Original Articles

The retinal pigment epithelium

Chemical composition and structure

E. R. Berman, H. Schwell, and L. Feeney

Chemical and morphologic studies were carried out on bovine retinal pigment epithelial (RPE) cells that were free of contamination by outer segments or other tissues. Both melanotic and amelanotic cells were present, the latter originating from areas overlying the tapetum lucidum. Cells near the tapetum lucidum contain large melanolysosomes suggesting that melanin produced in this marginal area is destroyed by autophagy. Otherwise, bovine RPE cells resemble those of other species. The protein content of RPE was somewhat lower than that of whole retina, and both in turn contained less than half of that in rat liver. RPE has about the same lipid content as whole retina and in both tissues, 55 to 60 per cent of the total lipids are phospholipids. While both phosphatidyl ethanolamine and phosphatidyl choline were readily identified in retina and liver, only PC could be detected in pigment cells. The fatty acids of RPE phospholipids consisted of 16:0 (31 per cent), 20:4 (17 per cent), and 18:0, 18:1, and 18:2 (each approximately 14 per cent). Long-chain polyenoates such as 22:5 and 22:6, which comprise a major portion of retinal phospholipid fatty acids were not clearly detectable in RPE. The content of retinol plus retinyl ester in pigment cells was 1.9 μg per eye, or 481 μg per gram of wet tissue. The latter value is about five times higher than bovine liver and 70 times higher than whole retina. The DNA content of RPE is about the same as that of whole retina, and both are approximately three times higher than liver. Cytophotometric analyses showed, however, that RPE nuclei are diploid.

Key words: retinal pigment epithelium, retinol, retinyl esters, phospholipids, DNA, neutral lipids, phosphatidyl choline, phosphatidyl ethanolamine, phagolysosomes, fatty acids.

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The importance of the pigment epithelium in both normal retinal function and in the pathogenesis of retinal disease has been stressed by several authors.[1-3] Yet, accurate information about this tissue, especially its chemical composition, is very limited. This may be attributed to several factors. One is the difficulty in obtaining sufficient material for reliable chemical analyses and another is the absence of suitable criteria for judging the purity of the starting material. Contamination with even small amounts of retinal or choroidal tissue could lead to gross errors in the

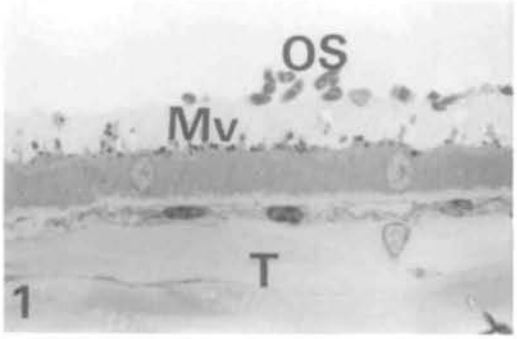

Fig. 1. Plastic section of bovine amelanotic RPE in situ showing an area where outer segment fragments (OS) remained adherent to the apical microvilli following dissection of the neural retina from the pigment epithelium. Toluidine blue stain, ×250. *Abbreviations used in illustrations:* CC, choriocapillaris; C, Colgi apparatus; JC, junctional complex; Me, melanin; Mv, microvilli; N, nucleus; OS, outer segment(s); Ph, phagolysosomes; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; T, tapetum lucidum.

interpretation of analytic data. The present study was undertaken to provide basic information for long-range investigations on the biochemistry and function of the RPE.* Some of these findings have been reported in preliminary form.[4]

Methods and materials

Isolation of pigment cells. From 20 to 40 cattle eyes were used for each experiment, all operations being carried out at normal levels of indoor illumination. Eyes were freed of adhering tissue and rinsed in saline to remove extraneous blood. They were then opened, and the anterior portions discarded. The neural retina was then gently removed. Microscopic sections of the tissue at this stage of dissection showed that portions of rod outer segment often remained within the apical microvilli of the RPE cells (Fig. 1). The pigment epithelial cells were then brushed out[5] and suspended in 30 to 50 ml. of 0.25 M sucrose to 0.01 M Tris buffer, pH 7.2. After centrifugation in a Sorvall RC2-B centrifuge (HB-4 rotor) at 112 × g for 10 minutes, the black and turbid supernatant was drawn off. It was found to consist of melanin granules, erythrocytes, broken and intact rod outer segments, occasional pigment epithelial cells (mainly of the amelanotic type), as well as other structures whose origin could not be determined. The 112 × g pellet contained a mixture of melanotic and amelanotic pigment epithelial cells some of which had rod outer segment fragments still inserted in their apical villous processes (Figs. 2, 3, and 4). After four to five sucrose washings, the cells could be freed of all the above mentioned contaminants. The final pellet consisted of a mixture of clean melanotic and amelanotic intact cells (Figs. 5 and 6). Some were present as isolated cells, while others were in the form of clusters or sheets, the individual cells held firmly together at their junctional complex as in the native state.

Light and electron microscopy. Light and electron microscopic preparations were made of (1) intact posterior segments of bovine eyes; (2) posterior segments stripped of neural retina; (3) isolated pigment epithelium from tapetal (amelanotic) and nontapetal (melanotic) areas; and (4) washed, pooled pigment epithelial cells. Specimens were fixed initially in paraformaldehyde-glutaraldehyde.[6] Wet preparations of cell suspensions were stained with toluidine blue and photographed by light microscopy. Centrifuged pellets of cells were incorporated into agar blocks prior to osmic acid fixation. Pellets and tissues were further prepared for electron microscopy using standard methods.[6]

Measurements of wet and dry weights. The four to five times washed pellet was suspended in 0.9 per cent NaCl and transferred to a preweighed test tube. The suspension was centrifuged at 2,000 r.p.m. for 10 to 15 minutes, the saline drawn off, and the washing repeated. The last traces of fluid were then removed, and the wet weight of the cells obtained by difference. The weight of pigment cells isolated in this manner from 20 cattle eyes usually varied from about 80 to 110 mg. Dry weights were obtained after freeze-drying the pellets. Wet weights of rat liver and cattle retina were obtained by standard procedures and dry weights by freeze drying the minced tissues.

Analytic methods. Lipids were extracted in chloroform-methanol (2:1) according to Folch, Lees, and Sloane-Stanley.[7] After partition of the water-methanol phase, the lower chloroform layer was evaporated under nitrogen and the total lipids determined gravimetrically.

For thin-layer chromatography, the dried lipid extract was dissolved in a small volume of chloroform and 5 to 10 μl samples were applied to precoated sheets of silica gel G (Eastman chromagram, Rochester, N. Y.). For separation of neutral lipids, the sheets were developed in petroleum ether:diethyl ether:acetic acid (70:30:1) and the lipids detected with iodine vapor. For separation of phospholipids, the sheets were developed in chloroform:methanol:water (65:25:4) and the phospholipids identified by spraying first with ninhydrin and then with molybdenum blue reagent.[8]

*Abbreviations used in text. RPE, retinal pigment epithelium; PC, phosphatidyl choline (lecithin); PE, phosphatidyl ethanolamine; Sph, sphingomyelin; LysPC, lysophosphatidyl choline; and LysPE, lysophosphatidyl ethanolamine.

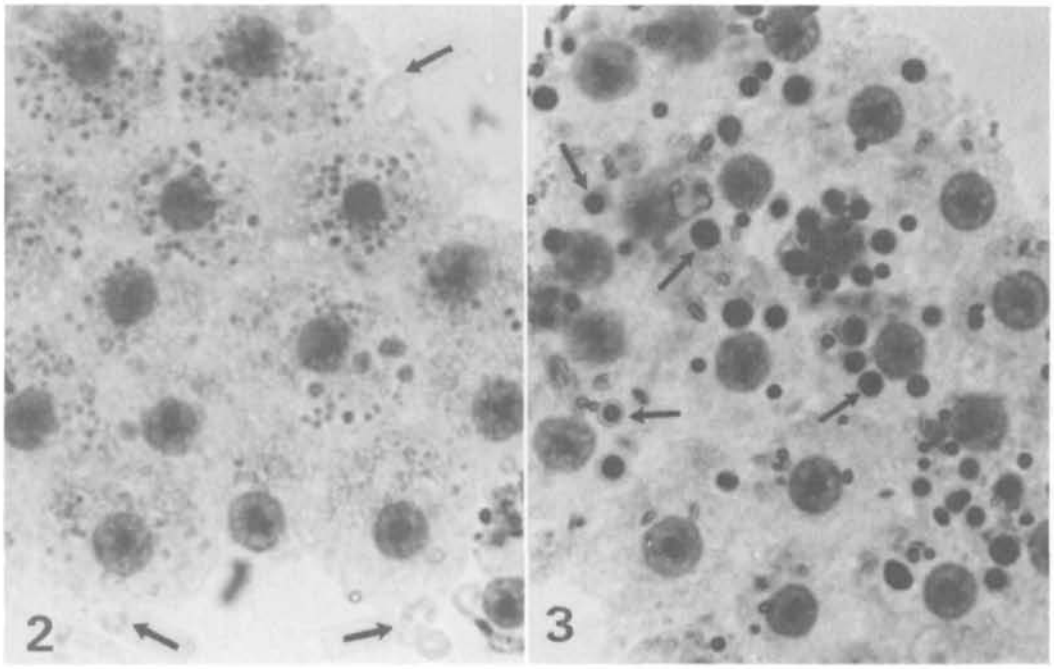

Fig. 2. Isolated, unwashed bovine RPE. Amelanotic cells with contaminating outer segment fragments (arrows). Cytoplasmic granules are lysosomes and mitochondria. Methylene blue-stained wet preparation, ×800.

Fig. 3. Isolated, intermediate type cells, unwashed. The cytoplasm contains some elliptical melanin granules, large melanolysosomes (arrows), smaller lysosomes of various types, and mitochondria. Contaminating OS are above the plane of focus. Methylene blue-stained wet preparation, ×800.

Protein was assayed according to Lowry and co-workers[9] and DNA according to Burton.[10] Lipid phosphorus was determined by a modification of the Bartlett procedure[11] using 0.5 ml. of 72 per cent $HClO_4$ for digestion of the samples. Phospholipid content was calculated from the phosphorus values assuming an average phosphorus content of 4 per cent.

For cytophotometry, smears of bull sperm, bovine neural retina, and pigment epithelial cells were prepared and stained by the Feulgen procedure using the method of Deitch.[12] The staining was quantitated with an integrating digital cytophotometric system.[13]

Retinol and retinyl esters were analyzed together by the following procedure. An 0.6 ml. aliquot of tissue homogenized in water was agitated with 3 ml. of ethanol on a Vortex mixer. Afterward, 4 ml. of cyclohexane was added, the mixture was shaken vigorously for 30 seconds, and centrifuged. The fluorescence intensity of the upper cyclohexane layer was measured in a Turner fluorometer Model 111 (G. K. Turner Associates, Palo Alto, Calif.) equipped with a high-sensitivity conversion kit (No. 110-865). Primary excitation at 325 nm. was obtained using a Wratten 34-A (unmounted) gelatin plus a 7-54 filter; emission at 485 nm. was provided with a 110-817 secondary filter. The results were calculated as retinol equivalents, using a standard of all-trans-retinyl acetate (Distillation Products Industries, Rochester, N. Y.). To estimate the relative proportion of retinol to retinyl esters, cyclohexane extracts were concentrated, and thin-layer chromatography performed according to Drujan, Castillon, and Guerrero.[14]

Fatty acid methyl esters from phospholipids of RPE and rod outer segments were prepared from the chloroform phase of the washed Folch[7] extract. After concentrating under N_2, the extract was streaked on silica gel G and the chromatogram developed in cyclohexane:petroleum ether:ethyl ether (7:3:1). The phospholipids, which remain at the origin in this solvent system, were eluted with chloroform-methanol (2:1) and the fatty acids converted to methyl esters using 15 per cent boron trifluoride in methanol.[15] The methyl esters were extracted with petroleum ether and analyzed on a Packard Model 7400 gas chromatograph equipped with a flame ionization detector. The column, consisting of 8 feet by ⅛ inch glass packed with 15 per cent DEGS on 80/100 Chromosorb W (Applied Science Laboratories, Inc.), was operated

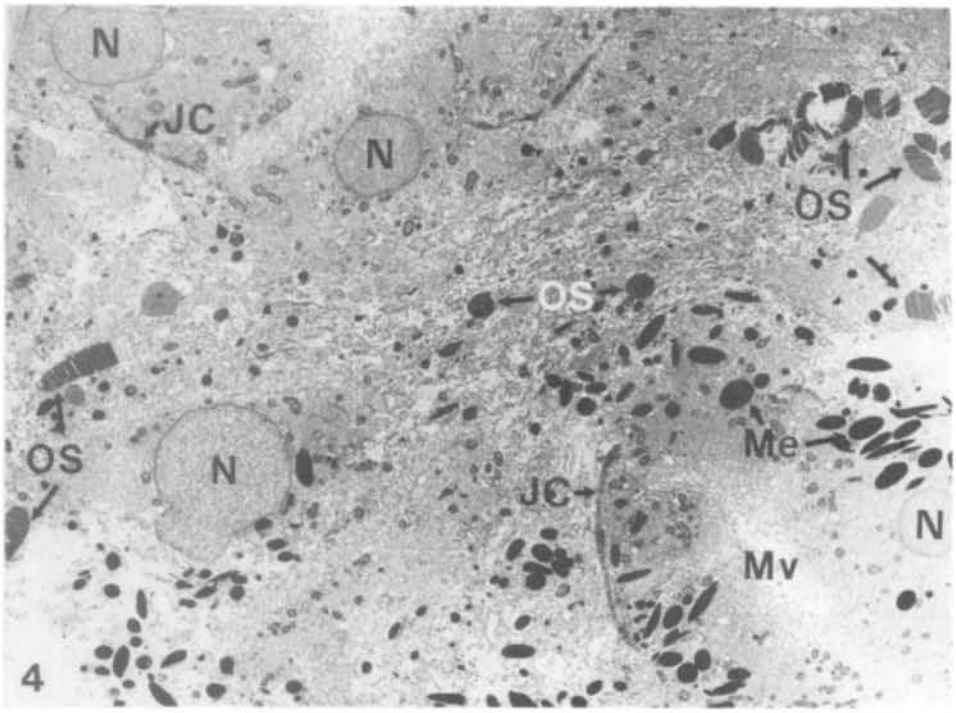

Fig. 4. Electron micrograph of a pellet of unwashed bovine RPE. Both melanotic and amelanotic cells are included in the field and some are held together by junctional complexes. Numerous outer segment fragments adhere to the epithelial cells. ×3,200.

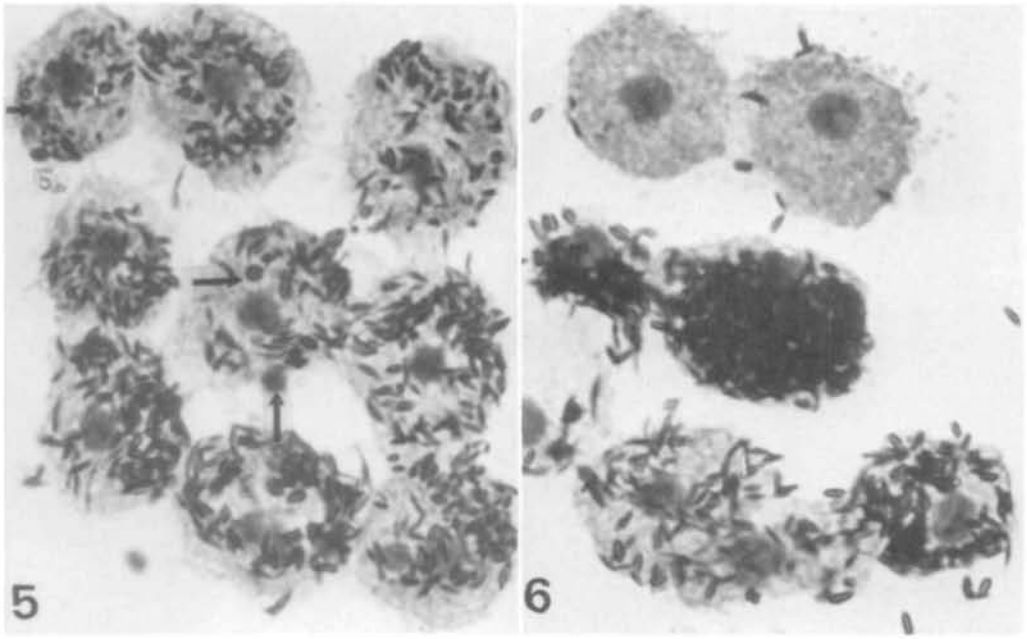

Fig. 5. Group of washed melanotic cells, also containing some melanolysosomes (arrows). Toluidine blue-stained wet preparations. Melanin appears yellow, lysosomes pink, and melanolysosomes as yellow-cored pink bodies. (See also, Fig. 8, inset). No contaminating OS are apparent.

Fig. 6. Specimen showing the three types of washed cells: amelanotic (upper), melanotic (middle), and intermediate (lower). ×800.

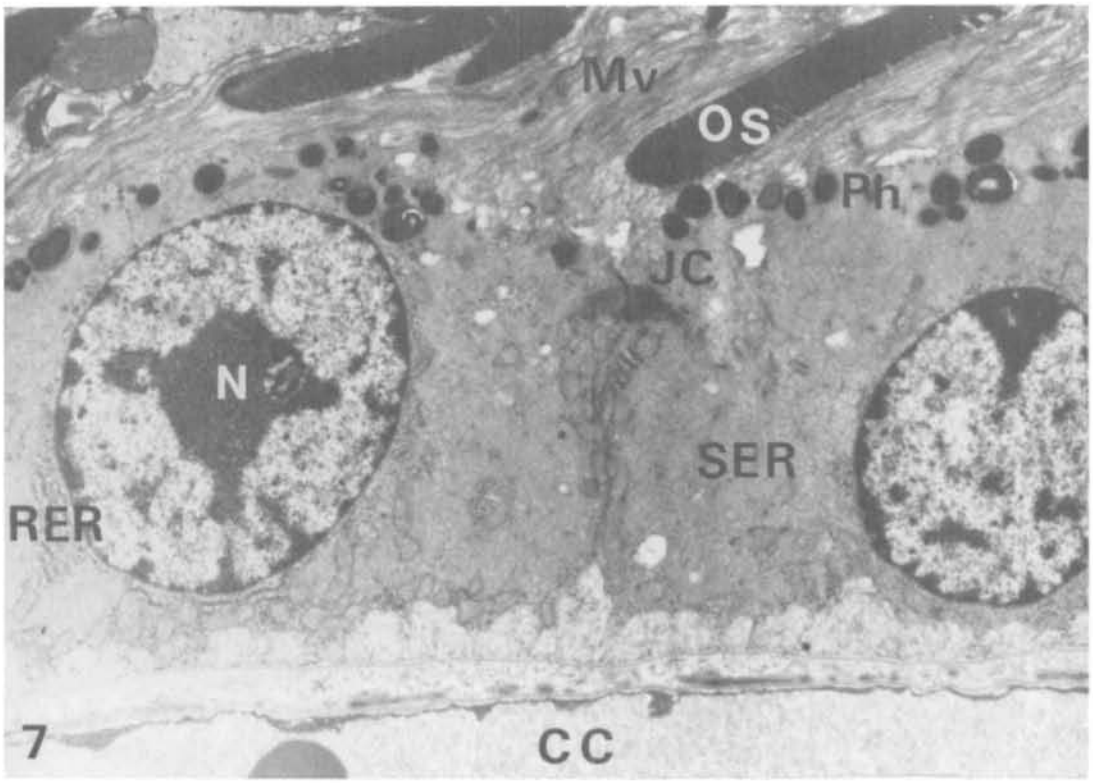

Fig. 7. Electron micrograph of amelanotic cells overlying the tapetum lucidum and fixed in situ. The photoreceptor outer segments are closely enveloped by the apical microvilli of the pigment epithelial cell. See text for detailed description. ×7,500.

at a temperature of 180° C. Fatty acids used for calibration were purchased from Applied Science Laboratories, Inc.

Results

Cell populations and morphology. Three distinct types of pigment epithelial cells occur in the bovine eye: amelanotic, melanotic, and an intermediate type. Amelanotic cells overlie the tapetum lucidum and are characterized by their lack of melanin, and the clearly visible phagolysosomal particles in the cytoplasm (Figs. 1, 2, 4, and 7). Melanotic cells overlie nontapetal areas and contain numerous elliptical melanin granules (Figs. 5, 6, and 8). The intermediate types of cell lie at the margin of the tapetal area and are characterized by their content of large heterogeneous melanin-containing bodies (Figs. 3 and 6). With the toluidine blue stain these bodies consist of a yellow melanin core surrounded by a halo of metachromatic (pink) material. By electron microscopy (Fig. 8, inset) they resemble autophagic vacuoles, i.e., melanin in the process of being digested within lysosomes.

Other morphologic features of bovine pigment epithelial cells are similar to those of other species (Figs. 7 and 8). Unfixed cells measure about 23 by 12 μm, excluding apical microvilli. Each contains a large (8 to 10 μm in diameter) nucleus having a large central mass of heterochromatin in which the nucleolus appears embedded. The cytoplasm is virtually filled with smooth endoplasmic reticulum (SER). There are isolated stacks of rough ER often associated with the nuclear envelope. Mitochondria are found predominantly in the basal cytoplasm, whereas numerous heterogeneous lysosomal particles are found mainly in the apical cytoplasm (Figs. 4, 7, and 8). Golgi apparati are small and indistinct. Adjacent cells are attached by

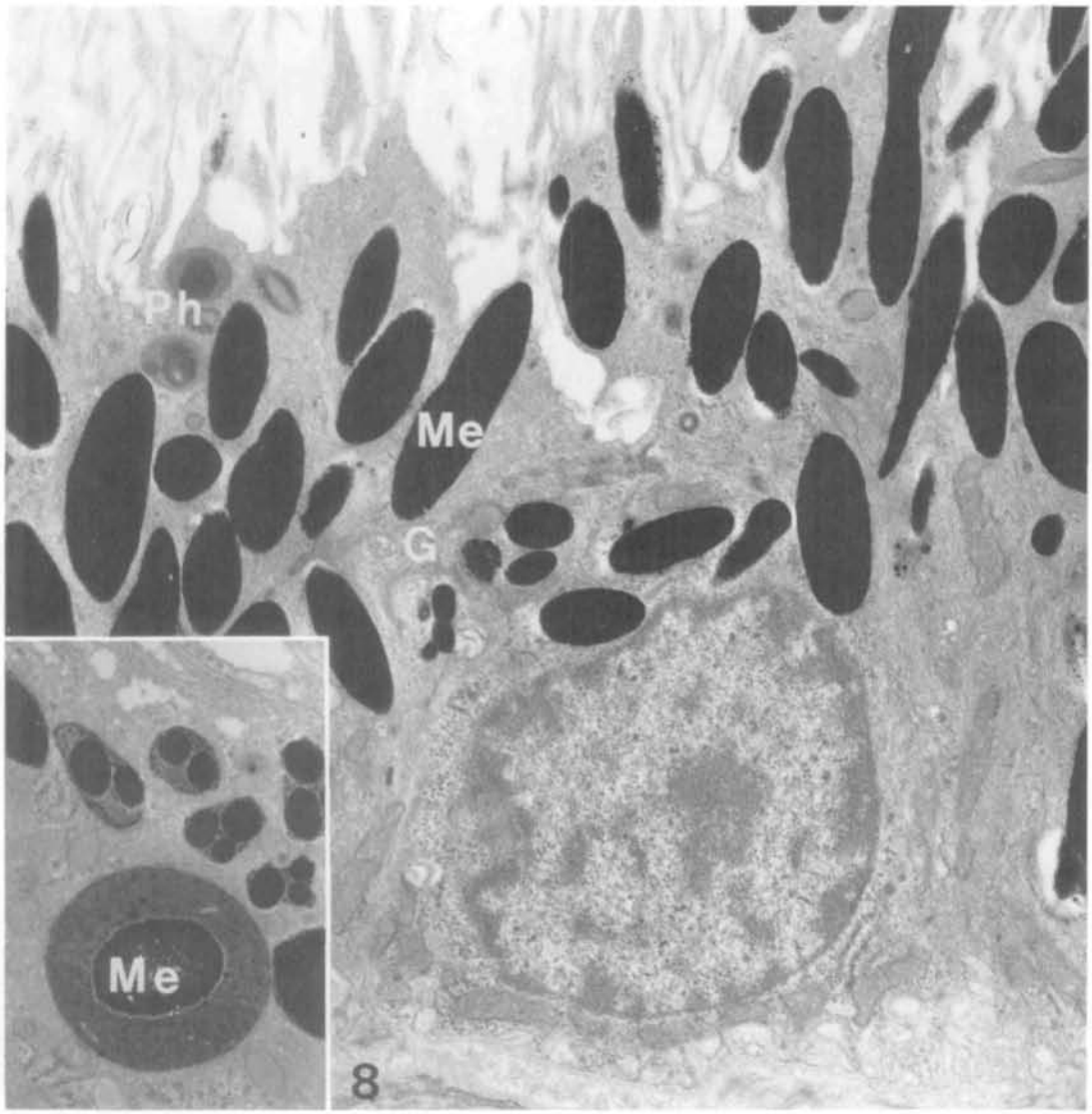

Fig. 8. Melanotic cell of nontapetal area fixed in situ. The overlying neural retina has been removed. Elliptical melanin granules are mainly oriented toward the apical surface and some lie within microvilli. Phagolysosomes also lie in the apical cytoplasm. ×9,000. *Inset:* melanolysosomes from intermediate-type cell. Single and multiple melanin granules are contained in lysosomes. ×20,000.

tight junctional complexes. The plasma membrane at the basal surface is highly infolded and the apical surface is covered with microvilli that envelop the rod outer segments.

Chemical composition. The wet and dry weight compositions of bovine RPE are given in Tables I and II, respectively. Water content (81.4 per cent), although considerably higher than liver, is nearly the same as that of retina and brain tissue, as reported elsewhere.[16] The percentages of all other components (protein, total lipid, phospholipids, and DNA) are remarkably similar in RPE and whole retina. For comparative purposes, literature values on the dry weight composition of rod outer segments are shown in Table II. The unique lipid (and phospholipid) composition of outer segments is well known and is espe-

Table I. Chemical composition of bovine RPE in comparison with other tissues (wet weights)

Component	*Pigment epithelium*	*Retina*	*Liver (rat)*
	(Gm./100 Gm. wet weight)		
Water	81.4 ± 2.0 (4)	80.6 ± 3.9 (8)	71.0 ± 0.6 (4)
Protein	8.1 ± 1.9 (12)	7.7 ± 1.1 (4)	17.9 ± 1.0 (7)
Total lipid	3.00 ± 0.72 (5)	2.74 ± 0.29 (5)	4.20 ± 0.35 (3)
Phospholipids	1.59 ± 0.26 (9)	1.67 ± 0.20 (5)	2.40 ± 0.43 (3)
DNA	0.72 ± 0.17 (9)	0.79 ± 0.08 (6)	0.23 ± 0.04 (5)

Figures shown are the means ± the S.D. Numbers in parentheses indicate the number of determinations.

Table II. Chemical composition of bovine RPE in comparison with other tissues (dry weights)

Component	*Pigment epithelium*	*Retina*	*Rod outer segments*	*Liver (rat)*
Dry weight (Gm./100 Gm. wet tissue)	*18.6*	*19.4*	—	*29.0*
	(Per cent of dry weight)			
Protein	43.5	39.7	41°	61.7
Total lipid	16.1	14.1	36° 48°	14.5
Phospholipids	8.5	8.6	31.5† 39† 24.7; 31.8‡	8.3
DNA	3.9	4.1	—	0.79

°Nielson, et al.[18]
†Borggreven, et al.[17]
‡Anderson and Sperling.[19]

cially striking when compared with whole retina and with other tissues such as RPE and liver.

The DNA content of RPE is slightly lower than that of whole retina, and both are about five times higher than that of rat liver. The differences between pigment epithelium and whole retina on the one hand, and rat liver, on the other, are even more striking when related to the protein content of these tissues. Thus, the ratio of micrograms of DNA per milligram of protein in RPE, whole retina, and rat liver are 88, 102, and 13, respectively. To explain the relatively high DNA content of RPE cells and of retina, the possibility of polyploidy was considered. Cytophotometric analyses of Feulgen-stained tissues showed that the nuclei of the photoreceptors and of the pigment epithelium contained twice as much DNA as sperm when corrections are made for heterochromatic packing in the haploid sperm nucleus. On this basis, both the retinal cells and the RPE appear to be diploid.

Lipid composition. The only neutral lipids detected in pigment epithelial cells by thin-layer chromatography were free fatty acids (Fig. 9). The two prominent bands in the retinal extract with mobilities intermediate between those of fatty acids and cholesterol may be diacylglycerols, recently identified in toad retina,[20] but not previously noted in cattle retina. None of these components were detectable in lipid extracts from RPE.

It is apparent that in the solvent system used to identify neutral lipids, most of the lipids in RPE remained at the origin suggesting that they consist of phospholipids. Using a chloroform-methanol-water solvent system, three components were detectable when sprayed with molybdenum blue reagent (Fig. 10, *B*). The major spot, with

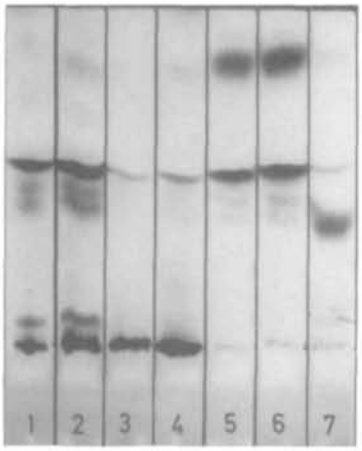

Fig. 9. Thin-layer chromatogram of neutral lipids. (1) retina (150 μg); (2) retina (300 μg); (3) pigment epithelium (200 μg); (4) pigment epithelium (400 μg); (5) standard mixture of oleic acid and triolein (100 μg); (6) standard mixture of oleic acid and triolein (200 μg); and (7) cholesterol standard.

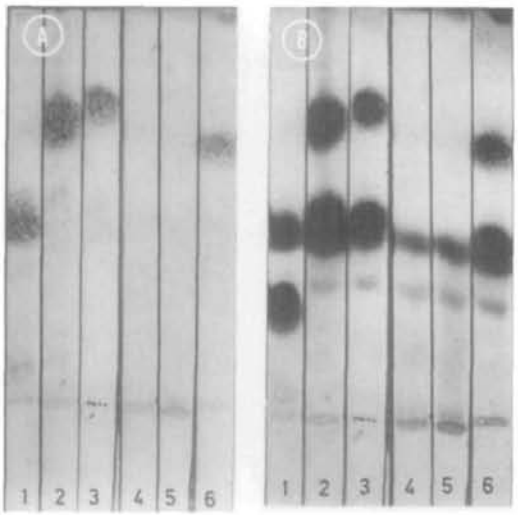

Fig. 10. Thin-layer chromatogram of phospholipids. (1) standard mixture of lysPE and lysPC; (2) standard mixture of PE and PC; (3) retina (150 μg); (4) pigment epithelium (200 μg); (5) pigment epithelium (400 μg); and (6) rat liver (150 μg). The sheets were sprayed first with ninhydrin (A) and then with molybdenum blue reagent (B).

the highest R_f, had the same mobility as both PC and lysPE which do not separate well in this system nor in others that were tested. However, since this component did not stain with ninhydrin (Fig. 10, *A*), it is tentatively concluded that the major phospholipid of pigment epithelial cells is PC. The minor phospholipid has a mobility similar to that of Sph, although the possibility that it may represent lysPC cannot be excluded. The most conspicuous feature of the thin-layer chromatogram shown in Fig. 10 is the absence of PE in the pigment epithelium. Even if present in small amounts, it could have been detected using 400 μg of lipid extract (Fig. 10, *B*, No. 5). We were unable to identify the material remaining at the origin.

Table III. Fatty acid composition of phospholipids of bovine RPE and rod outer segments

Fatty acids	*RPE*	*Rod outer segments*		
		Present study	*Borg-greven et al*[17]	*Nielson et al*[18]
		(Weight per cent)		
12:0	T	—	—	
14:0	—	T	—	2.4
16:0	30.7	19.9	19.4	15.6
16:1	2.6	1.2	0.8	0.7
16:2	—		—	—
18:0	14.9	21.7	23.1	21.6
18:1	13.1	6.3	6.4	4.7
18:2	14.9	1.9	1.4	—
20:4	16.6	7.8	6.0	8.3
22:4	T	3.5	1.5	—
22:5	—	8.2	1.2	
22:6	T	29.4	34.3	37.6
Unknowns	*	†		

T, trace.
*Three unknowns (7.2 per cent of the total methyl esters) have retention times between 18:2 and 20:4.
†Two unknowns (in trace amounts) have retention times between 18:2 and 20:4.

The gas-liquid chromatograms of fatty acid esters from RPE and rod outer segment phospholipids are shown in Fig. 11; per cent distribution data are summarized in Table III. In agreement with previous analyses, the present study shows that rod outer segments contain an unusually high proportion of long-chain polyenoic fatty acids, the principal one being docosahexaenoic acid (22:6). In contrast to this, C_{20} is the longest fatty acid chain in RPE phospholipids, although several small peaks

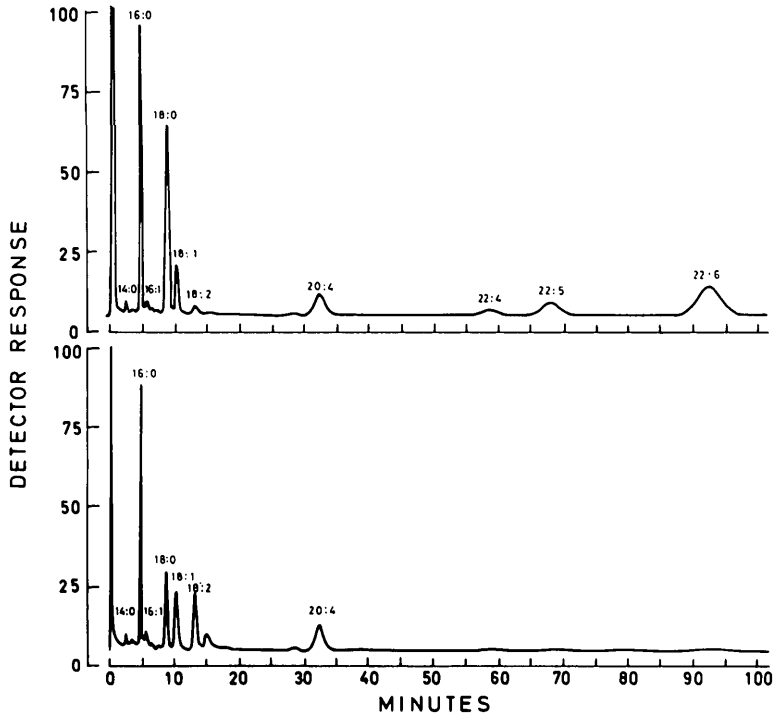

Fig. 11. Gas-liquid chromatograms of methyl esters of phospholipid fatty acids from rod outer segments (upper) and RPE (lower). The numbers above the peaks identified refer to chain length:number of double bonds.

Table IV. Retinol content of various tissues

Tissue	Species	Retinol* (μg/eye)	Retinol* (μg/Gm. wet tissue)	Relative proportions§ of Retinol	Relative proportions§ of Retinol ester
Pigment epithelium	Cattle	1.9 ± 0.4 (5)	481 ± 98 (5)	Not detectable	100
Choroid†	Cattle	3.0 ± 0.9 (3)	—	90	10
Retina	Cattle	3.8 ± 1.2 (6)	7.1 ± 2.3 (6)	80	20
Liver‡	Rat	—	116 ± 15 (5)	NA	NA
Liver	Cattle	—	88.4 ± 14.5 (5)	NA	NA

NA, Not analyzed.
*Numbers shown are the mean ± S.D. Figures in parentheses are the number of determinations. Data given are the total of retinol plus retinyl ester.
†Obtained (after removal of pigment cells) by teasing the choroid and Bruch's membrane from the sclera.
‡Male rats weighing 200 to 250 grams.
§Estimations based on an average of two experiments.

appear to be present at the elution positions of C_{22} polyenoates. The major saturated fatty acids of RPE phospholipids are 16:0 and 18:0, while the unsaturated ones consist of 16:1, 18:1, 18:2, and 20:4.

Retinol analyses. Only one report is known to us on the retinol content of bovine RPE.[21] The preparations, termed "pigmented layers," consisted of RPE plus choroid, and contained approximately 6.0 μg of retinol plus retinyl ester per eye. This value is about three times higher than the one shown in Table IV for pure pigment cells. The discrepancy appears to lie in the inclusion of choroid in the previous study[21] which, as shown in Table IV, contains 1.5 times more retinol per eye than pigment cells. The retinol content of bovine retina

found in the present study is in good agreement with previously reported values.[21, 22] Under the conditions of normal indoor illumination used for preparing the retinas, most of the retinol was present as the alcohol; in contrast to this, essentially all of the RPE retinol was esterified. When calculated on a weight basis (Table IV), the retinol content of bovine RPE is nearly 70 times greater than that of retina, and four to five times higher than that of bovine or rat liver. The latter was chosen for analysis in order to compare known literature values while the former was analyzed in order to obviate discrepancies due to species differences. Despite the highly variable retinol content of various tissues (especially liver), our data for both rat[14, 23] and cattle[23, 24] liver are in good agreement with most reported values.

Discussion

One of the major obstacles preventing reliable chemical analyses of RPE in the past has been the lack of a suitable method for preparing the cells in good yield and free of contaminating tissue fragments. This has now been achieved by modifying the procedure originally used by Glocklin and Potts[25] and later by others.[5, 26-28] A centrifugal force of 112 × g for 10 minutes in 0.25 M sucrose is sufficient to sediment essentially all of the melanotic cells and most of the amelanotic ones in the crude preparation. Under these conditions the principal contaminants (rod outer segments, red blood cells, and melanin granules) remain suspended in the supernatant and can be readily decanted or aspirated. Although a number of rod outer segment fragments remain inserted in the villous processes of the RPE in the early stages of purification, they are not present when the washing is completed. The final preparation consists of a mixture of melanotic, amelanotic, and an intermediate type of cell.

Morphologically, the different cell types appear to be alike except for their melanin content. Cells overlying the tapetum lucidum contain no melanin, which is not surprising since melanin would interfere with the light-reflecting properties of this layer. The mechanism by which these RPE cells achieve the amelanotic state is unknown, but cells at the margin of the tapetum appear to dispose of melanin granules by autophagic digestion. A similar mechanism is utilized by albino mice during ontogeny of the pigment epithelium; accumulated unmelanized premelanosomes are destroyed by autophagy.[29] The presence of the large melanophagosomes in the intermediate type cells may represent a means for removal of mistakenly synthesized melanosomes from the tapetal margin rather than the principal mechanism for achieving amelanosis in the cells overlying the tapetum.

The present study has also brought to light several unusual features in the chemical composition of bovine RPE. Although the total lipid content of RPE is similar to that of whole retina and rat liver, only a limited number of components are present. Thin-layer chromatography revealed just one neutral lipid whose mobility was similar to that of free fatty acids. Phospholipids comprise above 55 per cent of the total lipids in RPE, as well as in whole retina and rat liver. However, among the phospholipids of RPE, PC was the main component. The only other one that could be detected has tentatively been identified as Sph, or possibly lysPC, components which are also considered to be present in retina.[30] The absence of PE in pigment cells was conspicuous, although this phospholipid was readily apparent in the same thin-layer chromatogram in liver and in whole retina using considerably less starting material.

If only small amounts of neutral lipid are present, and about half of the total lipids are phospholipids, then a considerable amount of the chloroform-methanol extractable lipid has not been accounted for. Excluding retinol and retinyl ester (which in any case account for less than 0.05 per cent of the wet tissue), there may be other lipids in RPE, specifically those that

remain at the origin after thin-layer chromatography (Fig. 10, *B*). Lipofuscin granules, i.e., the residual bodies of lysosomes, are a prominent component of human RPE cells,[31] and exist to a lesser extent in animal RPE cells as well. Although previously thought to be insoluble in most lipid solvents, recent studies have shown that lipids can in fact be extracted from lipofuscin bodies isolated from brain (but not from liver).[32] The major component in brain is a nonpolar lipid polymer that does not migrate in any of the solvent systems tested. It is tempting to speculate that a similar substance may have been present in our RPE extracts, although there was insufficient material for characterization.

The fatty acids of RPE phospholipids are distinctly different from those of rod outer segments. In the latter, the polyunsaturated fatty acids 18:1, 20:4, 22:4, 22:5, and 22:6 make up about 55 per cent of the total, while saturated fatty acids (16:0 and 18:0) comprise about 40 per cent. Docosahexaenoic acid is the most abundant of the polyenoates—accounting for about one-third of the total fatty acids—and may play an important role in visual excitation.[33] The only other mammalian tissue with such an unusually high content of 22:6 is brain where the docosahexaenoate appears to be concentrated in the synaptic plasma membranes.[34] In RPE, as in rod outer segments, the principal saturated fatty acids are 16:0 and 18:0, which make up 45 per cent of the total. The major difference between RPE and rod outer segments is in the composition of the unsaturated fatty acids. In RPE, these consist almost entirely of 18:1, 18:2, and 20:4; faint suggestions of longer chain polyenoates may be construed from the gas-liquid chromatograms shown in Fig. 11.

The fatty acid composition of PC, the major phospholipid of the pigment epithelium, is similar in some respects, and different in others, from that of rod outer segment PC, which has been studied extensively by Anderson and co-workers.[19, 30] Thus, the percentages of 16:0, 16:1, 18:0, and 18:1 are remarkably similar in PC from both sources. However, the PC of pigment epithelium contains essentially no C_{22} polyenoates, but instead has relatively much higher percentages of 18:2 and 20:4 than PC of rod outer segments. One can only speculate at the present time on the metabolic fate of docosahexaenoate, which is an important component of the rod outer segment discs phagocytized by pigment cells.[35] Whether it is degraded to 18:2 and 20:4, two of the most abundant polyenoates of pigment epithelial PC, or polymerized further to form lipofuscin is a subject for future investigation.

The retinol plus retinyl ester content of 1.9 μg per eye found in the RPE in the present study is about one-third of the value reported previously[21] in "pigmented layers" of cattle eyes. The preparations examined at that time consisted of particulate fractions of pigment epithelial cells together with choroid. Our studies have shown that choroid has more retinol per eye than RPE. The present data are based on fluorometric analyses, whereas Krinsky[21] used the colorimetric antimony trichloride reaction. However, differences in chemical procedures are probably not the main reason for the discrepancy between Krinsky's data and ours. The major source of error appears to be in analyzing the two tissues (choroid plus RPE) together. This results not only in an overestimation of the total retinol content (free alcohol plus ester), but also leads to errors in evaluating the relative amounts of the two forms present in RPE since essentially all of the choroidal retinol is present as the free alcohol (Table IV), probably bound to the retinol-binding protein of plasma trapped in the choriocapillaris.[36] In contrast to this, nearly all of the intracellular RPE retinol is esterified. Preliminary studies have led to the tentative identification of 16:0, 17:0 (or 16:2), 18:0, and 18:2 as the principal fatty acids of RPE retinyl esters (ER Berman, unpublished data). They were present in approximately equal amounts, and together accounted for about 60 per cent of the

fatty acids detected by gas chromatography. Thus, the fatty acid composition of RPE retinyl esters appears to be distinctly different from that of the retina which consists mainly of palmitate esters.[21-23]

That the RPE serves as an important storage depot for retinol has often been speculated but never directly proved. The present study allowed a direct comparison, on a weight basis, to be made between RPE and other bovine tissues. The finding that the concentration of retinol is about 70 times greater in RPE than in retina, and four to five times greater than in liver, strengthens the commonly held view that on a weight basis, RPE is indeed a major storage depot.

The DNA content of mammalian cells is, in general, highly variable when calculated on a weight basis, being as low as 0.22 Gm. per 100 Gm. of fresh tissue in cattle pancreas and as high as 2.2 Gm. per 100 Gm. of tissue for thymus.[16] The value of 0.72 Gm. per 100 Gm. of wet tissue for RPE lies between these two extremes. The only DNA parameter that is constant among various somatic cells is the nuclear concentration, which in cattle is 6.3 to 6.4 pg. per nucleus.[16] Cytophotometric measurement of DNA in RPE gave a value consistent with the assumption that RPE cells are diploid. A similar observation has been made for cultured human pigment epithelium.[37]

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