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LIPID COMPOSITION OF BOVINE RETINAL OUTER SEGMENT FRAGMENTS

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SUMMARY

The lipid content and composition of highly purified preparations of bovine retinal outer segment fragments are given. Almost half of the retinal outer segment fragments is lipid on a weight basis; most of the lipid is phospholipid. Phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine are the major retinal outer segment phospholipids, although small but significant amounts of phosphatidylinositol and sphingomyelin are also present. The retinal outer segment fatty acids contain an unusually high concentration (approx. 50%) of highly unsaturated long-chain fatty acids. The predominant fatty acid, docosahexaenoic acid (22:6), accounts for 37% of the fatty acids; docosatetraenoic acid (22:4), 7% and arachidonic acid (20:4), 8%. Palmitic acid (16:0) and stearic acid (18:0), the most abundant saturated fatty acids, account for at least 10 and 16%, respectively.

INTRODUCTION

Rod outer segments are believed to be the primary photoreceptors in the eye for black and white vision. These organelles measure approx. $2 \times 20\,\mu$ and consist of stacks of approx. 1000 membraneous discs[1]. The visual pigment is contained in these membranes[2]. Recently, a highly purified preparation from bovine retina has been described[3]. In this communication we present the lipid composition of this preparation. A preliminary account of this study has already been presented[4].

METHODS

Highly purified bovine retinal outer segment fragments were isolated as described previously[3]. Protein was determined by the method of LOWRY *et al.*[5] using bovine plasma albumin for calibration. In order to measure the dry weight of retinal outer segment preparations, aliquots (1–2 ml) containing approx. 20–30 mg protein were dialyzed to remove sucrose. Dialysis was carried out in a dark coldroom using four changes of deionized water, 2 l each change. Aliquots of the dialyzate were then transferred to tared flasks and lyophilized to remove water from the sample. After lyophilization, the flasks were transferred to a desiccator and evacuated to remove

remaining moisture. The vacuum was released with N_2, and the flasks were weighed. In the same manner, the flasks were reweighed several times to constant weight.

The lipids were extracted from undialyzed suspensions of retinal outer segments, using chloroform–methanol (2:1, v/v) with appropriate care to exclude O_2 (ref. 6). Butylated hydroxytoluene was added at the beginning of the extraction to further insure against lipid peroxidation[7]. Approx. 0.2 % butylated hydroxytoluene per expected weight of lipid was added. Nonlipid material in the extract was removed either by passing the lipids through Sephadex G-25 (ref. 8), or by back-extraction according to FOLCH *et al.*[9]. Finally, the lipids were evaporated to a moist residue and resuspended in a small volume of chloroform–methanol (2:1, v/v). All operations were carried out under N_2 atmosphere[6].

A small aliquot of the extract was used to determine the dry weight of lipid in the retinal outer segment preparations. A sample was placed in a small tared aluminum foil cup and evaporated with a gentle stream of N_2 to insure even drying. Any remaining solvent was removed by placing the sample in a vacuum for 10 min. The vacuum was released with N_2, and the sample was weighed using a Model 6 Cahn gram electrobalance. The sample was re-evacuated and reweighed several times in order to obtain a constant weight[6]. Phosphorus was determined on another aliquot by a modification of CHEN *et al.*[10].

The phospholipid composition of the retinal outer segments was determined using two-dimensional thin-layer chromatography[11]. Thin-layer plates were spread with a Desaga spreader. Adsorbant for five plates contained 20 g silica acid (Silica gel H, E. Merck, Darmstadt, Germany, dist. by Brinkmann) suspended in a solution of magnesium acetate (1.5 g per 60 ml water[12]). Chloroform–methanol–ammonia (65:25:5, by vol.) was used in the first dimension, and chloroform–acetone–methanol–acetic acid–water (3:4:1:1:0.5, by vol.) in the second. This system gave complete separation of all observable retinal outer segment phospholipids and was therefore used for quantitation. After chromatography, the plates were air-dried and the components observed by charring with 3 % formaldehyde in concentrated H_2SO_4 (ref. 6). The spots which appeared on the plates after charring were transferred quantitatively to digestion flasks and the phosphorus contained in each spot determined[11]. The phospholipid composition is expressed as a percent of the total phosphorus mounted on the plate. Quadruplicate determinations were made on each retinal outer segment preparation.

Identification of the phospholipids was made on the basis of known migration of phospholipids in the two-dimensional chromatography system. Two other solvent systems using silica-gel plates containing 10 % magnesium silicate were also used for identification[11]. Sphingomyelin and phosphatidylinositol were cochromatographed with the retinal outer segment lipids for further verification of these minor components. The phosphatidylinostinol used was isolated from beef heart mitochondria using DEAE-cellulose column chromatography[13] and the brain sphingomyelin was a gift from Dr. George Rouser. A ninhydrin spray was employed to identify spots on the plates containing phospholipids with amino groups[6]. The elution sequence on DEAE-cellulose columns was also used to further verify the indentity of the phospholipids[6].

Differences in the infrared spectra of phospholipids is often adequate to provide a unique identification of a specific phospholipid. Infrared spectra on fractions dissolv-

ed in CS_2, using NaCl cells of 1-cm path length were obtained. The infrared spectrum was recorded using a Model 521 Perkin-Elmer spectrophotometer (courtesy of Dr. Garry Nelson, Biomedical Division, Lawrence Radiation Laboratory, University of California, Livermore, Calif.).

Fatty acid methyl esters of the retinal outer segment lipids were prepared using 10 % BF_3 in methanol[14]. The fatty acid methyl esters were also hydrogenated[15]. An aliquot of the fatty acid methyl esters was first evaporated almost to dryness using a gentle stream of N_2, then resuspended in 1 ml of methanol. A pinch of palladium black was added and the mixture was bubbled with H_2 for 5 min. The suspension was sealed with a cap containing a Teflon liner and heated for 10 min at 50°. After cooling, 1 ml of water was added to the sample. The hydrogenated methyl esters were partitioned from this suspension by extracting 3 times with 0.5 ml of hexane. Finally, the extract was concentrated using a gentle stream of N_2.

The methyl esters were analyzed using a Model 5000 Barber Coleman gas chromatograph equipped with a flame-ionization detector. The outputs from electrometers were recorded on a two-channel Texas Instrument Servoriter II with 1-mV sensitivity. Two 6 ft × 0.125 inch glass chromatographic columns were used. One column contained 3 % OV-1, a nonpolar liquid phase, on 60–80 mesh Gas-Chrom Q (Applied Science). The other column was packed with 10 % ECNSS-S on 100–120 mesh Gas-Chrom P (Applied Science). ECNSS-S is a polar liquid-phase which is particularly efficient at resolving unsaturated fatty acid methyl esters[16]. Both columns, polar and nonpolar, were run simultaneously and were maintained at 185°. The temperature of the injectors was 200° and the detectors were maintained at 245°. The flow rate of the carrier gas (N_2) was adjusted so that the retention time for methyl stearate was approx. 10 min for the OV-1 column and 6 min for the ECNSS-S column. Retention time was expressed relative to that of methyl stearate (18:0). Identification of peaks was by comparison with relative retention times of methyl ester mixtures of known composition. These were obtained from the Fatty Acid Project, The Hormel Institute, University of Minnesota, Austin, Minn. and from Applied Science Laboratories, State College, Pa. Plots of the log of relative retention times *versus* number of carbons for several series of fatty acid methyl esters were used to characterize unknowns[17]. The amount of each fatty acid methyl ester in the total mixture was expressed as the percent of its peak area relative to the sum of the area under all peaks. The retention time multiplied by the peak height was used to calculate the area under each peak[18]. The gas–liquid chromatographic analysis of the hydrogenated methyl esters served to substantiate the identification of the unhydrogenated methyl esters. Thus, analysis was performed on each sample using both a polar and a nonpolar column.

Mass spectroscopy further aided in the identification of several methyl esters. A Model 9000 LKB mass spectrophotometer equipped with a two foot glass gas–liquid chromatographic column was used for mass spectroscopy (courtesy of Chemistry Dept., Vanderbilt University). The retinal outer segment methyl esters were separated using a 3 % SE-30 on 100–120 mesh Gas Chrom Q. The column was maintained at 185° until arachidonic acid was eluted, and then the temperature was raised at 3° per min until all methyl esters were eluted from the column. The mass spectra were taken near the peak as the methyl esters were eluted from the column. The temperature in the ion chamber was 250° and the energy of the electrons was 70 eV. Perfluorokerosene was used as a mass marker.

RESULTS

Nearly 50% of the retinal outer segment fragments dry weight is lipid (Table I). Bovine serum albumin served as the protein standard for the procedure of LOWRY[5]. A higher value is obtained when the percent lipid in retinal outer segments is calculated using the protein content obtained this way. The determination of protein obtained by dry weight (*i.e.* total dry weight *minus* lipid dry weight) is 1.13–1.25 times greater than the protein content obtained using the procedure of LOWRY (Table I). The procedure of LOWRY gives an arbitrary value relative to a standard (bovine serum albumin). The dry weight method is more reliable if the only components present are lipid and protein. We have not determined whether our preparations contain other components such as carbohydrate. In an earlier report[4] we calculated the lipid content

TABLE I

LIPID CONTENT OF BOVINE OUTER SEGMENT DISC FRAGMENTS

	Prep. I	*Prep. II*	*Prep. III*	$\bar{X} \pm$ S.E.***
μg total phosphorus per mg protein (Folin)*	38.9	40.0	37.7	38.9 ± 1.6
μg lipid phosphorus per mg protein (Folin)*	33.6	36.0	33.4	34.3 ± 2.3
mg lipid per mg dry wt.	0.48	0.47	0.50	0.48 ± 0.020
mg lipid per mg protein (dry wt.)**	0.92	0.89	1.00	0.94 ± 0.08
mg lipid per mg protein (Folin)*	1.17	1.11	1.27	1.18 ± 0.10
μg phosphorus per mg lipid	30.9	30.4	29.2	30.2 ± 1.3
Ratio of protein value§	1.25	1.24	1.13	

* Protein was determined by the procedure of LOWRY *et al.*[5] using bovine plasma albumin as the standard[6].

** Protein was determined by subtracting the weight of lipid from the total retinal outer segment dry wt.

*** Mean ± standard error for the three preparations; S.E. $= \left(\frac{\Sigma X^2 - (\Sigma X)^2/n}{n-1}\right)^{1/2}$.

§ Protein as determined by dry weight (**) /protein determined by the procedure of LOWRY *et al.*[5] (*).

TABLE II

THE PHOSPHOLIPID COMPOSITION OF BOVINE ROD OUTER SEGMENT FRAGMENTS

Phospholipid	% *of total phosphorus*			
	*Prep. I**	*Prep. II**	*Prep. III**	$\bar{X} \pm$ S.E.**
Phosphatidylserine	13.7 ± 0.1	14.5 ± 0.3	13.1 ± 0.2	13.8 ± 2.1
Phosphatidylinositol	1.5 ± 0.1	1.8 ± 0.2	1.8 ± 2.1	1.7 ± 1.2
Sphingomyelin	1.5 ± 0.1	1.4 ± 0.2	1.3 ± 1.4	1.5 ± 1.1
Phosphatidylcholine	39.8 ± 1.6	39.2 ± 1.0	36.6 ± 2.3	38.8 ± 4.9
Phosphatidylethanolamine	43.0 ± 1.3	42.3 ± 0.2	42.8 ± 1.7	47.2 ± 2.0
Nonpolar lipids	0.5 ± 0.2	0.8 ± 0.1	3.9 ± 1.5	1.7 ± 4.6
Phosphorus recovery***	100.6	98.0	100.3	

* Mean ± S.E. (4 determinations).
** Mean ± S.E. (the total of 12 determinations was used in the calculations).
*** % Recovery phosphorus from the plate.

TABLE III

FATTY ACID COMPOSITION OF BOVINE ROD OUTER SEGMENT FRAGMENTS

Data expressed as the average percent ± S.E. Three different retinal outer segment preparations were used.

*Fatty acid**	*Percent of total fatty acids*			
	Nonpolar column		*Polar column*	
	Unhydrogenated (n = 9)	*Hydrogenated (n = 7)*	*Unhydrogenated (n = 9)*	*Hydrogenated (n = 8)*
< 16	2.4 ± 0.9	2.0 ± 0.3	1.0 ± 0.4	1.6 ± 1.5
16:0	15.6 ± 1.2	15.0 ± 0.9	10.8 ± 0.9	14.4 ± 3.1
16:1	0.7 ± 0.01	—	0.4 ± 0.4	—
16:2	—	—	0.3 ± 0.1	—
18:0	21.6 ± 1.6	25.6 ± 0.5	16.2 ± 2.2	25.2 ± 0.9
18:1	4.7 ± 1.2***	—	4.0 ± 1.4	—
18:2	—	—	1.0 ± 0.1	—
18:3	—	—	0.4 ± 0.2	—
20:0	—	9.1 ± 0.1	—	8.9 ± 8.6
20:4	8.3 ± 2.2	—	8.5 ± 2.6	—
22:0	—	41.6 ± 1.4	—	39.9 ± 5.3
22:4	—	—	7.2 ± 3.5	—
22:6	37.6 ± 4.9	—	36.8 ± 5.3	—
Unknowns**	9.1 ± 1.8	6.7 ± 1.8	13.4 ± 4.4	10.0 ± 0.6
X_1	1.1 ± 0.5	0.9 ± 0.4	1.1 ± 0.3	—
X_2	1.0 ± 0.7	—	3.9 ± 0.8	2.6 ± 0.7
X_3	0.6 ± 0.1	—	2.7 ± 1.4	1.7 ± 0.1
X_4	4.4 ± 0.1	5.3 ± 0.5	4.1 ± 1.2	5.1 ± 0.1

*Number carbons: number double bonds.

**Includes all unknowns. The major unknown components are X_1 to X_4 (*cf.* text for more details). Other unknowns are minor components between 16:0 and 18:3.

***Includes other C_{18} unsaturated fatty acids as well.

using a protein value determined spectrophotometrically. Approx. 90 % of the phosphorus in the retinal outer segment preparation is due to phospholipid. Moreover, most of the lipid is phospholipid as judged from the μg phosphorus/mg lipid ratio. In this regard, it should be noted that the μg phosphorus/mg lipid is somewhat lower than that reported for beef heart mitochondria. Yet the percent phospholipid of both bovine heart mitochondria and retinal outer segments is approximately the same since the average number of retinal outer segment fatty acid carbons is 20 (Table III) whereas the average number for the mitochondrial fatty acids is 18 (ref. 19).

Two-dimensional thin-layer chromatography resolves the retinal outer segment phospholipids into six spots (*cf.* Fig. 1). However, two of the spots are phosphatidylcholine (discussed below) so there are only five phospholipid classes detectable in the retinal outer segment lipids. Phosphatidylethanolamine and phosphatidylcholine are the major phospholipids. The phosphatidylethanolamine content is somewhat greater than phosphatidylcholine (Table II) and together they account for about 80 % of the phospholipids. Much of the remaining phospholipid is phosphatidylserine, although small amounts of phosphatidylinositol and sphingomyelin are routinely observed in the retinal outer segment fragments. On occasion, a trace amount of diphosphatidylglycerol was found in the retinal outer segment phospholipids. Since most retinal outer

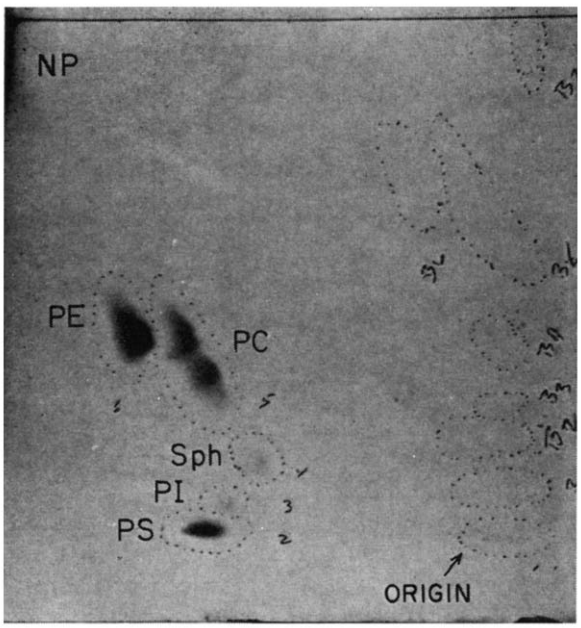

Fig. 1. Two-dimensional thin-layer chromatography of retinal outer segment lipids. The adsorbant was silica gel H mixed with magnesium acetate. Chloroform–methanol–ammonia (65:25:5, by vol.) was used in the first dimension, and chloroform–acetone–methanol–acetic acid–water (3:4:1:1:0.5, by vol.) in the second. Abbreviations: NP, nonpolar lipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine; Sph, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine. The spots were outlined as shown as well as areas of equivalent size to serve as blanks (right of the plate). The outlined areas are aspirated, digested and quantitated for phosphorus[11,12].

segment preparations did not contain diphosphatidylglycerol, those which did were not used for quantitation. The presence of cardiolipin is a good indication of contamination by mitochondria[19].

Two distinct spots are routinely observed in thin-layer chromatography of retinal outer segment phospholipids which are referable to phosphatidylcholine (*cf.* Fig. 1). These have been separated in part by column chromatography. Phosphatidylcholine and sphingomyelin are first separated from the rest of the retinal outer segment phospholipids on a DEAE-cellulose column[6]. The CM 9-1 fraction from the DEAE-cellulose column containing phosphatidylcholine is then rechromatographed on silicic acid. The phosphatidylcholine is eluted from the silicic acid by CM 3-2. Two fractions are resolved with some overlap. Thin-layer chromatography of the CM 3-2 fractions showed that each fraction was mainly responsible for only one of the two lecithin spots observed in the retinal outer segment phospholipids. The infrared spectra of both fractions were indicative of phosphatidylcholine, *i.e.* both fractions had an absorption maxima at 10.5 cm^{-1}. The high absorption at this wavelength is due to the quaternary amine of the choline moiety in phosphatidylcholine[20].

The most striking characteristics of the fatty acids of retinal outer segment lipids is the unusually high concentration of long-chain and unsaturated fatty acids (Table III). Almost 60 % of the retinal outer segment fatty acids are unsaturated. Nearly 55 % of the fatty acids contain twenty or more carbon atoms and four or more double bonds. Approximately two-thirds of the highly unsaturated fatty acids are 22:6.

At least 11 % of the fatty acids are palmitic acid (16:0) and another 17 % are stearic acid (18:0). Less than 0.5 % of the retinal outer segment fatty acids is myristic acid (14:0).

22:4 and 22:6 separate only slightly on the nonpolar column. The main peak is 22:6 and the trailing shoulder is the 22:4. In the quantitation of peaks the shoulder was not taken into consideration. The area under the shoulder is estimated at several percent and thus 22:6 + 22:4 approximates the value obtained for 22:0 of the hydrogenated methyl esters using the nonpolar column.

The identification of the major retinal outer segment fatty acids was confirmed using mass spectroscopy. Peaks due to the molecular ions of 16:0, 18:0, 20:4 and 22:6 were identified in their respective mass spectra. In addition the mass spectra of these compounds were typical of the respective type of fatty acid. The peaks due to the molecular ions were large in the 18:0 and 16:0 spectra but small in the cases of 20:4 and 22:6. A prominent peak at $m/e = 91$ was observed in the mass spectra of both 20:4 and 22:6. This peak is characteristic of the highly unsaturated fatty acids, and is thought to be due to tropylium ions formed by cyclization and rearrangement upon fragmentation of the molecules[21]. A mass spectrum of 22:4 was not obtained since this relatively minor component is not appreciably separated on SE-30 under the conditions used. The identification of this peak relies solely upon its relative retention time on the ECNSS-S column using plots of log relative retention times *versus* number of carbons.

We have been unable to identify a number of components among the retinal outer segment methyl esters (Table III). Most of these compounds (X_1 to X_4) appear to be large molecules with relative retention times greater than that for 20:4. Of the unidentified compounds, X_4 is the only one studied in any detail. X_4 accounts for about 5 % of the retinal outer segment methyl esters. It behaves like 24:0 on the ECNSS-S column giving the same relative retention time whether or not it has been hydrogenated. However, the relative retention time of unhydrogenated X_4 on OV-1 column is appreciably less than that for 24:0, *i.e.* it behaves like an unsaturated twenty-four-carbon fatty acid. Hydrogenation results in a methyl ester that has the same retention time as 24:0 in the OV-1 column.

The mass spectrum of X_4 contains a base peak at $m/e = 369$. A prominent peak about half the intensity of the base peak occurred at $m/e = 370$. The two peaks may be due to two different molecular ions. Hence, the X_4 fraction of the nonpolar column may be composed of several similar compounds. We feel that it is unlikely that X_4 is 24:6 although the mass spectra data of X_4 might be compatible with this.

DISCUSSION

Several other investigators have studied the lipid composition of retinal outer segments. We find a significantly higher lipid content (47–50 %) than has previously been reported for bovine retinal outer segments[23–25]. ADAMS[23] and SJÖSTRAND[24] report that about 40 % of bovine retinal outer segments is lipid, and EICHBERG AND HESS[25] find that nearly 41 % of the weight of frog retinal outer segments is lipid. The percent of phospholipid in our retinal outer segment extracts is similar to that of the other reports.

The retinal outer segment preparations used in our studies have been fragmented

to release contaminants[3]. The fragmentation process may have resulted in preferential loss of protein. If this is so, then the greater lipid values reported here for retinal outer segment fragments is compatible with the lower values obtained by others for intact retinal outer segments.

Only five phospholipids are detectable in the retinal outer segments, two of which are minor components. The retinal outer segment lipids are practically devoid of lysophosphatides, phosphatidic acid and diphosphatidylglycerol; the latter would be indicative of mitochondrial contamination[13]. The phospholipid composition of retinal outer segments has been reported by other laboratories. Our phospholipid analyses agree more closely with those recently reported by BONTING[22]. They find that the major retinal outer segment phospholipids are 43 % phosphatidylethanolamine, 36 % phosphatidylcholine and 12 % phosphatidylserine. They also report minor amounts of phosphatidylinositol, diphosphatidylglycerol, lysophosphatidylcholine, lysophosphatidylethanolamine and sphingomyelin. Of the minor components which they report, we find only phosphatidylinositol and sphingomyelin. POINCELOT AND ZULL[26] and SJÖSTRAND[24] have also investigated the bovine retinal outer segment phospholipid composition. SJÖSTRAND[24] carried out his phospholipid analysis before phospholipid analysis technology was greatly improved and found 51 % phosphatidylethanolamine (Cephalin A), 31 % phosphatidylcholine, 12 % phosphatidylserine (Cephalin B) and 6 % sphingomyelin. POINCELOT AND ZULL[26] report significantly more phosphatidylcholine than phosphatidylethanolamine (13 %). However, their determination of phosphatidylcholine was based on choline analysis of an unfractionated phospholipid extract. The phospholipid composition of frog retinal outer segments appears to be significantly different from bovine retinal outer segments. EICHBERG AND HESS[25] reported twice as much phosphatidylcholine as phosphatidylethanolamine in the frog rod outer segments. Like the bovine retinal outer segments, the most prominent phospholipids of frog retinal outer segments are phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine.

A striking feature of the retinal outer segment lipids is the unusually high concentration of long-chain polyunsaturated fatty acids. Approx. 60 % of the bovine retinal outer segment fatty acids are 20:4, 22:4 or 22:6. Most of the polyunsaturated fatty acid is 22:6 which accounts for 36 % of the fatty acids. Synaptic plasma membrane is the only other purified cell organelle which was found to contain nearly as high a 22:6 content as bovine retinal outer segment. COTMAN *et al.*[27] report that phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol from synaptic plasma membrane contain 60 % 20:4, 22:4 and 22:6. Approx. 32 % of the synaptic plasma membrane fatty acids from these three phospholipid classes is 22:6. In contrast with the mentioned phospholipids, phosphatidylcholine was found to contain only 3.4 % 22:6. The retinal outer segment lipids contain a greater amount of 22:6 than synaptic plasma membrane (calculated from their data) since in the retinal outer segment lipids, phosphatidylcholine as well as phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol contain high percentages of 22:6 (S. FLEISCHER AND N. C. NIELSEN, unpublished observations,.

Brain mitochondria from rats normally contain greater amounts of 22:6 (10–15 %) than mitochondria from liver (2–3 %). However, it is clear from these studies of WITTING *et al.*[29] that the fatty acid composition of mitochondria can be dramatically altered with diet. The 22:6 content of liver mitochondria from rats maintained on a

cod liver oil rich diet for 5 months increases to 14 %; this fatty acid increases only to 17 % in brain mitochondria from the same animals.

In a review[19], we called attention to basically two types of membrane structures having different turnover properties and lipid composition. Myelin represents the metabolically more stable membrane, has a high cholesterol and sphingomyelin content and many long-chain monoenoic and saturated fatty acids. The mitochondrion represents the metabolically active membrane structure, with low sphingomyelin and cholesterol content, and containing many unsaturated fatty acids. The retinal outer segment membrane appears to fit best into the latter class. It is metabolically unstable, has highly unsaturated fatty acids, and contains little cholesterol[4] and sphingomyelin. Retinal outer segments contain large amounts of long-chain polyunsaturated fatty acids as compared with mitochondria. It may be that membranes which are involved in generating or transmitting nerve impulses all have high quantities of 22:4 and 22:6. These fatty acids may somehow be involved with the function of these membranes.

It is clear from the elegant work of HUBBARD AND WALD[28] that the primary event in black and white vision involves rhodopsin, the visual pigment of retinal outer segment. Rhodopsin is a lipoprotein which is intimately associated with the membranous sacs of retinal outer segments[2]. Photons cause an isomerization of 11-*cis* double bond of the rhodopsin chromophore to form the all-*trans*-retinaldehyde. A basic problem in receptor physiology is how this primary event is converted to a nerve impulse. It is probable that the organization of the membranous sacs is basic to this transduction and that a knowledge of the phospholipid composition of retinal outer segment membranes is a step to this understanding.

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