[50,67] particularly in the male.[50] In fetal EFA deficiency, a reduction in lipid concentration of the brain has been reported.[74]

B. *Lipid Composition*

There was apparently no change in phospholipids even after feeding EFA deficient diet for two generations,[3,62,67] but Galli *et al.*[50] reported a 10% decrease in brain phospholipid content occurring in the male rat brain while the change was less pronounced in the female.

Concentration of cerebrosides was lower in the EFA deficient group up to 30 days postnatally in both males and females; afterwards, such a change was seen only in males.[3] The decrease in cerebroside concentration becomes less significant statistically in the adult.[3,62] A progressive reduction in brain cerebrosides and sphingomyelin has been reported by Odutuga[82] after prolonged feeding.

Gangliosides in the deficient group did not differ at birth but by 10 days the increase in these was smaller than the controls ($P < 0.01$).[62] A more severe reduction of ganglioside concentration in the forebrains of 21 day rats was reported by Merat and Dickerson.[76] Berra *et al.*[12] also demonstrated that different dietary fats can modify postnatally the ganglioside pattern. Recently Morgan *et al.*[78] showed that lack of EFA in the maternal diet during gestation and lactation depressed ganglioside levels in the progeny.

Myelin-specific components like galactolipid and proteolipid protein were depressed by 40 days (21% and 23%, respectively) compared to control values.[70]

C. *Fatty Acid Composition*

Comparisons of different animal species have shown that although liver and adipose tissue lipids reflect food selection practices, the fatty acid composition of the brain is remarkably similar regardless of species or of the wide differences in food patterns.[18,92] The fatty acid spectrum of the human brain changes from fetal stage all through development; the change is seen in polyunsaturated fatty acids (PUFA) during fetal growth, whereas the change in long chain saturated and monounsaturated fatty acids is seen during myelination.[18] Cellular and synaptosomal material is particularly rich in arachidonic (20:4(n-6)), adrenic (22:4(n-6)) and cervonic acids (22:6(n-3)). Brain lipids contain less than 1% of linoleic acid.[20]

The influence of the composition of dietary fat on the fatty acid spectrum of structural lipids in the brain has been established by the work from several laboratories. Generally a reduced intake of EFA results in the accumulation of fatty acids of the oleic acid series (trienes) and a reduction of polyunsaturated fatty acids of the linoleic acid series (tetraenes).[2,3,44,50,61,72,74,76a] The extent of these changes in the brain depend upon the time of onset of deficiency. Maximum level of 20:3(n-9) and 22:3(n-9) was found in the newborn brains which remained constant throughout the suckling period.[3] EFA deficiency induced by dietary excesses of oleic acid in the presence of limited linoleic acid produced brain fatty acid compositional changes earlier than diets containing only saturated fatty acids.[22,72,75] Compositional changes are reported in myelin[70,72,86,101,103,104] which parallel or even exceed that observed in whole brain, especially in the ethanolamine phosphoglyderides. Also of relevant interest is the decrease in odd chain fatty

acids in the myelin ethanolamine phosphoglycerides in EFA deficient rats.[72] Similarly, microsomes, mitochondria, synaptosomes[72,86,101,103,104] and endothelial cells[69] also show alterations in fatty acid pattern.

Docosapentaenoic acid (22:5(*n*-6)) is practically absent in tissue phospholipids but, when a diet high in linolenic acid ratio is fed to rats, an accumulation of this acid with a corresponding decrease in 22:6(*n*-3) content has been reported in the brain.[49,66,67,69] Galli *et al.*[44] have proposed to consider the ratio 22:5(*n*-6)/22:6(*n*-3) in tissue lipids as an index of relative linolenic acid deficiency. In linolenic acid deficiency, 22:5(*n*-6) is found to be increased.[52,107] Linolenic acid supplementation raised the proportion of 22:6(*n*-3) in brain glycerophosphatides.[107,108] The source of linolenic acid for the developing brain is considered to be milk and Sinclair and Crawford[96] have reported 3% 18:3(*n*-3) in dam's milk on the third postnatal day. Combined with its metabolites, the total *n*-3 fatty acids in milk is reported to be almost 8%. Our investigation failed to show any trace of 18:3(*n*-3) or its metabolites in milk when examined on the 12th day.[75]

D. *Origin of Eicosatrienoic Acid in the Developing Brain*

Galli *et al.*[41] have proposed the possibility that the brain is accumulating trienoic acid produced in the liver. The time course of the change in concentration of *n*-6 and *n*-9 series of fatty acids in the rat liver and brain was investigated. They found that in normal brains tetraenes accumulated during the first 40 days of life and there was no further deposition when brain growth was practically completed. In the EFA deficient brains, trienes were low during this time, after which there was an increase. The level of trienes did not reach that of tetraenes even after 100 days. During the course of active deposition of *n*-6 polyenes in the brain, a concomitant depletion of these compounds occurred in the liver. Hence, they have concluded that brain accumulates *n*-6 polyenes as long as they are available and the accumulation of *n*-9 polyenes start only when the former is no longer available.

To check whether the developing brain is capable of synthesizing 20:3(*n*-9), we injected [1-^{14}C]18:1 intracranially into 12 day old EFA deficient brains with a triene/tetraene ratio of 0.25 $\pm$ 0.08. The distribution of radioactivity in the fatty acids after 24 hr is given in Table 1. The data shows that developing brain is capable of synthesizing 20:3(*n*-9) from 18:1 in EFA deficiency.

TABLE 1. The Distribution of Radioactivity in Brain Fatty Acids after Intracranial Injection of [1-^{14}C]Oleic Acid

Fatty acid	Percent distribution of radioactivity
16:0	25.6
18:0	12.3
18:1	47.9
20:1	4.5
20:3(*n*-9)	9.7

E. *Biochemical Recovery of the Brain*

White *et al.*[114] studied the recovery of the developing brain from EFA deficiency and reported that animals deficient in EFA (even during the most actively growing period of the brain) were able to recover completely on restoration to the control diet for a sufficiently long period. On substitution of the control for the deficient diet, the *n*-6 family rebounded in a manner such that values for 20:4, 22:4 and 22:5 exceeded comparable figures in control animals. Concomitantly, the *n*-9 family receded below control levels and the *n*-3 acids remained or returned to normal. Odutuga[82] also found that, if the deficient diet is fed for not more than 7 weeks, the brain weight, as well as fatty acid

composition returns to normal values upon rehabilitation. On the other hand, 37 weeks of feeding a deficient diet reduced brain weight by 33% from which the brain did not recover even after long term rehabilitation.[83]

IV. METABOLISM OF ESSENTIAL FATTY ACIDS IN THE BRAIN

Extensive work from our laboratory has shown that fatty acids derived from diet or synthesized in other locations in the body are readily transported into the brain and rapidly incorporated into various lipids.[23,32 35,71] The preferred form of transport into the brain seems to be by way of free fatty acids rather than complex lipids.[31] Certain fatty acids can be synthesized by the developing brain itself and both the endogenous and exogenous fatty acids are β-oxidized, chain-elongated and desaturated in the brain.[24 26,94,111] Brain is endowed with $\Delta 5$, $\Delta 6$ and $\Delta 9$ desaturases.[27,121] Since $\Delta 8$ desaturase is lacking in the developing brain, the preferred route of synthesis of arachidonic acid from linoleic acid in the brain is: $18:2 \rightarrow 18:3 \rightarrow 20:3 \rightarrow 20:4$.[26] The rate-limiting step in the conversion of linoleate to arachidonate in the brain *in vivo* is the initial desaturation to γ-linolenic acid.[53] This desaturation activity in rat brain is very low soon after birth.[100]

Metabolism of intracerebrally injected ^{14}C labeled arachidonic acid, oleic acid and ^{3}H stearic acid in membrane phosphoglycerides has also been studied in the adult mouse brain.[102,105,120] Labeled free fatty acids are distributed among the synaptosomal-rich fraction, microsomal, myelin and cytosol fractions at 1 min after injection. However, incorporation into phospholipids and triacylglycerols occurred mainly in the microsomal and synaptosomal-rich fractions. Arachidonic acid was preferentially incorporated into the diacyl glycerophosphoinositols in the synaptosomal-rich fraction. *In vitro* studies on the incorporation of radioactive fatty acids and glucose into phospholipids of subsynaptosomal fractions of cerebral cortex suggest intrasynaptosomal phospholipid transport.[6] Lyles *et al.*[68] studied the effect of EFA deficiency upon the uptake of either of the above substances. The final incorporation levels were higher in deficient rats, especially with arachidonic acid.

Linolenic acid is regarded as an essential fatty acid since mammalian organism is incapable of its synthesis.[59a,89a] Some species require this acid growth but not the rat.[107] Linolenic acid need not be essential for general growth but since its major metabolite, 22:6(n-3), is distributed preferentially in nerve and synaptic endings of the brain, it may serve a specific function in these excitable tissues.[36] The lack of it is associated with learning impairment.[65,67] Recently Holman *et al.*[59b] have reported a case of human linolenic acid deficiency involving neurological abnormalities and the correction of the attendant symptoms by a preparation containing linolenic acid.

The metabolism of linolenic acid has been studied extensively in cultured brain cells,[121,122] in the developing rats after intraperitoneal injections[24,25,27,96] and after oral feeding.[53,94] Experiments tend to show that there is an initial β-oxidation of 18:3(n-3) in the developing brain but the nonoxidized 18:3(n-3) is converted to 22:6(n-3) which is retained in the brain as such for a longer time.[24] This unique sparing of n-3 fatty acids during the most rapid brain growth and myelination is probably to meet the membrane structural requirements of the developing brain.

Dwyer and Bernshon[36,37] studied the incorporation of [1-^{14}C]linolenate into lipids of 21 day old rat-brain during EFA deficiency and reported that the incorporation of n-4 fatty acids into phospholipids is not altered but fatty acid metabolism is affected. The incorporation of radioactivity into 22:6(n-3) was significantly higher in deficient animals while incorporation into saturated and monounsaturated fatty acids was less than controls, suggesting that the rate of degradation of linolenic acid and its metabolites was in some way reduced in the deficiency state. Conversion of linolenate to docosahexaenoate also proceeds by a pathway that does not require Δ^8 desaturase (i.e. by first desaturating 18:3 to 18:4 before elongation) since the developing brain lacks the Δ^8 desaturase.[28]

Dietary fatty acids 20:4 and 22:6 are also incorporated as such into the developing rat brain and the uptake of radioactivity is considerably greater than the uptake of label from either 18:2 or 18:3.[94,95] The faster turnover and oxidation of the short chain polyunsaturated fatty acids and the preferential uptake of preformed long chain polyunsaturated fatty acids by the brain can account for this observation.

V. MATERNAL EFA AND FETAL DEVELOPMENT

A. *Fetal Brain Growth*

When we produced fetal EFA deficiency through long term maternal dietary fatty acid imbalance,[74] there was a decrease in weight of 22.7, 31.7 and 18.5% in placenta, fetal body and fetal brain, respectively, in full term fetuses. The growth of 21 day old fetuses from rats on high oleic-low linoleic acid diet group was roughly equal to that of 18 day old controls. There was no significant difference in the concentration (mg/g) of total lipids. EFA deficiency and intrauterine growth and development have not been examined extensively.

B. *Effect on Fatty Acid Composition and Possible Role in Fetal Brain Metabolism*

The fetuses of dams on a high oleic acid diet accumulated eicosatrienoic acid in the fetal body, brain and placenta. The ratio of 20:3(*n*-9)/20:4(*n*-6) was more than the EFA deficiency index value of 0.4 in all these organs. The maternal brain contained only a very small amount of 20:3(*n*-9) as compared to fetal brain. As a result, the ratio of 20:3/204 was well below the deficiency index in maternal brain.

The levels of 18:1 in brain phospholipids, triglycerides and cholesteryl esters of term fetus was significantly elevated. Maternal brain was spared of this effect. The increase in 18:1 content of fetal body and brain must have resulted from the large excess of 18:1 in maternal circulation. From this, 20:3(*n*-9) could be synthesized in the fetus itself. Here again, the time of onset of EFA deficiency seems to be the deciding factor. When pregnant rats were fed a similar diet during the last week of gestation and the progeny continued on the same diet for a period of 3 months, we did not find an increase in 18:1 in the brain except in the EPG component of myelin; this indicates that the onset of EFA deficiency during the gestational period has a major influence on the newborn.

The stock diet as well as the oleic acid diet contained less than 0.5% of *n*-3 precursor acid, 18:3(*n*-3). The fetal brains from rats on oleic acid diet contained only a third of the 22:6(*n*-3) found in the brains from the control group. However, the EFA deficient diet did not alter the level of 22:6(*n*-3) in the maternal brain significantly (Fig. 2). Figure 2 shows the percent decrease in *n*-6 and *n*-3 families of fatty acids in the deficient group compared to the controls. The EFA deficient diet decreased the 22:6(*n*-3) and 20:4(*n*-6) fatty acids in the maternal plasma and this resulted in corresponding deficits in the fetus since maternal plasma is the only source of nutrients to the fetus. The decrease in the two families of fatty acids was not identical in the placenta, fetal body and brain. Placenta seemed to hold on to *n*-6 fatty acids, i.e. percent decrease was relatively smaller compared to fetal body and brain. Similarly, an attempt to conserve *n*-3 fatty acids by fetal brain was also observed.

Experiments (*in vivo*) by Sanders and Naismith[90,91] showed that normal fetal brain has the capacity to convert linoleate to arachidonate and linolenate to docosahexaenoate and a high Δ^6 desaturase activity is present in fetal brain as early as the 18th day of pregnancy. Thus, the fetal brain is not totally dependent on preformed polyunsaturated fatty acids. Crawford *et al.*[19] orally administered ^{14}C linoleic and linolenic acids to guinea pigs during gestation and found that there was a corresponding fall in the proportion of short-chain polyenoic acids and an increase in the proportion of long-chain polyenoic acids

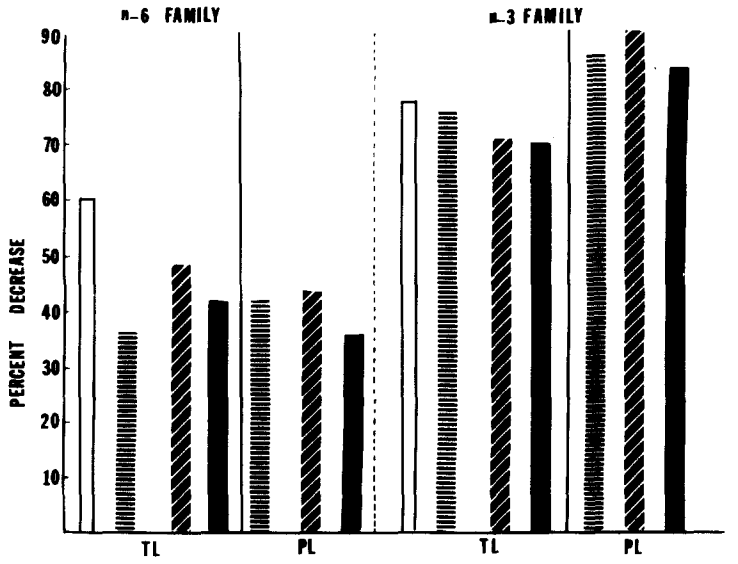

FIG. 2. Percent decrease in *n*-6 and *n*-3 families of fatty acids in the fetus. Values are percent decrease in the two families of fatty acids in the oleic acid diet group compared to controls. □ Maternal plasma total lipids, ▤ placenta ▨ fetal body ■ fetal brain.

from maternal blood to the fetal brain. A similar trend is found in human fetuses as well. They have proposed a selective screening of molecular species depending on the needs of the tissues concerned. Crawford *et al.*[19] have also proposed that fetal brain accumulates docosahexaenoic acid by a process of "biomagnification" and that the maternal liver, placenta and fetal liver and brain each augment the supply of this compound derived from less unsaturated derivatives of *n*-3. The tendency of the placenta to retain 20:4(*n*-6) at term may be for meeting the increased demand for prostaglandins needed for labor and other vascular changes.

C. *Gangliosides*

Although the precise physiological role of gangliosides remains unclear, their importance in the developing brain has been recognized. Gangliosides have been studied in relation to brain development.[11,12,76,123,124] Gangliosides undergo significant changes in fetal rat brain as well.[12,13] Female rats were maintained on a low (0.74% of total calories) and high (4.78% of total calories) EFA diet. The fetal brains were analyzed for gangliosides on the 15th and 20th day of gestation. In comparison with normal fed controls, no differences were found for the fetuses from rats on a high EFA diet, while in the low EFA group, the amount of gangliosides was significantly lower ($P < 0.001$) at the two gestational periods. The most striking differences were noticed by the 15th day for mono- and disialogangliosides while for the polysialoganglioside, GT_{1b}, the variations were evident only at day 20. This suggests that multiplication of neurons and glial cells is affected by maternal EFA deficiency. It is speculated that since gangliosides are thought to play a role as membrane receptors, cell-surface markers, in cell-cell interactions and cell-social behavior, these findings have a deeper physiological role.

VI. MATERNAL EFA DEFICIENCY AND LIPOGENESIS IN THE DEVELOPING BRAIN

Volpe and associates[109,110] have done considerable work on the development of brain fatty acid synthetase activity from fetal to weanling stages in normal rats and nutritional influences upon this enzyme. Synthetase activity in the brain was considerably higher in

fetal and suckling rats than in older animals. Until the second week of postnatal life, the total activity of the synthetase in brain is greater than the total activity in the liver. This enzyme system is unlike most of the others concerned with lipid biosynthesis in the nervous system since its activity is greatest in the fetal and young suckling animals and does not change markedly during the onset of myelination. Unlike the liver, the synthetase activity in the developing brain did not respond to the quantity of dietary fat in their short-term experiments. In the long-term experiments from birth to 62 days, the synthetase activity in the brain of rats on a high-fat diet containing 57% hydrogenated vegetable oil was 15% less than the activity in the brains of animals on a fat-free diet. The activity in the brains of animals on the high-fat diet and stock diet did not vary significantly whereas there was 40% higher activity ($P < 0.001$) in the brains of animals on the fat-free diet compared to the stock diet group. Thus, the fat-free diet, over the long term, produced an increase in the activity of fatty acid synthetase in the brain.

It is clearly established by the work of Smith and Abraham[98] that hepatic lipogenic activity, which is normally low in suckling pups, can be prematurely increased by weaning onto a fat-free diet on or after day 16 post-partum. Pups ingesting linoleate-deficient milk during days 6–15 showed greater hepatic lipogenesis than pups ingesting linoleate-rich milk. *De novo* synthesis of fatty acids in adipose tissues,[67a] lactating mammary glands[17] and testes[115] was increased in fat-deficient rats. In all the above studies, the animals were exposed to linoleate deficiency only during postnatal development stages or late in gestation. Under similar circumstances, the response of the developing brain was also minimal.[109] To study the effect of EFA deficiency produced in utero on the lipogenic capacity of the developing brain, female rats were started on a 5% fat diet (18:1 = 69%; 18:2 = 3.5%) two weeks before mating. They were switched to a more EFA deficient (18:1 = 90–95%; 18:2 = 2–2.5%) from the first day of gestation. Fifteen to twenty day old pups were given an intracranial injection of either [U-^{14}C]glucose or [3-^{14}C]β-hydroxybutyrate and sacrificed at 1, 4 and 24 hr intervals. In the group receiving a high oleic acid diet, decreases were observed in body, brain and liver weights and in brain total lipids (Table 2).

TABLE 2. Weights of Organs as Percent of Controls

	Term fetus	Postnatal day 15	Postnatal day 20
Body	68.3	66.3	65.7
Brain	81.4	86.1	88.8
Liver	—	63.0	74.0
Total brain lipids	94.0	71.5	86.4

Number of samples used, fetus 24; day 15 = 12; day 20 = 9.

At the initial 1 hr period, lipogenesis in the brain decreased significantly from both injected precursors in the high oleic acid diet group compared to age-matched controls on stock diet (Figs. 3 and 4).

During 4 and 24 hr, incorporation of ^{14}C from glucose in the test group increased steadily while the controls showed maximum incorporation at 1 hr and steadily decreased afterwards (Fig. 3).

The incorporation in the deficient brain reached control values in the 24 hr period. There was a continuous increase in the incorporation of ^{14}C from β-hydroxybutyrate in the controls whereas, in the animals on a high oleic acid diet, the incorporation increased between 1 and 4 hr but decreased at 24 hr (Fig. 4). A similar pattern was seen in fatty acid methyl esters and cholesterol (Figs 5 and 6).

Chain elongation based on radioactivity ratio in 16:0/18:0 decreased by 27% following glucose injection and 14.3% after hydroxybutyrate administration in the high oleic acid diet group (Table 3). Similarly, there was a 23% and 35% increase in Δ^9 desaturation

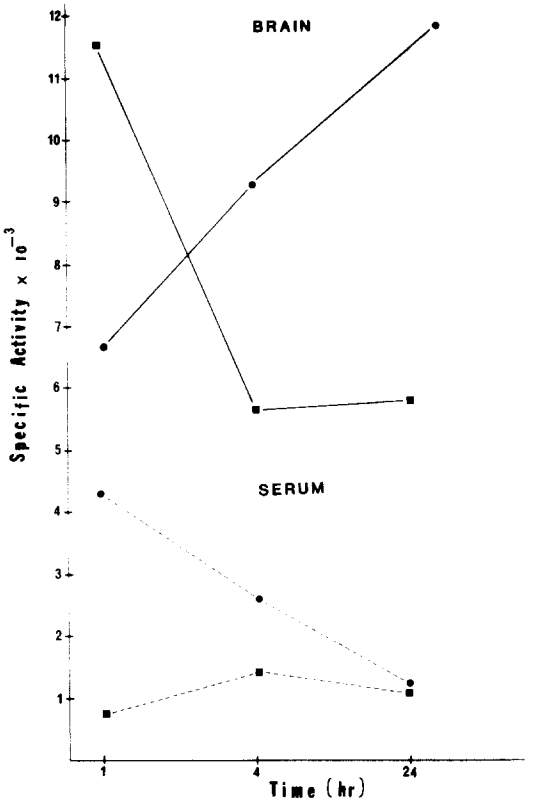

FIG. 3. Incorporation of ^{14}C from [U-^{14}C]glucose into brain and serum lipids. Fifteen day old rat pups were injected with 33 μCi of [U-^{14}C]glucose intracerebrally. Three samples were pooled for each time period. ■——■ control, ●——● oleic acid diet for brain, ■- - -■ control, ●- - -● oleic acid diet serum.

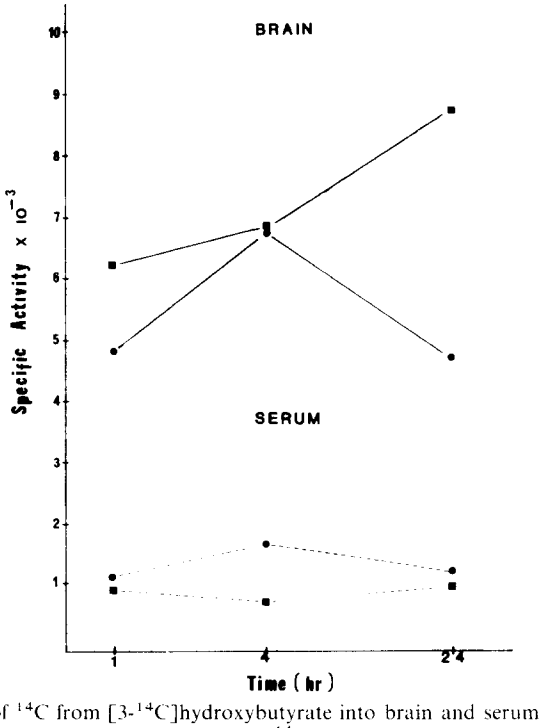

FIG. 4. Incorporation of ^{14}C from [3-^{14}C]hydroxybutyrate into brain and serum lipids. Twenty day old rat pups were injected with 24 μCi of [3-^{14}C]hydroxybutyrate intracerebrally. Four samples were pooled for each time period. ■——■ control, ●——● oleic acid diet for brain; ■- - -■ control, ●- - -● oleic acid diet for serum.

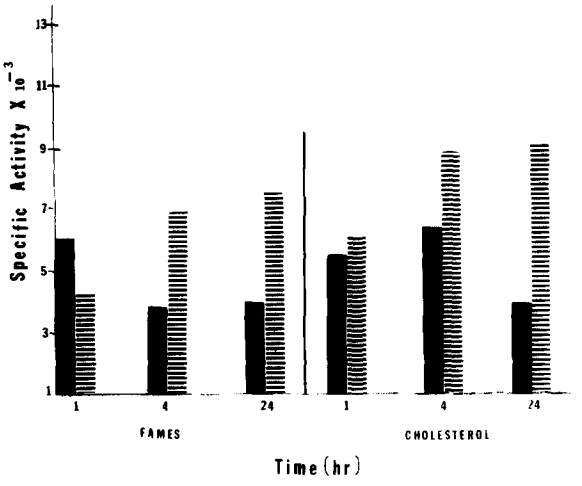

FIG. 5. Specific activity of fatty acids and cholesterol following injection of [U-^{14}C]glucose. Experimental conditions same as for Fig. 3. □ control, ▤ oleic acid diet.

following glucose and β-hydroxybutyrate, respectively (Table 4). These results clearly indicate a profound influence of EFA deficiency on brain lipogenesis in the progeny at a time when lipogenic enzymes are at peak activity. The triene/tetraene ratio in the brain was only 0.25 ± 0.08. The labeling patterns from glucose and β-hydroxybutyrate were not the same. The acetyl CoA formed does not go into the same homogeneous pool emphasizing the compartmentalization of the brain.[29,78,87] Incorporation of ^{14}C from hydroxybutyrate into fatty acids and cholesterol was inhibited throughout the study period whereas a significant decrease at the initial period of 1 hr was noticed with glucose. Thus, chain elongation and desaturation, the two primary mechanisms responsible for building up the entire membrane fatty acid architecture, are also affected. Δ^9 desaturate activity was shown to be increased in the liver in EFA deficiency.[21] A similar effect was seen in the brain also.

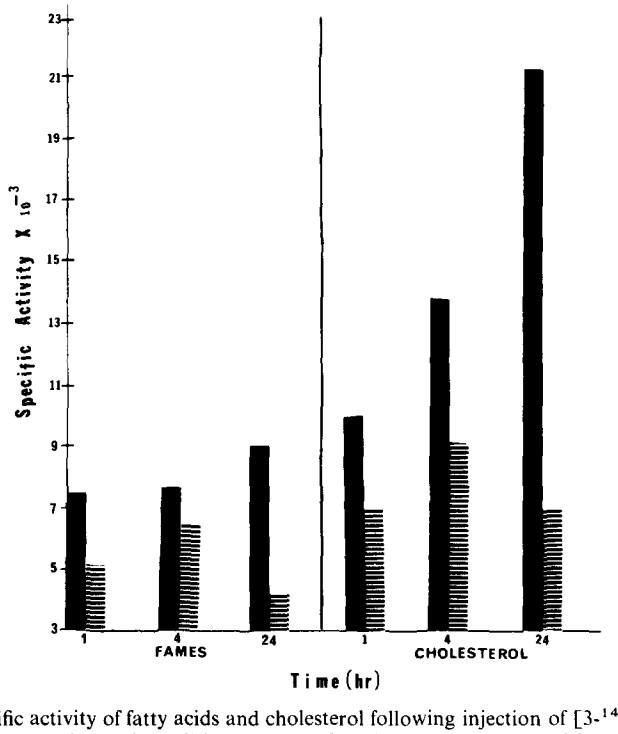

FIG. 6. Specific activity of fatty acids and cholesterol following injection of [3-^{14}C]hydroxybutyrate. Experimental conditions same as for Fig. 4. □ control, ▤ oleic acid diet.

TABLE 3. Index of Chain Elongation in the Brain

Tracer injected	Time (hr) Control	Ratio 16:0/18:0	Tracer injected	Time (hr) Control	Ratio 16:0/18:0
				1	1.4
	4	1.5		4	1.4
[U-^{14}C]Glucose	24	1.5	[3-^{14}C]Hydroxybutyrate	24	1.4
	Oleic Acid Diet			Oleic Acid Diet	
				1	1.6
	4	1.9		4	1.6
	24	1.9		24	1.6

The ratio of total activity (average counts/min/whole brain in 16:0 and 18:0 is taken as the index of chain elongation. Three brains were pooled for each time periods.

TABLE 4. Index of Desaturation in the Brain

Tracer injected	Time (hr) Control	Ratio 18/18:1	Tracer injected	Time (hr) Control	Ratio 18/18:1
				1	1.3
	4	1.3		4	1.3
[U-^{14}C]Glucose	24	1.3	[3-^{14}C]Hydroxybutyrate	24	1.3
	Oleic Acid Diet			Oleic Acid Diet	
				1	0.8
	4	1.0		4	0.9
	24	1.0		24	0.9

The ratio of total activity (average counts/min/whole brain) in 18:0 and 18:1 is taken as the index of desturation. Three brains were pooled for each time period.

The primary mechanisms responsible for triggering changes in lipogenic activity are still not known. Our results show that in EFA deficiency, the brain is incapable of regulating lipogenesis by adaptive enzyme synthesis like the liver. The dams or the pups did not show physical symptoms (scaly skin, etc.) of EFA deficiency. The triene/tetraene ratio in the brain was also below the EFA deficiency index value. The observed decrease in lipogenesis may be due to the inhibition of acetyl CoA carboxylase activity since this is one of the rate-limiting steps[87] in fatty acid synthesis.

VII. EFA DEFICIENCY AND LEARNING

It is highly speculative to associate chemical changes in structural lipids of the brain with specific changes in behavioral patterns. However, several attempts have been made in this direction.[42,66,67,78] Omission of EFA from the maternal diet during pregnancy and/or lactation was less detrimental to the physical, reflexological and neuromotor development of the progeny than did lack of energy, protein or pyridoxine[66] but such a diet irreversibly impaired learning behavior of the progeny; lactational deprivation tended to impair learning but differences from control were not significant; post-weaning EFA deficiency was without effect on learning performance.[67] This learning deficit induced by gestational lack of EFA was not reversible by supplementary EFAs during lactation or weaning. Recently Morgan *et al.*[78] also have shown that maternal dietary deprivation of EFA irreversibly impaired learning behavior of the progeny and suggested an association between brain *N*-acetylneuraminic acid and behavior. Thus, it seems that a relationship exists between prenatal exposure to EFA deficiency and learning potential of the progeny. It is difficult to correlate the poor learning ability of the mature progeny with the fatty acid profile at that time. In these animals, deprivation of EFA during gestation resulted in higher levels of saturates and monoenes and lower levels of *n*-6 and *n*-3 PUFA in brain EPG at birth. These rats were not EFA deficient by the triene/

tetraene ratio criterion. It is suggested that minimum proportions of phosphatides containing *n*-6 and *n*-3 PUFA are necessary for normal development of the brain in the prenatal period to ensure optimum learning ability.

Caldwell and Churchill[16] also found that feeding a fat-free diet to the dams throughout pregnancy exhibited impaired learning in the progeny which could not be reversed by a standard diet at parturition. They suggested that brain maturation was irreversibly impaired by fat deprivation during postnatal period and cited pathological evidences for a decrease in size of ganglionic cells from the frontal lobe of the brain. Paoletti and Galli[86] also observed impaired avoidance behavior in progeny from dams fed a fat-free diet during the final week of gestation.

Discrimination learning potential of rat progeny born to dams consuming diets with high and low 18:2/18:3 ratio has also been studied by Lamptey and Walker.[65] The diets contained 10% safflower oil (18:2/18:3 = Lamptey and Walker.[65] The diets contained 10% safflower oil (18:2/18:3 = 258) or 10% soybean oil (18:2/18:3 = 6) and were fed to female rats 6 weeks before mating. Progeny were continued on the diet for 210 days. There were no differences in the parameters employed in assessing the physical development of the progeny. There was no delay in the onset of normal reflexes and neuromotor coordination. The brain weight did not differ in the two groups. The low linolenate containing safflower oil diet lowered the level of 22:6(*n*-3) fatty acids of brain ($P < 0.001$) choline phosphoglycerides, EPG and mixed serine-inositol glycerophosphatides from birth through 210 days. This lowering of 22:6(*n*-3) was compensated for by higher proportions of 22:5(*n*-6) ($P > 0.001$) of the linoleate family. In the soybean oil fed group, 22:6(*n*-3) predominated at the expense of 22:5(*n*-6). The most significant observation of the study was the apparent lower proficiency in discrimination-learning in the mature rats on the safflower oil diet. In a similar unpublished observation quoted by the same authors rats raised on a corn oil containing diet where the 22:6(*n*-3) content of the brain EPG was intermediate between the safflower and soybean oil groups, the performance in the Y-maze test was also intermediate; while rats fed linoleate and linolenate-deficient hydrogenated coconut-oil exhibited the Y-maze performances most inferior to the three groups referred to above. Since linolenic acid metabolites are associated with the electrical responses of the photoreceptor membranes of the eye,[4,9] the above results may also reflect an impairment in the visual process of the rat.

From these data, it could be summarized that fat-free diet, diet with high 18:2/18:3 ratio all produced learning abnormalities in the progeny. Importance of balance in fatty acid composition in the diet fed during the critical period of brain development is implied. The protein content of the brains of these animals[67] were not determined and it is reported that the brain weight did not change but there was a decrease in lipid content at the time of testing. This would, in fact, alter the lipid:protein ratio of the brain similar to what we observed in brain microsomes. It would be interesting to see if there is any correlation between the altered lipid–protein ratio and the learning abnormalities.

VIII. MISCELLANEOUS

A. *Protein Utilization in EFA Deficiency*

The changes in membrane lipids seen after EFA deficiency would be expected to affect the function of proteins imbedded in such membranes. Changes in brain enzymes following EFA deficiency are described in a different section of this review. Curiously enough, when EFA deficiency was produced in the brains of rats which had been exposed to high oleic-low acid diet from the last week of gestation, the lipid to protein ratio in the brain microsomes increased.[72] This was due to an increase in protein rather than a decrease in lipids. The size or weight of the brain, however, was not affected. Rats raised on fat-free diet but not on purely EFA deficient diet have been reported to have increased rates of liver microsomal transformations of unsaturated fatty acids[99] but no such study has been carried out with brain microsomal preparations. Recently Henry *et al.*[55] induced EFA

deficiency by feeding 7% w/w hydrogenated coconut oil for 63 days. Protein utilization of the whole carcass measured as net protein utilization showed a fall. The deficient animals also had a high metabolic rate. They concluded that there is a reduction in protein utilization and the effect is secondary to the elevated metabolic rate.

B. *Sensitivity to Alcohol*

To determine whether or not an alteration in brain fatty acid composition could influence the responsiveness of mice to alcohol, Koblin and Deady[64] started mice on a 9% saturated fatty acid diet (with no significant 18:2 or 18:3) one week prior to mating and the offspring continued on the diet for 9 months. Decrease in 22:6(*n*-3) and increase in 20:3(*n*-9), 22:3(*n*-9) and 22:5(*n*-6) were noted. Ethanol-induced sleep onset times, sleep times and blood alcohol levels upon awakening were measured and compared to purina chow controls. The changes in fatty acids did not influence the sensitivity of the nervous system to alcohol.

On the other hand, John *et al.*[60] fed 10% coconut oil (w/w) to mice from mating and the diet was continued for the offspring throughout development for 35–40 days postnatally. Despite the absence of any diet-related differences in brain membrane phospholipids, a difference was noted in ethanol tolerance. The animals on a saturated fat diet required a greater blood ethanol concentration to produce loss of righting reflex than did mice receiving the control or herring oil supplemental diets. There was an apparent influx of saturated fatty acids into the brain membrane phospholipids after ethanol administration. More conclusive evidence *in vivo* is needed to correlate membrane saturation with the development of alcohol tolerance.

C. *Dietary EFAs and Brain Prostaglandins(PG)*

$PGF_{2\alpha}$, PGE_2, PGD_2 and Thromboxane B_2 (TXB_2) have been detected and measured in the central nervous system.[1,15,117,118] Formation of prostaglandins and thromboxanes from endogenous fatty acid percursors have been demonstrated in isolated brain tissue.[81,117,118] Since the precursor pool in the brain is affected by the dietary EFA, diet may modify the PG formation in the brain. Formation of $PGF_{2\alpha}$ was reduced in the brains of rats fed diets containing linseed oil and saturated fat from gestation through 3 months of age, as opposed to animals fed a diet containing olive oil.[45] The reduction was greater with linseed oil than with saturated fat. This was probably due to the interference in conversion of linoleic to arachidonic acid by linolenic acid.[59]

Brain free fatty acid levels increase during ischemia,[5,7] after treatment with convulsant drugs,[39] and after electroshock.[8] These reactions may be due to the possible activation of phospholipase probably involving cyclic AMP. Galli *et al.*[43,48] studied the release of free fatty acids especially arachidonic acid in brain during ischemia in EFA deficient rats. Levels of arachidonic acid were 40 and 70% lower before and 5 min after ischemia in the EFA deficient brains compared to controls. The increase of free arachidonic acid during ischemia was about 50% less than that of controls. Thus, in EFA deficiency, reduced stimulation of lipid hydrolysis occurs in the brain under conditions of ischemia. Galli and Spagnuolo[43] also found that during ischemia a slightly increased release of 20:3(*n*-9) also occurs which was less pronounced than that of 20:4, suggesting that with a given chain length tetraenes are preferentially released rather than the trienes. In other tissues, 18:1 and 20:3(*n*-9) are shown to be potent inhibitors of prostaglandin synthetase *in vitro*.[85,106,125] All these suggest reduced synthesis of PG formation in the brain during EFA deficiency. But the release of arachidonic acid and the synthesis of its prostaglandin metabolites are not always closely correlated in the brain.[46,47,84] Moreover, prostaglandin production in membrane phosphoglycerides does not strictly depend upon the total level of the precursor, arachidonic acid. Hassam *et al.*[54] have reported that when rabbits were fed EFA deficient diet for 8 weeks, there was no reduction in dihomo-γ-linolenic acid and arachidonic acid levels in the brain but there was a substantial reduction of PG

contents. (Dihomo-γ-linolenic acid is the precursor of PG, and arachidonic acid is the precursor of PGE_2 and $PGF_{2\alpha}$). Weston and Johnston[112] also found a reduction in brain PG synthesis in EFA deficient rats despite no significant difference in arachidonic acid. Galli *et al.*[45] reported that the reduction in PG production is greater than the changes in arachidonic acid levels in tissue phospholipids induced by diet. The regulatory mechanisms involved in the formation of prostaglandins from EFA is not yet fully understood. Hence, it is suggested that the dihomo-γ-linolenic acid and arachidonic acid utilized for PG production are derived from a metabolic pool which is more directly related to dietary EFA input rather than the principal membrane structural lipids.[54]

D. *EFA and Other Membrane-Bound Enzymes in the Brain*

Bernsohn and Spitz[10] studied the effect of EFA deficiency on monoamine oxidase [MAO, EC 1.4.3.4]in mitochondria, 5'-mononucleotidase [EC 3.1.3.5] in brain homogenates and glucose-6-phosphatase ([G6-Pase; EC 3.1.3.9] in microsomes, all of which are localized in various major membranes of the central nervous system. Rats were started on a fat-free diet at 10–15 days of gestation and continued for 130–140 days and showed EFA deficiency symptoms. There was a significant reduction of up to 57% MAO and up to 37% in 5'-mononucleotidase activity in the EFA deficient animals compared to controls. Supplementation of the diet with 18:2 or 18:3 increased the MAO activity to control or above control values, respectively. 5'-Mononucleotidase activity was restored to control values with 18:3 but 18:2 had not effect. Thus, it may be possible that the rate of synthesis of this enzyme is dependent on a member of the 18:3(*n*-3) series either directly or through prostaglandins, or the binding of the enzyme to the membrane in the brain requires this family of fatty acids. A fat-free diet did not reduce G-6-phosphatase activity. In fact, addition of 18:2 or 18:3 showed a significant inhibitory effect which could be due to an allosteric interaction of the enzyme with some metabolite or through the modulatory effect of prostaglandin.

Binaglia *et al.*[14] assayed ethanolamine-transphophatidylase activity in cultured brain cells with differing concentrations of 18:2 and 18:3 and found that the activation energy for the reaction was directly proportional to the arachidonate present in the membrane ethanolamine phosphoglycerides.

The activity of myelin marker enzyme, 2',3'-cyclic nucleotide-3'-phosphodiesterase (CN-Pase; EC 3.1.4.37), which is known to be decreased in protein and zinc deficiencies,[80,89] was unaffected in EFA deficiency in the developing rat brain.[70]

Sun and Sun[103] have reported an increase in the activity of (Na + K)-ATPase (EC 3.6.1.4) in total brain homogenates as well as isolated synaptosomal membranes from EFA deficient rats. The activity of this enzyme is dependent on the presence of membrane phospholipids and on the arrangement of the acyl side chain of the phospholipid molecules. Due to the fatty acid compositional changes produced in EFA deficiency, an increase in this enzyme activity may be necessary for compensatory purposes in order to maintain a normal level of ion transport in the brain.

Wheeler *et al.*[113] studied the membrane transport of the putative neurotransmitter, glutamic acid, *in vitro* and reported an alteration in the transport mechanism in cortical synaptosomes but the differences were not statistically significant.

Lack of EFA in the maternal diet during gestation and lactation depressed the activities of sialidase [EC 3.2.1.18] and cytidine monophosphate *N*-acetylneuraminic acid synthetase.[78]

E. *Requirement of Linoleic Acid*

The optimum requirement of EFA for growth and other cellular and biochemical functions has been set at 1–2% calories for human infants,[51] as well as rats.[58] The EFA deficiency index ratio of 0.4 is reached when dietary levels of linoleic acid provides 1% of total energy. The need to reassess the EFA deficiency index ratio of 0.4 is stressed by

Hassam *et al.*;[52] according to these workers, 3–4% of total energy is more realistic. We feel that the fatty acid composition of the whole diet is important in deciding the minimum requirement of linoleate. We have found that when saturated fatty acids are the sole source of fat with just 0.1% calories provided by 18:2, there was no accumulation of 20:3(*n*-9) in the brains even after 90 days of feeding, but in the presence of excess oleic acid, even 1% of calories provided by 18:2 is not effective in preventing the accumulation of 20:3(*n*-9).[75]

For humans, the FAO/WHO consultation[38] concluded that 3% of the dietary energy was required by way of EFA. Crawford[18] has evaluated the EFA requirement in pregnancy as 4.5% and in lactation as 5–7% of the dietary energy in an adequately nourished population.

F. *Vitamin E and EFA*

The demand for EFA is indirectly affected by low intake of vitamin E which normally protects PUFA against oxidation.[116] Recently it has been shown that vitamin E deficiency produces classical symptoms of EFA deficiency in growing rats.[30] By postnatal day 20, the growth was reduced by 50% of controls. This decrease correlated well with the disappearance of vitamin E in plasma. The liver showed significant decreases in polyenes of *n*-3 and *n*-6 series. There was accumulation of 20:3(*n*-9). In the brain, this effect was less pronounced and there was no accumulation of 20:3(*n*-9).

G. *Trans Fatty Acids*

The *trans* isomers of monoenoic acids have been shown to be deposited in animal tissues when fed in the diet,[88,93] and they intensify EFA deficiency in adult rats.[56] When male rats were fed margarine stock diet containing 45.8% *trans* for 195 days, brain fatty acids contained 7% *trans*. The triene/tetraene ratio in the brain increased significantly compared to hydrogenated coconut oil.

When female rats were fed a diet containing 5% partially hydrogenated corn oil (52.2% elaidate, 8.6% linoleate providing 1% calories) two weeks before making, and continued through pregnancy and lactation, the fetal brains contained less than 1% elaidic acid. This trend continued for 21 days postnatally.[73]

Experiments with radioactive *trans* fatty acids indicate uptake by the brain of 10–15 day old rats[63] and a maternal fetal transport.[77]

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