Uptake of Retinol and Retinoic Acid from Serum Retinol-binding Protein by Retinal Pigment Epithelial Cells*

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Isolated pigment epithelial cells from bovine eyes were shown to be capable of taking up retinol and retinoic acid. The uptake was dependent on the ligands being presented to the cells as complexes with serum retinol-binding protein. Neither free retinol nor retinol or retinoic acid presented as serum albumin complexes were taken up by the pigment epithelial cells. The transport of retinol and retinoic acid was a relatively rapid, temperature-dependent process. The uptake of retinol and retinoic acid was accompanied by dissociation of the ligand-retinol-binding protein complex, with the ligand being taken up by the cells while the carrier protein remained outside the cell.

Retinol which was taken up by the pigment epithelial cells was found in the cytosol as retinol. About a third of the transported retinol was found to be bound to a high molecular weight protein (>1.5 x 10^6) while the rest was found as free retinol. These results were similar to those previously obtained by the direct addition of retinol to pigment epithelial cytosol (Heller, J. (1976) J. Biol. Chem. 251, 2952). The retinoic acid which was taken up by the pigment epithelial cells was found as retinol-like material, partly bound to a high molecular weight cytosol protein and partly free. This retinol-like material was not retinol since it was not incorporated into rhodopsin in vivo. Fresh leukocytes and red cells from either human or bovine blood did not show any uptake of either retinol or retinoic acid when presented to the cells as their respective serum retinol-binding protein complexes.

Retinol (vitamin A), a polyene lipid, is transported in the blood as a tight complex with a specific protein, the retinol-binding protein (1, 2). Retinol, in the form of the aldehyde retinal, acts as the chromophoric prosthetic group of all known visual pigments and thus is found in appreciable amounts in photoreceptor cells. The retina shares with the brain the permeability barrier which prevents blood proteins and most other low molecular weight substances from entering the retinal interstitial space. The blood retina barrier is created by the retinal pigment epithelium, a monolayer of cells interposed between the rich choroidal blood supply and the neural retina. We have recently reported that pigment epithelial cells have a specific receptor for serum retinol-binding protein (3). This receptor is confined to the choroidal surface of the pigment epithelial cell and is not found in photoreceptors (4).

It was shown previously that retinoic acid (vitamin A acid) is capable of replacing retinol in vitamin A-depleted animals as far as general health and growth functions are concerned (5). Interestingly enough, dietary vitamin A acid is incapable of providing eye tissue with the necessary retinol (or retinal); thus, vitamin A-depleted animals which are supplied with retinoic acid thrive, although their vision is lost (6, 7). Goodman and his colleagues (8) have shown that dietary retinoic acid is carried as a complex with serum albumin and apparently is not found as a complex with serum RBP although it is well established that retinoic acid can combine in vitro with apo-RBP to form a stable retinoic acid-RBP complex. Since retinol is normally found in serum only as a complex with RBP (1), we were interested in investigating the uptake of retinol and retinoic acid by pigment epithelial cells.

Experiments using 125I-labeled RBP have previously indicated that the RBP molecule does not enter the pigment epithelial cells. This was shown both by in vitro binding experiments (3) and by in vivo injection of the labeled protein followed by autoradiography (4). Previous experiments in our laboratory have shown that when retinol is added in vitro to the cytosol from pigment epithelial cells, the chromophore is bound to a high molecular weight lipoglycoprotein (9). In the present series of experiments, we report the uptake of retinol and retinoic acid by isolated pigment epithelial cells from their respective holo-RBP complexes. We also found that isolated human white and red blood cells do not show any uptake of retinol or retinoic acid under similar conditions. Additionally, it was found that retinol which was taken up by the cells was present in the cytosol as retinol bound to a high molecular weight protein.

**EXPERIMENTAL PROCEDURES**

*Preparation of Pigment Epithelial Cells* – Pigment epithelial cells were prepared from freshly obtained bovine eyes as previously described (3).

*Preparation of Human White and Red Blood Cells* – Ten milliliters of freshly drawn blood from healthy adult humans were mixed with 1 ml of 0.9% (w/v) sodium citrate and 2 ml of 3% (w/v) glucose and 3% (w/v) Ficoll in 0.9% (w/v) of NaCl solution. The blood was then centrifuged at 22°C for 45 min at about 10 x g. The supernatant (plasma) was discarded. The blood was then centrifuged at 22°C for 45 min at about 10 x g. The supernatant (plasma containing white blood cells) was centrifuged at about 200 x g for 15 min at 22°C and the clear supernatant (plasma) was discarded. The white and red blood cells were rinsed twice in 8 ml of Hank's, 10 mM Hepes-buffered salt solution, pH 7.4. Bovine blood cells were pre-

*The abbreviations used are: RBP, retinol-binding protein; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.*
pared similarly from freshly obtained bovine blood.

Preparation of \(^{3}H\)Retinol and \(^{125}I\)Retinoic Acid-RBP Complexes—Apo-RBP was prepared from human retinol-RBP as previously described (10). All-trans-retinoic acid, tritiated in position 11 or 12, had a specific activity of 1.24 Ci/mmol and was a gift from Hoffman-La Roche. Tritated retinol was prepared from the labeled retinoic acid by reduction according to previously published procedures (11). The reduction of the acid was complete as judged by spectrophotometric data. \(^{3}H\)Retinol-RBP and \(^{125}I\)Retinoic acid-RBP complexes were prepared as previously described (12). The concentration of the complexes was determined from their absorption spectra using a figure of \(e_{\text{max}} = 46,000\) for all-trans-retinol-RBP, \(e_{\text{max}} = 45,000\) for all-trans-retinoic acid-RBP at pH 7.4, and \(e_{\text{max}} = 40,400\) M\(^{-1}\) cm\(^{-1}\) for \(^{125}I\) retinoic acid-RBP. Radioactivity was measured in a liquid scintillation counter and expressed as disintegration per minute after correcting for counting efficiency and subtracting background counts.

Uptake Assay—In a typical experiment, pigment epithelial cells from 25 bovine eyes were incubated with 25 ml of Hanks' salt solution buffered with 10 mM Hepes, pH 7.4, and containing 0.1% glucose. The incubation mixture was brought to the appropriate temperature in a water bath and labeled holo-RBP (generally about 10-10 mole in 1 to 10 \(\mu l\) containing about 3 \(\times\) 10\(^{10}\) dpm) was added. At various times, samples of 1 ml were taken out of the incubation mixture into 12-ml centrifuge tubes containing 600-fold molar excess of untrated retinol-RBP in 1 ml of buffered Hanks' salt solution. The samples were kept at 22°C for 5 min and then centrifuged at about 150 \(\times\) g for 5 min at 22°C. The clear supernatant was removed and aliquots were counted for radioactivity. The pigment epithelial cells were washed with 2 ml of buffered Hanks' salt solution, transferred in 0.5 ml of distilled water, and counted for radioactivity.

Iodination of RBP—RBP was iodinated with \(^{125}I\) as described previously (3). Similar iodination was performed with nonradioactive NaI to check the effect of iodination. Specific activity of \(^{125}I\)-labeled RBP was 5.9 \(\times\) 10\(^{9}\) cpm/mmol.

Preparation of Retinol- and Retinoic Acid-Bovine Serum Albumin Complexes—In a typical experiment, 10 mg of crystalline bovine serum albumin (Sigma) was extracted twice with 2 ml of absolute alcohol and taken to dryness under nitrogen. Diluted bovine serum albumin was then dissolved in 1 ml of 0.1 M NaCl, 0.033 M sodium phosphate buffer, pH 7.0, and dialyzed against two changes of 125 ml of 2 mM Tris/HCl buffer, pH 9.0, for 48 h. To separate any possible RBP in the preparation from bovine serum albumin, the dialysate was applied to a Sephadex G-100 column (1.5 \(\times\) 82 cm) equilibrated with 2 mM Tris/HCl buffer, pH 9.0. A single symmetrical peak in the elution profile was found at a position corresponding to bovine serum albumin. The purified protein was coupled with retinol or retinoic acid either radioactive or nonradioactive forms as described in the preparation of \(^{3}H\)retinol-RBP \(^{125}I\)retinoic acid-RBP. Bovine serum albumin and \(^{125}I\)retinoic acid-bovine serum albumin had a specific activity of 1.28 mCi/mmol and 15.15 mCi/mmol, respectively.

Gel Filtration Chromatography—Gel filtration of the supernatant from the incubation mixture was performed as described previously (9) using a Sephadex G-100 column (1.5 \(\times\) 88 cm) equilibrated in 0.033 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl at 4°C. Flow rate was approximately 6 ml/h and fractions of about 1.7 ml were collected.

Preparation of Cytools from Pigment Epithelium after Incubation with \(^{3}H\)Retinol or \(^{125}I\)Retinoic Acid—Isolated pigment epithelial cells were incubated with \(^{3}H\)retinol-RBP or \(^{125}I\)retinoic acid-RBP at 37°C for 5 min. Fresh \(^{3}H\)retinol-RBP or \(^{125}I\)retinoic acid-RBP was added and incubated for another 5 min and this process was repeated twice more. After incubation, extracellular medium was obtained by centrifuging the incubation mixture at 170 \(\times\) g for 10 min. The cells were washed twice with Hanks'/Hepes buffer and homogenized, and clear cytools was obtained as described previously (9).

Extraction and Determination of Chromophores—Cytools was extracted with volumes of chloroform:methanol mixture (3:1). The lower organic phase was removed and reextracted twice with an equal volume of distilled water. The organic phase was evaporated to dryness under nitrogen. Quantitative thin layer chromatography was carried out on EM Laboratory precoated silica gel plates (5 \(\times\) 20 cm) and developed with ethyl ether/petroleum ether (30-60° b.p.) (3:2). The plates were scanned in parallel with samples and visualized after iodine-vapor staining. Appropriate spots were removed from the plate and counted in a toluene-based scintillation mixture.

RESULTS

Uptake of \(^{3}H\)Retinol by Pigment Epithelial Cells—When pigment epithelial cells were incubated with \(^{3}H\)retinol-RBP complex, there was a rapid uptake by the cells of the labeled retinol (Fig. 1). The uptake of retinol was rapid for the first 2 to 3 min and was about 80% complete in that time. The loss of \(^{3}H\)retinol in the extracellular medium corresponded to the gain of \(^{3}H\)retinol in the cells (Fig. 1).

When the uptake experiment was performed by adding free \(^{3}H\)retinol (instead of \(^{3}H\)retinol-RBP complex) to the pigment epithelial cells, there was no uptake of labeled material by the cells (Fig. 2). These experiments indicate that retinol-RBP complex is an essential component of the uptake process by the pigment epithelial cells and that free retinol cannot be transported in this system.

Effect of Addition of Fresh Retinol-RBP—As seen in Fig. 1, the uptake of \(^{3}H\)retinol by pigment epithelial cells leveled off after about 10 min of incubation at 37°C. To establish whether this leveling off was due to saturation of the pigment epithelial cells by retinol or to exhaustion of the retinol-RBP in the medium, the pigment epithelial cells after 10 min of incubation were recovered by centrifugation at 170 \(\times\) g for 5 min and incubated in additional retinol-RBP. As shown in Fig. 2, the uptake of retinol by pigment epithelial cells following this procedure was as effective as during the first period of incubation. The addition of fresh retinol-RBP to pigment epithelial cells was repeated four times, resulting in a similar uptake of retinol following each addition. Moreover, in vivo injection of \(^{3}H\)retinol-RBP led, after 5 days, to a total retinol uptake by pigment epithelial cells that was about 100-fold larger than that seen after 10 min of in vitro incubation. On the other hand, addition of fresh pigment epithelial cells to the supernatant from the first incubation did not result in any further uptake of retinol by the fresh pigment epithelial cells (Fig. 2). These experiments indicated that the reduced uptake of retinol by the pigment epithelial cells after 10 min of incubation at 37°C was due to depletion of retinol-RBP complex in the medium and not due to saturation of the pigment epithelial cells by retinol.

Since the supernatant medium after 10-min incubation with \(^{3}H\)retinol-RBP still contained about 80% of the initial \(^{3}H\)counts, we investigated the fate of the retinol-RBP complex in the extracellular medium. When the radioactive supernatant at the termination of the incubation was subjected to gel filtration chromatography, the counts appeared as a low molecular weight (<1000) substance, while no radioactivity appeared at the position of retinol-RBP complex. Addition of excess protein (egg albumin) to the incubation medium did not affect the extent of RBP degradation. The conclusion drawn from this experiment was that the \(^{3}H\)retinol-RBP complex in the incubation mixture was progressively broken down.

Effect of Temperature on Retinol Uptake—As shown in Fig. 3, very little uptake was observed at 4°C and there was only a slight difference between uptake at 37°C and 22°C. When the pigment epithelial cells were incubated for 10 min at 4°C and then raised to 37°C, there was an increase in retinol uptake, without any lag period and with an uptake pattern similar to that shown by pigment epithelial cells that were not kept in the cold. This experiment illustrates that the cold treatment did not affect the uptake potential of the cells and that when the incubation temperature was raised back to 37°C, the retinol uptake seemed to be normal. In other words, the transport of

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retinol into pigment epithelial cells is a temperature-dependent process.

Is Retinol or Retinol-RBP Complex Transported into Pigment Epithelial Cells?—In previous publications, it was shown that the iodinated RBP molecule was bound to the pigment epithelial cell surface receptors and yet was not found within the cells even after 1-h incubation in vitro (3) or after in vivo injection (4). Because of the overlapping energy spectrum of $^3$H and $^{125}$I, it was not possible to perform a direct double labeling experiment to show that $[^3$H]retinol actually penetrates the cells while RBP stays outside. From our previous studies of various modifications of RBP, we learned that modifications such as acetylation change the binding properties of the protein molecule and thus it was not possible to use other labeling isotopes such as $^{13}$C. To circumvent this technical difficulty, we performed parallel experiments using parallel experiments using $[^3$H]retinol and $[^3$H]retinol-RBP of similar specific activity. As seen in Fig. 4 and Table I, the amount of

![Fig. 1](image1.png)

**Fig. 1.** Uptake of $[^3$H]retinol from $[^3$H]retinol-RBP by pigment epithelial cells. Bovine pigment epithelial cells (2.5 x $10^9$ cells) were incubated at 22$^\circ$ with $[^3$H]retinol-RBP (2.5 x $10^9$ dpm) in a final volume of 25 ml. Aliquots of 1.0 ml were taken at various intervals and processed as described under "Experimental Procedures." One hundred per cent uptake was taken as the difference between the counts of the cellular fraction at zero time and after 60-min incubation. One hundred per cent loss was taken as the difference between the counts after 60-min incubation and at zero time in the extracellular medium which was obtained by centrifuging the incubation mixture at 170 x g for 10 min. O, uptake by the pigment epithelial cells; C, loss in the extracellular medium.

![Fig. 2](image2.png)

**Fig. 2.** Effect of addition of fresh $[^3$H]retinol-RBP on the uptake of retinol. Bovine pigment epithelial cells were incubated with $[^3$H]retinol-RBP at 22$^\circ$ and uptake of retinol was measured for 10 min as described in Fig. 1. After 10 min, incubation cells were centrifuged at 170 x g for 10 min and resuspended in buffer of appropriate volume to obtain the initial cell concentration and either $[^3$H]retinol-RBP or free $[^3$H]retinol was added as before and samples were taken for an additional 10 min. In a parallel experiment, fresh pigment epithelial cells were added to the supernatant from the first 10 min of incubation (no additional $[^3$H]retinol-RBP was added). O, first incubation; C, second incubation of pigment epithelial cells with additional $[^3$H]retinol-RBP; x, second incubation of pigment epithelial cells with free $[^3$H]retinol; A, fresh pigment epithelial cells incubated in supernatant from first incubation.

![Fig. 3](image3.png)

**Fig. 3.** Effect of temperature on the uptake of retinol. Bovine pigment epithelial cells were incubated with $[^3$H]retinol-RBP at various temperatures and the uptake of $[^3$H]retinol was measured as described in Fig. 1. For temperature shifting experiment (from 4-22$^\circ$), fresh $[^3$H]retinol-RBP was added to the cell suspension after 10 min at 4$^\circ$ and incubated at 37$^\circ$. The amount of uptake was expressed as picomoles of retinol uptake/1 x $10^9$ cells.

![Fig. 4](image4.png)

**Fig. 4.** Binding of retinol-$^{125}$I labeled RBP and uptake of $[^3$H]retinol by pigment epithelial cells. The binding of retinol-$^{125}$I-labeled RBP was measured by incubating cells (2.5 x $10^9$) with retinol-$^{125}$I labeled RBP (2.2 x $10^9$ dpm) as described in Fig. 1. O, binding of retinol-$^{125}$I-RBP; C, uptake of $[^3$H]retinol-$^{125}$I-RBP. A, uptake of $[^3$H]retinol from $[^3$H]retinol and the binding of $^{125}$I-RBP were measured for displacement with 600-fold molar excess of unlabeled retinol-RBP as described under "Experimental Procedures."

**Table I**

| Complex used | Addition of RBP* | Number of receptors* | Molar binding or uptake* | Number of binding or uptake per receptor |
|--------------|-----------------|---------------------|-------------------------|----------------------------------------|
| $[^3$H]retinol-RBP | + 2.5 12.69 5.1 |                      |                          |                                        |
| $[^3$H]retinol-1-RBP | + 2.5 15.27 6.1 |                      |                          |                                        |
| $[^3$H]retinol-1-RBP | - 2.5 13.97 5.6 |                      |                          |                                        |
| Retinol-$^{125}$I-labeled RBP | + 2.5 0.46 0.2 |                      |                          |                                        |
| Retinol-$^{125}$I-labeled RBP | - 2.5 2.81 1.1 |                      |                          |                                        |

* Displacement at the end of incubation period with 600-fold nonradioactive retinol-RBP as described under "Experimental Procedures."

* Calculated assuming 5.0 x $10^9$ molecules/pigment epithelial cell as reported in Ref. 3.

* Values taken after 10-min incubation at 22$^\circ$.
tors/pigment epithelial cell (3), we show (Table I) that only 1.1 mol of RBP were bound per mol of RBP receptor, while 5 to 6 mol of retinol were taken up by the cells during the same period per mol of RBP receptor. To eliminate the possibility that the lower absorption of \[\text{retinol}\] as compared to the uptake of \[\text{retinol}\] was due to damage caused by the iodination procedure, we have iodinated \[\text{retinol}\] RBP with nonradioactive NaI under identical conditions as those used for iodination with \[\text{NaI}\]. As seen in Fig. 4 and Table I, the \[\text{retinol}\] labeled RBP was as effective in the delivery of retinol to the cells as the noniodinated retinol RBP.

These experiments show that there is a dissociation between retinol uptake and RBP binding by the pigment epithelial cells. Additionally, while retinol could not be displaced from the pigment epithelial cells once it was taken up, the RBP moiety could be displaced rapidly by the addition of excess retinol RBP, providing further evidence that the retinol and the RBP are dissociated during the transport process and are then present in two distinct compartments.

Cell Specificity of Binding of Retinol-RBP and Uptake of Retinol—Fig. 5, A and B, demonstrated that there was neither binding of retinol RBP nor uptake of retinol by bovine leukocytes or red blood cells. By using retinoic acid RBP, again we found no binding and uptake by bovine cells (Fig. 5C). Similar results were found in the human white and red blood cells.

Uptake of Retinoic Acid from Bovine Serum Albumin Complex—Both retinol and retinoic acid bind in vitro to serum albumin forming a relatively stable complex. Goodman and his colleagues (8) have recently shown that unlike retinol, which is normally carried in plasma as a complex with RBP, retinoic acid seems to be complexed with serum albumin. To check the specificity of RBP in the transport of retinol and retinoic acid into pigment epithelial cells, we tested the ability of pigment epithelial cells to take up either compound when presented to the cells as a complex with bovine serum albumin. Neither retinol nor retinoic acid were taken up by pigment epithelial cells when presented to the cells as a complex with bovine serum albumin (Fig. 6). To show that there was no inhibition by bovine serum albumin of the transport mechanism, we have added either retinol RBP complex and the normal, prompt uptake of either retinol or retinoic acid was observed (Fig. 6). The conclusion drawn from these experiments was that RBP is an obligatory carrier protein for uptake by pigment epithelial cells and that retinoic acid (which is normally carried by serum albumin) cannot be utilized by the pigment epithelial cells.

Cell Filtration Chromatography of Cytosol after Retinol or Retinoic Acid Uptake—When the cytosol prepared from pigment epithelial cells that have been loaded with \[\text{retinol}\] (supplied as \[\text{retinol}\] RBP) was examined, it was found that radioactivity was present in this fraction. To establish the nature of the radioactive compound, the labeled cytosol was subjected to gel filtration chromatography. As seen in Fig. 7A, about a third of the radioactivity was bound to a protein which appeared in the column's void volume, while the rest of the label appeared as a low molecular weight (<1000) material. These results were similar to those observed in a previous study (9) in which free retinol was added to cytosol from isolated pigment epithelial cells.

When this experiment was repeated after \[\text{retinoic acid}\] uptake by the pigment epithelial cells, the chromatographic pattern of the cytosol was similar to that obtained after retinol uptake.

J. Heller, unpublished results.

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Fig. 5. Uptake of retinol by pigment epithelial cells and bovine white and red blood cells. White and red blood cells were prepared as described under "Experimental Procedures. "Uptake assay for \[\text{retinol}\] and \[\text{retinoic acid}\] binding of \[\text{RBP}\] was performed as described in Figs. 1 and 4. The number of cells in each incubation mixture was similar. A, binding of \[\text{retinol}\] labeled RBP to pigment epithelial cells and white or red blood cells. B, uptake of \[\text{retinol}\] by pigment epithelial cells and white or red blood cells. C, uptake of \[\text{retinol}\] labeled RBP from \[\text{retinol}\] RBP complex by pigment epithelial cells and white or red blood cells. All incubation was performed at 37°C. PE, pigment epithelial cells; WBC, white blood cells; RBC, red blood cells.

Fig. 6. Uptake of retinol and retinoic acid as either complexes with bovine serum albumin or RBP. \[\text{RBP}\] Retinol and \[\text{retinoic acid}\] were used to form a complex with bovine serum albumin as described under "Experimental Procedures" and were then incubated with pigment epithelial cells at 37°C as described in Figs. 1 and 7. After 10 min of incubation, a sample was taken, the pigment epithelial cells were recovered by centrifugation, and then suspended in fresh medium containing either \[\text{RBP}\] or \[\text{RBP}\] complexes, and the incubation was carried out for a further 10 min. A, experiment with \[\text{RBP}\] bovine serum albumin and \[\text{RBP}\] complexes. B, experiment with bovine serum albumin and \[\text{RBP}\] complexes. BSA, bovine serum albumin.
uptake by the cells (Fig. 7B). In neither case was there any label in the chromatographic position of \(^{3}H\)-labeled chromophore RBP.

These experiments indicate that the label from both \(^{3}H\)retinol and \(^{3}H\)retinoic acid uptake was found in the cytosol and, in both cases, about a third of the radioactivity was bound to a high molecular weight protein.

Identification of Cytosol-labeled Material After Retinol or Retinoic Acid Uptake—Thin layer chromatography of the extracted cytosol retinol-protein complex showed that about 95% of the recovered counts were found in a spot corresponding to retinol. No radioactivity was found on the chromatogram in spots corresponding to retinoid acid, retinal or retinyl esters. Total recovery of radioactivity from the plate was 75% of the counts in the cytosol. Similar results were obtained when the low molecular weight material found after gel filtration chromatography (Fig. 7A) was extracted and subjected to thin layer chromatography.

When the pigment epithelial cytosol obtained from cells that had taken up \(^{3}H\)retinoic acid (from the RBP complex) was examined in the same way, 98% of the radioactivity in the chromatophore-protein complex (Fig. 7B) was due to retinol-like material as judged by thin layer chromatography. The low molecular weight radioactive fraction from the gel filtration chromatography (Fig. 7B) was also found to be retinol-like. To exclude the possibility that this reduction of \(^{3}H\)retinoic acid happened during the extraction of the cytosol and the thin layer chromatography, equal amounts of labeled cytosol were mixed with \(^{3}H\)retinoic acid-RBP, extracted with organic solvents, and subjected to thin layer chromatography as above. Forty-four per cent of the counts were found in the retinol fraction and 54% in the retinoic acid fraction. It is thus clear that retinoic acid was not reduced to retinol during extraction and thin layer chromatography.

The conclusion drawn from this series of experiments was that retinoic acid uptake (from RBP complex) by pigment epithelial cell is followed by the reduction of the acid. This retinol-like material is not vitamin A itself since it is not incorporated into rhodopsin in vivo. In contrast, retinol (provided as retinol-RBP complex) is rapidly incorporated as the chromophore of rhodopsin following injection into the living frog.

**DISCUSSION**

The polyene lipid retinol is transported in plasma as a complex with a specific protein, the retinol-binding protein. We have previously shown that pigment epithelial cells in bovine (3) and rat (13) possess a specific cell surface receptor for RBP. This receptor is found only on one side of the cell, the side facing the systemic (choroidal) blood supply. In the present study we have utilized pigment epithelial cells isolated from bovine eyes to investigate the transport of retinol to pigment epithelial cells.

The experiments reported in this paper show that retinol is taken up by pigment epithelial cells only if presented as a retinol-RBP complex, while neither free retinol nor retinol bovine serum albumin complex were sequestered by the cells. The uptake of retinol (from retinol-RBP complex) was rapid, temperature-dependent, and did not show saturation at the level employed in this study. The reduced uptake of retinol after a few minutes of incubation was due to the breakdown of retinol-RBP complex and was not due to saturation of the cells by retinol (Fig. 2). Since the amount of retinol taken up by the cells was 5- to 8-fold larger than the number of cell surface RBP receptors (as determined by binding studies) (Table I), we conclude that there was a rapid exchange at the receptor site of retinol-RBP molecules with apo-RBP molecules (molecules that have already delivered their retinol complement to the cell). In this connection, it is interesting to note that previous binding studies have indicated that apo-RBP is less firmly bound to the RBP receptor site than are retinol-RBP molecules (3). Maraini and Gouzoli (14) have shown that RBP is essential for retinol "binding" by pigment epithelium. Rask and Peterson (15) have also shown that retinol is taken up in vitro by intestinal mucosal epithelial cells when supplied as the retinol-RBP complex.

Previous work by Dowling and Wald (6) has shown that while dietary retinoid acid can maintain vitamin A-deficient animals in good health, it did not supply the chromophoric group (retinol or retinal) for the visual pigments and the animals became blind. Goodman and his colleagues have shown that dietary retinoid acid is normally bound to serum albumin and not to RBP (8). The experiments reported in the present study show that when retinoid acid is used to form a complex with RBP, it is taken up by pigment epithelial cells as effectively as retinol (Fig. 6), at least during the incubation periods which were employed in this study. In a recent study, we have found that although retinoid acid is taken up by pigment epithelial cells following an in vivo injection of retinoid acid-RBP complex, the internalized retinoid acid is not incorporated into rhodopsin. In contrast, retinol (derived from retinol-RBP) is effectively incorporated as the chromophore (retinal) of rhodopsin.

It is interesting to note that neither white nor red blood cells show any uptake of retinol or retinoid acid from their respective RBP complexes. Whether this is due to the absence of RBP receptors on the surface of these cells or to the RBP receptor being at so low a concentration as to be detected is not known at the present time.
acid by pigment epithelial cells, the chromophores were found in the cytosol partly free and partly as a complex with a high molecular weight protein. In previous experiments in which retinol was added directly to cytosol from bovine pigment epithelial cells, it was also found that the chromophore was bound to a high molecular weight lipoglycoprotein (9). In other words, retinol which was internalized by intact pigment epithelial cells from retinol-RBP in the medium was bound to a similar high molecular weight protein as is free retinol added to isolated cytosol from pigment epithelial cells. Whether these two intracellular cytosol retinol-binding proteins are actually one and the same remains to be established.

We have previously shown that photoreceptor cells do not have a receptor for RBP (3, 4). Consequently the pigment epithelial cells would seem to be the only avenue for entry of retinol from the blood. The retinol (or retinal) which is found in the photoreceptor would thus come from retinol taken up by the pigment epithelial cells. The mode of retinol transport between the pigment epithelium and the photoreceptors remains to be studied.

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REFERENCES
1. Kanai, M., Raz, A., and Goodman, DeW. S. (1968) J. Clin. Invest. 47, 2025–2044
2. Glover, J. (1973) Vitam. Horm. 31, 1–42
3. Heller, J. (1975) J. Biol. Chem. 250, 3613–3619
4. Heller, J., and Bok, D. (1976) Am. J. Ophthalmol. 81, 93–97
5. Dowling, J. E., and Wald, G. (1958) Proc. Natl. Acad. Sci. U. S. A. 44, 648–661
6. Dowling, J. E., and Wald, G. (1960) Proc. Natl. Acad. Sci. U. S. A. 46, 587–608
7. DeLuca, H. F., and Zile, M. (1975) Acta Derm.-Venereol. Suppl. 74, 13–20
8. Smith, J. E., Milch, P. O., Muto, Y., and Goodman, D. S. (1973) Biochem. J. 132, 831–837
9. Heller, J. (1976) J. Biol. Chem. 251, 2962–2975
10. Heller, J., and Horwitz, J. (1976) J. Biol. Chem. 248, 6308–6316
11. Schwarzkopf, O., Cahnmann, H. J., Lewis, A. D., Swidins, J., and Wuest, H. M. (1949) Helv. Chim. Acta 32, 443–452
12. Horwitz, J., and Heller, J. (1973) J. Biol. Chem. 248, 6317–6324
13. Bok, D., and Heller, J. (1976) Exp. Eye Res. 22, 395–402
14. Maraini, G., and Gozzoli, F. (1975) Invest. Ophth. 14, 768–787
15. Rask, L., and Peterson, P. A. (1976) J. Biol. Chem. 251, 6360–6366
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