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THE ESSENTIALITY OF N-3 FATTY ACIDS FOR THE DEVELOPMENT AND FUNCTION OF THE RETINA AND BRAIN

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INTRODUCTION

During the past 15 years, evidence has accumulated for the nutritional essentiality of n-3 fatty acids, including alpha-linolenic acid (18:3n-3)[1] and its

[1]Fatty acid nomenclature: the first number indicates the length of the carbon chain; the second number (following the colon) specifies the number of double bonds; the third number, after n- (or omega-), gives the number of carbons from the first double bond to the methyl end. Abbreviations: DHA = docosahexaenoic acid, 22:6n-3; EPA = eicosapentaenoic acid, 20:5n-3; 18:2n-6 = linoleic acid; 18:3n-3 = linolenic acid; 20:4n-6 = arachidonic acid.

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longer-chain derivatives. In the past, n-3 fatty acids were included as essential fatty acids only because of their very limited ability to ameliorate some of the classic symptoms of essential fatty acid deficiency, such as dermatitis, growth retardation, and reproductive failure (80). All of these symptoms were completely corrected by fatty acids of the n-6 series (linoleic and arachidonic acids), and so the role of the n-3 series was seen, at best, as secondary. However, this viewpoint ignored the possibility that n-3 fatty acids have their own distinct role, as suggested by their special prominence in neural tissues. Recent work has increasingly distinguished the functions of n-3 fatty acids from those of the n-6 series. This review focuses on the newly defined and specific role of n-3 fatty acids in the function of the retina and nervous system. Other active areas of research on n-3 fatty acids have been reviewed by others and are not covered here. These include their hypolipidemic and antithrombotic effects (74, 75), their immunological and anticarcinogenic actions (88, 92), and their possible effect on gestation time (115). At least some of these effects are mediated by the ability of n-3 fatty acids to serve as precursors or inhibitors of prostanoid, thromboxane, or leukotriene synthesis (79, 98, 164).

N-3 and n-6 fatty acids are defined by the position of the double bond closest to the terminal methyl group of the fatty acid molecule, i.e. for n-3 fatty acids the first double bond occurs between the third and fourth carbons, whereas in the n-6 series it is between the sixth and seventh carbons (Figure 1). Within each series, elongation and desaturation occur without altering the methyl end of the molecule. These basic structures cannot be synthesized de novo by vertebrate animals and the n-3 and n-6 series are not interconvertible. Therefore, both series must be obtained from the diet. Precursor molecules from both series, namely linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3), compete for the same desaturation and elongation enzymes to form 20- and 22-carbon polyunsaturates, principally arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3 or DHA). These end products are incorporated mainly into the 2-position of phospholipids via de novo synthesis and acylation-deacylation mechanisms. The detailed metabolic pathways have been reviewed extensively (22, 81, 83, 103, 121, 122, 134, 136, 148) and are not elaborated here.

The 18-carbon fatty acids 18:3n-3 and 18:2n-6 are usually the primary dietary sources on land, as they are synthesized by many plants. The longer-chain members of each family may be biosynthesized or may be obtained directly from animal foods of land or marine origin. The longer-chain n-3 fatty acids, DHA and eicosapentaenoic acid (20:5n-3 or EPA), are especially rich in fish and other marine animals, as they are synthesized by phytoplankton at the base of the aquatic food chain (Table 1). The cat family is the only

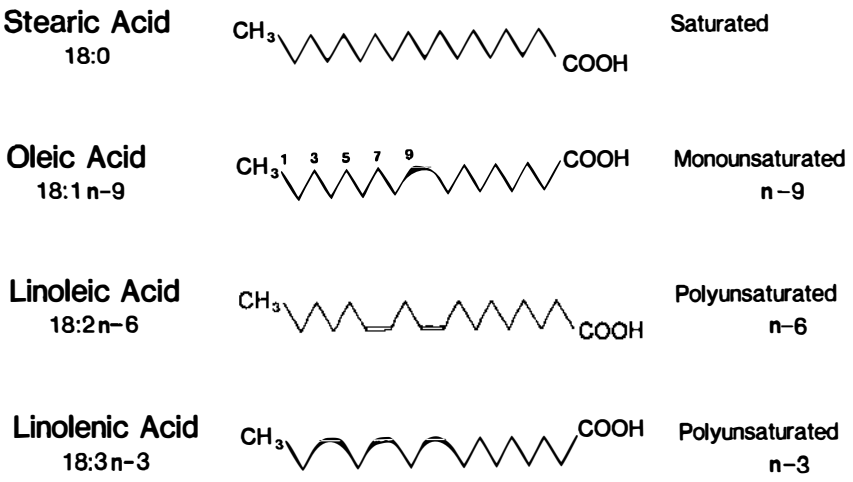

Figure 1 Schematic structures of representative saturated, monounsaturated, and polyunsaturated n-6 and n-3 fatty acids.

animal group that requires preformed longer-chain n-6 and n-3 fatty acids, because it lacks the Δ6-desaturase enzyme (128).

TISSUE DISTRIBUTION

Longer-chain fatty acids of both the n-3 and n-6 series are important components of tissue lipids, especially cell membrane phospholipids (Table 1). Arachidonic acid (20:4n-6) is present in all biological membranes and represents approximately 5–15% of the total fatty acids in most tissue phospholipids. DHA (22:6n-3) has a more specific tissue distribution. Although it makes up only a few percent of the fatty acids in most tissue lipids, DHA is present at very high levels in the retina, cerebral cortex, testis, and sperm (154). These high levels are found consistently in mammalian species, despite disparities in dietary intakes of n-3 fatty acids (54, 57). This specific and consistent tissue distribution provided the first evidence for an important role of DHA in these tissues.

In cerebral gray matter, DHA makes up approximately one third of the total fatty acid content of ethanolamine and serine phospholipids (112, 149). Among subcellular fractions of brain tissue, the highest levels of DHA are found in synaptosomes (53), synaptic vesicles (32), mitochondria, and microsomes. These cellular membranes can all be characterized as fluid and metabolically active. In contrast, white matter and its myelin fraction contain low levels of polyunsaturated fatty acids and high levels of saturated and monoun-

Table 1 Dietary sources, tissue distribution, and metabolic relationships of the common unsaturat fatty acids

Fatty acid series	Major members of series	Tissue distribution in mammals	Dietary sources
n-3	α-Linolenic acid 18:3n-3	Minor component of tissues	Some vegetable oil (soy, linseed, rapeseed) and leafy vegetables
	Eicosapentaenoic acid 20:5n-3	Minor component of tissues	Fish and shellfish
	Docosahexaenoic acid 22:6n-3	Major component of membrane phospholipids in retinal photoreceptors, cerebral gray matter, testes, and sperm	Fish and shellfish
n-6	Linoleic acid 18:2n-6	Component of most tissues	Most vegetable oil:
	Arachidonic acid 20:4n-6	Major component of most membrane phospholipids	Meat, liver, brain
	Docosapentaenoic acid 22:5n-6	Very low in most normal tissues, except testes, rabbit and guinea pig retina, and rabbit brain; replaces 22:6n-3 in n-3 fatty acid deficiency	None
n-9	Oleic acid 18:1n-9	Major component of many tissues, including white matter and myelin	Animal and vegetable fats
	Eicosatrienoic acid 20:3n-9	Accumulates in essential fatty acid deficiency	None

saturated fatty acids, especially oleic acid (18:1). These membranes also contain more cholesterol and sphingomyelin and are more stable and rigid (64).

Levels of DHA in the retina as a whole are similar to those in gray matter (3, 104). However, within the retina, much higher levels are found in photoreceptor cells, and more specifically in photoreceptor outer segments. These unique specialized structures consist of stacks of disks, each composed of a pair of membrane bilayers. Rod photoreceptor outer segments typically contain 500–2000 such membranous disks. The lipid composition of outer segment membranes is unusual in several respects. Their cholesterol content is only 8–10 mol% of total lipid, lower than in most other tissue membranes, whereas their content of phospholipid is very high, comprising 80–90 mol% of total lipid (65). In addition, the component phospholipids contain an exceptionally high level of polyunsaturated fatty acids. This lipid profile

creates a high degree of membrane fluidity (65). The percentage of DHA is higher than in any other neural subcellular component, comprising 30–65% of the total fatty acids in both phosphatidylethanolamine and phosphatidylserine in species from frog to human (6, 9, 146). In the 2-position of these phospholipids, which is characteristically occupied by polyunsaturated fatty acids, DHA accounts for 75–100% of the total fatty acids (10, 161). More surprisingly, the 1-position, which usually is occupied by saturated or monounsaturated fatty acids (10), also contains 5–25% DHA, depending on the species (4, 161). As can be inferred, this means that a substantial proportion of phospholipid molecules are "supraenes" containing DHA in both positions (15, 161). A number of other "supraene" molecular species, with various combinations of polyunsaturated fatty acids in the two positions, have also been identified in bovine rod outer segments (15).

Recent tracer studies with [^{3}H]glycerol and [^{14}C]-labeled n-3 fatty acids have revealed an active metabolism of n-3 fatty acids in the retina (19, 20, 85, 131, 166, 170) and brain (100, 113, 116, 129), and of the supraene species in the retina in particular (16, 73, 131, 132). Thus, didocosahexaenoyl species may have an important functional role.

A few animal species provide interesting exceptions to the stated generalizations about retinal fatty acid composition. Guinea pigs and young rabbits have lower levels of DHA (~20% of total fatty acids) in outer segment membranes, but these are balanced by higher levels of 22:5n-6, the most highly polyunsaturated member of the n-6 series (94, 109). A similar pattern occurs in rabbit cerebral cortex (96). Levels of 22:5n-6 are normally very low in the retina and neural tissues of most species. However, as described below, they are elevated in n-3 fatty acid deficiency.

Recently, it has been found that there are polyunsaturated fatty acids of the n-3 and n-6 series in nervous tissue with chain lengths considerably greater than 22 carbons. Aveldano and Sprecher have characterized 24–36 carbon species, containing 4–6 double bonds, in rod outer segment phosphatidylcholine (14, 17). These species comprised 13 mol% of total fatty acids, which suggests that they may serve a function rather than simply representing runaway elongation products. Abnormally elevated levels of very-long chain polyenes have been found in the brain of patients with Zellweger's syndrome (125), a condition in which peroxisomes are absent.

ACCUMULATION DURING DEVELOPMENT

In the rat, the major brain growth spurt occurs postnatally. DHA and arachidonic acid increase rapidly during the period of rapid brain cell division, between birth and 20 days of age (89, 142). The accumulation of myelin

lipids and their constituent monounsaturated fatty acids peaks later, between days 20 and 40. In human and nonhuman primates, on the other hand, a significant proportion of brain development occurs prenatally (42, 123). In rhesus monkeys at birth, the percentage of DHA in the retina and cerebral cortex is approximately half the adult value (104). During human brain development, the DHA and arachidonic acid content of the cerebrum and cerebellum increases 3–5 times during the last trimester, and a similar percentage increase takes place again during the first 12 postnatal weeks (45, 46, 99). There is a corresponding decrease in 22:5n-6 with age.

Blood levels of DHA and arachidonic acid are higher in human and monkey fetuses than in the pregnant mothers, despite lower blood levels of the precursors, linolenic and linoleic acids (56, 108). Crawford et al (56) described the progressive "biomagnification" of the percentage concentration of DHA from maternal liver to placenta to fetal liver to fetal brain. Thus, processes exist to enrich the fetal supply of DHA and to incorporate it selectively into the developing brain. However, the mechanisms for DHA synthesis, transport, and metabolism during gestation are poorly understood.

Cook (50, 51) examined the capacity of the developing rat brain to synthesize DHA and arachidonic acid from their precursor molecules by assaying desaturation and elongation activities in brain homogenate and microsomes, respectively. He found that the developing brain has a high capacity for desaturation. Despite this capacity, it is not clear to what extent brain DHA is derived specifically from brain metabolism of 18:3n-3, because there appears to be placental transfer of DHA to the fetus, and the liver also is able to synthesize DHA from 18:3n-3 (50, 111).

After birth, the milk of most mammals serves as a source of preformed DHA. The form in which dietary DHA reaches the brain is not known. Some DHA may be derived directly from the diet through the action of brain lipoprotein lipase on trigylceride in circulating chylomicrons or other triglyceride-rich lipoproteins (41). However, most of the circulating DHA in the developing rat is present in phospholipid, not triglyceride (141), and there may be direct transfer of intact phospholipid molecules into the brain. Albumin-bound free fatty acids have also been hypothesized as a DHA source for the brain (60).

An interesting finding has emerged from studies of cultured fetal brain cells. Bourre et al (28) found that such cells responded mitogenically to 18:n-3 and DHA in the medium. Although this effect was also seen with polyunsaturated fatty acids of the n-6 family, it may be significant, since n-3 fatty acids have not previously been associated with growth, except in fish (143).

DIETARY DEFICIENCY

In a wide range of animals, from insects to birds and mammals, fat-free diets or those lacking polyunsaturated fatty acids produce several easily observed pathologies, including retarded growth, reproductive failure, and changes in many organs including the skin, liver, and kidneys (36, 37, 80). These signs of classic essential fatty acid (EFA) deficiency are completely prevented or reversed by dietary n-6 fatty acids. Although n-3 fatty acids may produce very partial amelioration of some signs, they do not eliminate them and are not specifically required (66, 80).

Essential fatty acid deficient diets result in greater reductions in tissue levels of n-6 fatty acids than of n-3 fatty acids (1, 7, 24, 69, 119) and so do not induce a specific n-3 fatty acid deficiency. Substantial and specific depletion of tissue DHA requires diets with both low levels of n-3 fatty acids and high levels of n-6 fatty acids, which result in an abnormally high n-6:n-3 ratio. The degree of tissue DHA depletion is largely determined by this ratio (71, 102, 150) because high levels of linoleic acid suppress the metabolism of linolenic acid to DHA through competitive inhibition of Δ6-desaturase. Therefore, the diets most effective in lowering tissue DHA levels are those using pure linoleic acid as the only fat, or those based on natural fats such as safflower, sunflower, or peanut oil, in which the n-6:n-3 ratio exceeds 150:1.

A specific lack of dietary n-3 fatty acids produces an overt deficiency syndrome in some species of fish; symptoms include impaired growth and reproduction and a stress-induced shock syndrome that occasionally proves fatal (95, 143). Dietary n-3 fatty acids are also required by some species of insects for normal adult emergence and wing development (58, 145). In mammals, however, the effects of a specific dietary deficiency of n-3 fatty acids have been more difficult to detect and define. Even after dietary deprivation of n-3 fatty acids for two to four generations, rats show no skin changes or other gross pathology and no reduction in fertility, birth weight, or postnatal growth (91, 157). Some investigators have found increases in perinatal mortality (76) but others have not (1, 157). However, in recent years, a number of studies using more specific tests of retinal and brain function have led to the definition of a distinct set of effects of n-3 fatty acid deprivation (Table 2).

Studies in Rats

TISSUE COMPOSITION Tinoco and coworkers, in their important early studies of specific n-3 fatty acid deficiency, fed rats diets containing 1.25% purified linoleic acid as the only fat source. Among the many tissues examined, the brain and retina were most resistant to alterations in n-3 fatty acid

Table 2 Functional effects of n-3 fatty acid deficiency in experimental animals

Species	Effect	Reference
Rhesus monkey	Reduced visual acuity in infants	108
	Prolonged electroretinographic recovery time and delayed latencies	104, 106
Rat	Reduced electroretinogram amplitudes	24, 110, 160
	Impaired brightness discrimination	91, 165
Fish (trout)	Impaired growth and reproduction, stress-induced shock syndrome	143
Insects (moth)	Failure of normal adult emergence and wing development	58, 145

levels, and two to three generations of deprivation were required to produce maximal DHA depletion (155, 156). DHA levels in the retina and brain fell to 30–50% of control values in the first generation and to 8–20% in the second generation (5, 155, 156). As first observed by Mohrhauer & Holman (102), decreases in DHA were compensated by increases in the n-6 fatty acids 22:5n-6 and, to a lesser extent, 22:4n-6. Consequently, the total level of 22-carbon polyunsaturated fatty acids remained roughly constant, and the degree of polyunsaturation changed only slightly. This reciprocal replacement of DHA by long-chain n-6 fatty acids is a consistent finding in studies of n-3 fatty acid deficiency. It is particularly striking because, with a few exceptions, 22:5n-6 is normally present at very low levels in animal tissues. Therefore, the ratio of 22:5n-6 to 22:6n-3 in blood and tissues has been suggested as an index of n-3 fatty acid deficiency (70). The existence of a homeostatic mechanism for preserving chain length and polyunsaturation suggests that these properties are important for the normal function of retinal and neural membranes. The tissue content of the major phospholipid classes is not affected by n-3 fatty acid deprivation (91, 155), despite the change in fatty acid composition within each class.

Similar changes in tissue fatty acid composition have been found by a variety of other investigators using diets based on natural oils low in linolenic acid (11, 29, 71, 91). In rats fed sunflower oil for two generations, Bourre et al (29) assessed the relative impact on different brain cells and subcellular fractions at 15 and 60 days after birth. All brain components showed substantial reductions in DHA levels. However, isolated neurons, astroglia, and synaptosomes, which normally have high levels of DHA, showed less DHA depletion than oligodendrocytes or myelin, which normally have lower DHA levels. Cranial and peripheral nerves also appear to be relatively less resistant to DHA depletion (153). A complete compensatory increase in long-chain n-6 fatty acids occurred in all fractions except neurons, which showed an overall

reduction in the percentage composition of polyunsaturated fatty acids at 60 days of age (29).

When n-3 fatty acid deficient dams were transferred to a linolenic-acid-rich soybean oil diet on day 15, levels of DHA in all subcellular fractions increased slowly and returned to control values by day 90, while 22:5n-6 levels decreased (169). Initial increases in DHA occurred within a few days for synaptosomal, microsomal, and mitochondrial fractions but were delayed for about 20 days in myelin and sciatic nerve (30, 169).

N-3 fatty acid deficiency does not lead to elevated blood and tissue levels of 20:3n-9 or the ratio of 20:3n-9 to 20:4n-6, which are established indexes of total essential fatty acid deficiency (80). These changes occur only when both n-6 and n-3 fatty acids are in short supply, so that they no longer block the desaturation of oleic acid (18:1n-9).

RETINAL FUNCTION Several investigators have used the electroretinogram to assess changes in retinal function in n-3 fatty acid deficient rats. The electroretinogram measures the physiological response evoked from the retina by brief flashes of light. Benolken et al (24) were the first to correlate changes in retinal DHA levels with the amplitude of the electroretinogram in essential fatty acid deficient rats. Similar effects have since been reported in specifically n-3 fatty acid deficient rats fed diets based on peanut oil (110) or safflower oil (114) for two or more generations. Curiously, even in adult rats, periods of essential fatty acid deprivation as short as 24 days produced diminutions in the electroretinographic a-wave and b-wave (160); supplementation with linolenic acid, but not linoleic or oleic acids, maintained normal electroretinographic responses. The mechanism for this effect is not understood, because such brief deprivation produced no detectable change in the DHA content of rod outer segment membranes. The relationship of these electroretinographic changes to functional vision is not known, because none of these studies assessed the vision of deficient animals by direct behavioral methods.

In contrast to these studies, Leat and coworkers (94) found no changes in electroretinogram amplitude after three or four generations, either in albino rats fed fat-free diets supplemented with small amounts of 18:2n-6, or in guinea pigs fed diets based on sunflower oil. Unfortunately, all of the control rats, and many of those fed the experimental diets, suffered from an age-related retinal degeneration of unknown origin, so that electroretinogram amplitudes were small and variable in all groups. Lighting conditions may have been responsible, as albino rats are susceptible to retinal damage induced by normal levels of artificial indoor lighting. The parallel studies of guinea pigs are also difficult to interpret, but for a different reason. As noted above, guinea pigs and rabbits are the only two species known to have relatively low levels of DHA and high levels of 22:5n-6 in their photoreceptor outer

segment membranes (109). The biochemical data of Leat et al show that DHA comprises only 8% of total fatty acids in the whole retina of the guinea pig, while 22:5n-6 makes up 6%. It is possible, therefore, that in this species the shift in retinal composition induced by n-3 fatty acid deficiency may have less impact on function than in the rat and other species, which normally have far higher levels of retinal DHA.

BEHAVIOR Lamptey & Walker (91) evaluated several aspects of behavior in rats fed safflower oil diets for two generations. Levels of DHA in whole-brain phospholipids were reduced by 80–90% compared to soybean-oil-fed control rats. Reflexes and motor abilities developed normally but, when placed in a novel environment, the deficient rats showed fewer exploratory behaviors. They also performed more poorly in a maze-learning task that required discrimination between black and white stimuli. It is unclear whether this result was due to an effect on learning ability or on vision, although only a severe visual deficit would impair the ability to discriminate black from white.

Recently, Yamamoto et al (165) carried out a similar study of brightness discrimination learning using different testing methods. They also found poorer discrimination in n-3 fatty acid deficient rats, but, again, did not distinguish effects on learning from those on vision.

Studies in Nonhuman Primates

Rats and other small mammals have serious limitations as models for human nutritional deficiencies. Nonhuman primates can provide a much closer approximation to human metabolism and nutritional requirements, the rate and timing of human developmental processes, and the anatomy and functional capacities of the retina and brain. Rats are particularly poor models for the study of abnormalities in the human retina and vision, whereas higher primates are very similar to humans in their retinal anatomy and visual capacities. The most critical shared features are a central retina rich in cone photoreceptors and a specialized fovea, both of which underlie high levels of visual acuity.

TISSUE COMPOSITION When safflower oil diets were fed to rhesus monkeys throughout pregnancy and to their infants from birth, tissue DHA depletion was similar to that seen in rats after two generations of n-3 fatty acid deprivation. Around the time of birth, DHA levels in phosphatidylethanolamine were reduced to 50% of control levels in the retina and 25% in the cerebral cortex (104). By approximately two years of age, DHA levels in both tissues were 15–20% of normal. During this postnatal period, the proportion of DHA in retinal and brain phospholipids doubled in the control, soybean-oil-fed monkeys, but failed to show any increase in the deficient group. As in

rats, there was a quantitative substitution of 22:5n-6 for DHA in the tissues of deficient monkeys.

RETINAL FUNCTION AND VISION DHA depletion in these monkeys was associated with changes in the electroretinogram and in visual function. In contrast to the findings in rats, the amplitudes of both rod and cone electroretinograms were not diminished, but changes were found in the peak latencies of both rod and cone responses (105). A change was also seen in the recovery of the amplitude of the dark-adapted electroretinogram after an initial bright flash (104). In the deficient monkeys, the time required for full recovery was nearly doubled. These results suggest that n-3 fatty acid deficiency slows the retinal processes involved in both the generation of the electroretinogram response and the recovery of responsiveness.

A direct behavioral test of visual acuity was also used to assess the effects of n-3 fatty acid deficiency in these monkeys. The visual acuity of the deficient infants was 25% poorer than that of control infants at 4 weeks and 50% poorer at 8 and 12 weeks of age (108). This finding suggests that n-3 fatty acids are necessary for normal development of a functionally important aspect of vision. It is not known whether the deficit is due to changes in the retina, central visual system, or both.

BEHAVIOR In contrast to the visual loss found in n-3 fatty acid deficient infant monkeys, no effect was seen on performance in a spatial reversal learning task (107). Unlike the tests of learning used in the rat studies described above, this task did not require a visual discrimination. Additional tests of learning and other behaviors in these animals are called for.

REVERSIBILITY The reversibility of the biochemical and functional manifestations of n-3 fatty acid deficiency was tested in 10–24-month-old monkeys, after the deficiency was well established and brain growth was virtually complete (47). Following supplementation of the deficient diet with a fish oil mixture rich in EPA and DHA, levels of DHA in cerebral cortex phospholipids increased rapidly and reached control levels within 12 weeks, while levels of 22:5n-6 declined. Thus, even late in development, DHA is avidly incorporated and brain DHA composition can be altered by dietary manipulation. However, the abnormal peak latencies and recovery time of the electroretinographic response did not improve, even after 9 months of supplementation. This finding suggests that a lack of DHA during prenatal and early postnatal development irreversibly alters retinal function.

ESSENTIAL FATTY ACID DEFICIENCY-LIKE SYNDROME A syndrome resembling total essential fatty acid deficiency, including lesions of the skin and liver, was reported in juvenile *Cebus* monkeys fed corn oil diets that were

moderately low in linolenic acid and also, at least in some cases, deficient in vitamin D (63). However, these symptoms have not been seen by other investigators in *Cebus* monkeys fed corn-oil-based diets for longer periods of time (97, 124). Furthermore, these abnormalities do not occur in rhesus monkeys fed safflower-oil-based diets throughout gestation and infancy, despite much greater effects on blood and tissue levels of n-3 fatty acids (104).

Human Case Studies

Holman et al (82) reported a case study of peripheral neuropathy and intermittent blurred vision in a six-year-old child receiving total parenteral nutrition with a safflower oil emulsion as the only source of lipid. Changing the lipid source to a soybean oil emulsion, relatively rich in linolenic acid, correlated with the disappearance of clinical symptoms. The authors attributed the symptoms to alpha-linolenic acid deficiency, but similar problems can result from other nutritional or metabolic imbalances induced by long-term total parenteral nutrition (31, 84).

Bjerve et al (26, 27) have reported dermatitis and low blood levels of n-3 fatty acids in elderly nursing-home patients on long-term gastric tube feeding. The skin condition improved after treatment with 18:3n-3 or oil mixtures containing both n-3 and n-6 fatty acids. However, the initial diet appeared to be low in all essential fatty acids; the patients' baseline plasma levels of 18:2n-6 were low, while 20:3n-9 was elevated, as is characteristic of total essential fatty acid deficiency. After supplementation, erythrocyte levels of both n-3 and n-6 fatty acids increased. Therefore, the dermatologic symptoms may have been due to n-6 fatty acid deficiency. The authors did not evaluate the effects of supplements of pure n-6 fatty acids.

A series of recent papers has identified low levels of DHA in the plasma of patients with some types of retinitis pigmentosa, a retinal degenerative disease (8, 23, 49). The relationship of this finding to the disease process is unknown. In animal models of inherited retinal degenerations, reductions in retinal DHA levels either do not occur or appear to be a secondary effect of the degeneration (18, 138).

FUNCTIONAL EFFECTS IN BIOLOGICAL MEMBRANES

The functional importance of high levels of DHA in excitable membranes of the retina and nervous system is poorly understood. Several roles have been proposed. First, DHA influences the biophysical properties of membranes via its high polyunsaturation and perhaps via other specific properties such as the shape of the molecule. Second, DHA may modulate a number of aspects of lipid-protein interactions, including the activities of membrane-bound en-

zymes and receptors and the kinetics of membrane transport systems (33, 144, 147, 167). Third, DHA may be a precursor for functionally important lipoxygenase products. On the other hand, DHA may increase the vulnerability of membranes to the damaging effects of lipid peroxidation.

Biophysical Properties of DHA-rich Membranes

The presence of polyunsaturated fatty acids in the phospholipids of biological membranes produces increased membrane fluidity, compressibility, and permeability. DHA, with six double bonds, is unique in biological tissue in its high degree of polyunsaturation. Therefore, it has sometimes been assumed that its presence would produce membranes with maximum fluidity. However, membrane fluidity does not appear to be monotonically related to the number of double bonds. For example, studies of artificial phosphatidylcholine membranes with different fatty acids in the 2-position have shown increases in membrane fluidity and decreases in melting temperature only with addition of the first and second double bond. Further increases in the number of double bonds—e.g. substitution of 20:4n-6 or DHA for 18:2n-6—failed to produce additive effects and, in fact, produced changes in the opposite directions (52, 61). Rod outer segment membranes are exceptionally high in both DHA content and fluidity, but the latter is the combined result of several aspects of their lipid composition, not DHA content alone.

DHA's role may involve biophysical properties other than fluidity, such as the physical flexibility or compressibility of the membrane. Dratz et al (62) proposed that the presence of DHA in the 2-position of membrane phospholipids results in a membrane that is relatively thin but is able to thicken to accommodate changes in the conformation of integral membrane proteins, such as the visual pigment rhodopsin in photoreceptor outer segment membranes. He hypothesized that DHA takes a helical shape in the membrane that can uncoil and thereby allow for membrane expansion. Such expansion occurs during the conversion of metarhodopsin I to metarhodopsin II, a critical step in the visual excitation process (90).

Applegate & Glomset (12) performed sophisticated computer modeling of the conformation and packing properties of DHA, and concluded that it may promote tight, regular acyl chain packing arrays. These computer calculations must be viewed with caution, however, because the model only examines interactions between acyl chains; it cannot yet take into account other important contributors to lipid packing dynamics, such as the phospholipid head group, membrane cholesterol, or the aqueous medium.

Dratz and coworkers have also studied 1-16:0,2-22:6-phosphatidylcholine by ^{2}H-NMR in artificial membranes (59, 61, 118). They found evidence for a stronger interaction of rhodopsin with this species than with the same phospholipid containing 16:1n-7 in the 2-position, despite similar fluidity and

phase transition temperatures. They also found that the order of the 1-position chain, as well as the deformability of the membrane bilayer, was lower for the species containing DHA in the 2-position. Recently the new technique of spectral editing of the ^{1}H-NMR signal from samples of excised rat brain has demonstrated that the terminal methyl group has greater mobility in DHA than in any other fatty acid (13). ^{13}C-NMR detected considerable conformational motion of DHA's methylene units in bovine rod outer segment membranes (101).

Another approach to studying the physical chemistry of DHA in membranes is the use of fluorescent probes, such as diphenyl hexatriene. Van Blitterswijk et al (159) found, by measuring fluorescence polarization in well-defined liposomes, that phospholipids containing unsaturated fatty acyl chains, such as DHA, may create very fluid membrane domains, in part because of their poor association with cholesterol. Fluorescence anisotropy measurements with the same probe in lipid vesicles showed increased disorder for 1-16:0,2-22:6-phosphatidylcholine compared to the same phospholipid containing 20:4n-6 or 18:1n-9 in the 2-position (48). Increased disorder was also found in phosphatidylcholine isolated from the livers of fish-oil-fed rats, which have elevated levels of DHA (48).

Effects on Enzyme Activity

The activity of a number of membrane-bound enzymes is affected by changes in membrane fatty acid composition (33, 147). In addition, some of the enzymes involved in brain lipid metabolism show selectivity toward n-3 fatty acids as substrates. Some of the more interesting and recent work in these areas is summarized below.

5'-NUCLEOTIDASE Bernsohn & Spitz (25) found that the activity of this enzyme was lowered considerably in brain homogenates of rats fed a fat-free diet. Activity could be restored by dietary linolenic, but not linoleic, acid. However, the conclusion that this enzyme may be specifically sensitive to the membrane concentration of n-3 fatty acids is not supported by the results of Tinoco et al (155), which show no effect of n-3 fatty acid deficiency on enzyme activity.

β-N-ACETYL-D-GLUCOSAMINIDASE Orlacchio et al (117) found that the specific activity of lysosomal β-N-acetyl-D-glucosaminidase was higher when rat brain primary cell cultures were incubated with 18:3n-3 vs 18:2n-6. This higher activity was accompanied by a higher membrane unsaturation index.

ACETYLCHOLINESTERASE Preparations of synaptosomal membranes from rats fed a sunflower oil diet showed slightly higher activity of this enzyme than those from rats fed linolenic-acid-rich soybean oil (68). The possibility

of a specific interaction with n-3 fatty acids has been strengthened by the discovery that substantial quantities of 22:5 are covalently linked to this enzyme from erythrocytes (130). The 22:5 was evidently the n-3 isomer (W. L. Roberts, personal communication). Efforts are under way to confirm this observation in enzyme from nervous tissue.

ACYL-CoA SYNTHETASE Much work has been done in the laboratory of N. G. Bazan to characterize the acyl-CoA synthetase found in brain microsomes (22, 126, 127). There is apparently only one synthetase in the microsomes, and it displays a higher affinity for the more unsaturated substrates, such as 20:4n-6 and DHA. Inhibition studies revealed that the K_m for DHA was lower by a factor of four than that for 20:4n-6.

PHOSPHATIDYLETHANOLAMINE METHYLTRANSFERASE Tacconi & Wurtman (151) found in rat synaptosomal preparations that three fourths of the phosphatidylcholine newly synthesized from phosphatidylethanolamine by this enzyme contained pentaenes or hexaenes, compared to less than 5% in the bulk phosphatidylcholine in the membrane. This raises the possibility that the enzyme is specific for phosphatidylethanolamine containing DHA. Young rats fed a safflower- or sunflower-oil-based formula for 24 days had much higher activity of this enzyme in synaptic plasma membranes than animals fed a soybean-oil-based formula (77).

Ca^{2+}-ATPase Infante (86) has hypothesized that DHA-containing phosphatidylcholine is involved in the oligomerization of this enzyme in retina.

(Na^+,K^+)-ATPase This enzyme in vitro is activated by lipid. The enzyme from bovine cerebral cortex is activated better by DHA-rich bovine brain phosphatidylserine than by phosphatidylserine from egg yolk (152). The activation by brain phosphatidylserine was also much better than that obtained with any lecithin (e.g. from bovine brain, egg yolk, or synthetic dipalmitoyl lecithin) or any phosphatidylethanolamine (egg yolk or synthetic dipalmitoyl) that was tested. This raises the possibility of a specific interaction between this enzyme and phosphatidylserine containing DHA. There is evidence that this and other membrane proteins selectively associate with DHA-containing phosphatidylethanolamine and phosphatidylserine in synaptic and erythrocyte plasma membranes (134, 158). A model for this type of protein-phospholipid interaction is discussed by Salem et al (134).

Lipoxygenase Products of DHA

Lipoxygenase products of DHA, including mono- and dihydroxy derivatives, have now been identified in both canine retina (21) and rat brain homogenate (134). Furthermore, cultured retinoblastoma cells have been shown to retro-

convert some DHA to EPA, which suggests that DHA may provide an intracellular storage pool for the formation of EPA and thus its eicosanoid metabolites (166). These reports are especially interesting in light of recent experiments implicating lipoxygenase products of 20:4n-6 as potential second messengers in neurons (120, 137). It is tempting to speculate that the n-3 derivatives may also play a role, especially since the dietary balance of n-6 vs n-3 fatty acids is known to alter cerebral prostaglandin synthesis (35).

Lipid Peroxidation

It should be noted that a high content of DHA in tissues can have important biological disadvantages. In particular, it results in increased vulnerability to lipid peroxidation. In Down's syndrome fetuses, abnormally high brain levels of DHA have been observed, accompanied by increased lipid peroxidation (34). In the retina, the potential for oxidative damage and membrane disruption is compounded by exposure to light, high oxygen tension, and the presence of large quantities of retinoids, as a component of the visual pigment. The retinal degenerative changes induced by oxidants or by constant light exposure in rats are accompanied by a selective loss of DHA from outer segment membranes (162, 163). To counteract this vulnerability, the retina contains a variety of established or putative antioxidant and membrane-stabilizing substances, including vitamin E, vitamin C, selenium and glutathione, superoxide dismutase, zinc, taurine, and xanthophylls. Furthermore, outer segment membranes are continually renewed, with complete replacement occurring approximately every 10 days (168). It seems reasonable to speculate that the increased vulnerability of DHA-rich membranes, as well as the metabolic costs involved in antioxidant protection and membrane renewal, must be outweighed by imporant functional advantages such as increased speed and efficiency of signal transduction.

CONCLUSIONS AND IMPLICATIONS

A growing body of evidence from animal studies indicates that n-3 fatty acids are essential for normal development of the retina and perhaps the central visual system. Fewer studies of brain function and behavior are available, but they also support the functional importance of n-3 fatty acids. The direct clinical evidence for n-3 fatty acid deficiency in humans is less convincing, because it involves seriously ill patients supported by total parenteral or enteral feeding, and such cases are often difficult to interpret. The animal work suggests that a new approach to human studies is needed, one that focuses on prenatal and postnatal development and that uses more specific measures of retinal and brain function. Such studies are now being conducted in at least two laboratories (40, 158a).

With regard to the implications of experimental studies for human nutrition, the major concern is for the diets of infants, pregnant or lactating women, and patients maintained on synthetic diets. Total parenteral or enteral nutrition, which is required by many premature infants, can pose a special risk of fatty acid depletion because it provides high levels of glucose that inhibit the mobilization of fatty acids from adipose tissue stores (133).

After birth, infants may have a limited capacity to metabolize linolenic and linoleic acids to DHA and arachidonic acid, respectively. Sinclair (140) has shown that preformed dietary DHA is incorporated into developing brain at ten times the rate of linolenic acid. It is not known whether direct dietary sources of these longer-chain fatty acids are important for optimal postnatal development. Both DHA and arachidonic acids are usually present in human milk at levels sufficient to account for their postnatal accumulation in the brain (44). However, the maternal diet influences the fatty acid composition of the milk. Thus, mothers consuming vegetarian diets with no dietary sources of DHA have only a third as much DHA in their milk as women eating diets that include meat and fish (135). On the other hand, a high maternal intake of fish and fish oils, which are rich in EPA and DHA, produces large increases in the DHA content of the milk (78). Synthetic infant formulas generally use only vegetable oils as their lipid sources and thus contain only 18-carbon polyunsaturates. Even when these formulas supply ample linolenic acid, DHA levels in the infants' erythrocyte membrane phospholipids are much lower than in infants receiving either human milk or formulas supplemented with sources of longer-chain n-3 fatty acids (39, 40, 55). Such changes in erythrocytes membranes have been used as an index of fatty acid changes in the brain because, at least in rats, the fatty acid compositions of the two tissues change in parallel after dietary manupulation (38).

N-3 fatty acids are now widely regarded as essential nutrients, and recommendations for human dietary intakes have been proposed (66). However, no requirement has been officially established. Neither the *Recommended Dietary Allowances* of the National Research Council Food and Nutrition Board (67) nor the American Academy of Pediatrics guidelines for infant formulas (2) contain a recommendation for n-3 fatty acid intake. The available experimental studies have identified effects of very low intakes of n-3 fatty acids, but have not attempted to determine a precise minumum requirement. They do, however, provide a basis for estimates of advisable minimum intake. The ratio of n-6 to n-3 fatty acids is a useful way of expressing a recommendation because, within the usual range of fat intakes, this ratio appears to have a close relationship to tissue DHA levels. N-6:n-3 ratios of 4:1 to 10:1 appear to be optimal, whereas ratios above 150:1, as in safflower, sunflower, and peanut oils, produce substantial DHA tissue depletion and

retinal abnormalities in experimental animals. Ratios above 50:1, as in corn oil, still produce clear effects on tissue DHA. Therefore, if the linoleic acid content of the diet is approximately 6–8% of total calories, as in most human diets, a minimum linolenic acid content of 1% would appear to be advisable. If the diet contains preformed DHA, as in fish and shellfish, then lower intakes may be sufficient.

In human milk, n-3 fatty acids normally represent 1.5–2.5% by weight of total fatty acids or 0.7–1.3% of calories, and the n-6:n-3 ratio is 4:1 to 10:1 (66, 72, 87). We would recommend that these values be used as a guideline for the composition of human infant formulas. Human infant formulas prepared with soy oil (n-6:n-3 ratio of 7:1) as a major fat source supply ample amounts of linolenic acid. However, those with polyunsaturates supplied solely by corn oil (n-6:n-3 ratio of ~ 50:1) may provide less than an optimum level.

There is now considerable evidence that n-3 fatty acids are essential nutrients, with their own specific functions distinct from those of n-6 fatty acids. However, many questions remain to be answered. The effects of n-3 fatty acids on basic molecular mechanisms within biological membranes are poorly understood. The developmental period of vulnerability to the functional effects of n-3 fatty acid deprivation has not been defined, nor the capacity for recovery at different ages. It is not clear whether dietary DHA is specifically required for optimal retinal and brain development. And little has yet been done to explore the effects of n-3 fatty acids on brain function and behavior. These and many other aspects of n-3 fatty acids are fruitful areas for future research.

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Literature Cited

1. Alling, C., Bruce, A., Karlsson, I., Svennerholm, J. 1974. The effect of different dietary levels of essential fatty acids on lipids of rat cerebrum during maturation. *J. Neurochem.* 23:1262–70
2. American Academy of Pediatrics Committee on Nutrition. 1976. *Pediatrics* 57:278–85
3. Anderson, R. E. 1970. Lipids of the ocular tissues. IV. A comparison of the phospholipids from the retina of six mammalian species. *Exp. Eye Res.* 10:339–44
4. Anderson, R. E., Andrews, L. D. 1982. Biochemistry of retinal photoreceptor membranes in vertebrates and invertebrates. In *Visual Cells in Evolution,* ed. J. Westfall, pp. 1–22. New York: Raven
5. Anderson, R. E., Benolken, R. M., Jackson, M. B., Maude, M. B. 1977. The relationship between membrane fat-

ty acids and the development of the rat retina. In *Function and Biosynthesis of Lipids*, ed. N. G. Bazan, R. R., Brenner, N. M. Giusto, pp. 547–59. New York: Plenum

6. Anderson, R. E., Benolken, R. M., Dudley, P. A., Landis, D. J., Wheeler, T. G. 1974. Polyunsaturated fatty acids of photoreceptor membranes. *Exp. Eye Res.* 18:205–13
7. Anderson, R. E., Maude, M. B. 1971. Lipids of ocular tissue. VIII. The effects of essential fatty acid deficiency on the phospholipids of the photoreceptor membranes of rat retina. *Arch. Biochem. Biophys.* 151:270–76
8. Anderson, R. E., Maude, M. B., Lewis, R. A., Newsome, D. A., Fishman, G. A. 1987. Abnormal plasma levels of polyunsaturated fatty acid in autosomal dominant retinitis pigmentosa. *Exp. Eye Res.* 44:155–59
9. Anderson, R. E., Risk, M. 1971. Lipids of ocular tissue. IX. The phospholipids of frog photoreceptor membranes. *Vis. Res.* 14:129–31
10. Anderson, R. E., Sperling, L. 1971. Lipids of ocular tissues. VII. Positional distribution of the fatty acids in the phospholipids of bovine retina rod outer segments. *Arch. Biochem. Biophys.* 144: 673–77
11. Anding, R. H., Hwang, D. H. 1986. Effects of dietary linolenate on the fatty acid composition of brain lipids in rats. *Lipids* 21:697–701
12. Applegate, K. R., Glomset, J. A. 1986. Computer-based modeling of the conformation and packing properties of docosahexaenoic acid. *J. Lipid Res.* 27:658–80
13. Arus, C., Westler, W. M., Barany, M., Markley, J. L. 1986. Observation of the terminal methyl group in fatty acids of the linolenic series by an new ^{1}H NMR pulse sequence providing spectral editing and solvent suppression. Application to excised frog muscle and rat brain. *Biochemistry* 25:3346–51
14. Aveldano, M. I. 1987. A novel group of very long chain polyenoic fatty acids in dipolyunsaturated phosphatidylcholines from vertebrate retina. *J. Biol. Chem.* 262:1172–79
15. Aveldano, M. I., Bazan, N. G. 1983. Molecular species of phosphatidylcholine, -ethanolamine, -serine, and -inositol in microsomal and photoreceptor membranes of bovine retina. *J. Lipid Res.* 24:620–27
16. Aveldano, M. I., Pasquare de Garcia, S. J., Bazan, N. G. 1983. Biosynthesis of molecular species of inositol, choline, serine, and ethanolamine glycerophospholipids in the bovine retina. *J. Lipid Res.* 24:628–38
17. Aveldano, M. I., Sprecher, H. 1987. Very long chain (C_{24} to C_{36}) polyenoic fatty acids of the n-3 and n-6 series in dipolyunsaturated phosphatidlycholines from bovine retina. *J. Biol. Chem.* 262:1180–86
18. Batey, D. W., Mead, J. F., Eckhert, C. D. 1986. Lipids of the retinal pigment epithelium in RCS dystrophic and normal rats. *Exp. Eye Res.* 43:751–57
19. Bazan, H. E. P., Careaga, M. M., Sprecher, H., Bazan, N. G. 1982. Chain elongation and desaturation of eicosapentaenoate to docosahexaenoate and phospholipid labeling in the rat retina in vivo. *Biochim. Biophys. Acta* 712:123–28
20. Bazan, H. E. P., Sprecher, H., Bazan, N. G. 1984. De novo biosynthesis of docosahexaenoyl-phosphatidic acid in bovine retinal microsomes. *Biochim. Biophys. Acta* 796–11–19
21. Bazan, N. G., Birkle, D. L., Reddy, T. J. 1984. Docosahexaenoic acid (22: 6n-3) is metabolized to lipoxygenase reaction products in the retina. *Biochem. Biophys. Res. Commun.* 125: 741–47
22. Bazan, N. G., Reddy, T. S., Bazan, H. E. P., Birkle, D. L. 1986. Metabolism of arachidonic and docosahexaenoic acids in the retina. *Prog. Lipid Res.* 25:595–606
23. Bazan, N. G., Scott, B. L., Reddy, T. S., Pelias, M. Z. 1986. Decreased content of docosahexaenoate and arachidonate in plasma phospholipids in Usher's syndrome. *Biochem. Biophys. Res. Commun.* 141:600–4
24. Benolken, R. M., Anderson, R. E., Wheeler, T. G. 1973. Membrane fatty acids associated with the electrial response in visual excitation. *Science* 182:1253–54
25. Bernsohn, J., Spitz, F. J. 1974. Linoleic and linolenic acid dependency of some brain membrane-bound enzymes after lipid deprivation in rats. *Biochem. Biophys. Res. Commun.* 57:293–98
26. Bjerve, K. S., Mostad, I. L., Thoresen, L. 1987. Alpha-linolenic acid deficiency in patients on long-term gastric-tube feeding: estimation of linolenic acid and long-chain unsaturated n-3 fatty acid requirement in man. *Am. J. Clin. Nutr.* 46:570–76
27. Bjerve, K. S., Fischer, S., Alme, K. 1987. Alpha-linolenic acid deficiency in man: Effect of ethyl-linolenate on plasma and erythrocyte fatty acid composi-

tion and biosynthesis of prostanoids. *Am. J. Clin. Nutr.* 46:570–76
28. Bourre, J. M., Faivre, A., Dumont, O., Nouvelot, A., Loudes, C., et al. 1983. Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. *J. Neurochem.* 41:1243–42
29. Bourre, J. M., Pascal, G., Durand, G., Masson, M., Dumont, O., 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.* 43:343–48
30. Bourre, J. M., Youyou, A., Durand, G., Pascal, G. 1987. Slow recovery of the fatty acid composition of sciatic nerve in rats fed a diet initially low in n-3 fatty acids. *Lipids* 22:535–38
31. Bozian, R. C., Moussavian, S. N. 1982. Human linolenic acid deficiency. *Am. J. Clin. Nutr.* 36:1252–54
32. Breckenridge, W. C., Morgan, I. G., Zanetta, J. P., Vincendon, G. 1973. Adult rat brain synaptic vesicles. II. Lipid composition. *Biochim. Biophys. Acta* 320:681–86
33. Brenner, R. R. 1984. Effect of unsaturated acids on membrane structure and enzyme kinetics. *Prog. Lipid Res.* 23:69–96
34. Brooksbank, B. W. L., Martinez, M., Balazs, R. 1985. Altered composition of polyunsaturated fatty acyl groups in phosphoglycerides of Down's syndrome fetal brain. *J. Neurochem.* 44: 869–74
35. Brown, M. L., Marshall, L. A., Johnston, P. V. 1984. Alterations in cerebral and microvascular prostaglandin synthesis by manipulation of dietary essential fatty acids. *J. Neurochem.* 43:1392–1400
36. Burr, G. O., Burr, M. M. 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem.* 82:345–67
37. Burr, G. O., Burr, M. M. 1930. On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* 86:587–621
38. Carlson, S. E., Carver, J. D., House, S. G. 1986. High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. *J. Nutr.* 116:718–25
39. Carlson, S. E., Rhodes, P. G., Ferguson, M. G. 1986. Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *Am. J. Clin. Nutr.* 44:798–804
40. Carlson, S. E., Rhodes, P. G., Rao, V. S., Goldgar, D. E. 1987. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. *Pediatr. Res.* 21:507–10
41. Chajek, T., Stein, O., Stein, Y. 1977. Pre- and postnatal development of lipoprotein lipase and hepatic triglyceride hydrolase activity in rat tissues. *Atherosclerosis* 26:549–61
42. Cheek, D. B., ed. 1975. *Fetal and Postnatal Cellular Growth: Hormones and Nutrition,* pp. 3–22. New York: Wiley
43. Ciba Foundation Symposium. 1972. *Lipids, Malnutrition and the Developing Brain.* Amsterdam: Elsevier
44. Clandinin, M. T., Chappell, J. E., Heim, T. 1982. Do low weight infants require nutrition with chain elongation-desaturation products of essential fatty acids? *Prog. Lipid Res.* 21:901–4
45. Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R., Chance, G. W. 1980. Intrauterine fatty acid accretion rates in human brain: Implications for fatty acid requirements. *Early Hum. Dev.* 4:121–29
46. Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R., Chance, G. W. 1980. Extrauterine fatty acid accretion in infant brain: Implications for fatty acid requirements. *Early Hum. Dev.* 4:131–38
47. Connor, W. E., Neuringer, M., Lin, D. 1985. The incorporation of docosahexaenoic acid into the brain of monkeys deficient in omega-3 essential fatty acids. *Clin. Res.* 33:598A
48. Conroy, D. M., Stubbs, C. D., Belin, J., Pryor, C. L., Smith, A. D. 1985. The effects of dietary (n-3) fatty acid supplementation on lipid dynamics and composition in rat lymphocytes and liver microsomes. *Biochim. Biophys. Acta* 861:457–62
49. Converse, C. A., Hammer, H. M., Packard, C. J., Shepherd, J. 1983. Plasma lipid abnormalities in retinitis pigmentosa and related conditions. *Trans. Ophthalmol. Soc. UK* 103:508–12
50. Cook, H. W. 1978. In vitro formation of polyunsaturated fatty acids by desaturation in rat brain: some properties of the enzymes in developing brain and comparisons with liver. *J. Neurochem.* 30:1327–34
51. Cook, H. W. 1982. Chain elongation in the formation of polyunsaturated fatty acids by brain: some properties of the

microsomal system. *Arch. Biochem. Biophys.* 214:695–704
52. Coolbear, K. P., Bearde, C. B., Keough, K. M. W. 1983. Gel to liquid-crystalline phase transitions of aqueous dispersions of polyunsaturated mixed-acid phosphatidylcholines. *Biochemistry* 22:1466–73
53. Cotman, C., Blank, M. L., Moehl, A., Synder, F. 1969. Lipid composition of synaptic plasma membranes isolated from rat brain by zonal ultracentrifugation. *Biochemistry* 8:4606–12
54. Crawford, M. A., Casperd, N. M., Sinclair, A. J. 1976. The long chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. *Comp. Biochem. Physiol.* 54B:395–401
55. Crawford, M. A., Hassam, A. G., Hall, B. M. 1977. Metabolism of essential fatty acids in the human fetus and neonate. *Nutr. Metab.* 21:187–88
56. Crawford, M. A., Hassam, A. G., Williams, G. 1976. Essential fatty acids and fetal brain growth. *Lancet* 1:452–53
57. Crawford, M. A., Sinclair, A. J. 1972. Nutritional influences in the evolution of mammalian brain. See Ref. 43, pp. 267–87
58. Dadd, R. H. 1983. Long-chain polyenoics and the essential dietary fatty acid requirement of the waxmoth, *Galleria mellonella. J. Insect Physiol.* 29:779–86
59. Deese, A. J., Dratz, E. A., Dahlquist, F. W., Paddy, M. R. 1981. Interaction of rhodopsin with two unsaturated phosphatidylcholines: a deuterium nuclear magnetic resonance study. *Biochemistry* 20:6420–27
60. Dhopeshwarkar, G. A. 1973. Uptake and transport of fatty acids into the brain and the role of the blood-brain barrier system. *Adv. Lipid Res.* 11:109–42
61. Dratz, E. A., Deese, A. J. 1986. The role of docosahexaenoic acid (22:6n-3) in biological membranes: examples from photoreceptors and model membrane bilayers. See Ref. 139, pp. 319–51
62. Dratz, E. A., Ryba, N., Watts, A., Deese, A. J. 1987. Studies of the essential role of docosahexaenoic acid (DHA), 22:6ω3, in visual excitation. *Invest. Ophthalmol. Vis. Sci.* 28(Suppl. 3): 96 (Abstr.)
63. Fiennes, R. N. T., Sinclair, A. J., Crawford, M. A. 1973. Essential fatty acid studies in primates: linolenic acid requirements of Capuchins. *J. Med. Primatol.* 2:155–69
64. Fleischer, S., Rouser, G. 1965. Lipids of subcellular particles. *J. Am. Oil Chem. Soc.* 42:588–607
65. Fliesler, S. J., Anderson, R. E. 1983. Chemistry and metabolism of lipids in the vertebrate retina. *Prog. Lipid Res.* 22:79–131
66. Food and Agriculture Organization. 1977. Food and Nutrition Paper 3: *Dietary Fats and Oils in Human Nutrition.* Rome: Food Agric. Org.
67. Food and Nutrition Board, National Research Council. 1980. *Recommended Dietary Allowances,* pp. 33–35. Washington, DC: Nat. Acad. Sci. 9th ed.
68. Foot, M., Cruz, T. F., Clandinin, M. T. 1983. Effect of dietary lipid on synaptosomal acetylcholinesterase activity. *Biochem. J.* 211:507–9
69. Futterman, S., Downer, J. L., Hendricksen, A. 1971. Effect of essential fatty acid deficiency on the fatty acid composition, morphology, and electroretinographic response of the retina. *Invest. Ophthalmol.* 10:151–56
70. Galli, C., Agradi, E., Paoletti, R. 1974. The (n-6) pentaene: (n-3) hexaene fatty acid ratio as an index of linolenic acid deficiency. *Biochim. Biophys. Acta* 369:142–45
71. Galli, C., Trzeciak, H. I., 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* 248:449–54
72. Gibson, R. A., Kneebone, G. M. 1981. Fatty acid composition of human colostrum and mature breast milk. *Am. J. Clin. Nutr.* 34:252–57
73. Giusto, N. M., De Boschero, M. I., Sprecher, H., Aveldano, M. I. 1986. Active labeling of phosphatidylcholines by [1-^{14}C]docosahexaenoate in isolated photoreceptor membranes. *Biochim. Biophys. Acta* 860:137–48
74. Glomset, J. A. 1985. Fish, fatty acids, and human health. *N. Engl. J. Med.* 312:1253–54
75. Goodnight, S. H., Harris, W. S., Connor, W. E., Illingworth, D. R. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis* 2:87–113
76. Guesnet, P., Pascal, G., Durand, G. 1986. Dietary α-linolenic acid deficiency in the rat. I. Effects on reproduction and postnatal growth. *Reprod. Nutr. Dev.* 26:969–85
77. Hargreaves, K. M., Clandinin, M. T. 1987. Phosphatidylethanolamine methyltransferase: evidence for influence of diet fat on selectivity of substrate

for methylation in rat brain synaptic plasma membranes. *Biochim. Biophys. Acta* 918:97–105
78. Harris, W. S., Connor, W. E., Lindsey, S. 1984. Will dietary omega-3 fatty acids change the composition of human milk? *Am. J. Clin. Nutr.* 40:780–85
79. Herold, P. M., Kinsella, J. E. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* 43:566–98
80. Holman, R. T. 1968. Essential fatty acid deficiency. *Prog. Chem. Fat* 9:275–348
81. Holman, R. T. 1986. Nutritional and biochemical evidences of acyl interaction with respect to essential polyunsaturated fatty acids. *Prog. Lipid Res.* 25:29–39
82. Holman, R. T., Johnson, S. B., Hatch, T. F. 1982. Human linolenic acid deficiency. *Am. J. Clin. Nutr.* 35:617–23
83. Horrocks, L. A., Harder, H. W. 1983. Fatty acids and cholesterol. *Handbook Neurochem.* 3:1–16
84. Howard, L., Michalek, A. V. 1984. Home parenteral nutrition. *Ann. Rev. Nutr.* 4:69–99
85. Hyman, B. T., Spector, A. A. 1981. Accumulation of n-3 polyunsaturated fatty acids by cultured human Y79 retinoblastoma cells. *J. Neurochem.* 37: 60–69
86. Infante, J. P. 1987. Docosahexaenoate-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in Ca^{2+}-ATPase calcium transport. *Mol. Cell. Biochem.* 74:111–16
87. Jansson, L., Akesson, B., Holmberg, L. 1981. Vitamin E and fatty acid composition of human milk. *Am. J. Clin. Nutr.* 34:8–13
88. Karmali, R. A. 1987. Omega-3 fatty acids and cancer: A review. See Ref. 93, pp. 222–32
89. Kishimoto, Y., Davies, W. E., Radin, N. S. 1965. Developing rat brain: changes in cholesterol, galactolipids, and the individual fatty acids of gangliosides and glycerophosphatides. *J. Lipid Res.* 6:532–36
90. Lamola, A. A., Yamave, T., Zipp, A. 1974. Effects of detergents and high pressures upon the metarhodopsin I to metarhodospin II equilibrium. *Biochemistry* 15:738–45
91. Lamptey, M. S., Walker, B. L. 1976. A possible essential role for dietary linolenic acid in the development of the young rat. *J. Nutr.* 106:86–93
92. Lands, W. E. M. 1986. *Fish and Human Health*. Orlando: Academic
93. Lands, W. E. M., ed. 1987. *Polyunsaturated Fatty Acids and Eicosanoids*. Champaign: Am. Oil Chemists' Soc.
94. Leat, W. M. F., Curtis, R., Millichamp, N. J., Cox, R. W. 1986. Retinal function in rats and guinea-pigs reared on diets low in essential fatty acids and supplemented with linoleic or linolenic acids. *Ann. Nutr. Metab.* 30:166–74
95. Leray, C., Nonnotte, G., Roubaud, P., Leger, C. 1985. Incidence of (n-3) essential fatty acid deficiency on trout reproductive processes. *Reprod. Nutr. Dev.* 25:567–81
96. Lin, D. S., Connor, W. E., Anderson, G. J. 1988. Fish oil prevents the naturally occurring pseudo n-3 fatty acid deficiency in rabbit brain and retina. *Fed. Proc.* 2:A434 (Abstr.)
97. Mann, G. V. 1970. Nutritional requirements of *Cebus* monkeys. In *Feeding and Nutrition of Nonhuman Primates*, ed. R. S. Harris, pp. 143–57. New York: Academic
98. Marshall, L. A., Johnston, P. V. 1982. Modulation of tissue prostaglandin synthesizing capacity by increased ratios of dietary alpha-linolenic acid to linoleic acid. *Lipids* 17:905–13
99. Martinez, M., Conde, C., Ballabriga, A. 1974. Some chemical aspects of human brain development. II. Phosphoglyceride fatty acids. *Pediatr. Res.* 8:93–102
100. Masuzawa, Y., Sugiura, T., Ishima, Y., Waku, K. 1984. Turnover rates of the molecular species of ethanolamine plasmalogen of rat brain. *J. Neurochem.* 42:961–68
101. Millett, F., Hargrave, P. A., Raftery, M. A. 1973. Natural abundance ^{13}C nuclear magnetic resonance spectra of the lipid in intact bovine retinal rod outer segment membranes. *Biochemistry* 12: 3591–92
102. Mohrhauer, H., Holman, R. T. 1963. Alteration of the fatty acid composition of brain lipids by varing levels of dietary essential fatty acids. *J. Neurochem.* 10:523–30
103. Naughton, J. M. 1981. Supply of polyenoic fatty acids to the mammalian brain. *Int. J. Biochem.* 13:21–32
104. Neuringer, M., Connor, W. E., Lin, D. S., Barstad, L., Luck, S. J. 1986. Biochemical and functional effects of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci. USA* 83:285–94
105. Neuringer, M., Connor, W. E., Luck, S. J. 1985. Docosahexaenoic acid depletion in rhesus monkeys: Persistent effect

on recovery time of electroretinogram. *Soc. Neurosci. Abstr.* 22:70 (Abstr.)

106. Neuringer, M., Connor, W. E., Luck, S. J. 1985. Suppression of ERG amplitude by repetitive stimulation in rhesus monkeys deficient in retinal docosahexaenoic acid. *Invest. Ophthalmol. Vis. Sci.* 26(Suppl. 3):31 (Abstr.)
107. Neuringer, M., Van Petten, C., Connor, W. E., Barstad, L. 1983. Dietary deprivation of linolenic acid in developing rhesus monkeys: Effect on visual function but not discrimination learning. *Soc. Neurosci. Abstr.* 9:667 (Abstr.)
108. Neuringer, M., Connor, W. E., Van Petten, C., Barstad, L. 1984. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.* 73:272–76
109. Nielson, J. C., Maude, M. B., Hughes, H., Anderson, R. E. 1986. Rabbit photoreceptor outer segments contain high levels of docosapentaenoic acid. *Invest. Ophthalmol. Vis. Sci.* 27:261–64
110. Nouvelot, A., Dedonder, E., Dewailly, P., Bourre, J. M. 1985. Influence des n-3 exogènes sur la composition en acides gras polyinsaturés de la rétine, aspects structural et physiologique. *Cah. Nutr. Diet.* 20:123–25
111. Nouvelot, A., Delbart, C., Bourre, J. M. 1986. Hepatic metabolism of dietary alpha-linolenic acid in suckling rats, and its possible importance in polyunsaturated fatty acid uptake by the brain. *Ann. Nutr. Metab.* 30:316–23
112. O'Brien, J. S., Sampson, E. L. 1965. Fatty acid aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin. *J. Lipid Res.* 6:545–51
113. Ojima, A., Nakagawa, Y., Sugiura, T., Masuzawa, Y., Waku, K. 1987. Selective transacylation of 1-*O*-alkylglycerophosphoethanolamine by docosahexaenoate and arachidonate in rat brain microsomes. *J. Neurochem.* 48:1403–10
114. Okuyama, H., Saitoh, M., Naito, Y., Hori, T., Hashimoto, A., et al. 1987. Re-evaluation of the essentiality of alpha-linolenic acid in rats. See Ref. 93, pp. 296–300
115. Olsen, S. F., Hansen, H. S., Sorensen, T. I. A., Jensen, B., Secher, N. J., et al. 1986. Intake of marine fat, rich in (n-3)-polyunsaturated fatty-acids, may increase birthweight by prolonging gestation. *Lancet* 2:367–69
116. Onuma, Y., Masuzawa, Y., Ishima, Y., Waku, K. 1984. Selective incorporation of docosahexaenoic acid in rat brain. *Biochim. Biophys. Acta* 793:80–85
117. Orlacchio, A., Maffei, C., Binaglia, L., Porcellati, G. 1981. The effect of membrane phospholipid acyl-chain composition on the activity of brain β-N-acetyl-D-glucosaminidase. *Biochem. J.* 195: 383–88
118. Paddy, M. R., Dahlquist, F. W. 1985. Simultaneous observation of order and dynamics at several defined positions in a single acyl chain using ^2H NMR of single acyl chain perdeuterated phosphatidylcholines. *Biochemistry* 24:5988–95
119. Paoletti, R., Galli, C. 1972. Effects of essential fatty acid deficiency on the central nervous system in the growing rat. See Ref. 43, pp. 121–32
120. Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S. A., Kandel, E. R., et al. 1987. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature* 328:38–43
121. Porcellati, G., Arienti, G. 1983. Metabolism of phosphosglycerides. *Handb. Neurochem.* 3:133–61
122. Porcellati, G., Goracci, G. 1983. Lipid turnover. *Hand. Neurochem.* 5:277–94
123. Portman, O. W., Alexander, M., Illingworth, D. R. 1972. Changes in brain and sciatic nerve composition with development of the rhesus monkey *(Macaca mulatta). Brain Res.* 43:197–213
124. Portman, O. W., Andrus, S. B., Pollard, D., Bruno, D. 1961. Effects of long-term feeding of fat-free diets to *Cebus* monkeys. *J. Nutr.* 74:429–40
125. Poulos, A., Sharp, P., Singh, H., Johnson, D., Fellenberg, A., Pollard, A. 1986. Detection of a homologous series of C_{26}-C_{38} polyenoic fatty acids in the brain of patients without peroxisomes (Zellweger's syndrome). *Biochem. J.* 235:607–10
126. Reddy, T. S., Bazan, N. G. 1985. Long-chain acyl CoA synthetase in microsomes from rat brain gray matter and white matter. *Neurochem. Res.* 10:377–86
127. Reddy, T. S., Sprecher, H., Bazan, N. G. 1984. Long-chain acyl-coenzyme A synthetase from rat brain microsomes: kinetic studies using [1-^{14}C]docosahexaenoic acid substrate. *Eur. J. Biochem.* 145:21–29
128. Rivers, J. P. W., Sinclair, A. J., Crawford, M. A. 1975. Inability of the cat to desaturate essential fatty acids. *Nature* 258:171–73
129. Robert, J., Montaudon, D., Hugues, P. 1983. Incorporation and metabolism of exogenous fatty acids by cultured nor-

mal and tumoral glial cells. *Biochim. Biophys. Acta* 752:383–95

130. Roberts, W. L., Rosenberry, T. L. 1985. Identification of covalently attached fatty acids in the hydrophobic membrane-binding domain of human erythrocyte acetylcholinesterase. *Biochem. Biophys. Res. Commun.* 133:621–27
131. Rotstein, N. P., Aveldano, M. I. 1987. Labeling of lipids of retina subcellular fractions by [1-^{14}C]eicosatetraenoate (20:4(n-6)) docosapentaenoate (22:5(n-3)) and docosahexaenoate (22:6(n-3)). *Biochim. Biophys. Acta* 921:221–34
132. Rotstein, N. P., Aveldano, M. I. 1987. Labeling of phosphatidylcholines of retina subcellular fractions by [1-^{14}C]eicosatetraenoate (20:4(n-6)), docosapentaenoate (22:5(n-3)) and docosahexaenoate (22:6(n-3)). *Biochim. Biophys. Acta* 921:235–44
133. Rudman, D., Williams, P. J. 1985. Nutrient deficiencies during total parenteral nutrition. *Nutr. Rev.* 43:1–13
134. Salem, N., Kim, H.-Y., Yergey, J. A. 1986. Docosahexaenoic acid: membrane function and metabolism. See Ref. 139, pp. 263–317
135. Sanders, T. A. B., Ellis, R. R., Dickerson, T. W. T. 1978. Studies of vegans: the fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue, and breast milk, and some indicators of susceptibility to ischemic heart disease in vegans and omnivore controls. *Am. J. Clin. Nutr.* 31:805–13
136. Sastry, P. S. 1985. Lipids of nervous tissue: composition and metabolism. *Prog. Lipid Res.* 24:69–176
137. Schaad, N. C., Schorderet, M., Magistretti, P. J. 1987. Prostaglandins and the synergism between VIP and noradrenaline in the cerebral cortex. *Nature* 328:637–40
138. Scott, B. L., Reddy, T. S., Bazan, N. G. 1987. Docosahexaenoate metabolism and fatty-acid composition in developing retinas of normal and *rd* mutant mice. *Exp. Eye Res.* 44:101–13
139. Simopoulos, A. P., ed. 1986. *Health Effects of Polyunsaturated Fatty Acids in Seafoods*. New York: Academic
140. Sinclair, A. J. 1975. Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rat. *Lipids* 10:175–84
141. Sinclair, A. J. 1975. Long-chain polyunsaturated fatty acids in the mammalian brain. *Proc. Nutr. Soc.* 34: 287–91
142. Sinclair, A. J., Crawford, M. A. 1972. The accumulation of arachidonate and docosahexaenoate in the developing rat brain. *J. Neurochem.* 19:1753–58
143. Sinnhuber, R. O., Castell, J. D., Lee, D. J. 1972. Essential fatty acid requirement of the rainbow trout, *Salmo gairdneri. Fed. Proc.* 31:1436–41
144. Spector, A. A., Yorek, M. A. 1985. Membrane lipid composition and cellular function. *J. Lipid Res.* 26:1015–35
145. Stanley-Samuelson, D. W., Dadd, R. H. 1984. Polyunsaturated fatty acids in the lipids from adult *Galleria mellonella* reared on diets to which only one unsaturated fatty acid had been added. *Insect Biochem.* 3:321–27
146. Stone, W. L., Farnsworth, C. C., Dratz, E. A. 1979. A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segments. *Exp. Eye Res.* 28:387–97
147. Stubbs, C. D., 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
148. Sun, G. Y. 1983. Enzymes of lipid metabolism. *Handb. Neurochem.* 4: 367–83
149. Svennerholm, L. 1968. Distribution and fatty acid composition of phosphoglycerides in normal human brain. *J. Lipid Res.* 9:570–79
150. Svennerholm, L., Alling, C., Bruce, A., Karlsson, I., Sapia, O. 1972. Effects on offspring of maternal malnutrition in the rat. See Ref. 43, pp. 141–57
151. Tacconi, M., Wurtman, R. J. 1985. Phosphatidylcholine produced in rat synaptosomes by N-methylation is enriched in polyunsaturated fatty acids. *Proc. Natl. Acad. Sci. USA* 82:4828–31
152. Tanaka, R. 1969. Comparison of lipid effects on K^+-Mg^{2+} activated *p*-nitrophenyl phosphatase and Na^+-K^+-Mg^{2+} activated adenosine triphosphatase of membrane. *J. Neurochem.* 16: 1301–7
153. Tarozzi, G., Barzanti, V., Biagi, P. L., Lodi, R., Maranesi, M., Turchetto, E. 1986. The effect of diet upon the fatty acid composition of optic and trigeminal nerve lipids. *Prog. Lipid Res.* 25:619–23
154. Tinoco, J. 1982. Dietary requirements and functions of alpha-linolenic acid in animals. *Prog. Lipid Res.* 21:1–45
155. Tinoco, J., Babcock, R., Hincenbergs, I., Medwadowski, B., Miljanich, P.

1978. Changes in fatty acid patterns in female and male rats raised on a linole-nic acid-deficient diet for two generations. *Lipids* 13:6–17
156. Tinoco, J., Miljanich, P., Medwadowski, B. 1977. Depletion of docosahexaenoic acid in retinal lipids of rats fed a linolenic acid-deficient, linoleic-acid containing diet. *Biochim. Biophys. Acta* 486:575–78
157. Tinoco, J., Williams, M. A., Hincenbergs, I., Lyman, R. L. 1971. Evidence for nonessentiality of linolenic acid in the diet of the rat. *J. Nutr.* 101:937–46
158. Tyurin, V. A., Gorbunov, N. V. 1983. Fatty acid composition of aminophospholipids in protein microenvironment of plasmatic synaptic membranes of the brain in rat. *J. Evol. Biochem. Physiol.*, pp. 591–94 (in Russian)
158a. Uauy, R., Saitua, M. T., Gil, A. 1986. Changes in plasma and red blood cell membrane fatty acids for neonates in the first day of life. *Pediatr. Res.* 20: 419A
159. van Blitterswijk, W. J., van der Meer, B., Hilkmann, H. 1987. Quantitative contributions of cholesterol and the individual classes of phospholipids and their degree of fatty acyl (un)saturation to membrane fluidity measured by fluorescence polarization. *Biochemistry* 26:1746–56
160. Wheeler, T. G., Benolken, R. M., Anderson, R. E. 1975. Visual membranes: Specificity of fatty acid precursors for the electrical response to illumination. *Science* 188:1312–14
161. Wiegand, R. D., Anderson, R. E. 1983. Phospholipid molecular species of frog rod outer segment membranes. *Exp. Eye Res.* 37:159–73
162. Wiegand, R. D., Joel, C. D., Rapp, L. M., Nielsen, J. C., Maude, M. B., Anderson, R. E. 1986. Polyunsaturated fatty acids and vitamin E in rat rod outer segments during light damage. *Invest. Ophthalmol. Vis. Sci.* 27:727–33
163. Wiegand, R. D., Rapp, L. M., Anderson, R. E. 1985. Ferrous ion-induced retinal degeneration: Biochemical changes in photoreceptor membranes. *Invest. Ophthalmol. Vis. Sci.* 26(Suppl. 3):65 (Abstr.)
164. Willis, A. L. 1981. Nutritional and pharmacological factors in eicosanoid biology. *Nutr. Rev.* 39:289–301
165. Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., Okuyama, H. 1987. Effect of dietary alpha-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* 28:144–51
166. Yorek, M. A., Bohnker, R. R., Dudley, D. T., Spector, A. A. 1984. Comparative utilization of n-3 polyunsaturated fatty acids by cultured human Y-79 retinoblastoma cells. *Biochim. Biophys. Acta* 795:277–85
167. Yorek, M. A., Strom, D. K., Spector, A. A. 1984. Effect of membrane polyunsaturation on carrier-mediated transport in cultured retinoblastoma cells: alterations in taurine uptake. *J. Neurochem.* 42:254–61
168. Young, R. W. 1967. The renewal of photoreceptor cell outer segments. *J. Cell Biol.* 33:61–72
169. Youyou, A., Durand, G., Pascal, G., Piciotti, M., Dumont, O., Bourre, J. M. 1986. 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.* 46:224–28
170. Zimmerman, W. F., Keys, S. 1986. Acyl transferase and fatty acid coenzyme A synthetase activities within bovine rod outer segments. *Biochem. Biophys. Res. Commun.* 138:988–94