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Retinoid metabolism in cultured human retinal pigment epithelium

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Uptake, esterification and release of all-*trans*-retinol in primary cultures of human retinal epithelium were studied. Cultured cells were supplemented with ^{3}H-labelled 11,12-all-*trans*-retinol, using fatty-acid-free albumin as the carrier. This led to incorporation of retinol and the formation of all-*trans*- and 11-*cis*-retinyl palmitate. The metabolism of the all-*trans* ester was monitored in a medium containing various concentrations of foetal-bovine serum (FBS). In 20% (v/v) FBS, the ester was hydrolysed, and all-*trans*-retinol was released into the culture medium. In the absence of FBS, little ester was hydrolysed and no retinol was found in the medium. Dialysed or heat-inactivated FBS or fatty-acid-free albumin was as effective as FBS in provoking ester hydrolysis and retinol release. The concentration-dependency of this effect on FBS was matched by the corresponding concentrations of albumin alone. A linear relationship was also found between interphotoreceptor retinoid-binding protein and retinoid release. Haemoglobin, which does not bind retinoids, is ineffective in this capacity. It is concluded that lipid-binding substances, mainly albumin, in FBS act as acceptors for retinol and drain the cultured cells of this molecule. The release of the retinol is coupled to the hydrolysis of retinyl esters in the cell, so that there is little or no net hydrolysis of ester if there is no acceptor for retinol in the culture medium. This effect explains why cultured human retinal epithelial cells are depleted of their stores of retinoids when maintained in medium supplemented with FBS.

INTRODUCTION

The role of the retinal pigment epithelium (RPE) in vision is well established (Bok, 1985). This epithelial monolayer incorporates all-*trans*-retinol from the serum by a presumed membrane-receptor-mediated process (Pfeffer *et al.*, 1986). The all-*trans*-retinol is both esterified and, as recently shown in frog RPE (Bernstein *et al.*, 1987), isomerized to 11-*cis*-retinol before esterification. This 11-*cis*-ester pool can be hydrolysed (Blaner *et al.*, 1987), and the alcohol formed is then oxidized by a stereospecific dehydrogenase (Lion *et al.*, 1975) to 11-*cis*-retinaldehyde, the latter being facilitated by cellular retinaldehyde-binding protein (CRALBP) (Mertz *et al.*, 1987*b*). 11-*cis*-Retinaldehyde is transferred to the outer segments of photoreceptors, this process presumably being facilitated by an interphotoreceptor retinoid-binding protein, IRBP (Chader & Wiggert, 1984; Bridges & Adler, 1985). This 11-*cis*-retinoid metabolism must be specific to RPE, because this molecule is not needed elsewhere in the body; it is a molecule unique to vision. Therefore scientists interested in the molecular basis of hereditary diseases that produce malfunction of photoreceptors and no other systemic abnormality have been interested in the 11-*cis*-retinoid metabolism of RPE.

RPE has been successfully cultured for decades (Albert *et al.*, 1972; Mannagh *et al.*, 1973; Edwards, 1978; DelMonte, 1979; Flood *et al.*, 1980; Boulton *et al.*, 1983; Pfeffer & Newsome, 1983). We have been examining the retinoid metabolism of human RPE in tissue culture in order to determine how much of the unique retinoid metabolism of these cells *in vivo* is maintained *in vitro* (Flood *et al.*, 1982, 1983). In previous studies it has been found that human RPE in culture loses its relatively large retinoid stores after only a few days in culture. This depletion can be offset by exposing the cultured cells to albumin-bound all-*trans*-retinol, which they incorporate and esterify. If not continuously supplemented with retinol, however, the cells continued to deplete their retinoid stores in culture. At that time it was suspected that this depletion might be due to the absence of the neural retina with contiguous photoreceptors (Flood *et al.*, 1983).

In the present study we demonstrate that the depletion of retinoids from cultured human RPE is due to the presence of serum used to supplement the nutrient medium of the cultured cells. The depletion is linearly related to the serum concentration and can be reproduced by serum albumin alone, a protein known to bind retinoids. Proteins not capable of binding retinoids, such as haemoglobin, are ineffective in this capacity. During the course of these experiments we have found that cultured human RPE not only incorporates and esterifies retinol, hydrolyses the esters and releases the retinol formed into the medium, but also forms detectable amounts of 11-*cis*-retinyl palmitate, indicating that much of the retinoid metabolism specific to RPE is maintained *in vitro*. Parts of this work have already been published in abstract form (Das *et al.*, 1986, 1987).

Abbreviations used: FBS, foetal-bovine serum; RPE, retinal pigment epithelium; CRALBP, cellular retinaldehyde-binding protein; IRBP, interphotoreceptor retinoid-binding protein; BSA, bovine serum albumin; BHT, butylated hydroxytoluene; MEM, Eagle's minimal essential medium; CRBP, cellular retinol-binding protein.

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EXPERIMENTAL

Materials

Post-mortem donor eyes from humans ranging in age from 54 to 89 years were received through the New York Eye Bank for Sight Restoration, National Diabetes Research Interchange and the Retinitis Pigmentosa Donor Eye Program. ^{3}H-labelled 11,12-all-*trans*-retinol was from Amersham Corp., Des Plaines, IL, U.S.A. Purified fatty-acid-free bovine serum albumin (BSA), haemoglobin, Trizma Base (Tris), glucose and butylated hydroxytoluene (BHT) were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A. Dioxan, diethyl ether, n-hexane, anhydrous Na_2SO_4 and Spectrapor dialysis membrane tubing were from Fisher Scientific, Fair Lawn, NJ, U.S.A. Eagle's minimal essential medium (MEM) was from Gibco Laboratories, Grand Island, NY, U.S.A. FBS was from Hyclone Laboratories Logan, UT, U.S.A., and 35 mm × 10 mm tissue-culture plates were from Corning Glass Works, Medfield, MA, U.S.A. Econofluor was from New England Nuclear Research Products, Boston, MA, U.S.A., and protein assay dye reagent was from Bio-Rad Laboratories, Richmond, CA, U.S.A. All solvents were h.p.l.c.-grade (Fisher). Authentic retinoids used as standards were generously given by Hoffmann–La Roche, Nutley, NJ, U.S.A., and Professor Nakanishi of Columbia University. Human IRBP was a gift from Dr. Hiroyuki Ishizaki, who purified it from human interphotoreceptor matrix (IPM) by using the procedure of Adler & Evans (1985).

Methods

Retinal epithelial cells obtained from human donor eyes were allowed to grow as a primary culture by using methods described elsewhere (Flood *et al.*, 1980). The '*in vitro*' age of the cultures used in the present study was between 2 and 3 weeks. In any one experiment, cultures from the same donor were used to diminish variability of the results. Tracer amounts of ^{3}H-labelled all-*trans*-retinol purified by h.p.l.c. and bound to fatty-acid-free BSA was used for retinol supplementation. Supplementation of retinol was done under dim red light, and extraction of retinoids was carried out in dim red light using amber glassware and an oxygen-free argon atmosphere. At 6 h before retinol supplementation, the regular medium [MEM supplemented with 0.5% glucose and 20% (v/v) FBS] was replaced by medium without FBS. This was done to maximize the uptake of ^{3}H-labelled all-*trans*-retinol by avoiding competition with the unlabelled all-*trans*-retinol present in the serum.

A 1 μCi portion of ^{3}H-labelled all-*trans*-retinol (sp. radioactivity 60 mCi·mmol^{-1}) per 35 mm × 10 mm culture plate containing the cells (generally 10^6 cells) was dissolved in 10 μl of ethanol and added to 2 ml of MEM containing 0.5% glucose and BSA (500 μg/ml). This supplementation medium was vortex-mixed and kept in the dark at 4 °C for 1 h before being added to the cells. To study the uptake and esterification of ^{3}H-labelled all-*trans*-retinol, cells were incubated in this medium for a maximum period of 24 h. Esterification at different time points was stopped by putting the cultures on ice. The supplementation medium was removed and the cells washed thrice with cold 10 mM-Tris/HCl, pH 7.0, and scraped into a Dounce glass/glass tissue grinder with a rubber policeman. Cells were homogenized on ice with 100 strokes in 2 ml of 10 mM-Tris/HCl, pH 7.0. A portion of the homogenate was used to determine the protein content, a Bio-Rad microassay kit being employed for this purpose.

To extract the retinoids, ethanol was added to the homogenate in the ratio 1:1 (v/v) and left on ice for 15 min. Extraction was done twice with 10 ml of n-hexane, followed by two extractions with 10 ml of diethyl ether. Extracts were pooled, washed with 20 ml of deionized water, dried over anhydrous Na_2SO_4 and evaporated to dryness under purified nitrogen. The recovery of the retinoids extracted ranged from 70 to 80%. Extracted retinoids were redissolved in 200 μl of n-hexane containing 25 μg/ml of BHT and stored under O_2-free argon at −20 °C before being analysed by h.p.l.c. Solvents used for extraction and analysis of retinoids contained BHT (25 μg/ml) to minimize oxidative degradation of retinoids.

In order to determine the metabolic fate of the cellular retinoids, we supplemented the cultures with ^{3}H-labelled all-*trans*-retinol for 18 h, thereby building up a labelled-retinoid store. Then we monitored the fate of this store under different media conditions. Incorporation of radioactivity due to uptake and esterification of ^{3}H-labelled all-*trans*-retinol was determined by extracting and analysing the retinoids by h.p.l.c. after 18 h of supplementation from one of a set of plates. The supplementation medium was removed from the remaining plates, and the cells were washed thrice with MEM before being replaced with MEM/0.5% glucose containing different concentrations of FBS and incubated at 37 °C for different time periods. The incubation was stopped and the retinoids extracted from both the cell homogenates and the media, the procedure described above being used.

FBS was heated at 80 °C in a water bath for 30 min. Heat-deactivated serum was then centrifuged in a Sorvall RC-5B refrigerated centrifuge (du Pont) at 2000 ***g***, and this supernatant was used as an experimental medium. Dialysis of FBS was done extensively against MEM in Spectrapor membrane tubing with M_r cut-off range of 12000–14000. This dialysed serum was also used as an experimental medium. Fatty-acid-free BSA was added to the serum-free media at 0.26, 1.3, 2.6 and 5.2 mg/ml, the amounts of albumin present respectively in 1-, 5-, 10- and 20%-(v/v)-FBS-containing media. Similar experiments were also carried out in the presence of haemoglobin in concentrations identical with those of albumin. We compared how different media conditions influence the radiolabelled-retinoid stores in these cells. When using FBS in the media the specific radioactivity of the retinoids is changed slightly, because FBS contains some unlabelled retinol. This change in retinoid activity appears to have no significant impact on the major results from the present study, because they are the same when albumin containing no retinoids is used to mimic the effects of FBS.

H.p.l.c. analysis of retinoids was done on two normal-phase 3.9 mm × 15 cm Resolve columns (Waters), packed with spherical silica (5 μm particle size), connected in series. The mobile phase used for the analysis of retinyl ester was 0.4% diethyl ether/n-hexane at 0.6 ml/min. For identification of retinol, the mobile phase used was 5% dioxane/0.4% diethyl ether (final concns.) in n-hexane at 0.6 ml/min. The eluate from the h.p.l.c. column was collected into 20 ml glass scintillation vials

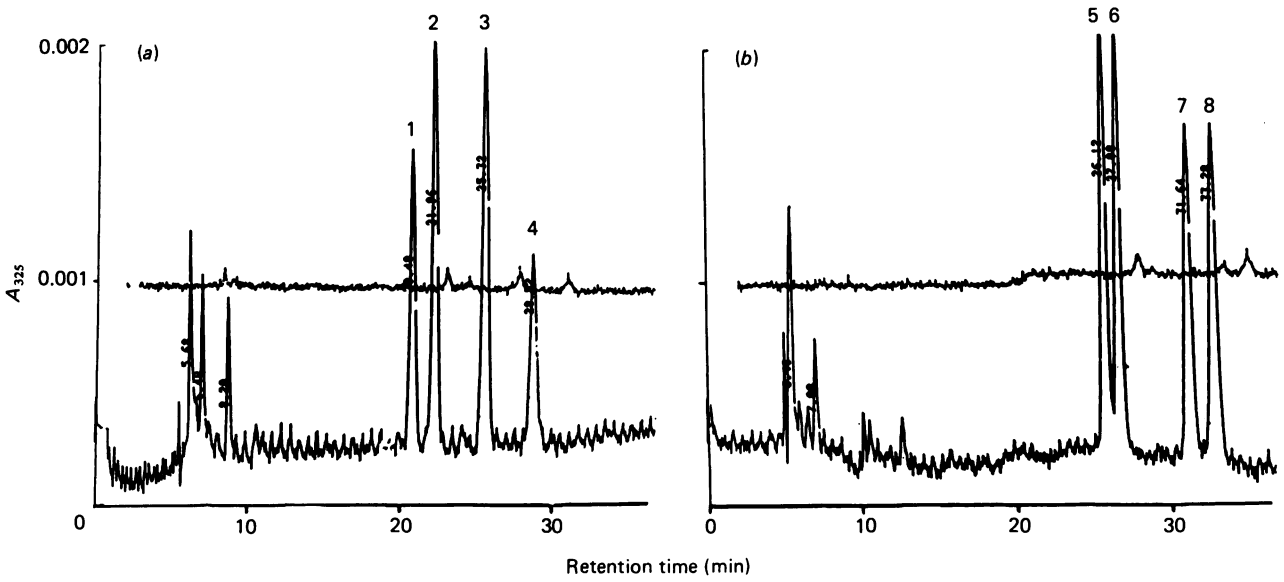

Fig. 1. H.p.l.c. chromatograms of (*a*) three *cis* isomers and all-*trans*-retinyl palmitate and (*b*) three *cis* isomers and all-*trans*-retinol

H.p.l.c. details: column, Resolve (Waters, 5 μm-particle-size spherical silica), two in series; mobile phase, n-hexane/diethyl ether (249:1, v/v); flow rate, 0.6 ml/min. Peak identifications: 1, 13-*cis*-retinyl palmitate; 2, 11-*cis*-retinyl palmitate; 3, 9-*cis*-retinyl palmitate; 4, all-*trans*-retinyl palmitate. (*b*) The column was the same as that used in the ester system; mobile phase, n-hexane/diethyl ether/dioxane (3784:1:125, by vol.); flow rate, 0.6 ml/min. Peak identifications: 5, 11-*cis*-retinol; 6, 13-*cis*-retinol; 7, 9-*cis*-retinol; 8, all-*trans*-retinol. The trace at $A = 0.001$ represents the output of the fluorescence detector set at 338 nm excitation and 460 nm emission. The exact retention times are written alongside each peak.

at 0.5 min intervals. A 10 ml portion of Econofluor was then added to each vial and radioactivity was counted in a LKB 1217 Rackbeta liquid-scintillation counter. Radioactivity emerging at the peaks corresponding to authentic retinoids was normalized for protein and used for the measurement of retinyl palmitate formed or hydrolysed, depending on the nature of the experiment. Free retinol was also measured in the same way. In all experiments amounts of radioactive retinyl palmitate and free retinol, either in the cells or in the media, are expressed as c.p.m./mg of protein present in RPE cells.

RESULTS

Separation of three *cis* isomers and all-*trans*-retinol as well as of retinyl palmitate in h.p.l.c. was achieved. Authentic retinol and retinyl palmitates were run in an h.p.l.c. system as described in the Experimental section. The chromatogram of retinyl palmitate isomers (Fig. 1*a*) shows a baseline separation of all four isomers, and especially the difference in retention times of 11-*cis* and 13-*cis* isomer by 1.5 min enabled us to distinguish these two isomers. Fig. 1(*b*) shows the chromatogram of retinol isomers. Although baseline separation could not be achieved, four distinctly separated peaks were obtained, which was not possible when retinoids were analysed with a single h.p.l.c. column at a mobile-phase flow rate of 1 ml/min (Das *et al.*, 1986).

Uptake and esterification of ^{3}H-labelled all-*trans*-retinol was studied by supplying all-*trans*-retinol bound to BSA to primary cultures of human retinal epithelium. The h.p.l.c. profile of the radioactive retinyl palmitates formed after supplementation for 6 h with ^{3}H-labelled all-*trans*-retinol shows a major peak of all-*trans*-retinyl palmitate and a smaller, but definitely detectable, peak of 11-*cis*-retinyl palmitate (Fig. 2). A small peak of all-*trans*-retinyl oleate was also detected. Formation of ^{3}H-labelled all-*trans*-retinyl palmitate was observed within 1 h of supplementation. The amount of retinyl palmitate formed increased progressively in time over a total incubation period of 24 h (Fig. 3).

Metabolism of labelled all-*trans*-retinyl palmitate stored in the cells after 18 h of incubation with ^{3}H-labelled all-*trans*-retinol was monitored at different time points covering a period of 24 h in regular medium containing 20% (v/v) FBS. The amount of all-*trans*-retinyl palmitate decreased considerably within the first 2 h. After 6 h the amount of all-*trans*-retinyl palmitate decreased less rapidly and appeared to level off at 24 h (Fig. 4). When the cellular retinyl palmitate store was monitored in the absence of FBS, the loss of ester was much less, only 25% compared with an 82% loss at 2 h in serum-containing medium. The most dramatic effect of FBS was on the amount of ^{3}H-labelled all-*trans*-retinol in the cells. The decrease in cellular retinol content is 95% when incubation was in the presence of FBS, compared with only 4% when incubation was in FBS-free medium for 2 h (Fig. 4). As the cellular concentration of retinol decreased, a time-dependent increase in the concentration of ^{3}H-labelled all-*trans*-retinol was observed in the FBS-containing medium (Fig. 5).

No retinyl palmitate was detected in the medium, indicating that the decrease in cellular retinyl palmitate content is due to its hydrolysis and subsequent release of the all-*trans*-retinol into the medium. Very little retinol was found in the medium devoid of FBS.

In our initial efforts to identify the factor in the serum responsible for this enhanced hydrolysis of retinyl ester and cellular release of retinol, both dialysed and heat-treated serum tested at a concentration of 20% (v/v)

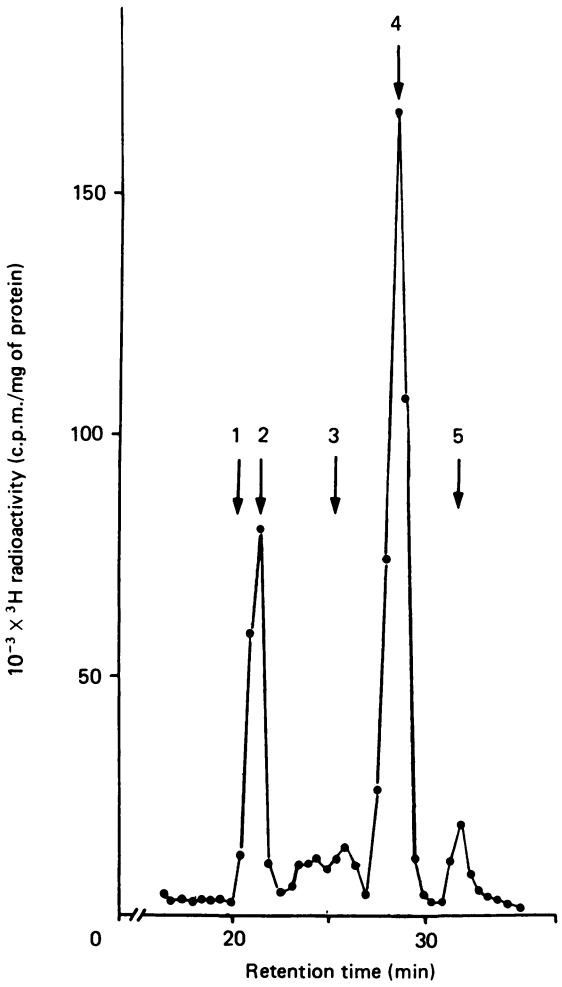

Fig. 2. H.p.l.c. elution profile of radioactivity due to retinyl esters formed in primary cultures of human retinal epithelium supplemented with 1 μCi (specific radioactivity 60 Ci·mmol^{-1}) of ^{3}H-labelled 11,12-all-*trans*-retinol/plate for 6 h

^{3}H-labelled all-*trans*-retinol in 10 μl of ethanol was preincubated with 2 ml of MEM containing 0.5% glucose and BSA (500 μg/ml) at 4 °C for 1 h before being added to each 35 mm × 10 mm culture plate. Extraction of retinoids with n-hexane was monitored by h.p.l.c. analysis as described in the legend to Fig. 1. The arrows indicate the retention times of authentic standards: 1, 13-*cis*-retinyl palmitate; 2, 11-*cis*-retinyl palmitate; 3, 9-*cis*-retinyl palmitate; 4, all-*trans*-retinyl palmitate; 5, all-*trans*-retinyl oleate. The ordinate indicates the radioactivity (c.p.m./mg of protein) in the RPE cells in this and all subsequent Figures.

were found to be as effective as 20% (v/v) serum-containing medium. Bovine serum albumin at a concentration (5.2 mg/ml) equivalent to the amount present in 20% (v/v) FBS-containing medium was virtually as effective as serum in causing the hydrolysis of all-*trans*-retinyl palmitate and the release of all-*trans*-retinol (Fig. 6).

Release of ^{3}H-labelled all-*trans*-retinol from the cultured cells, on incubation with FBS-containing medium for 4 h depended on the concentration of FBS present in the medium (Fig. 7). Fig. 7 shows a similar correlation between the amount of ^{3}H-labelled retinol released into

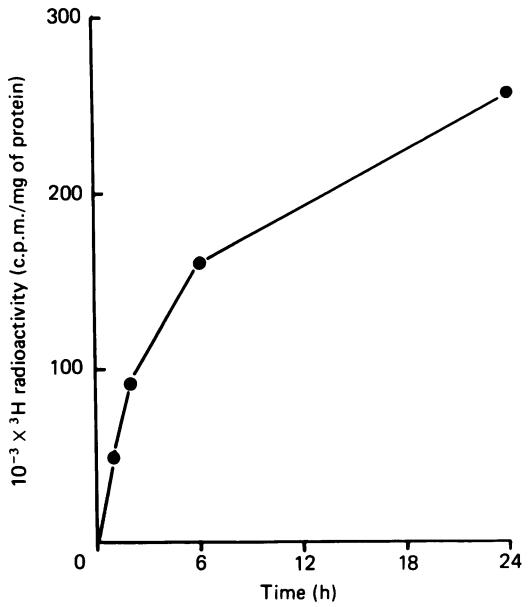

Fig. 3. Time-dependence of formation of all-*trans*-retinyl palmitate in cultured human retinal epithelium after being incubated at 37 °C with ^{3}H-labelled 11,12-all-*trans*-retinol over a maximum period of 24 h

Incubation of the cells with ^{3}H-labelled all-*trans*-retinol was performed as described in the legend to Fig. 2.

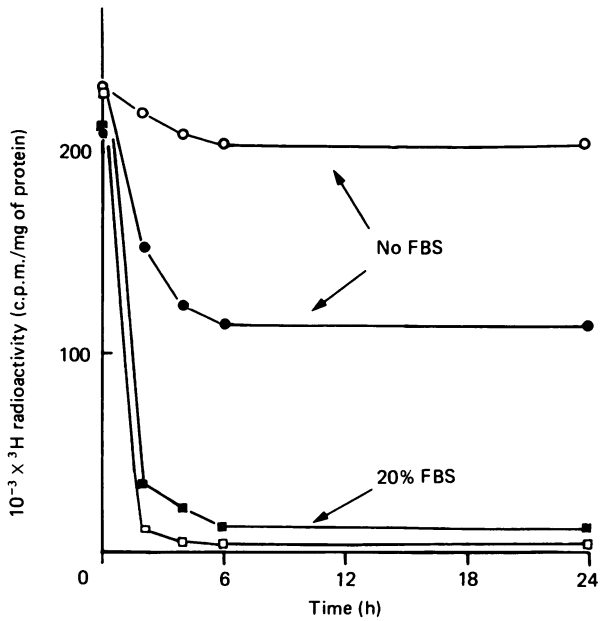

Fig. 4. Decline in radioactive all-*trans*-retinyl palmitate content of retinal epithelium after being incubated with (■) and without (●) FBS in the medium

After initial incubation for 18 h at 37 °C with ^{3}H-labelled all-*trans*-retinol as described in legend to Fig. 2, the cells were washed with MEM and then incubated at 37 °C over a maximum period of 24 h in MEM containing 0.5% glucose together with, or without, FBS. After the second incubation the cells were washed, harvested, and the retinoids extracted and analysed by h.p.l.c. as described in the legend to Fig. 1. The ^{3}H-labelled-all-*trans*-retinol content in the cells after they had been incubated with (□) and without (○) FBS in the medium is also shown.

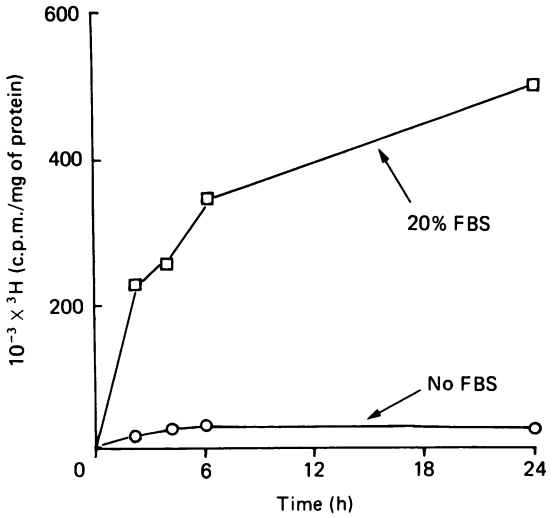

Fig. 5. Secretion of free ^{3}H-labelled all-*trans*-retinol into the medium after cells had been incubated with (□) or without (○) FBS in the medium

After initial supplementation of ^{3}H-labelled all-*trans*-retinol, the retinoids in the media were analysed at different time points as described in the legend to Fig. 4. The retinoids were extracted from the media and analysed by h.p.l.c. as described in the legend to Fig. 1. No retinyl esters were found in the media (results not shown). ^{3}H-labelled retinol appears mainly in the media containing FBS.

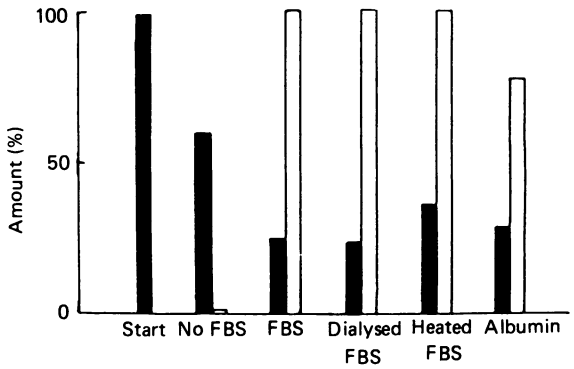

Fig. 6. Effect of serum, modified serum and serum albumin on the hydrolysis of all-*trans*-retinyl palmitate and release of all-*trans*-retinol by cultured retinal epithelium

After initial supplementation of ^{3}H-labelled all-*trans*-retinol for 18 h, the retinoids in the cells and the media were analysed after being incubated for 4 h in the presence of MEM and 0.5% glucose only, and also with 20% (v/v) FBS, 20% (v/v) dialysed (using Spectrapor membrane with an M_r cut-off range of 12000–14000) serum, 20% (v/v) heat-deactivated (at 80 °C for 30 min) serum or 5.2 mg of purified fatty-acid-free bovine serum albumin/ml added to it. The amount of radioactive all-*trans*-retinyl palmitate present after the initial incubation of the cells with ^{3}H-labelled all-*trans*-retinol for 18 h and the amount of all-*trans*-retinol found in the medium after incubation for 4 h in the presence of 20% (v/v) FBS were assumed to be 100%. The amount of ^{3}H-labelled all-*trans*-retinyl palmitate (solid bars) in the cell greatly diminished, and ^{3}H-labelled all-*trans*-retinol (open bars) in the medium increased in the presence of treated or untreated serum and serum albumin.

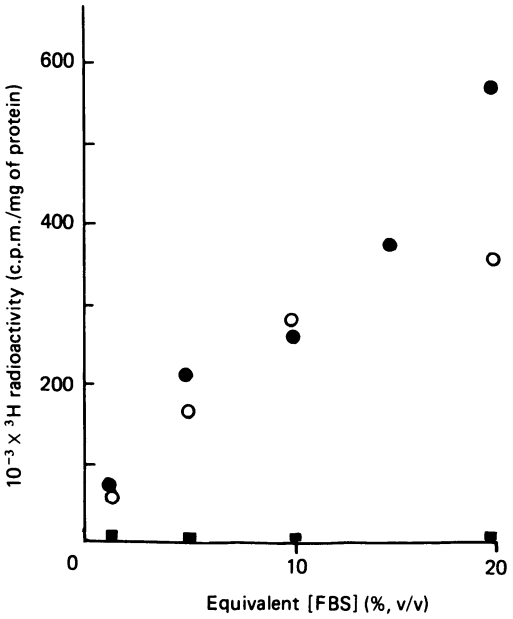

Fig. 7. Dependence of release of ^{3}H-labelled all-*trans*-retinol by cultured retinal epithelium on FBS concentrations in the media (●), the amount of albumin present in corresponding concentrations of FBS (○) and haemoglobin in concentrations identical with that of albumin (■)

After supplementation of ^{3}H-labelled all-*trans*-retinol 18 h, the retinoids were monitored in MEM and 0.5% glucose containing 1, 5, 10, 15 and 20% (v/v) FBS for 4 h (●). In one experiment, 18 h of all-*trans*-retinol supplementation was followed by incubation in presence of MEM and 0.5% glucose containing 0.26, 1.3, 2.6 and 5.2 mg of fatty-acid-free bovine serum albumin/ml, the amounts present in 1, 5, 10 and 20% (v/v) FBS-containing media respectively (○). Similar experiments were carried out with 0.26, 1.3, 2.6 and 5.2 mg of haemoglobin/ml (■).

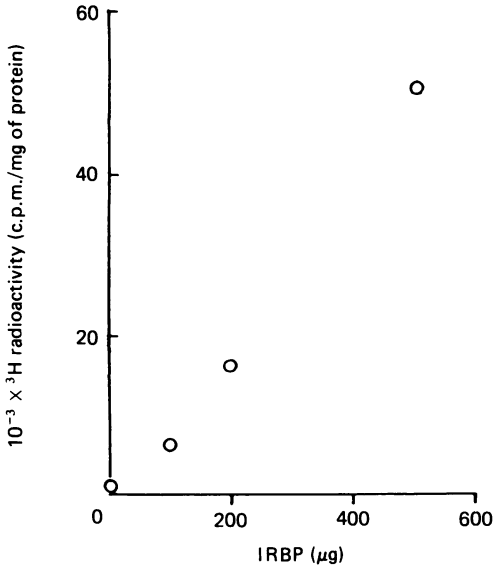

Fig. 8. IRBP-concentration-dependent release of ^{3}H-labelled all-*trans*-retinol by cultured human RPE

After initial supplementation of ^{3}H-labelled all-*trans*-retinol for 18 h, the release of all-*trans*-retinol was monitored after incubation of cells for 4 h in the presence of 100, 200 and 500 μg of purified human IRBP.

the medium and the amount of albumin present. When a similar experiment was carried out in the presence of haemoglobin, one of the major components of serum, release of ^{3}H-labelled all-*trans*-retinol into the medium was not observed.

In a similar experiment IRBP added in physiological amounts (Fong *et al.*, 1984) to the medium provoked the release of ^{3}H-labelled all-*trans*-retinol from cultured RPE cells and this effect was also concentration-dependent (Fig. 8).

DISCUSSION

In a previous study, Flood *et al.* (1983) were unable to detect any isomerization of all-*trans* to 11-*cis* retinoid by cultured human RPE. By increasing the sensitivity of detecting retinoids using radioisotopes, we began to detect the formation of the 11-*cis* isomer. At that time we were not sure whether it was the 11- or the 13-*cis* isomer, owing to the limitation of the h.p.l.c. resolution capacity. By using two columns in series and altering the mobile-phase flow rate we were able to separate these two *cis* isomers, which enabled us to show conclusively that cultured human retinal epithelium, devoid of any neural retina, can form 11-*cis* retinoid when presented with ^{3}H-labelled all-*trans*-retinol. However, the relative amount of 11-*cis* retinoid formed compared with the all-*trans* isomer is small compared with their concentrations *in vivo* (Das *et al.*, 1986). Whether the '*in vitro*' age of the cultures or the retinoid status of the cultured epithelium affects the formation of 11-*cis* retinoid *in vitro* remains to be elucidated. Recently Bernstein *et al.* (1987) have demonstrated an enzyme in frog RPE homogenates that isomerizes all-*trans*- to 11-*cis*-retinol. It will be interesting to know whether such an enzyme is responsible for the formation of 11-*cis* retinoid we detect *in vitro* and whether the levels of this enzyme *in vitro* resembles that *in vivo*.

However, the most interesting finding of the present study is that the rapid loss *in vitro* of the large retinoid store that RPE maintains *in vivo* is due to the presence of serum in the media and, in particular, the albumin component of the serum which removes retinol, presumably from the cell membrane. Although it is known that serum can have profound influence on cultured cells (Tyson Tildon & Stevenson, 1984), there have only been rare reports of the influence of serum on cultured RPE (Flaherty & Edwards, 1986) and none which examined the influence of serum on retinoid metabolism. Our data show that serum provokes the release of retinol formed by the hydrolysis of the retinyl ester in the cultured RPE. This effect of serum on hydrolysis of retinyl ester and release of retinol resembles a similar effect of serum on cholesterol, a non-polar lipid like retinol. Cholesterol is also incorporated and stored as fatty acid esters by cultured cells. These esters are hydrolysed, with free cholesterol being released if there is a suitable acceptor in the media. A variety of substances like high-density lipoprotein, whole serum, thyroglobulin and erythrocytes (Ho *et al.*, 1980) can serve as acceptors. Interestingly, albumin does not act as an acceptor for cholesterol. The hydrolysis of cholesteryl ester is linked to the release of cholesterol. Hydrolysis of the ester does not occur if there is no release of cholesterol. A similar coupling between retinol release and hydrolysis of retinyl ester appears to occur in cultured RPE. The fact that albumin, which binds retinoids non-specifically, can stimulate release of retinol from the cultured RPE suggests that other retinoid-binding molecules, such as IRBP, can provoke the release of retinol and the further hydrolysis of retinyl ester in RPE *in vivo*. This release of retinoids, especially retinol and/or retinal, by extracellular retinoid-binding proteins may play a role in the physiological movement of 11-*cis*-retinal from RPE to the outer segment of photoreceptors *in vivo*. IRBP could facilitate the release of retinal and/or retinol from both RPE and outer-segment plasma membrane, allowing these molecules to diffuse along their concentration gradients resulting from the effects of light on the photoreceptors. Rod outer segments are dissimilar to lipid vesicles and microsomal membranes in resisting the incorporation of retinal, possibly because of their abnormally high content of polyunsaturated fatty acyl side chains (Groenenduk *et al.*, 1984). Perhaps IRBP facilitates not only the release of retinal from the RPE, but also its entry into the outer segment, thereby playing a unique role in the visual cycle. Such an hypothesis seems testable by tissue-culture techniques.

Several enzymes involved in stereospecific retinoid metabolism are known to be present in RPE. Blaner *et al.* (1987) have shown the presence of one such activity which hydrolyses 11-*cis*-retinyl palmitate. The activity of this enzyme appears to diminish *in vitro* (Ishizaki *et al.*, 1986). Another enzyme is a dehydrogenase specific for 11-*cis*-retinol (Lion *et al.*, 1975), which we have found to be present in human RPE. This enzyme oxidizes 11-*cis*-retinol to 11-*cis*-retinaldehyde. The oxidation was found to be facilitated by CRALBP (Mertz *et al.*, 1987*b*). The levels of these two proteins have not been measured in cultured RPE. CRBP levels in cultured RPE were shown to be 89% of their levels *in vivo* (Blaner *et al.*, 1985). However, CRBP mRNA levels in RPE cells were shown to remain stable *in vitro*. Moreover, comparable actin mRNA levels of confluent primary cultures of RPE with that of fresh suggests that the RPE cells maintain an '*in vivo*'-like genotype in primary culture (Mertz *et al.*, 1987*a*). From our present data we believe that, by controlling the composition of the culture medium, it will be possible to prevent the loss of endogenous retinoids from the cultured RPE. Once it is achieved, it will be valuable to examine the levels of various specific enzyme activities related to retinoid metabolism under conditions where the retinoid levels in these cells can be controlled.

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