Synthesis of Docosahexaenoic Acid by Retina and Retinal Pigment Epithelium†

Nan Wang‡ and Robert E. Anderson*,‡,§

Department of Biochemistry and Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

Received June 10, 1993; Revised Manuscript Received August 9, 1993®

ABSTRACT: Rod outer segments of vertebrate retinas are highly enriched in docosahexaenoic acid (22:*n*–3), a fatty acid that is essential for optimal retinal function. The high levels of retinal 22:6*n*–3 are maintained through conservation (recycling) within the eye and incorporation from the circulation. The liver is thought to be a major source of 22:6*n*–3 through synthesis from appropriate *n*–3 precursors and delivery to target tissues via plasma lipoproteins. The contribution of other tissues to the total body pool of 22:6*n*–3 is not known. We investigated the synthesis of 22:6*n*–3 from [1-^{14}C]18:3*n*–3 or [3-^{14}C]22:5*n*–3 in frog retina and retinal pigment epithelium (RPE). RPE cells rapidly converted each precursor to 22:6*n*–3, which contained about 23 and 35%, respectively, of the label after 8 h. Significant labeling of 24:6*n*–3 and 24:5*n*–3 occurred when [3-^{14}C]22:5*n*–3 was the substrate. In contrast, the major end products of retinas incubated with [1-^{14}C]18:3*n*–3 and [3-^{14}C]22:5*n*–3 were 18:4*n*–3 and 20:5*n*–3, respectively, neither of which is found in retinal lipids. Less than 5% of the radioactivity from either precursor was in 22:6*n*–3 after an 8-h incubation. Our results demonstrate an active *in vitro* synthesis of 22:6*n*–3 in frog RPE, but not in the retina. The labeling of 24:5*n*–3 and 24:6*n*–3 is consistent with the proposal of Voss et al. [Voss, A., Reinhart, M., Sankarappa, S., & Sprecher, H. (1991) *J. Biol. Chem. 266*, 19995–20000] that they are intermediates in the conversion of 22:5*n*–3 to 22:6*n*–3. Since frog RPE contains measurable amounts of 18:3*n*–3, 20:5*n*–3, and 22:5*n*–3, which are readily converted to 22:6*n*–3 in these cells, we suggest that the RPE is a source of 22:6*n*–3 for the retina.

Docosahexaenoic acid (22:6*n*–3), an *n*–3 essential fatty acid derived from elongation and desaturation of linolenic acid (18:3*n*–3), comprises about 50 mol % of the fatty acids in retinal rod outer segment (ROS)[1] membrane phospholipids of most vertebrate species (Fliesler & Anderson, 1983). The reason for the high levels of 22:6*n*–3 in retinal membranes is not known, although changes in retinal function have been reported in rats (Benolken et al., 1973; Wheeler et al., 1975; Lamptey & Walker, 1976; Yamamoto et al., 1987), monkeys (Neuringer et al., 1984; 1986; 1988), and humans (Holman et al., 1982; Bjerve et al., 1987a; 1987b; 1988; Uauy et al., 1990; Birch, et al., 1992; Hoffman et al., 1992) fed diets deficient in *n*–3 fatty acids. Given some putative role in maintaining optimal retinal function, it is not surprising that the vertebrate retina conserves 22:6*n*–3 during *n*–3 fatty acid deprivation (Anderson & Maude, 1972; Futterman et al., 1971; Stinson, et al., 1990; Tinoco, 1982; Wiegand et al., 1991). However, 22:6*n*–3 turnover occurs in the retina (Stinson et al., 1991), necessitating a dietary supply of 22:6*n*–3 or its shorter chain precursors.

The liver is thought to be the primary source of 22:6*n*–3 in the body, either through packaging 22:6*n*–3 derived from the diet into lipoproteins or via synthesis from shorter-chain precursors (Brenner, 1971; Sinclair & Crawford, 1972; Sinclair, 1975; Naughton, 1981; Nouvelot et al., 1986; Sprecher, 1990; Scott & Bazan, 1989; Li et al., 1992). The role of other tissues in 22:6*n*–3 synthesis is generally considered to be minor (Scott & Bazan, 1989; Bazan et al., 1992). However, the issue of 22:6*n*–3 synthesis in extrahepatic tissues is not resolved. When labeled 18:3*n*–3 (Wetzel et al., 1991), 20:5*n*–3 (Bazan, et al., 1982), and 22:5*n*–3 (footnote 2) were injected into the vitreous humor, circumventing the blood–ocular barrier, labeled 22:6*n*–3 was recovered in retinal lipids. Astrocytes incubated with radioactive 18:3*n*–3 incorporated significant amounts of label into 22:6*n*–3 (Moore et al., 1991). Thus, it appears that some neuronal tissues can synthesize 22:6*n*–3 if provided the appropriate precursors.

Retinal pigment epithelial (RPE) cells form part of the posterior blood–ocular barrier and are intimately involved in the provision of blood-derived nutrients to the retina (Steinberg & Miller, 1979; Cunha-Vaz, 1980; Berman, 1991). We tested the hypothesis that frog retina and RPE can synthesize 22:6*n*–3 from two radioactive precursors, [1-^{14}C]18:3*n*–3 and [3-^{14}C]-22:5*n*–3. The results of our *in vitro* studies show that the RPE, but not the retina, actively converts both precursors to 22:6*n*–3.

EXPERIMENTAL PROCEDURES

Animals. Adult *Rana pipiens* (30–40 g) were purchased from J. M. Hazen (Alburg, VT) and maintained in a plastic chamber with constant running water. Animals were fed crickets weekly (Fluker's Cricket Farm, Baton Rouge, LA) and regulated to a light cycle of 14-h light and 10-h dark of ceiling fluorescent illumination of 250 lx at cage level. Frogs used in this study were adapted to these conditions for 1–2 months. All procedures were conducted according to the *NIH Guide for the Care and Use of Laboratory Animals* and the *ARVO Resolution on the Use of Animals in Research*. This research protocol was approved by the institutional Animal Protocol Review Committee.

† This work was supported by grants from the National Eye Institute (EY00871/04149/07001/02520), the National Retinitis Pigmentosa Foundation, Research to Prevent Blindness, Inc., and the Retina Research Foundation.

* To whom correspondence and reprint requests should be addressed.

‡ Department of Biochemistry.

§ Cullen Eye Institute.

® Abstract published in *Advance ACS Abstracts*, November 15, 1993.

[1] Abbreviations: PUFA, polyunsaturated fatty acid; ROS, rod outer segments; RPE, retinal pigment epithelium; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin; TG, triglycerides; DG, diglycerides; PL, phospholipids; FFA, free fatty acids.

[2] R. A. Alvarez and R. E. Anderson, unpublished results.

0006-2960/93/0432-13703$04.00/0

Tissue Preparation. Frogs were dark-adapted overnight, killed by decapitation, and pithed, and their eyes were immediately enucleated under dim red light and placed on ice. All procedures were carried out under dim red light until RPE cells were isolated. The anterior segments were removed and the eyecups containing retinas were incubated for 10–20 min at room temperature in Ca^{2+}-free Ringer Krebs medium containing 118 mM NaCl, 4.7 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 5.6 mM D-glucose, 35 mM $NaHCO_3$, and 1.0 mM ethylenediaminetetraacetate (EDTA) (pH = 7.4) (Salceda, 1986). The retina was then gently removed from the eyecup with a pair of jeweler's forceps. The retinas and resulting eyecups were kept in ice-cold Ca^{2+}-plus Ringer containing 118 mM NaCl, 4.7 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 5.6 mM D-glucose, 35 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and antibiotic cocktail (100 units/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B; GIBCO, Grand Island, NY) (pH = 7.4) until all dissections were completed.

Incubation Conditions. The retinas and eyecups (usually 3–4 retinas/mL or 4–5 eyecups/mL) were preincubated for 30 min in Ca^{2+}-plus Ringer (usually 2–3 mL for retinas, 6 mL for RPE) at 24 ± 1 °C under a constant stream of humidified O_2/CO_2 (95:5) with mild shaking. The incubation was started by the addition of an aliquot (usually 20 μL/mL of buffer) of 50 mM $NaHCO_3$ containing [1-^{14}C]18:3*n*–3 (53.0 mCi/mmol, New England Nuclear, Boston, MA) or [3-^{14}C]22:5*n*–3 (52.0 mCi/mmol, New England Nuclear, Boston, MA) conjugated with bovine serum albumin (BSA, 2:1 molar ratio). The final concentrations of [1-^{14}C]18:3*n*–3 and [3-^{14}C]22:5*n*–3 in the incubation buffer were 19–38 and 19 μM, respectively. After incubating for the indicated time periods, retinas and eyecups were washed three times with ice-cold Ca^{2+}-free Ringer Krebs medium containing 10 μM BSA. RPE cells were isolated as previously described (Chen et al., 1992a). Retinas and RPE cells were homogenized in (Tris buffered saline, TBS) and aliquots were taken for lipid analysis and protein determination (BCA protein assay reagent, Pierce Chemical Co., Rockford, IL).

Lipid Extraction and Lipid Class Separation. Total lipids were extracted from retinas and RPE cells according to Bligh and Dyer (1959) and washed once with Folch theoretical upper phase (Folch et al., 1957). An aliquot of total lipids was separated into phospholipids (PL), diglycerides (DG), free fatty acids (FFA), and triglycerides (TG) by one-dimensional thin-layer chromatography (TLC) on silica gel 60 (E. Merck, Darmstadt, Germany) using a solvent system of hexane/diethyl ether/glacial acetic acid (75:25:1, v/v/v). Lipid spots were visualized by iodine vapor. After sublimation, gel containing the lipid spots was scraped and counted for radioactivity with BCS cocktail (Amersham, Arlington Heights, IL).

Fatty Acid Phenacyl Ester (FAPE) Preparation and Analysis. Lipids, with a known amount of heptadecanoic acid (17:0) added as internal standard, were saponified (Wetzel et al., 1991), and free fatty acids were converted to FAPEs (Wood and Lee, 1983). FAPEs were dissolved in methanol and separated by HPLC on a LC-18 reverse-phase column [25 cm × 4.6 mm (I.D.), Supelco Inc., Bellefonte, PA] using a linear gradient of acetonitrile/water from 80:20 (v/v) to 92:8 at 2 mL/min for 45 min, followed by holding at 92:8 for 10 min. The mobile phase was then brought back to 80:20 in 5 min. FAPEs were monitored by UV absorbance at 242 nm. The identity of individual fatty acids was made by comparing elution times to those of FAPEs prepared from fatty acid standards (Nu Chek Prep. Inc., Elysian, MN) and analyzing the HPLC eluents by gas-liquid chromatography (Chen and Anderson, 1992b) after methylation. Fatty acid standards 24:5*n*–3 and 24:6*n*–3 were kindly provided by Dr. Howard Sprecher (Department of Medical Biochemistry, Ohio State University). The mass of individual fatty acids was determined as previously reported (Chen & Anderson, 1992b) and the radioactivity profile was monitored by an on-line radioactivity flow detector (Flo-One\Beta, Radiomatic, Tampa, FL) using Flo-scint A (Packard Instrument Co., Inc., Meriden, CT) at 2.5:1 (v/v) ratio of cocktail to mobile phase.

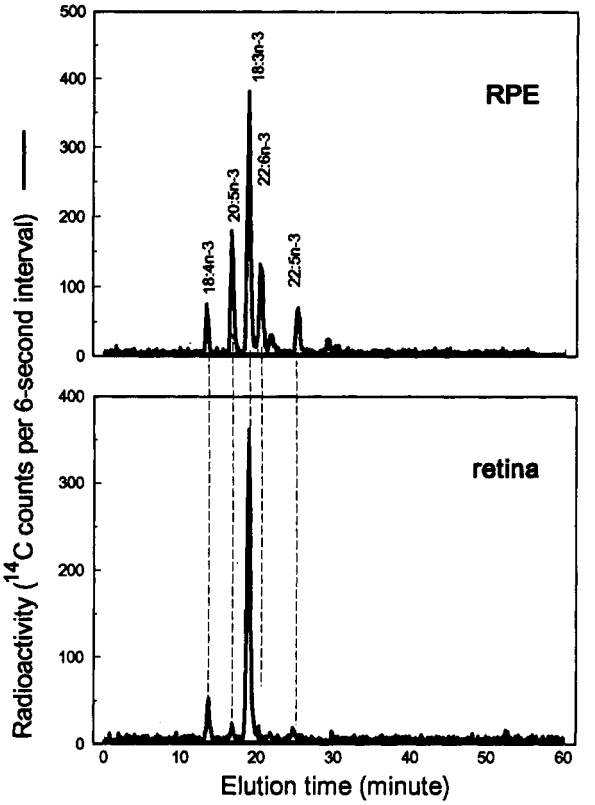

FIGURE 1: HPLC elution profiles of FAPE radioactivity from frog RPE and retina total lipids incubated with [1-^{14}C]18:3*n*–3. Retinas and eyecups were incubated with 19 μM [1-^{14}C]18:3*n*–3 for 3 h. FAPE of total lipids were separated by reverse-phase HPLC and radioactivity determined by an on-line radioisotope counter.

Catalytic Hydrogenation and HPLC Analysis of Hydrogenated Products. FAPE peaks were collected and FAPEs were extracted two times with equal volumes of hexane, dried, and dissolved in 2 mL ethanol/hexane (2:1). Approximately 6–10 mg of platinum oxide (Matheson Coleman & Bell Manufacturing Chemists, Norwood, OH) was added and the FAPE solution was bubbled with hydrogen for 10 min. The catalyst was pelleted by centrifugation and the pellet was washed two times with hexane. The pooled supernatant and washes were rephenacylated and analyzed on the same column described above using 100% acetonitrile as mobile phase over 35 min.

RESULTS

Elongation and Desaturation of [1-^{14}C]18:3n–3. Figure 1 shows a representative HPLC tracing of the radioactive fatty acids formed by frog retinas and RPE during a 3-h incubation with [1-^{14}C]18:3*n*–3. Labeled compounds were identified by comparing their retention times to those of authentic standards except 18:4*n*–3, which was compared to the retention of its methyl ester reported earlier (Moore et al., 1990, 1991). Peak identities were further confirmed by reverse-phase HPLC analysis of the hydrogenation products. There was an active

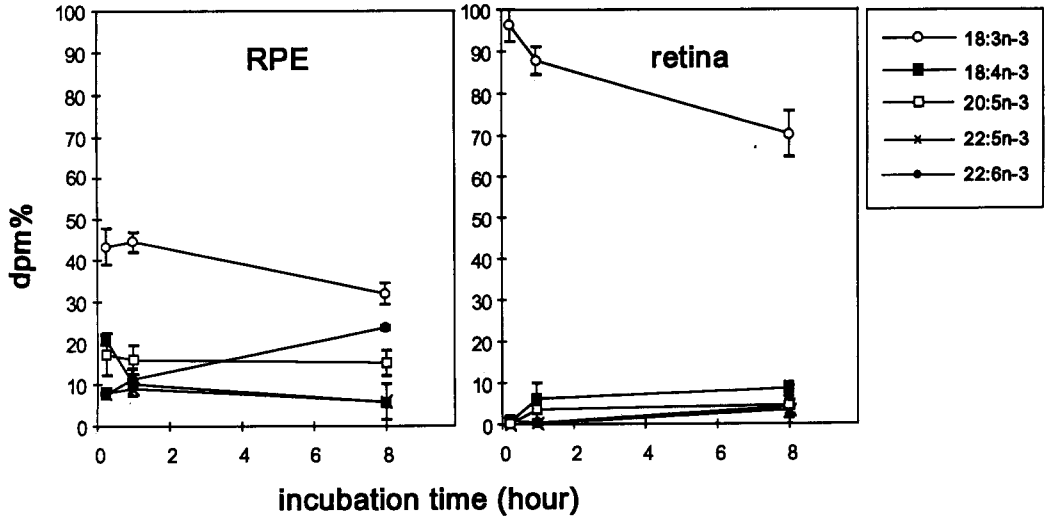

FIGURE 2: Time-dependent formation of radioactive metabolic products in frog RPE and retina incubated with [1-^{14}C]18:3*n*–3. Retinas and eyecups were incubated with 19–38 μM [1-^{14}C]18:3*n*–3 from 15 min to 8 h. FAPEs of total lipids were prepared and analyzed by HPLC. Each data point represented an average of two observations ± range.

uptake and metabolism of 18:3*n*–3 by RPE cells (Figure 2). At 15 min, over one-half of the label was already in other fatty acids, 18:4*n*–3 and 20:5*n*–3 being the most heavily labeled products. With time, the relative amount of label in 18:4*n*–3 declined substantially and almost 25% of the label had accumulated in 22:6*n*–3 after 8 h. In contrast, 18:3*n*–3 was metabolized to a much lesser extent in the retina. The major product of the 8-h incubation was 18:4*n*–3 and the relative amount of label that accumulated in 22:6*n*–3 was never over 4%.

Elongation and Desaturation of [3-^{14}C]22:5n–3. When retinas and eyecups were incubated with [3-^{14}C]22:5*n*–3, radioactivity was recovered in 20:5*n*–3, 22:5*n*–3, 22:6*n*–3, 24:5*n*–3, and 24:6*n*–3 (Figure 3). Identification of these fatty acids was described above. The relative distribution of radioactivity (dpm%) following up to 8-h incubation with [3-^{14}C]22:5*n*–3 is shown in Figure 4. The dpm% of 22:6*n*–3 in RPE increased steadily with time and reached 37% after 8 h. Among the metabolites of 22:5*n*–3, 22:6*n*–3 had the highest dpm% in RPE at all time points. In contrast, the value for 22:6*n*–3 in retina never reached 5%. The amount (dpm/μg protein) of each labeled fatty acid in RPE and retina is shown in Figure 5. The RPE incorporated more radioactivity than retina at all time points. Compared to retina, RPE has 15–25-fold more labeled 22:6*n*–3 and 24:6*n*–3, 3–5-fold more labeled 22:5*n*–3 and 20:5*n*–3, and 2-fold more labeled 24:5*n*–3.

The specific activities (dpm/nmol fatty acid) of four FAPEs from RPE isolated from eyecups incubated with [3-^{14}C]22:5*n*–3 are plotted against time in Figure 6 (top left). Data for 24:5*n*–3 are not included because an accurate determination of mass could not be made due to coelution of 24:5*n*–3 and 16:0 (Figure 3). Since the endogenous levels of 20:5*n*–3 and 24:6*n*–3 are low in RPE, the calculated specific activities are minimum values because any unlabeled fatty acids coeluting with either would increase their apparent mass without necessarily increasing their radioactivity. The specific activity of 24:6*n*–3 in the RPE was less than that of 22:5*n*–3, but 26–28 times higher than that of 22:6*n*–3 at all time points.

The relative incorporation (dpm%) of labeled 22:5*n*–3 into 24:5*n*–3, 24:6*n*–3, and 22:6*n*–3 in RPE is depicted in Figure 6 (bottom left). At the earliest time points, 60% of the label was in 22:6*n*–3, 30% in 24:6*n*–3, and 10% in 24:5*n*–3. With time, the dpm% in 22:6*n*–3 increased while the percentages

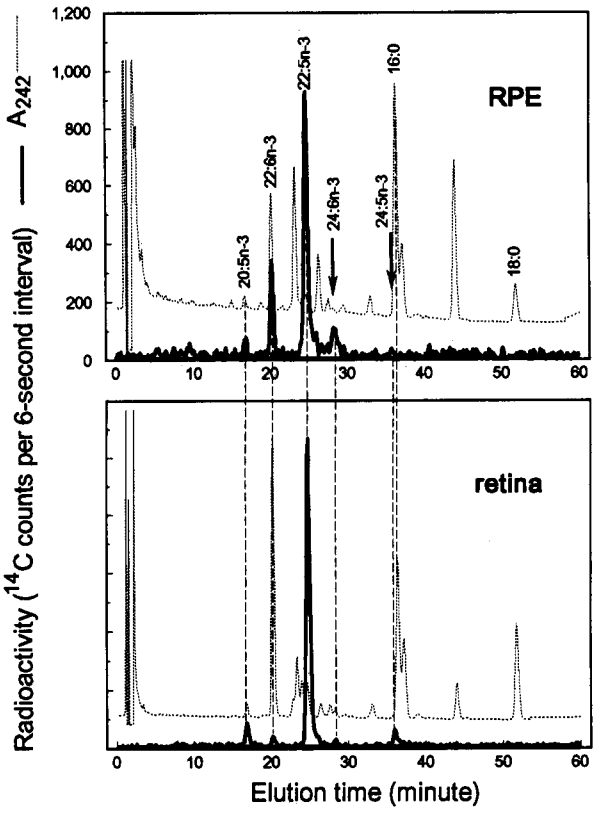

FIGURE 3: HPLC elution profiles of FAPE radioactivity from frog RPE and retina total lipids incubated with [3-^{13}C]22:5*n*–3. Retinas and eyecupes were incubated with 19 μM [3-^{14}C]22:5*n*–3 for 2 h. FAPE of total lipids were separated by reverse-phase HPLC and radioactivity determined by an on-line radioisotope counter. The mass tracing is shown with dotted line.

in 24:5*n*–3 and 24:6*n*–3 decreased, consistent with the latter two being intermediates and 22:6*n*–3 an end product of fatty acid anabolism.

The specific activities of retinal fatty acids derived from [3-^{14}C]22:5*n*–3 are plotted against time in Figure 6 (top right). The absolute values are less than those of the RPE fatty acids (including that of the precursor), probably due to a larger dilution by endogenous fatty acids. The product with the highest specific activity was 20:5*n*–3, which was formed by retroconversion of 22:5*n*–3. The relative distribution of the label in 24:5*n*–3, 24:6*n*–3, and 22:6*n*–3 (Figure 6, bottom

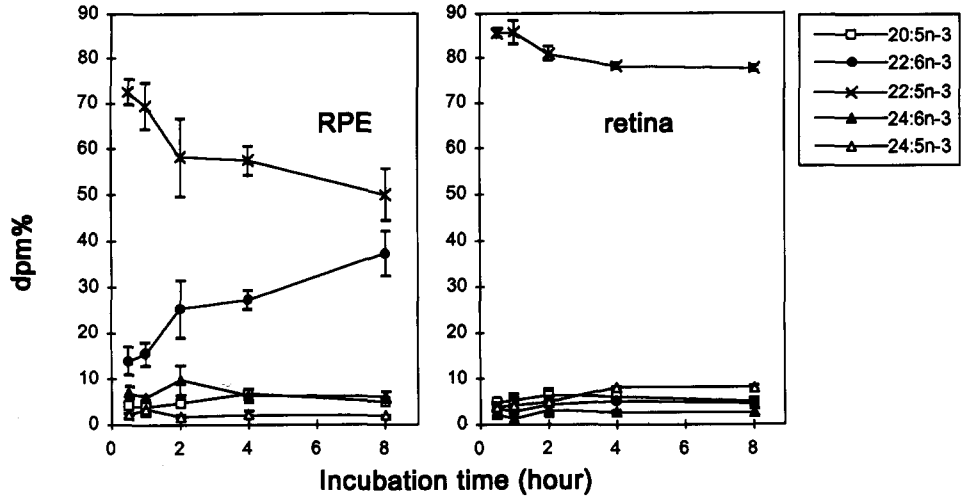

FIGURE 4: Time-dependent formation of radioactive metabolic products in frog RPE and retina incubated with [3-^{14}C]22:5*n*–3. Retinas and eyecups were incubated with 19 μM [3-^{14}C]22:5*n*–3 from 15 min to 8 h. FAPEs of total lipids were prepared and analyzed by HPLC. Each data point is mean of at least three independent determinations with the error bar indicating standard error of mean.

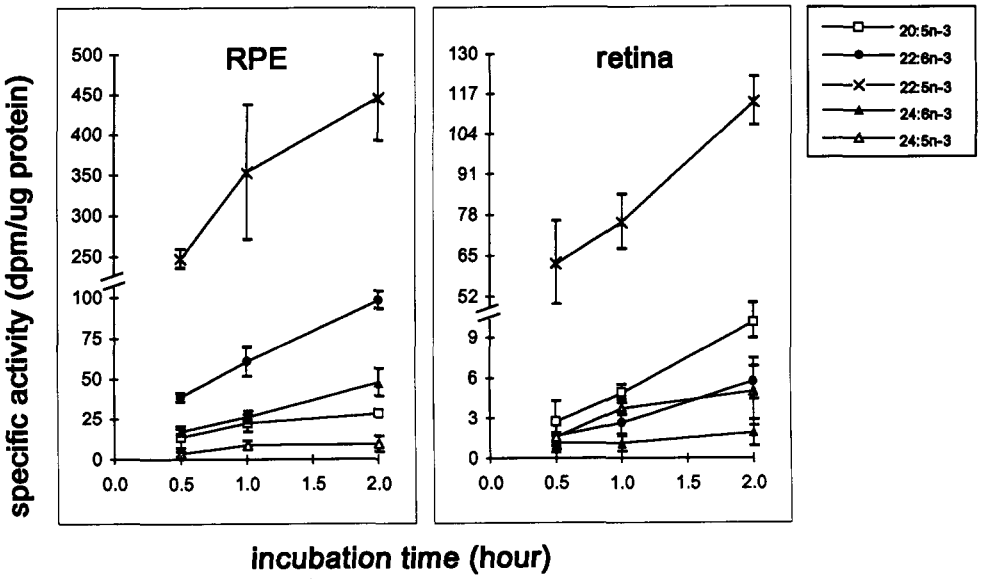

FIGURE 5: Specific activity (dpm/μg protein) in RPE and retina incubated with [3-^{14}C]22:5*n*–3. Retinas and eyecups were incubated with 19 μM [3-^{14}C]22:5*n*–3 for 0.5, 1, and 2 h, RPE cells were subsequently isolated and purified, and fatty acids were analyzed for both tissues. Each data point is expressed as average of three ± SEM.

right) shows a pattern different from that found for the RPE.

Distribution of Radioactivity among Lipid Classes. The relative distribution of radioactivity (dpm%) among different lipid classes is shown in Figure 7. Most of the radioactivity was found in phospholipids in retina, although free fatty acids contained a significant amount of label during the early time points (ca. 35% at 0.5 h). With time, the dpm% in phospholipids increased and accounted for more than 70% of the total radioactivity in the retina after 2 h. In contrast, 70–80% of the radioactivity in RPE was in triglycerides and only 15–20% in phospholipids.

DISCUSSION

Our results show that the RPE, but not the retina, actively synthesizes 22:6*n*–3 from available precursors. RPE cells incubated continuously with [1-^{14}C]18:3*n*–3 or [3-^{14}C]22:5*n*–3 at relatively high concentrations (19–38 μM) converted over half of the substrate to other polyunsaturated fatty acids, the major product being 22:6*n*–3. In contrast, the retina converted only modest amounts of the labeled precursors to 22:6*n*–3. Even after 8 h incubation with [1-^{14}C]18:3*n*–3, only 4% of the label was in 22:6*n*–3. The most heavily labeled fatty acid (other than 18:3*n*–3) was 18:4*n*–3 (8%), which has no measurable mass in the retina. When incubated with [3-^{14}C]22:5*n*–3, the fatty acid with the highest specific activity in the retina was 20:5*n*–3, a retroconversion product. After 8-h incubation, less than 5% of the label was in 22:6*n*–3. Thus, while the RPE seems quite capable of active synthesis of 22:6*n*–3, the retina shows limited ability under our experimental conditions. Previous studies have shown that 22:5*n*–3 and 20:5*n*–3, but not 22:6*n*–3, can be synthesized in cerebral and cerebellar neurons (Moore, et al., 1991; Spector & Moore, 1992) and brain microvessel endothelial cells (Moore, Yoder, & Spector, 1990). However, only cultured astrocytes were able to convert 18:3*n*–3 to 22:6*n*–3 (Moore et al., 1991). Brain astrocytes and retinal pigment epithelial cells are apparently serving a similar role in that each can synthesize 22:6*n*–3 from available precursors, while adjacent neuronal cells cannot.

In previous studies where precursors of 22:6*n*–3 were injected intravitrally, a significant amount of label was recovered in 22:6*n*–3 in the retina (Alvarez & Anderson, 1990; Bazan et al., 1982; Wetzel et al., 1991; footnote 2). Curiously, large

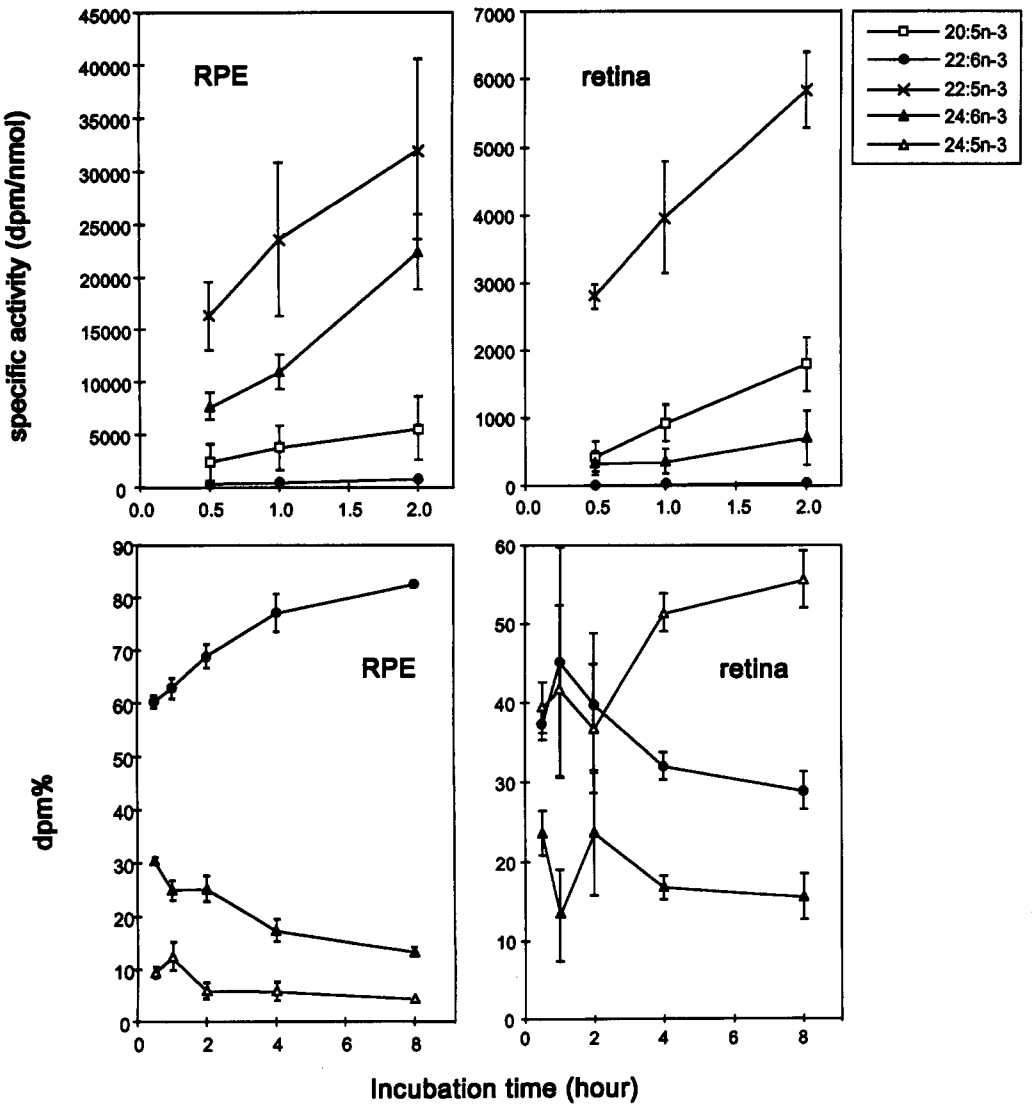

FIGURE 6: Specific activity (dpm/nmol fatty acid) and relative distribution (dpm%) of radioactive metabolic products in frog RPE and retina incubated with [3-^{14}C]22:5n–3. Retinas and eyecups were incubated with 19 μM [3-^{14}C]22:5n–3 for the indicated periods of time. The top panels contain the specific activity of individual fatty acids. The bottom panels contain the dpm% in 22:6n–3, 24:6n–3, and 24:5n–3. Each data point is mean of at least three independent determinations with the error bar indicating standard error of mean.

amounts of labeled precursors (18:3n–3 and 20:5n–3) were also incorporated unchanged into retinal glycerolipids. The ratio of label in 22:6n–3 to that in the precursors increased slowly with time (days). A conclusion from these studies was that the retina could synthesize 22:6n–3 from available precursors, but that the blood–ocular barrier prevented access to 18:3n–3 and 20:5n–3. The argument that the low endogenous levels of these precursors reflected a rapid conversion to 22:6n–3 was rejected because of the accumulation of labeled 18:3n–3 and 20:5n–3 in retinal glycerolipids. In these studies, it was assumed that the synthesis of 22:6n–3 occurred in the retina. In view of the present results, a different interpretation is suggested. Labeled precursors taken up by retina are incorporated unchanged into glycerolipids, while in the RPE they are rapidly converted to 22:6n–3, which is shuttled back to the retina. With time, as more precursor is provided to the RPE through the rhythmic shedding of ROS tips, the ratio of labeled 22:6n–3 to precursor in ROS slowly increases.

Since the RPE can synthesize 22:6n–3, its relative importance as a source of retinal 22:6n–3 depends in part on the availability of precursors in the RPE. Several recent studies in our laboratory address this issue. RPE cells isolated from frogs (Chen & Anderson, 1992a) contain measurable levels of 18:3n–3, 20:5n–3, and 22:5n–3. Rats fed diets containing 10% linseed oil (ca. 50% 18:3n–3) as the only source of fat incorporated 18:3n–3 (footnote 3), 20:5n–3 (Wang & Anderson, 1992), and 22:5n–3 (footnote 3) into RPE lipids. Since the RPE has the appropriate precursors of 22:6n–3, it seems reasonable to conclude that synthesis of 22:6n–3 in this tissue is a source of 22:6n–3 for the retina. The recycling of 22:6n–3 derived from phagocytized ROS tips provides a mechanism for 22:6n–3 synthesized in the RPE to be shuttled to the retina (Gordon & Bazan, 1990; Gordon et al., 1992; Bazan et al., 1992; Anderson et al., 1992; Chen et al., 1992b).

The retina and RPE retroconverted significant amounts of 22:5n–3 to 20:5n–3. In fact, more radioactivity was incorporated into 20:5n–3 than into 22:6n–3 in the retina. Since the level of 20:5n–3 in retina phospholipids is <0.1% (Fliesler & Anderson, 1983), the significance of the retroconversion activity is not clear. One possibility is that retroconversion provides a source of shorter chain polyunsaturated fatty acids that may have other physiological functions. For example, 14:2n–6 and 14:1n–9 are esterified to the N-terminus of the α-subunit of transducin (Neubert et al., 1992; Kokame et al., 1992), the G-protein involved in visual transduction in the retina (Fung, 1987). Since animals cannot synthesize a fatty

[3] N. Wang and S. E. Anderson, unpublished results.

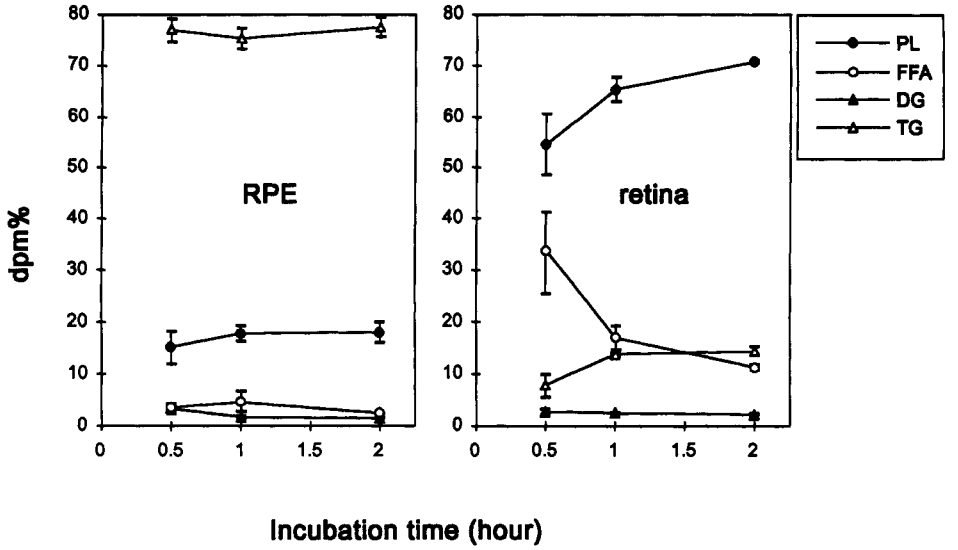

FIGURE 7: Relative distribution of radioactivity among lipid classes (dpm%) in RPE and retina incubated with [3-^{14}C]22:5*n*–3. Retinas and eyecups were incubated with 19 μM [3-^{14}C]22:5*n*–3 for up to 2 h. Lipids were extracted, lipid class separated by thin-layer chromatography, and radioactivity counted. PL, total phospholipids; DG, diacylglycerides; FFA, free fatty acids; TG, triglycerides. Each data point is expressed as an average of three independent determinations with the error bar indicating standard error of mean.

acid with a *n*–3 or an *n*–6 double bond, the most likely source of 14:2*n*–6 is from retroconversion of 18:2*n*–6. Indeed, Hiltunen et al. (1986) have demonstrated the retroconversion of 20:4*n*–6 to 14:2*n*–6 in rat liver peroxisomes. Likewise, 14:1*n*–9 must be derived from 18:1*n*–9 because the double bond in 14:1 produced by *de novo* synthesis is in the *n*–5 position [reviewed by Jensen et al. (1990); Hamosh & Bitman, 1992; Kolezko & Springer, 1992]. Since retroconversion is an active catabolic pathway in retina, it should be considered a potential source of these short-chain unsaturated fatty acids.

Until the recent report of Voss et al. (1991), it had been proposed but never proven that 22:6*n*–3 was formed directly by desaturation of 22:5*n*–3 via a δ-4 desaturase. Although other desaturases involved in conversion of 18-carbon *n*–3 and *n*–6 polyunsaturated fatty acids to longer chain products have been described (Bernet & Sprecher, 1975; Pugh & Kates, 1977; Brenner et al., 1980; Okayasu et al., 1981; Rosenthal, 1987; Leikin & Brenner, 1989), the δ-4 desaturase remained elusive. Voss et al. (1991) showed in rat liver hepatocytes that 22:5*n*–3 was first converted to 24:5*n*–3 which was then desaturated to 24:6*n*–3. This product was retroconverted to 22:6*n*–3, presumably through removal of the two-carbon unit by peroxisomal enzymes. The unique feature of this pathway is that the last desaturation involves δ-6 rather than a δ-4 desaturase.

Our results suggest that the pathway shown by Voss et al. (1991) for liver hepatocytes is operative in RPE cells. Eyecups incubated with [3-^{14}C]22:5*n*–3 incorporated radioactivity into 24:5*n*–3 and 24:6*n*–3. The specific radioactivity (dpm/nmol fatty acid) to 24:6*n*–3 was over 25 times that of 22:6*n*–3 at all time points. It may be argued that the low specific activity of 22:6*n*–3 relative to 24:6*n*–3 (see Figure 6, upper left) could be due to a greater endogenous dilution of 22:6*n*–3 when the total RPE lipids were extracted for HPLC analysis, since RPE has considerably more 22:6*n*–3 than 24:6*n*–3. However, this issue can be addressed in another way, by comparing the relative amounts of label in 22:6*n*–3, 24:5*n*–3, and 24:6*n*–3 (Figure 6, lower left). At the early time points, the dpm% of 24:5*n*–3 and 24:6*n*–3 was highest relative to 22:6*n*–3. At later incubation times, the proportion changed as the dpm% of 22:6*n*–3 increased. This pattern is consistent with 22:6*n*–3 being an end product of synthesis, and 24:5*n*–3 and 24:6*n*–3 being intermediates, and suggests that the pathway proposed by Voss et al. (1991) is operative in the RPE.

Retina and RPE actively synthesized glycerolipids from [3-^{14}C]22:5*n*–3. It is interesting that the major product in the retina is phospholipid while triglyceride is most prominent in the RPE. In the retina, there is an active daily renewal of ROS membranes which necessitates synthesis of large amounts of phospholipid. In contrast, the RPE phagocytizes shed ROS tips and digests the membranes to their small molecular components. 22:*n*–3 is conserved in the retina through recycling between the retina and RPE (Stinson et al., 1991; Gordon & Bazan, 1990; Gordon et al., 1992; Chen et al., 1992b). Fatty acids in the RPE are incorporated into triglycerides of the oil droplets (Gordon & Bazan, 1990; Gordon et al., 1992; Chen & Anderson, 1993), which we have proposed are temporary storage sites for the large amount of fatty acids that are processed each day by the RPE (Chen & Anderson, 1993). The present study supports this notion and accents the fundamentally different roles of the retina and the RPE in glycerolipid metabolism.

ACKNOWLEDGMENT

The authors thank Dr. Howard Sprecher (Department of Medical Biochemistry, Ohio State University) for his generous gift of 24:5*n*–3 and 24:6*n*–3 fatty acids and for helpful discussions of the experiments, and Huiming Chen for his critique of the manuscript.

REFERENCES

Alvarez, R. A., & Anderson, R. E. (1990) *Invest. Ophthalmol. Vis. Sci. 31* (*Suppl.*), 579.

Anderson, R. E., & Maude, M. B. (1972) *Arch. Biochem. Biophys. 151*, 270–276.

Anderson, R. E., O'Brien, P. J., Wiegand, R. D., Koutz, C. A., & Stinson, A. M. (1992) in *Advances in Experimental Medicine and Biology* (Bazan, N. G., Murphy, M. G., and Toffano, G., Eds.), Vol. 318, pp 285–294.

Bazan, H. E. P., Careaga, M. M., Sprecher, H., & Bazan, N. G. (1982) *Biochim. Biophys. Acta 712*, 123–128.

Bazan, N. G., Gordon, W. C., & Rodriguez de Turco, E. B. (1992) in *Neurobiology of Essential Fatty Acids* (Bazan, N. G., Murphy, M. G., and Toffano, G. eds), pp 295–306, Plenum Press, New York.

Benolken R. M., Anderson, R. E., & Wheeler T. G. (1973) *Science 182*, 1253.
Berman, E. R. (1991) in *Biochemistry of the Eye*, pp 309–467, Plenum Press, New York.
Bernet, J. T., & Sprecher, H. (1975) *Biochim. Biophys. Acta 398*, 354–363.
Birch, D. G., Birch, E. E., Hoffman, D. R., & Uauy, R. D. (1992) *Invest. Ophthalmol. Vis. Sci. 33*, 2365–2376.
Bjerve, K. S., Mostad, I. L., & Thoresen, L. (1987a) *Am. J. Clin. Nutr. 45*, 66–77.
Bjerve, K. S., Fischer, S., & Alme, K. (1987b) *Am. J. Clin. Nutr. 46*, 570–576.
Bjerve, K. S., Thoresen, L., & Børsting, S. (1988) *J. Parent. Enteral. Nutr. 12*, 521–525.
Bligh, E. G., & Dyer, W. J. (1959) *Can. J. Biochem. Physiol. 37*, 911–917.
Bok, D., & Young, R. W. (1979) in *The Retinal Pigment Epithelium* (Zinn, K. M., and Marmor, M. F., Eds.), pp 148–174, Harvard University Press, Cambridge.
Brenner, R. R. (1971) *Lipids 6*, 567–575.
Brenner, R. R., Garda, H., Leikin, A. I., & Pezzano, H. (1980) *Acta. Physiol. Lat. Am. 30*, 225–238.
Chen, H., & Anderson, R. E. (1993) *J. Lipid Res.* (in press).
Chen, H., & Anderson, R. E. (1992a) *Curr. Eye Res. 11*, 793–800.
Chen, H., & Anderson, R. E. (1992b) *J. Chromatogr. 578*, 124–129.
Chen, H., Wiegand, R. D., & Anderson R. E. (1992a) *Exp. Eye Res. 54*, 885–892.
Chen, H., Wiegand, R. D., Koutz, C. A., & Anderson, R. E. (1992b) *Exp. Eye Res. 55*, 93–100.
Cohen, S. R., & Bernsohn J. (1978) *J. Neurochem. 30*, 661–669.
Cook, H. W. (1978) *J. Neurochem. 30*, 1327–1334.
Cunha-Vaz, J. G. (1980) in *The Blood Retinal Barriers* (Cunha-Vaz, J. G., Ed.), pp 101–118, Plenum Press, New York.
Dhopeshwarkar, G. A., & Subramanian, C. (1976) *Lipids, 11*, 67–71.
Fliesler, S. J., & Anderson, R. E. (1983) in *Progress in Lipid Research* (Holman, R. T., Ed.), Vol. 22, pp 79–131, Pergamon Press Ltd., London, UK.
Folch, J., Lees, M., & Solanestanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
Fung, B. K. K. (1987) in *Progress in Retinal Research* (Osborne, N., and Chader, G., Eds.), Vol. 6, pp 151–177, Pergamon Press, New York.
Futterman, S., Downer, J. L., & Hendrickson, A. (1971) *Invest. Ophthalmol. 10*, 151–156.
Gordon, W. C., & Bazan, N. G. (1990) *J. Neurosci. 10*, 2190–2202.
Gordon, W. C., Rodriguez-de-Turco, E. B., & Bazan, N. G. (1992) *Curr. Eye Res. 11*, 73–83.
Hamosh, M., & Bitman, J. (1992) *Lipids 27*, 848–857.
Hiltunen, J. K., Karki, T., Hassinen, I. E., & Osmundsen, H. (1986) *J. Biol. Chem. 216*, 16484–16493.
Hoffman, D. R., Uauy, R., Birch, D. G., & Birch, E. E. (1992) *Invest. Ophthal. Vis. Sci. 33 (Suppl.)*, 1185.
Holman, R. T., Johnson, S. B., & Hatch, T. F. (1982) *Am. J. Clin. Nutr. 35*, 617–623.
Jenson, R. G., Ferris, A. M., Lammi-Keefe, C. J., & Henderson, R. A. (1990) *J. Dairy Sci. 73*, 223–240.
Kokame, K., Fukada, Y., Yoshizawa, T., Takao, T., & Shimonishi, Y. (1992) *Nature 359*, 749–752.
Koletzko, B., Thiel, I., & Springer, S. (1992) *Eur. J. Clin. Nutr. 46 (Suppl. 4)*, S45–S55.
Lamptey, M. S., & Walker, B. L. (1976) *J. Nutr. 106*, 86–93.
Leikin, A. I., & Brenner, R. R. (1989) *Lipids 24*, 101–104.
Li, J., Wetzel, M. G., & O'Brien, P. J. (1992) *J. Lipid Res. 33*, 539–548.
Miyamotor, K., Stephanides, L. M., & Bernsohn, J. (1967) *J. Neurochem. 14*, 227–237.
Moore, S. A., Yoder, E., & Spector, A. A. (1990) *J. Neurochem. 55*, 391–402.
Moore, S. A., Yoder, E., Murphy, S., Dutton, G., & Spector, A. A. (1991) *J. Neurochem. 56*, 518–524.
Naughton, J. M. (1981) *Int. J. Biochem. 13*, 21–32.
Neubert, T. A., Johnson, R. S., Hurley, J. B., & Walsh, K. A. (1992) *J. Biol. Chem. 267*, 18274–18277.
Neuringer, M., Connor, W. E., Van Petten, C., & Barstad, L. (1984) *J. Clin. Invest. 73*, 272–276.
Neuringer, M., Connor, W. E., Lin, D. S., Barstad, L., & Luck, S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4021–4025.
Neuringer, M., Anderson, G. J., & Connor, W. E. (1988) *Annu. Rev. Nutr. 8*, 517–541.
Nouvelot, A., Delbart, C., & Bourre, J. M. (1986) *Annu. Nutr. Metab. 30*, 316–323.
Okayasu, T., Nagao, M., Ishibashi, T., & Imai, Y. (1981) *Arch. Biochem. Biophys. 206*, 21–28.
Pugh, E. L., & Kates, M. (1977) *J. Biol. Chem. 252*, 68–72.
Robert, J., Mentaudon, D., & Hughes, P. (1983) *Biochim. Biophys. Acta 752*, 383–395.
Rosenthal, M. D. (1987) in *Progress in Lipid Research* (Holman, R. T., Ed.), Vol. 26, pp 87–124, Pergamon Press Ltd., London, UK.
Salceda, R. (1986) *Invest. Ophthalmol. Vis. Sci. 27*, 1172–1176.
Scott, B. L., & Bazan, N. G. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 2903–2907.
Sinclair, A. J. (1975) *Lipids 10*, 175–184.
Sinclair, A. J., & Crawford, M. A. (1972) *FEBS Lett. 26*, 127–129.
Spector, A. A., & Moore, S. A. (1992) in *Essential Fatty Acids and Eicosanoids* (Sinclair, A., and Gibson, R., Eds.), pp 4, American Oil Chemists' Society, Champaign, IL.
Sprecher, H. (1990) in *Health Effects of ω3 Polyunsaturated Fatty Acids in Seafoods* (Simopoulos, A. P., Kiffer, R. R., Martin, R. E., and Barlow, S. M., Eds.), pp 166–176, Karger, New York.
Steinberg, R. H., & Miller, S. S. (1979) in *The Retina Pigment Epithelium* (Zinn, K. M., and Marmor, M. F., Eds.) pp 205–225, Harvard University Press, Cambridge.
Stinson, A. M. (1990) Mechanisms of conservation of docosahexaenoic acid in rat retinas during essential fatty acid deprivation. Ph.D. Thesis, Baylor College of Medicine, Houston.
Stinson, A. M., Wiegand, R. D., & Anderson, R. E. (1991) *J. Lipid Res. 32*, 2009–2017.
Tinoco, J. (1982) *Prog. Lipid Res. 21*, 1–45.
Tocher, D. R., & Sargent, J. R. (1990) *J. Neurochem. 54*, 2118–2124.
Uauy, R.; Birch, D. G., Birch, E. E., & Hoffman, D. R. (1990) in *Health Effects of ω3 Polyunsaturated Fatty Acids in Seafood* (Simopoulos, A. P., Kifer, R. R., Martin, R. E., and Barlow, S. M., Eds.), pp 506, Karger, New York.
Voss, A., Reinhart, M., Sankarappa, S., & Sprecher, H. (1991) *J. Biol. Chem. 266*, 19995–20000.
Wang, N., & Anderson, R. E. (1992) *Cur. Eye Res. 11*, 783–791.
Wetzel, M. G., Li, J., Alvarez, R. A., Anderson, R. E., & O'Brien, P. J. (1991) *Exp. Eye Res. 53*, 437–446.
Wiegand, R. D., Koutz, C. A., Stinson, A. M., & Anderson, R. E. (1991) *J. Neurochem. 57*, 1690–1699.
Wheeler, T. G., Benolken, R. M., & Anderson, R. E. (1975) *Science 188*, 1312–1314.
Wood, R., & Lee, T. (1983) *J. Chromatogr. 254*, 237–246.
Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., & Okutama, H. (1987) *J. Lipid Res. 28*, 144–155.
Yavin, E., & Menkes, J. H. (1974a) *J. Lipid Res. 15*, 152–157.
Yavin, E., & Menkes, J. H. (1974b) *Lipids 9*, 248–253.