0145-6008/01/2512-1758$03.00/0
Alcoholism: Clinical and Experimental Research

Vol. 25, No. 12
December 2001

Ethanol Consumption Alters Electroretinograms and Depletes Neural Tissues of Docosahexaenoic Acid in Rhesus Monkeys: Nutritional Consequences of a Low n-3 Fatty Acid Diet

Robert J. Pawlosky, John Bacher, and Norman Salem, Jr

Background: Alcohol amblyopia is a rare neuropathy characterized by the development of blurred vision and a reduction in visual acuity. Further diagnostic details of this condition have shown abnormalities in the electroretinogram (ERG) that include an increase in implicit times in the a- and b-waves and a depression of b-wave amplitude.

Methods: Periodically, the ERGs and the fatty acyl composition of nervous tissue were analyzed from alcohol-consuming rhesus monkeys (*Macaca mulatta*) (mean consumption 2.6 g kg/day over a 5-year period) and controls that were maintained on a nutritionally sufficient diet that had low, yet adequate, amounts of linoleic acid but very low α-linolenic acid.

Results: Animals consuming alcohol had increased a- and b-wave implicit times and decreased b-wave amplitudes in their electroretinograms compared with those of the dietary control group at 2.5 and 5 years. The fatty acyl composition of brain specimens obtained by surgical biopsy at baseline, 2.5 years, and 5 years demonstrated that docosahexaenoic acid (DHA) had decreased in both groups of animals compared with baseline values. In the brains of the alcohol-treated animals, DHA was even further decreased (2.5 years: -20%; 5 years: -33%) compared with the diet controls. In the retinas of the alcohol-consuming animals at 5 years, there was a similar decrease in DHA (-35%) compared with controls. Generally, the n-6 fatty acid, docosapentaenoic acid (DPAn-6) increased in these tissues, apparently compensating for the loss of DHA.

Conclusions: A reciprocal change in the DHA/DPAn-6 ratio is known to be associated with abnormal electroretinograms in a number of species. Thus, a marginal intake of n-3 fatty acids in some alcohol abusers may, in part, be responsible for the biochemical changes that underlie the diminished retinal function associated with the visual abnormalities observed in alcohol-amblyopic patients.

Key Words: Electroretinogram, Amblyopia, Retina, Brain, Docosahexaenoic Acid.

ALCOHOL AMBLYOPIA IS a progressive neuropathy occurring in chronic alcohol abusers at a frequency of about 0.5% (Carroll, 1944,1966; Dang, 1981; Victor et al., 1965). It is characterized by the development of blurred vision and reduction in visual acuity, which may be accompanied by a peripheral neuropathy (Victor et al., 1965). Although the exact etiology in the development of the pathology is unknown, a nutrient insufficiency possibly related to years of alcohol abuse is thought to be a major factor in the progression of this disorder (Carroll, 1966; Dreyfus, 1965; Woon et al., 1995). In a recent Cuban epidemic, several case studies were described which detailed a similar neuropathy that may have been associated with high alcohol consumption and poor nutrition (Tucker et al., 1994).

Further diagnostic details of this syndrome have shown that alcohol consumption, alone or in combination with cigarette smoking (tobacco-alcohol amblyopia), frequently causes abnormalities in the electroretinogram (ERG), which may partially explain the reduction in visual acuity (Hennekes, 1982; van Lith and Henkes, 1979; Williams, 1981). Alterations observed in the ERGs usually include an increase in implicit times in the a- and b-waves and a depression of b-wave amplitude (Hennekes, 1982; van Lith and Henkes, 1979; Williams, 1981).

It is also known that chronic exposure to alcohol in utero can cause alterations in ERGs of children born with fetal alcohol syndrome (Hug et al., 2000) and in animal models of fetal alcohol syndrome (Katz and Fox, 1991). When female hooded-rats had been exposed to alcohol during the prenatal period, adult offspring were shown to have re-

From the Food Composition Laboratory (RJP), Beltsville Human Nutrition Research Center ARS, USDA, Beltsville, Maryland; Office Research Service (JB), Veterinary Resources Program, National Institutes of Health, Bethesda; and Laboratory of Membrane Biochemistry and Biophysics (NS), National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland.

Received for publication July 9, 2001; accepted October 3, 2001.

Reprint requests: Robert J. Pawlosky, Food Composition Lab, Bldg. 161, 10300 Baltimore Avenue, Beltsville, MD, 20705; Fax: 301-504-8314; E-mail: pawlosky@bhnrc.usda.gov.

duced rhodopsin content in the retina and several visual abnormalities, which included increases in a- and b-wave latencies and decreases in b-wave amplitudes. Whether, the ERG alterations observed here share a common biochemical pathology with the alcohol amblyopic condition is unknown. However, it is clear that prolonged continuous exposure to ethanol during fetal development has adverse affects on the retina that may persist into adulthood (Katz and Fox, 1991).

The precise nature of the proposed nutritional deficit in amblyopic patients that elicits biochemical alterations responsive to the ERG is not well understood (Dang, 1981; Dreyfus, 1965; Krumsiek et al., 1985a,b; Woon et al., 1995). Of the several possible nutritional insufficiencies, one proposed hypothesis is that a folic acid and/or a vitamin B12 deficiency may play a central role in the development of this pathology especially as this relates to the effects of chronic alcohol consumption on the supply of S-adenosylmethionine for neurotransmission processes (Dang, 1981). However, another plausible explanation that may directly pertain to the alterations in the ERG is that the function of the retina may be diminished by chronic exposure to ethanol and/or the byproducts of ethanol metabolism (van Lith and Henkes, 1979). As an example, short-lived free radical intermediates generated in the metabolism of ethanol via P-450 2E1 (Hansson et al., 1990; Montoliu et al., 1994; Nordmann et al., 1992; Upadhya et al., 2000; Warner and Gustafsson, 1994) can react with highly unsaturated fatty acids that are enriched in neuronal membranes and thereby generate lipid peroxide products (e.g., hydroxy-aldehydes) (He et al., 1998). The reactions of these aldehydes with other cellular constituents have been shown to alter metabolic processes in neural tissue (Keller et al., 1997; Mattson, 1998; Pedersen et al., 1999; Singhal et al., 1999). Moreover, it has been hypothesized that persistent conditions of oxidative stress due to chronic alcohol exposure can alter the fatty acyl composition of membrane phospholipids (Pawlosky et al., 1998; Pawlosky and Salem, 1999). In a previous study in felines, a 6-month ethanol exposure led to an increase in lipid peroxidation in the brain (Pawlosky et al., 1998) and a decrease in the highly unsaturated n-3 fatty acid, docosahexaenoic acid (DHA), in the brain and the retina (Pawlosky and Salem, 1995). Low amounts of DHA in the retina have been shown to have a negative impact on a- and b-waves of the ERG (Neuringer et al., 1986; Pawlosky et al., 1997a; Uauy et al., 1992). It has been proposed that for the optimal function of rhodopsin, a high concentration of DHA is required in the outer segments of rod and cone cells (Litman and Mitchell, 1996) and low amounts of DHA in these cells impair visual transduction processes.

Currently, no animal model of alcohol amblyopia has been identified. In a previous investigation, primates attained moderately high and sustainable blood alcohol concentrations (BACs) utilizing a self-select model of alcohol consumption when offered a palliative artificially sweetened alcohol solution (Pawlosky et al., 1997b; Pawlosky and Salem, 1999). In the present study, rhesus monkeys (*Macaca mulatta*) were used to study the effects of prolonged alcohol exposure on the ERG and lipid composition of neural tissues. Animals were maintained on a diet that contained adequate levels of antioxidants and had olive oil as the source of essential fatty acids (linoleic acid and α-linolenic acid). One group ($n = 5$) was given free access to an artificially sweetened 7%-ethanol solution over a period of 5 years, the other group ($n = 4$) served as a dietary control. Individual alcohol consumption together with BACs were monitored periodically to assess variations in ethanol intake. At 2.5 and 5 years, ERGs were obtained from animals after a brief period of dark adaptation. Brain specimens were obtained by surgical biopsy prior to beginning the dietary regimen and at 2.5 years. At the end of 5 years, animals were killed, brains and retinas were excised, and the fatty acyl composition of the tissues analyzed.

Table 1. The Nutrient Content and Fatty Acyl Composition of the Primate Diet

Nutrient	g/kg
Casein vitamin-free	176
Corn starch	425
Sucrose	201
Fat[1]	
Olive oil	40
Hydrogenated coconut oil	20
Alphacel non-nutritive bulk	30
Mineral mixture[2]	
Vitamin mixture[3]	
Vitamin E	40 IU/kg
Vitamin C	250 mg/kg

[1] Fatty acid, weight percent 12:0, 6.2; 14:0, 1.8; 16:0, 18.3; 16:1n7, 0.5; 18:0, 11.8; 18:1n9, 54.4; 18:1n7, 1.0; 18:2n6, 8.4; 18:3n6, 0.4; 18:3n3, 0.5.

[2] All values expressed in gm/kg of diet or otherwise noted: calcium, 5; chlorine, 4.6; chromium, 0.5 mg; copper, 3.8 mg; fluorine, 0.5 mg; iodine, 3.6 mg; iron, 0.249; manganese, 0.02; phosphorus, 3.76; potassium, 7.32; selenium, 0.06 mg; sodium, 3.37; sulfur, 0.67; zinc, 0.02; magnesium, 1.50.

[3] All values expressed in gm/kg of diet or otherwise noted: biotin 0.2 mg; pantothenate, 0.025; choline, 2.5; folic acid 6.35 mg, inositol, 0.52; menadione, 1.5 mg; niacin, 42 mg; pyridoxine, 4.0 mg; riboflavin, 8.0 mg; thiamine, 4.0 mg; vitamin A, 18000 IU; vitamin D3, 1300 IU, vitamin B12, 0.2 mg; para-aminobenzoic acid, 0.25.

MATERIALS AND METHODS

Animal Procedures. Animal procedures were carried out in accordance with the NIH animal welfare guidelines and the protocol was approved by the National Institute on Alcoholism Alcohol Abuse Animal Care and Use Committee. Nine adult male rhesus monkeys (8–16 kg) free of known pathogens were selected from the facility's primate colony, housed separately, and fasted before experimental procedures were carried out. Animals were acclimated to a semi-purified diet (Table 1) (Bio-Serv Inc., Frenchtown, NJ) and given access to food twice a day. After a month of dietary equilibration, five animals were given 24-hr access to an artificially-flavored 7%-ethanol solution sweetened with aspartame each day for 5 years. Peak BACs were determined using an alcohol dehydrogenase kit (Sigma Chemical Co., St. Louis, MO) for 4 hr following a period of alcohol consumption. Control animals were maintained on the same diet and provided with the same amount of food as the alcohol-exposed animals (540 kcal/day).

Diet. The composition of the experimental diet is given in Table 1. The fat content (14% of energy) of the diet consisted of a 2:1 ratio of olive oil/hydrogenated coconut oil and contained no 20- or 22-carbon polyun-

saturated fatty acids. There were 8.9 g of linoleic acid and 0.5 g of α-linolenic acid per 100 g of fat. The maximum amount of trans-unsaturated fatty acids in the hydrogenated coconut oil was less than 1.1% of the total fat, as determined by gas chromatographic analysis. The protein (22% of the energy) and carbohydrate (65% of the energy) content of the diet are also given in Table 1. The levels of vitamin C and E in the diet were 250 mg/kg and 40 IU/kg, respectively.

Procedures for the ERGs The procedure for obtaining the ERGs in felines has been reported previously (Pawlosky et al., 1997a) with additional modifications for similar determinations in primates. Alcohol was withheld from animals for 24 hr prior to beginning the procedure. Animals were fasted overnight and anesthetized with an intravenous injection of (ketamine hydrochloride/acepromazine, 20/2 mg/kg). Animals were placed on a heating pad and draped to maintain their body core temperature at 37°C. Each animal was dark adapted for a full 10 min prior to initiating the ERG procedure. Their heads were elevated approximately 15% from the plane of the table and an infant speculum was used to retain the eyelids. ERGs were obtained when animals were fully anesthetized without the use of additional pupillary dilation.

A sterile gold-leaf contact lens electrode (ERG-jet, 12-mm diameter, 7.90-mm radius; The Electrode Store, Enumclaw, WA) was placed on the cornea of the right eye and one or two drops of methyl cellulose were deposited between the contact lens and cornea to improve electrical contact. The eye was irrigated regularly with normal saline solution to prevent edema and a reference electrode (28 gauge x 12-mm long stainless steel needle electrode) was inserted beneath the skin folds above the left eyelid. The ERGs were obtained in a room under dim red-light conditions using a standard digital electrographic BPM-100 (Retinographics Inc., Norwalk, CT) with a stimulating flash that was composed of eight red-light emitting diodes (660 nm, having a half-power beam width of 30°) providing a total of 16 candela (cd) with full-field luminescence (Hogg, 1991). The flash duration was 3.5 msec with a 2-sec interval. The light was positioned exactly 2.5 cm in front of the right eye of each subject, and four individual recordings were averaged to produce a composite wave-form. The a- and b- wave implicit times were determined by selecting those intervals that corresponded to the minimum (a-wave) and maximum (b-wave) peaks of the waveform. The a-wave amplitude was calculated from the difference between baseline and a-wave peak minimum (in μvolts); and b-wave amplitude was calculated from the difference of a-wave peak minimum and b-wave maximum.

Procedure for Surgical Brain Biopsies

Brain specimens were obtained by biopsy from monkeys at baseline and 2.5 years after commencing the study. Animals were fasted 24 hr prior to surgery. Ketamine hydrochloride (10 mg/kg intramuscular) and atropine (0.04 mg/kg intramuscular) were given as preanesthetic agents. An Angiocath (20 gauge x 1.25 in, Deseret Medical Inc. Sandy, UT) was inserted into the saphenous or cephalic vein for hydration with sodium chloride solution (10 ml/kg per hour). The monkeys received Cephazolin sodium (500 mg intravenous and 500 mg dispersed in intravenous fluids; Ancef, SmithKline Beecham Pharmaceuticals, Philadelphia, PA) and 44,000 IU/kg intramuscular of a long acting penicillin (AMBI-PEN, G. C. Henford Mfg. Co., The Butler Co., Columbus, OH). Dexamethasone (0.7 mg/kg intravenous; Phoenix Scientific Inc. St. Joseph, MO) was given to reduce swelling of the brain. Sodium thiopental (2.5%) (Pentothal, Abbott Laboratories, North Chicago, IL) was administered intravenously, if needed, to allow for intubation with a 5-mm endotracheal tube (Mallinckodt Critical Care, Glens Falls, NY). Anesthesia was maintained via inhalation with Isoflurane (0.5–1.5%) (Aerrane, Anaquest, Madison, WI) and oxygen at a total flow rate of 2 liters/min during the surgical biopsy of the brain. The hair was clipped and the monkeys were positioned in a stereotaxic unit (David Kopf Instruments, Tujunga, CA) using the eye bars and mouthpiece to position the head in a horizontal plane. The surgical area was scrubbed with hexachlorophene foam (Vestal Laboratories, St. Louis, MO) and then draped for aseptic surgery.

A 4–5 cm midline incision was made over the frontal and parietal bones. The periosteum and temporal muscle on either the right or left side was reflected using a periosteal elevator to expose the cranium. The right and left sides were alternated for succeeding biopsies. A 5/8" or 3/4" trephine was used to make a burr hole through the skull just off the midline of the cranium to expose the cerebral hemisphere. An avascular area was selected and bipolar coagulating forceps were used to dissect out the biopsy sample. The sample was approximately 1 cm x 1 cm (200–250 mg). Bleeding was controlled by using bipolar coagulation or by squirting sterile hydrogen peroxide into the biopsy site. When all bleeding had ceased, the area was flushed with saline and the dura was closed using 5–0 PDS suture (Ethicon Inc., Somerville, NJ). The bone plug removed by trephination was replaced in the defect. The periosteum and temporal muscle was pulled over the bone plug and sutured back in place with a 4–0 Vicryl suture (Ethicon Inc.) Skin was closed in a continuous subcuticular pattern.

Fatty Acyl Analyses of Brain Specimens and Retina

Approximately 100 mg of fresh brain specimens were kept on ice and samples were homogenized in water (1 ml) using a ground glass handheld homogenizer. Under red-lamp conditions, the entire retina from the right eye was removed and homogenized in water (1 ml). An internal standard, 23:0 methyl ester, was added to the samples to quantify the absolute amounts of the fatty acids, and the tissues were extracted using the Bligh and Dyer total lipid extraction procedure (Bligh and Dyer, 1959). The lipid extracts were transmethylated with BF_3 in methanol (14% w/v), according to the procedure of Morrison and Smith (1961) for analysis by gas chromatography (GC)-flame ionization detection according to a previously described method (Pawlosky and Salem, 1995).

Data Reduction and Statistical Analyses

The Student's *t* test was used to determine differences in implicit times and amplitudes of the a- and b-wave in the ERGs at 2.5 and 5 years for these groups. A p value of 0.05 or less was considered significant. Concentrations of the brain and retina fatty acids are expressed in weight percent of the total fatty acids. Student's *t* test was used to determine significant differences in the weight percent of the fatty acids as a result of the dietary treatment and between groups at 2.5 and 5 years. A p value of 0.01 or less was considered to be significant.

RESULTS

Alcohol Consumption and General Health

Throughout the study, the general health of animals in either group was considered good and did not appear to be affected by the diet or diet-alcohol regimen. However, animals receiving the sweetened-alcohol solution appeared to show a strong preference for the containers that held the alcohol solution, which indicated that the sweetened solution provided a strong reinforcement for consumption of alcohol over the period of study. Animals consumed alcohol regularly over the 5-year period. The daily mean alcohol intake was 2.6 g/kg per day (range, 1.8–4.6 g/kg per day) or approximately 24% of the total daily calories (range, 16–42%). Peak BAC levels (18–30 mmol/L or 82–135 mg/dL) occurred approximately 2 hr after animals consumed between 180 and 250 ml of the alcohol solution. Food intake was monitored daily and animals in both groups generally consumed their daily food rations. Although, alcohol-consuming animals had an apparent

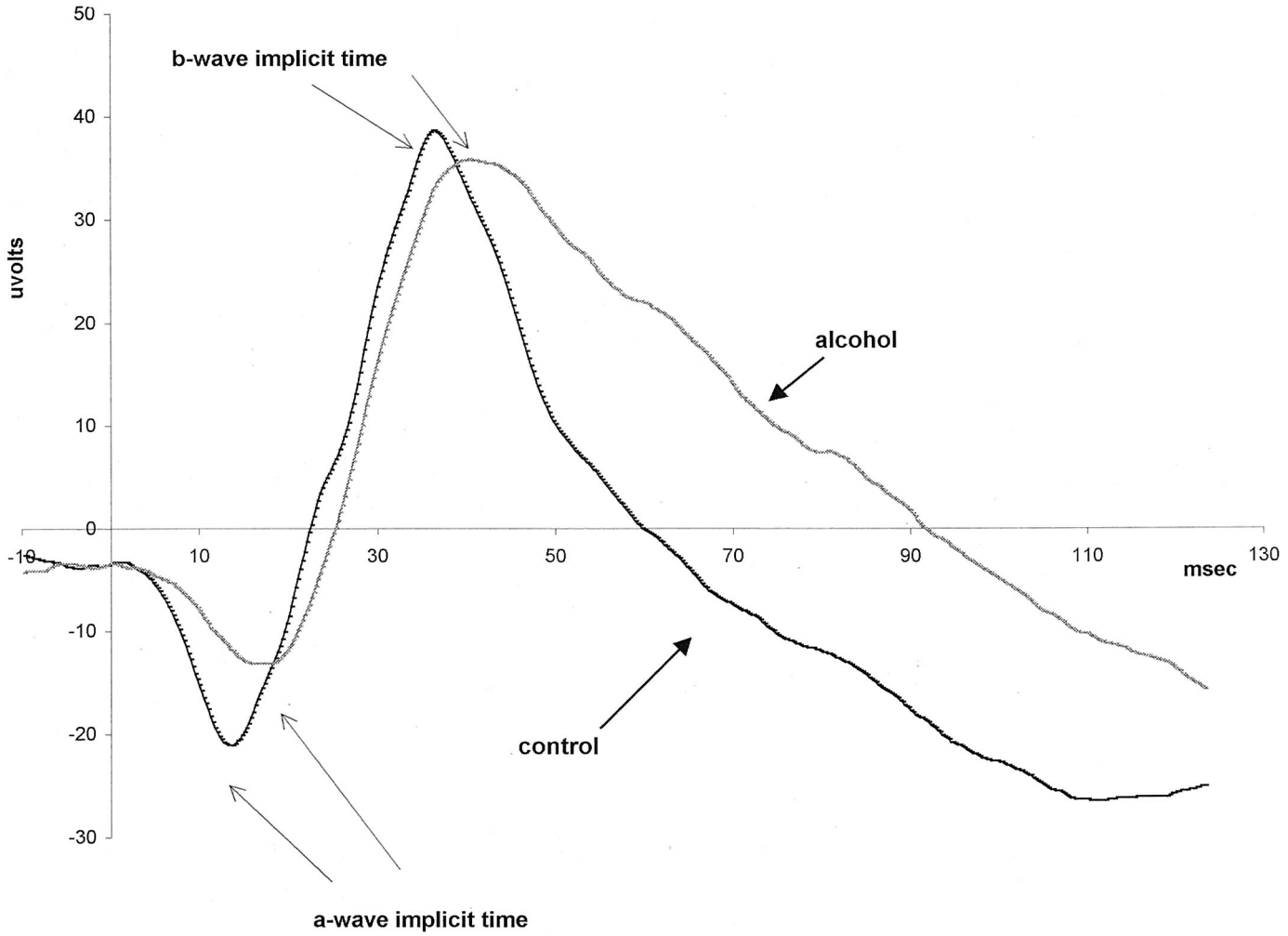

Fig. 1. Representative electroretinograms at full luminescence (16 cd) taken from dark-adapted rhesus monkeys after animals had been maintained on a low n-3 fatty acid diet or low n-3 fatty acid diet and alcohol regimen for 5 y.

greater caloric intake than the controls, this did not appear to have any clear positive effect on their body weights (Pawlosky et al., 1997b).

ERG Results

Typical and characteristic examples of the ERGs from a dark-adapted dietary control and an alcohol-treated monkey at 5 years using red LEDs at full-field luminescence are shown in Fig. 1. When interpreting the ERG results, it should be understood that red LEDs tend to elicit more cone than rod response compared with a full-field white light flash stimulus (Hogg, 1991). The ERG responses in each individual animal at 2.5 years were similar to those obtained at 5 years, indicating low intrasubject variability at these time points. Since no attempt was made to determine the effects of the dietary regimen alone on the ERGs, it was assumed that the differences between these groups resulted from an alcohol-diet interaction.

The alcohol-treated animals had significant increases in a- and b-wave latencies compared with the control animals at both the 2.5 and 5 year intervals (Table 2). Moreover, animals in the alcohol group tended to have lower b-wave amplitudes compared with controls, which was significant at 5 years. However, there were no significant differences in the a-wave amplitudes between these groups at either time point. As there were similar values in the implicit times and amplitudes in animals from the alcohol group at 2.5 and 5 years, this suggested that the main impact of the chronic alcohol consumption on the ERGs occurred prior to 2.5 years and thereafter remained unabated.

Fatty Acyl Composition of the Brain Specimens and Retina

Effects of Diet. During the course of the 5-year study, the low n-3 fatty acid diet was found to have a major influence on the fatty acyl composition of the brain in the control animals (Table 3). At 2.5 years, there was a significant decrease in DHA (from 14.0 to 11.4 wt%, equivalent to a 18% decrease) and an increase in DPAn-6 (from 1.1 to 3.8 wt%, or a 250% increase) in animals maintained on the control diet relative to baseline values (Table 3). At 5 years,

Table 2. Electroretinogram Determinations From Rhesus Monkeys After Being Maintained for 2.5 and 5 Years on a Low n-3 Fatty Acid Diet (Control) or Diet Together With Self-Administered Alcohol Consumption (Alcohol)

	2.5 y							
	a-wave				b-wave			
	Implicit time		Amplitude		Implicit time		Amplitude	
	msec	SD	μvolts	SD	msec	SD	μvolts	SD
Control	13.1	1.1	14.0	3.2	36.5	1.4	77.2	13.7
Alcohol	16.2[a]	0.5	10.6	2.8	42.4[b]	1.8	44.3	8.2
	5 y							
	a-wave				b-wave			
	Implicit time		Amplitude		Implicit time		Amplitude	
	msec	SD	μvolts	SD	msec	SD	μvolts	SD
Control	13.3	1.1	12.3	3.9	39.3	1.4	69.6	8.1
Alcohol	16.7[a]	0.5	9.5	2.6	43.9[b]	0.8	39.4[b]	6.2

The values are reported in milliseconds for implicit time determinations and μvolts for peak amplitudes for a- and b-wave components of the ERG.
[a] Significantly different from controls, $p < 0.01$ (Student's *t* test).
[b] Significantly different from controls, $p < 0.05$ (Student's *t* test).

Table 3. Fatty Acyl Composition of Tissues Obtained From Rhesus Monkeys That had Been Maintained on a Low n-3 Fatty Acid Diet or a Low n-3 Fatty Acid Diet for 2.5 y and 5 y

	Cortex Baseline		Cortex control		Cortex alcohol		Cortex control		Cortex alcohol		Retina control		Retina alcohol	
Fatty acid	y	SD	2.5 y	SD	2.5 y	SD	5 y	SD	5 y	SD	5 y	SD	5 y	SD
14:0	0.5	0.2	0.6	0.1	0.5	0.0	0.7	0.0	0.5	0.0	0.7	0.1	0.5[d]	0.1
16:0	18.5	0.6	19.0	0.8	18.8	1.4	19.3	0.3	18.7	1.6	17.2	0.9	19.2[d]	0.8
16:1	1.1	0.8	1.1	0.3	1.6	0.3	0.5	0.1	0.5	0.1	0.4	0.1	0.6	0.2
18:0	20.4	1.0	20.7	0.5	20.6	0.2	19.9	0.8	19.7	1.4	17.8	1.0	17.5	1.1
18:1n–9	12.2	0.9	12.0[a]	0.3	10.7[b]	0.4	13.1	1.2	13.0	1.0	16.2	0.9	15.6	1.2
18:1n–7	4.2	0.3	4.4	0.2	4.4	0.5	4.8	0.1	5.1	0.2	3.3	0.2	3.8	0.4
20:0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.1	0.2	0.0	1.0	0.1	0.8	0.0
20:1n–9	0.4	0.2	0.4	0.1	0.3	0.1	0.6	0.2	0.6	0.3	0.3	0.0	0.4	0.1
20:3n–9	0.2	0.0	0.6	0.2	0.6	0.1	0.5	0.1	0.6	0.2	0.6	0.1	0.2[d]	0.0
22:0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.2	0.1	1.2	0.1	0.4[d]	0.0
24:0	0.7	0.3	0.5	0.1	0.5	0.2	0.6	0.1	0.9	0.5	1.0	0.1	0.3[d]	0.1
24:1	2.0	0.9	2.0	0.4	1.0	0.5	2.3	0.5	2.5	1.5	2.7	0.3	0.5[d]	0.1
n–6 fatty acids														
18:2	0.7	0.1	0.4	0.0	0.5	0.0	0.5	0.2	0.4	0.1	3.0	0.7	1.4[d]	0.2
20:2	ND	–	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
20:3	0.7	0.1	0.6	0.1	0.6	0.0	0.8	0.3	0.8	0.2	1.2	0.2	1.4	0.1
20:4	6.9	0.5	6.9	0.3	7.1	0.3	6.9	0.2	6.6	0.9	14.2	0.3	11.2[d]	1.2
22:2	0.4	0.2	0.6	0.1	0.4	0.1	0.5	0.2	0.8	0.1	0.2	–	ND	–
22:4	4.2	0.2	4.1	0.2	4.2	0.1	3.8	0.5	4.2	0.4	1.8	0.1	2.0	0.3
22:5	1.1	0.2	3.8[a]	0.5	5.7[b]	0.8	6.4	0.7	6.1	1.7	2.3	0.2	4.4[d]	1.2
n–3 fatty acids														
20:5	0.3	0.1	0.3	0.1	0.2	0.1	0.2	0.0	ND	–	0.3	0.2	0.6	0.3
22:5	0.2	0.1	ND	–	0.1	0.0	0.2	0.1	0.1	0.0	0.6	0.1	0.3	0.0
22:6	13.9	0.2	11.3[a]	0.8	9.5[b]	0.3	9.4	1.5	7.1[c]	0.7	14.5	0.9	9.2[d]	1.6

Brain specimens were obtained by surgical biopsy at baseline (prior to low n-3 fatty acid diet) and at 2.5 y. Retinas were obtained at 5 years after beginning the study. The values are reported in weight percent of the fatty acid.
[a] Student's *t* test; significantly different BL vs control 2.5 y, ($p < 0.01$); [b] significantly different control vs alcohol ($p < 0.01$); [c] significantly different control vs alcohol, ($p < 0.01$); [d] significantly different control vs alcohol, $p < 0.01$; ND: not detected.

there was a further decrease in DHA (to 9.4 wt%, a 33% decrease from baseline values) and nearly a 400% increase in DPAn-6 (to 6.4 wt%). There was very little change in the proportion of any other n-6 fatty acid at either of the two intervals compared with baseline values (Fig. 2). As it is known that mammals are capable of synthesizing long-chain (22- and 24-carbon) polyunsaturated fatty acids in neural tissue (Delton-Vandenbrouke et al., 1997; Pawlosky et al., 1994,1996), the selectivity for DPAn-6 may be the result of preferential in regio biosynthesis rather than an increased uptake into the brain of this fatty acid.

Effects of Diet and Alcohol Interaction. The effect of 2.5 years of alcohol consumption on the fatty acyl composition of the brain resulted in marked decreases in the percentage of DHA and increases in DPAn-6 compared with control animals (Table 3). There was a 16% decrease in DHA in the brains of alcohol-treated animals compared with controls at this time point (control, 11.4 wt%; alcohol, 9.5 wt%). Similarly, in the brains of animals from the alcohol group, there was an approximately 50% increase in DPAn-6 (control, 3.8 wt%; alcohol, 5.7 wt%). Other than a small change (-10%) observed in the level of the fatty acid, 18:1n-9, 18:1n-9 in the alcohol-exposed animals, there were no other notable differences in the fatty acyl distributions at

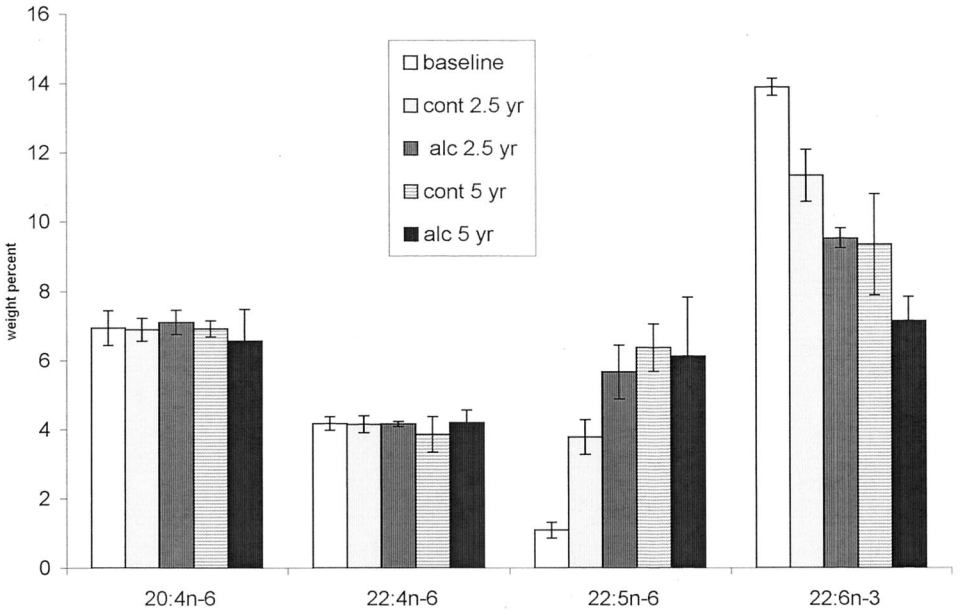

Fig. 2. Bar graph depicting the highly unsaturated fatty acids from brain specimens obtained from rhesus monkeys at baseline, and at 2.5 and 5 y after beginning a low n-3 fatty acid diet and/or alcohol-diet regimen.

2.5 years. At 5 years, the percentage of DHA in the brains of animals from the alcohol group had decreased by about 25% compared with values observed at 2.5 years, and this value was about 75% of that in the controls. In contrast, the proportion of DPAn-6 was similar in both groups (control, 6.4 wt%; alcohol, 6.1 wt%) at 5 years, indicating that that the reciprocal change in the concentration of this fatty acid no longer adequately compensated for the loss of DHA.

There was broader variation in the comparisons of the fatty acyl composition of the retinas in animals from these groups compared with the brain determinations (Table 3). This might possibly reflect a greater dynamic state of flux in the composition of the membrane phospholipids of the retina, which is consistent with the constant renewal of outer disk membranes of the rod and cone cells. In the retinas of the alcohol-exposed animals there were lower percentages (-18%) of linoleic acid (control, 3 wt%; alcohol, 1.4 wt%) and (-21%) arachidonic acid (control, 14.2 wt%; alcohol, 11.2 wt%) but a higher percentage (90%) of DPAn-6 (control, 2.3 wt%; alcohol, 4.4 wt%). The effect of the low n-3 fatty acid diet on the DHA content of the retina of animals in the control group (14.5 wt%) was apparent because these values, when compared with values that have been reported for many species, appear below normal (Salem, 1989). In the retinas of alcohol-exposed animals, the percentage of DHA was about 65% of that found in the controls at 5 years (control, 14.5 wt%; alcohol, 9.2 wt%). There were also notable decreases in plasma (-70%) and erythrocyte membranes (-50%) of animals that consumed alcohol compared with controls (Pawlosky and Salem, 1999). This suggests that the concentration of docosahexaenolic acid (22:6n-3) these tissues may be a useful indicator of an alcohol-induced n-3 fatty acid deficiency in humans.

DISCUSSION

Although it has been a longheld view that the syndrome known as alcohol amblyopia is associated with specific nutritional deficiencies (Carroll, 1944,1966; Dreyfus, 1965; Victor and Dreyfus 1965; Woon et al., 1995), other investigators have suggested that chronic alcohol consumption alone may be a direct cause of this pathology (Krumsiek et al., 1985a,b). Several therapeutic regimens have focused on administering B-vitamin complexes (thiamine, riboflavin, pyridoxine, and hydroxycobalamin) (Carroll, 1966; Dreyfus, 1965) or folic acid (Dang, 1981) supplements to patients. Administration of these interventions to amblyopic patients is consistent with the view that chronic alcohol consumption may depress the supply of S-adenosyl-methionine to nervous tissue and thereby inhibit neurotransmission processes (Dang, 1981). Despite the positive effects that these therapies have had on the visual outcomes of patients, the etiology of this pathology remains unknown (Dang, 1981; Krumsiek et al., 1985a,b). Further, ERG analyses of amblyopic patients have suggested that the early events in the visual transduction processes may be compromised by chronic exposure to ethanol (Hennekes, 1982; van Lith and Henkes, 1979; Williams, 1981).

In the present study, rhesus monkeys that were maintained on a nutritiously sufficient diet yet had very low levels of α-linolenic acid and had been regularly consuming alcohol over a period of 5 years were found to have alterations in the ERG similar to those reported in patients diagnosed with alcoholic amblyopia (Hennekes, 1982; van Lith and Henkes, 1979; Williams, 1981) and other subjects with alcohol-related visual abnormalities (Hug et al., 2000; Katz and Fox, 1991). The alcohol-exposed animals had longer a- and b-wave implicit times and depressed b-wave amplitudes compared with dietary control animals at 2.5

and 5 years. It was theorized that the alterations in the ERGs may be related to the chronic effects of alcohol metabolism on the generation of lipid peroxides in the retina and decreases in the polyunsaturated fatty acid content of the photoreceptor cells.

In our previous investigations, chronic alcohol consumption was associated with greatly elevated concentrations of the isoprostane, PGF2α, and 4-hydroxynonenal in the plasma of these same rhesus monkeys (Pawlosky et al., 1997a,b; Pawlosky and Salem, 1999) and in the brains of felines (Pawlosky et al., 1998). In the latter case, this may have been the result of ethanol metabolism by P-450 enzymes in neural tissues (Hansson et al., 1990; Nordmann et al., 1992; Upadhya et al., 2000; Warner and Gustafsson, 1994). The hydroxy-aldehydes that are generated in this way have been shown to affect a variety of neural processes, including the coupling of cell-surface receptors to GTP-binding proteins, analogous to processes that are involved in visual transduction sequences subsequent to rhodopsin activation (Mattson, 1998). Moreover, there is increasing evidence that oxygen-derived free radicals are being implicated in diseases of the retina (Anderson et al., 1985; Goss-Sampson et al., 1998; Handelman and Dratz, 1986). An increase in lipid hydroperoxides has been associated with a loss of regenerability of rhodopsin in vitro (Anderson et al., 1985) and, in rodents, a vitamin E deficiency induced losses of long-chain polyunsaturated fatty acids in the membranes of photoreceptor cells, which was associated with depressed a- and b-wave amplitudes and delayed latencies (Goss-Sampson et al., 1998).

Alcohol consumption has also been shown to decrease the concentration of DHA in the brains and retinas of felines maintained on well-controlled diets. The results reported here confirm our findings in felines that consumed alcohol for 6 months (Pawlosky and Salem, 1995). In conjunction with the loss of DHA, there occurred significant increases in the concentrations of DPAn-6. The near reciprocal change in the concentrations of DHA and DPAn-6 has also been observed in animals that are maintained on n-3 fatty acid deficient diets (Galli et al., 1977; Moriguchi et al., 2000; Pawlosky et al., 1997a; Salem, 1989).

The factors influencing the ERG outcome in the present study may be rationalized based on the effects that lipid peroxidation and the loss of DHA have on the function of rhodopsin in the photoreceptor cells. Rhodopsin, the transmembrane photo pigment, is sensitive to light through the interaction that occurs between a photon and 11-*cis* retinal. As a result of the photochemical reaction, the protein conformation is altered (rhodopsin undergoes a conformational change from MI to MII), initiating the sequence in the visual transduction process. It is interesting to note that the direct effect of ethanol on reducing the transition energy from MI to MII appears to contrast with the observations reported here on its chronic effects (Mitchell et al., 1996; Mitchell and Litman, 2000).

An alcohol-induced increase in lipid peroxides may diminish the photoreceptor function through a decrease in the regeneration of rhodopsin (Anderson et al., 1985) and through an inhibition in processes related to the phototransduction cascade (Mattson, 1998). The activity of rhodopsin is also known to be sensitive to the polyunsaturated fatty acid environment of the photoreceptor outer disk membranes (Goss-Sampson et al., 1998; Litman and Mitchell, 1996). Low amounts of DHA in these membranes have been associated with a decrease in rhodopsin function, which may have a direct impact on a-wave parameters and subsequently affect the b-wave outcome. Because, in this study, alcohol consumption had more pronounced effects on b-wave parameters compared with the a-wave, it is likely that signal transduction processes involving inner retinal neurons may also have been adversely affected by the chronic exposure to ethanol. Thus, the effects of chronic alcohol consumption on visual transduction processes initiated in the retinal photoreceptors may be mediated by an increase in lipid peroxidation and a loss of DHA-containing phospholipids. Although no measurement of alcohol-induced lipid peroxidation was made in the retinas of these animals, there is evidence from previous determinations in this same group of animals that chronic alcohol consumption increased the levels of lipid peroxidation products systemically over this 5-year period (Pawlosky et al., 1997a,b; Pawlosky and Salem, 1999). In summary, evidence from this study demonstrated that monkeys that were maintained on a low n-3 fatty acid diet had significant decreases in DHA in the brains at 2.5 and 5 years compared with baseline values. Moreover, alcohol was found to have a negative impact on the percentage of DHA in both the brain and retina. Animals that were consuming alcohol had increases in a- and b-wave implicit times and depressed b-wave amplitudes compared with controls on the same diet. Although, it was not possible in these animals to correlate the ERG findings to psychophysical components of vision (e.g., acuity determinations), it is notable that diminished retinal function in amblyopic patients was associated with other negative visual outcome parameters (Williams, 1984). It is hypothesized that the metabolism of ethanol in neural tissues and in the retina altered the fatty acyl composition of tissues and increased lipid peroxidation, which may be associated with diminished retinal function. The present study suggests that some alcohol abusers who have visual abnormalities, which include alterations in the ERG, may also have an insufficient intake of n-3 fatty acids. This, in turn, suggests that therapeutic measures designed for countermanding the effects of amblyopia should also include an analysis of the n-3 fatty acid status of the patient.

REFERENCES

Anderson RE, Maude MB, Nielsen JC (1985) Effect of lipid peroxidation on rhodopsin regeneration. Curr Eye Res 4:65–71.

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–916.

Carroll FD (1944) The etiology and treatment of tobacco-alcohol amblyopia. Am J Ophthalmol 7:713–718.

Carroll FD (1966) Nutritional amblyopia. Arch Ophthalmol 76:406–413.

Dang CV (1981) Tobacco-alcohol amblyopia: a proposed biochemical basis for pathogenesis. Med Hypotheses 7:1317–1328.

Delton-Vandenbrouke I, Grammas P, Anderson RE (1997) Polyunsaturated fatty acid metabolism in retinal and cerebral microvascular endothelial cells. J Lipid Res 38:147–159.

Dreyfus PM (1965) Blood transketolase levels in tobacco-alcohol amblyopia. Arch Ophthalmol 74:617–620.

Galli C, Galli G, Spagnuolo C, Bosisio E, Tosi L, Folco GC, Longiave D (1977) Dietary essential fatty acids, brain polyunsaturated fatty acids, and prostaglandin biosynthesis. Adv Exp Med Biol 83:561–573.

Goss-Sampson MA, Kriss T, Muller DP (1998) Retinal abnormalities in experimental vitamin E deficiency. Free Radical Biol Med 25:457–462.

Handelman GJ, Dratz EA (1986) The role of antioxidants in the retina and retinal pigment epothelium and the nature of prooxidant induced damage. Free Radical Biol Med 2:1–89.

Hansson T, Tindberg N, Ingelman-Sundberg M, Kohler C (1990) Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. Neuroscience 34:451–463.

He Q, Khanna P, Srivastava S, van Kuijk FJ, Ansari NH (1998) Reduction of 4-hydroxynonenal and 4-hydroxyhexenal by retinal aldose reductase. Biochem Biophys Res Commun 247:719–722.

Hennekes R (1982) Clinical ERG findings in tobacco-alcohol amblyopia. Graefes Arch Clin Exp Ophthalmol 219:38–39.

Hogg C (1991) The use of light-emitting diodes in electrophysiology and psychophysics, in *Principles and practice in electrophysiology of vision* (Heckenlively JR, Arden GB eds), pp 221–227. Mosby Year Book, St. Louis.

Hug TE, Fitzgerald KM, Cibis GW (2000) Clinical and electroretinographic findings in fetal alcohol syndrome. J AAPOS 4:200–204.

Katz LM, Fox DA (1991) Prenatal ethanol exposure alters scotopic and photopic components of adult rat electroretinograms. Invest Ophthalmol Vis Sci 32:2861–2872.

Keller JN, Pang Z, Geddes JW, Begley JG, Germeyer A, Waeg G, Mattson MP (1997) Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. J Neurochem 69:273–284.

Krumsiek J, Kruger C, Patzold U (1985a) Tobacco-alcohol amblyopia neuro-ophthalmological findings and clinical course. Acta Neurol Scand 72:180–187.

Krumsiek J, Kruger C, Wurster U, Patzold U (1985b) Tobacco-alcohol amblyopia. Clinical course in 33 patients. Fortschr Neurol Psychiatr 53:88–93.

Litman BJ, Mitchell DC (1996) A role for phospholipid polyunsaturation in modulating membrane protein function. Lipids 31S:S193–197.

Mattson MP (1998) Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity. Trends Neurosci 21:53–57.

Mitchell DC, Lawrence JTR, Litman BJ (1996) Primary alcohols modulate the activation of the G protein-coupled receptor rhodopsin by a lipid-mediated mechanism. J Biol Chem 271(32):19033–19036.

Mitchell DC, Litman BJ (2000) Effect of ethanol and osmotic stress on receptor conformation. J Biol Chem 275:5355–5360.

Montoliu C, Valles S, Renau-Piqueras J, Guerri C (1994) Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain: effect of chronic alcohol consumption. J Neurochem 63:1855–1862.

Moriguchi T, Sheaff Greiner R, Salem N Jr (2000) Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem 75:2563–2573.

Morrison WR, Smith LM (1961) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600–608.

Neuringer M, Connor WE, Lin DS, Barstad L, Luck S (1986) Biochemical and functional effects of prenatal and postnatal n-3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci USA 83:4021–4025.

Nordmann R, Ribier C, Rouach H (1992) Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radical Biol Med 12:219–240.

Pawlosky RJ, Barnes A, Salem N Jr (1994) Essential fatty acid metabolism in the feline: the relationship between liver and brain production of long chain polyunsaturated fatty acids. J Lipid Res 35:2032–2039.

Pawlosky RJ, Denkins Y, Ward G, Salem N Jr (1997a) Retinal and brain accretion of long-chain polyunsaturated fatty acids in developing felines: the effects of corn oil-based maternal diets. Am J Clin Nutr 665:465–472.

Pawlosky RJ, Flynn BM, Salem N Jr (1997b) The effects of a low essential fatty acid diet on alcohol-induced liver fibrosis in rhesus monkeys. Hepatology 26:1387–1392.

Pawlosky RJ, Gupta S, Salem N Jr (1998) Alcohol consumption in felines increases lipid peroxidation in the brain and stimulates polyunsaturated fatty acid metabolism, in *Essential Fatty Acids and Eicosanoids: Invited Papers from 4th International Congress* (Riemersma RA, Armstrong R, Kelly R, Wilson R eds), pp 317–320. AOCS Press, Champaign, IL.

Pawlosky RJ, Salem N Jr (1995) Ethanol exposure causes a decrease in docosahexaenoic acid and an increase in docosapentenoic acid in the feline brain and retina. Am J Clin Nutr 61:1284–1289.

Pawlosky RJ, Salem N Jr (1999) Alcohol consumption in rhesus monkeys depletes tissues of polyunsaturated fatty acids and alters essential fatty acid metabolism. Alcohol Clin Exp Res 23:311–317.

Pawlosky RJ, Ward G, Salem N Jr (1996) Essential fatty acid uptake and metabolism in the developing rodent brain. Lipids 31S:S103-S107.

Pedersen WA, Cashman NR, Mattson MP (1999) The lipid peroxidation product 4-hydroxynonenl impairs glutamate and glucose transport and choline asetyltransferase activity in NSC-19 motor neuron cells. Exp Neurol 155:1–10.

Salem N Jr (1989) Omega-3 fatty acids: molecular and biochemical aspects, in *New protective roles for selected nutrients* (Spiller GA, Scala J eds), pp 109–228. Alan R. Liss, New York.

Singhal SS, Godley BF, Chandra A, Pandya U, Jin GF, Saini MK, Awasthi S, Awasthi YC (1999) Induction of glutathione S-transferase hGST 5.8 is an early response to oxidative stress in RPE cells. Invest Ophthalmol Vis Sci 40:2652–2659.

Tucker K, Hedges TR (1993) Food shortages and an epidemic of optic and peripheral neuropathy in Cuba. Nutr Rev 51:349–357.

Uauy RD, Birch EE, Birch DG, Peirano P (1992) Visual and brain function measurements in studies of w3 fatty acid requirements of infants. J Pediatr 120:168–180.

Upadhya SC, Tirumalai PS, Boyd MR Mori T, Ravindranath V (2000) Cytochrome P4502E in brain: constitutive expression, induction by ethanol and localization by fluorescence in situ hybridization. Arch Biochem Biophys 373:23–34.

van Lith GHM, Henkes HE (1979) Low vision in alcohol abuse. Doc Ophthalmol 46:333–338.

Victor M, Dreyfus P (1965) Tobacco-Alcohol Amblyopia. Arch Ophthalmol 74:649–657.

Warner M, Gustafsson JA (1994) Effect of ethanol on cytochrome P450in the rat brain. Proc Natl Acad Sci USA 91:1019–1023.

Williams DE (1981) Visual electrophysiology and psychophysics in chronic alcoholics and in patients on tuberculostatic chemotherapy. Am J Optom Physiol Opt 61:576–585.

Woon C, Tang R, Pardo G (1995) Nutrition and optic disease. Semin Ophthalmol 10:195–202.