Interactions of Plasma Retinol-binding Protein with Its Receptor

SPECIFIC BINDING OF BOVINE AND HUMAN RETINOL-BINDING PROTEIN TO PIGMENT EPITHELIUM CELLS FROM BOVINE EYES*

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SUMMARY

Bovine and human retinol-retinol-binding protein (RBP) were iodinated to high specific activity. At least 34% of the iodinated material was native and retained its retinol chromophore as judged by its characteristic ability to bind to plasma prealbumin. Bovine and human retinol-125I-RBP were found to bind specifically to intact isolated pigment epithelium cells from bovine eyes. The specific binding was complete in about 1 min with an estimated second order rate constant of about $10^4$ M$^{-1}$ s$^{-1}$ (at 22°C). The specific binding was temperature dependent with the binding at 0°C being some 7-fold slower than at 22°C. The specific binding of both human and bovine retinol-125I-RBP was a linear function of the number of binding sites (number of cells) and was saturable with respect to retinol-125I-RBP. Bound iodinated retinol-RBP was rapidly displaced by the addition of unlabeled retinol-RBP, indicating that the specific binding process was a surface phenomenon and was not due to endocytosis. At saturation about 3.7 to 5.2 $\times 10^4$ molecules of either bovine or human retinol-125I-RBP was bound to one bovine pigment epithelium cell. The dissociation constant for the binding between retinol-125I-RBP and pigment epithelium receptor was estimated to be about $5 \times 10^{-12}$ M. Addition of human prealbumin (thyroxine-binding prealbumin) did not affect the binding of either human or bovine retinol-125I-RBP to pigment epithelium cells. Retinol-125I-RBP did not bind specifically to isolated bovine rod photoreceptor outer segments. Human apo-RBP was less effective in displacing bound retinol-125I-RBP than either native or reconstituted human retinol-RBP. These results suggest a mechanism whereby, after delivering its retinol to the cell, apo-RBP is displaced from the specific receptor on pigment epithelium cell by another retinol-RBP molecule. This postulated mechanism makes it possible to control the delivery of retinol to the target cell by the relative plasma concentrations of apo- and retinol-RBP and their relative affinities for the specific receptor binding site.

Retinol (vitamin A) is a polyene lipid which is involved in vision as the chromophoric group of visual pigments and possibly in the activation of sugars. Retinol is transported in the plasma from its storage site in the liver to the target organs as a specific complex with a unique protein (1). Retinol-binding protein has a molecular weight of 21,000, and it possesses a specific receptor for a single retinol molecule. Retinol is tightly bound to the native protein and can only be removed in vitro by extraction with organic solvents or by denaturing the protein (1-3). It was thus of interest to investigate the mode of release and delivery of retinol in vivo.

I began this investigation by asking whether retinol is delivered to the target cells as a retinol-RBP complex, and I tried to answer this question by showing that target cells have a specific receptor for retinol-RBP.

As target cells I have chosen the pigment epithelium from bovine retinas. The pigment epithelium is a single layer of cells lying next to the outer segments of the photoreceptor cells. The pigment epithelium and the neural retina share a common developmental origin from the optic vesicle. I was guided in my choice by the following considerations. The pigment epithelium (as well as the photoreceptor cells) has a high content of retinol (4, 5); bovine pigment epithelium cells can be obtained fresh in relatively large amounts; and intact individual pigment epithelium cells can be obtained by gentle methods without the use of proteolytic enzymes.

The present paper details some of the evidence for the existence of specific high affinity receptor sites for both bovine and human retinol-RBP on isolated intact bovine pigment epithelium cells. In another report we show by means of 125I autoradiographic techniques that the specific binding of retinol-RBP is indeed to pigment epithelium cells.

EXPERIMENTAL PROCEDURE

Retinol-binding Protein—Human retinol-binding protein was obtained from outdated plasma as previously described (3). The $A_{450}$/$A_{280}$ ratio of the pure material was 1.00 to 1.05 in different preparations. Bovine retinol-RBP was prepared from 4 liters of serum by essentially the same procedure as that used for the human material.

The abbreviations used are: KHP, retinol-binding protein, Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Aperetinol-binding Protein—Human apo-RBP was prepared from purified retinol-RBP as previously described (6, 8). The apo-RBP was free of retinol as determined by fluorescence and circular dichroism spectra. The apo-RBP was capable of full regeneration upon addition of freshly made all-trans-retinol (6, 7).

The apo-RBP was further purified by gel filtration chromatography on a column of Sephadex G-100 (1.5 × 89 cm) in 0.033 M sodium phosphate buffer, pH 7, containing 0.1 M NaCl in the presence of human prealbumin as described before (6). Since under these conditions only retinol-RBP binds to prealbumin, the last traces of the holoprotein were removed from the apo-RBP preparation.

Protein Concentrations—These were determined by absorption with the use of the known molar absorptivities of all-trans-retinol-RBP and apo-RBP (6). The various lower concentrations were made by proper dilution of known stock solutions. The absorptivity of bovine retinol-RBP was similar to that of the human material.

Pigment Epithelium—Bovine eyes were obtained fresh and were processed about 1 hour after slaughter. The eyes were kept in the dark during transfer to the laboratory. The anterior part of the eye was removed and the vitreous dislodged, after which the eye cup was incubated for 15 min at 22°C in Hanks'-balanced salt solution buffered with 10 mM of 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid, pH 7.4. Hanks' medium contained 142 mM NaCl, 5.36 mM KCl, 0.34 mM NaH2PO4, 0.44 mM KH2PO4, 0.81 mM MgSO4, 1.28 mM CaCl2, and 5.56 mM glucose, with a total osmolarity of about 305 mosM. The neural retina was then removed by careful dissection, the eye cup was gently rinsed with the same buffer and incubated for 15 min at 22°C. The cells were then collected by centrifugation up to and including the removal of the neural retina. The retina was then removed by careful dissection under dim light (Kodak Wratten no. 2 filter) to avoid bleaching the visual pigment, and consequent degradation of the photoreceptors. The pigment epithelium was then removed from the eye cup by gentle brushing with a soft brush and transferred to fresh Hanks'-Hepes medium. The intact pigment epithelium cells were collected by centrifugation for 5 min at 3000 × g at 4°C. The supernatant contained some of which had the inner segment still attached. A few nuclei were also seen but no pigment granules (melanosomes). About 1 × 10⁶ cells were used without further treatment. All processes including the later incubation with retinol-¹²⁵I-RBP were performed under dim red light.

Iodination of RBP—Iodination with NaI and chloramine-T was performed essentially as described by Hunter (8, 9). To 10 to 50 μg (0.48 to 3.28 × 10⁻¹⁴ mol) of either human or bovine retinol-RBP in 10 μl were added 20 μl of 0.05 M sodium phosphate buffer, pH 7.5, and 1 μCi (10 μl) of Na¹²⁵I (Amersham/Searle). To these were added 5 μl of freshly made 17.5 mM chloramine-T (in 0.05 M sodium phosphate buffer, pH 7.5). After 60 s at 22°C, 50 μl of freshly prepared Na₂S₂O₄ (in the above buffer) were added, and after a further 20 s, 250 μl of 0.05 M sodium phosphate buffer, pH 7.5, containing 0.1% ovalbumin (Sigma) were added. The whole iodination mixture was then applied to a column (1.5 × 6 cm) of Sephadex G-25 equilibrated with the sodium phosphate buffer containing 0.1% ovalbumin. The material which appeared following the void volume was collected. Over 90% of the radioactivity of the Na²¹²⁵I appeared in this material, and about 92 to 95% of the radioactivity of this peak was precipitated with trichloroacetic acid (final concentration, 7%). A previous experiment has shown that there was no binding between ovalbumin and retinol-RBP. Radioactivity was determined with a Nuclear-Chicago γ well counter.

Binding Assay—The iodinated retinol-RBP was adjusted to a final concentration of 100 ng per ml (4.75 × 10⁻¹⁷ M). The incubation mixture (final volume, generally 500 μl) of pigmented epithelium (generally 50 to 100 μl), retinol-¹²⁵I-RBP (generally 5 μg (2.4 × 10⁻¹⁰ mol), about 0.5 to 1.0 × 10⁶ cpm), and variable amounts of Hanks' balanced salt solution buffered with 10 mM Hepes, pH 7.4, and including 0.1% ovalbumin. To measure the nonspecific binding of retinol-¹²⁵I-RBP to the cells and to the filtration membrane, each experimental point included samples to which excess (generally 600 molar excess) unlabeled retinol-RBP was added. Each experimental point and the corresponding measurement for nonspecific binding were performed in triplicate. After incubation at 22°C for various times (generally 15 min) with rotary shaking (Eberbach rotary shaker, about 80 cycles per min), 2 ml of ice-cold Hanks'-Hepes medium was added to the incubation medium, and the whole mixture was filtered through a Millipore Celotate filter (EAWP) under vacuum. The Celotate filters were pre-soaked in Hanks' medium containing 0.1% ovalbumin. Each filter was washed twice with 2 ml of ice-cold Hanks' Heparin buffer. The filtration and washings took less than 5 s to complete. The filters were then placed in vials and counted.

In order to measure short incubation times, the above procedure was modified. The volume of the incubation mixture was increased, and after adding pigment epithelium cells, samples were taken and applied directly to the Celotate filter without prior dilution. This procedure enabled us to take reproducible samples at 15-s intervals.

The dissociation rate of iodinated RBP from pigment epithelial cells was measured in the following way. Pigment epithelium cells were incubated with bovine retinol-¹²⁵I-RBP for 15 min at 22°C. Other experimental conditions were identical with those described above. The cells were then collected by centrifugation at 5000 × g for 5 min, the clear supernatant was discarded, and the cells were dispersed in fresh Hanks'-Hepes medium containing 0.1% of ovalbumin, in a volume twice that of the original incubation medium. The temperature was 22°C. Samples were then taken at various time intervals and assayed for radioactivity after filtration as described above.

Specific Binding of ¹²⁵I-RBP—This was defined as that amount of radioactive RBP that was displaced (not bound) from the cells by the addition of excess nonradioactive RBP. The percentage of specific binding was calculated as the fraction of specific binding out of the total ¹²⁵I-RBP binding (including nonspecific binding).

RESULTS

Pigment Epithelium Preparation—The final preparation consisted of single pigment epithelium cells, which could be recognized by their characteristic hexagonal shape, small cell clumps, and a few single layer sheets of up to about 20 cells. A photomicrograph of a typical preparation is shown in Fig. 1. No free nuclei were observed, but there were many small pigment granules (melanosomes), erythrocytes, and an occasional photoreceptor outer segment. Because bovine pigment epithelium varies in its pigmentation according to its location in the eye, some cells were heavily pigmented while others were practically free of pigment granules (melanosomes). About 1 × 10⁶ cells were obtained from one eye. As judged by phase contrast microscopy, the cells retained their morphology intact for 24 hours when kept at 4°C in Hanks' balanced salt medium buffered with 10 mM Hepes, pH 7.4.

Properties of Iodinated Bovine Retinol-RBP—We have previously shown that only retinol RBP is bound to prealbumin. Removal of retinol, substitution of the chromophore with retinal and a number of chemical modifications of RBP lead to dissociation of the two proteins (6, 7, 10, 11). As can be seen from Fig. 2, when bovine retinol-¹³₁-RBP was subjected to gel filtration chromatography in the presence of excess prealbumin in a buffer of "physiological ionic strength," 34% of the recoverable counts were present in the first peak as a retinol-¹³₁-RBP-prealbumin complex. The efficacy of specific binding to pigment epithelium cells of this complex compared to that of the free retinol-¹³₁-RBP (second peak, Fig. 2) was found to be about...
Fm. 1. Bovine pigment epithelium preparation. Cells were prepared as detailed under "Experimental Procedure." Individual hexagonal cells and small cell clumps were commonly observed. Many small pigment granules (small arrow) and erythrocytes (large arrow) also were observed. Phase contrast photomicrograph, × 370.

Fig. 2. Gel filtration chromatography of bovine retinol-125I-RBP with excess prealbumin. Bovine retinol-125I-RBP, 3.3 × 10^{-12} mol (8 × 10^9 cpm), was mixed with human prealbumin, 10^{-8} mol. Solid NaCl was added to a final concentration of 0.1 M, and the material (total 0.5 ml) was applied to a Sephadex G-100 column (1.5 × 89 cm) equilibrated with 0.033 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl and 0.1% ovalbumin at 22°. The column was eluted with the same buffer at a flow rate of about 6 ml/hour. Fractions of 1.6 ml were collected. Eighty-six per cent of the total counts applied were recovered and 81 per cent of the recovered counts were found in the first peak (retinol-125I-RBP-prealbumin complex).

Fig. 3. Specific binding of bovine retinol-125I-RBP to pigment epithelium cells as a function of cell number. Incubation medium (final volume, 0.3 ml) contained bovine retinol-125I-RBP, 5 × 10^4 cpm (5 ng), and various amounts of pigment epithelium cells (7.5 × 10^6 cells/ml). Incubation was for 15 min at 22°. Nonspecific binding (measured in the presence of 600-fold molar excess of unlabeled bovine retinol-RBP) was subtracted for each point.

the same (Table I). The conclusion from this experiment (Table I) was that, for the purpose of binding studies which are reported in this paper, both fractions of iodinated RBP, namely that which did and that which did not bind to prealbumin, were equally effective in binding to pigment epithelium cells. To find out whether the free retinol-125I-RBP represents the fraction which is normally dissociated in the equilibrium retinol-

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RBP + prealbumin = retinol-125I-RBP-prealbumin complex, or represents a fraction that was unable to bind to prealbumin, the second peak (Ve = 90 ml) was pooled, concentrated, and rechromatographed under the same experimental conditions with a fresh sample of prealbumin. None of the retinol-125I-RBP did bind to prealbumin. The conclusion from this experiment was that although the "free" iodinated RBP fraction represented a modified form that was unable to bind to prealbumin, it was still capable of binding specifically to pigment epithelium cells in a manner that was indistinguishable from the iodinated RBP fraction that did bind to prealbumin (Table I).
In the various experiments reported in this paper, only about 5% of the total counts in the incubation mixture were bound to the cells. One possibility for this relatively low percentage was that a large amount of the material in the incubation mixture was denatured or somehow lost its capacity to bind to pigment epithelium. Because 34% of retinol-\(^{125}\)I-RBP was bound to prealbumin, at least this fraction retained its retinol content (only the holoprotein binds to prealbumin) and was "native" as judged by binding to prealbumin. Moreover, the free (dissociated) retinol-\(^{125}\)I-RBP was equally effective in binding to pigment epithelium cells (Table I). It seems more plausible that this represents the upper limit of binding sites available in our incubation system. If we assume that the number of binding sites per cell is about 5 \(\times 10^4\) (see above) and the incubation mixture contained an average of about 1.5 \(\times 10^4\) cells, this represents a total of 7.5 \(\times 10^5\) binding sites in the system. Each incubation mixture contained about 1.4 \(\times 10^{11}\) molecules of retinol-\(^{125}\)I-RBP or an excess of some 20-fold over the number of presumed binding sites. Because of the various experimental considerations (the specific activity of retinol-\(^{125}\)I-RBP and the difficulty of increasing the number of cells used in the incubation medium), it was not possible to change this ratio appreciably.

Specificity—Because the specific binding of retinol-\(^{125}\)I-RBP to pigment epithelium cells was defined as that amount of iodinated RBP which was displaced (not bound) in the presence of 600-fold molar excess of unlabeled RBP as compared to the binding in the absence of unlabeled RBP, I investigated the ability of several other proteins to displace iodinated RBP from the cells. A 600-fold molar excess of bovine hemoglobin, bovine serum albumin, horse liver alcohol dehydrogenase, horse myoglobin, and cytochrome c and human prealbumin did not affect the binding of iodinated RBP to the cells, i.e. the binding of retinol-\(^{125}\)I-RBP to pigment epithelium in the presence and absence of these proteins was identical. As shown below (Fig. 7), competitive binding by unlabeled RBP was about 80% effective at 1- to 2-fold molar excess as that at 600-fold molar excess.

Binding of Bovine Retinol-\(^{125}\)I-RBP to Bovine Rod Outer Segments—No specific binding between iodinated RBP and isolated purified rod photoreceptor outer segments was observed. When increasing amounts of outer segments (from 5 \(\times 10^6\) to 3.3 \(\times 10^7\) per incubation mixture) were added to a standard assay (similar to the one described in Fig. 3) in the absence and presence of 600-fold molar excess of unlabeled bovine RBP, only nonspecific binding which was identical for all the incubation mixtures was observed. The conclusion from this experiment was that the specific binding of retinol-\(^{125}\)I-RBP was not a general property of all tissues but a specific property shown in this case only by pigment epithelium cells.

Binding of Human Retinol-\(^{125}\)I-RBP to Bovine Pigment Epithelium—Because bovine and human RBP are very similar (amino acid composition, molecular weight, absorption and circular dichroism, spectra, binding to human prealbumin), it was of interest to investigated the binding of human retinol-\(^{125}\)I-RBP to bovine pigment epithelium. As seen from Fig. 4, human retinol-RBP did bind specifically to bovine pigment epithelium with about the same effectiveness as that of bovine retinol-RBP. After 15 min at 22°, about 1.8 \(\times 10^{-14}\) mol of human retinol were bound specifically to about 2 \(\times 10^4\) cells or about 5.4 \(\times 10^4\) molecules of retinol-RBP bound per cell. This surprisingly close agreement with the 5.2 \(\times 10^4\) molecules of the bovine retinol-RBP that were bound under similar conditions is probably fortuitous, yet it indicates that the binding of the bovine and human RBP to bovine pigment epithelium is about equally effective.

Rate of Binding—As seen from Fig. 5 the specific binding of bovine retinol-\(^{125}\)I-RBP was rapid and essentially complete after 1 min, with 85% of the maximal binding after about 30 s (at 22°). The nonspecific binding was only increasing very slowly, if at all, with time. After about 1 min the specific binding was constant and did not change essentially for the next 15 min (at 22°).

Because of the rapid binding rate it was difficult to determine accurately the exact kinetic constant, but it was possible to calculate some approximate figures. Assuming that the reaction between retinol-\(^{125}\)I-RBP and the receptor sites is bimolecular and obeys a second order rate equation, that it is complete by 1 min, and that each cell has about 5 \(\times 10^4\) binding sites, the concentration of receptor sites in the assay was about 5.6 \(\times 10^{-11}\) M. The concentration of retinol-\(^{125}\)I-RBP was 7.9 \(\times 10^{-10}\) M, out of which about 3.3 \(\times 10^{-11}\) M were specifically bound to the receptor in 60 s. With the use of these figures the second order binding rate constant was about 10^8 M⁻¹ s⁻¹ at 22°.

Dissociation Rate—The rate of dissociation of retinol-\(^{125}\)I-RBP bound to pigment epithelium cells followed first order kinetics with a half-life time of 24 min and a rate constant of 5 \(\times 10^{-12}\) s⁻¹.

The estimated dissociation constant of the retinol-\(^{125}\)I-RBP—pigment epithelium receptor complex, obtained by using the kinetic data for binding and dissociation obtained above, was about 5 \(\times 10^{-12}\) M. It is important to note that this dissociation constant is only an approximation because of the difficulty in determining accurately the kinetics of the rapid binding between iodinated RBP and the pigment epithelium cells. It is quite possible that the true binding rate is faster than the one estimated here indicating that the dissociation constant of the complex is even smaller than 5 \(\times 10^{-12}\) M.

Effect of Temperature on Binding Rate—While the nonspecific binding was temperature independent, the specific binding of retinol-\(^{125}\)I-RBP was markedly dependent on the incubation temperature. In the various experiments reported in this paper, only about 5% of the total counts in the incubation mixture were bound to the cells.
temperature (Fig. 5). Optimal specific binding was at 22°, while at 0° it was some 7-fold smaller (after 1 min).

Rate of Competitive Displacement—When excess unlabeled retinol-RBP was added to pigment epithelium cells that were previously incubated for 5 min (at 22°) with iodinated retinol-RBP, a rapid displacement of the labeled material and consequent decrease in specific retinol-125I-RBP binding took place (Fig. 6). Fifty per cent of the specifically bound retinol-125I-RBP was displaced after 45 s and 78% after 60 s. The displacement of the labeled retinol-RBP by 600-fold molar excess unlabeled retinol-RBP was complete after 3 min.

Competitive Displacement by Increasing Amounts of Unlabeled Retinol-RBP—Labeled retinol-RBP was progressively displaced from its receptor on pigment epithelium cells by the addition of increasing amounts of unlabeled retinol-RBP (Fig. 7). The unlabeled bovine and human retinol-RBP were about as effective in displacing the labeled bovine retinol-RBP from bovine pigment epithelium (Fig. 7).

The above experiments are important in showing that the binding of retinol-125I-RBP to pigment epithelium cells does not indicate endocytosis. If the binding process represented endocytosis it would not be possible to displace the labeled material from the cell by small amounts of unlabeled retinol-RBP.

Saturation of Specific Binding Sites—The process of specific binding of human retinol-125I-RBP to pigment epithelium cells was saturable while the nonspecific binding increased linearly with increasing amount of label (Fig. 8). In this particular experiment 6.7 \times 10^{-14} mol of human retinol-125I-RBP were bound to 1.1 \times 10^5 cells at the highest concentration employed. Under
these conditions, then, about 3.7 × 10^4 molecules of retinol-RBP were bound specifically to each pigment epithelium cell. This figure is in reasonably good agreement with the number of 5.2 × 10^4 molecules/cells obtained under different experimental conditions (Figs. 3 and 4).

**Competitive Displacement with Apo-RBP**—When human apo-RBP was tested for its ability to displace bovine retinol-125I-RBP from pigment epithelium, it was found to be less effective than native unlabeled human retinol-RBP (Fig. 9). That this was not due to the fact that apo-RBP was denatured was shown by taking a sample of the apo-RBP preparation and reconstituting it with fresh all-trans-retinol. The reconstituted retinol-RBP was as effective as native retinol-RBP in displacing the labeled material from pigmented epithelial cells (Fig. 9). It is important to realize that the nature of the experiment was such that apo-RBP was not less effective because it had any inhibitory or negative effect on the assay system. The normal specific binding of bovine retinol-125I-RBP still took place in its usual fashion. What was affected was the ability to competitively displace the labeled material from the binding site.

**Effect of Prealbumin**—In the plasma, retinol-RBP is tightly bound to prealbumin, a tetrameric protein, molecular weight of 54,000, which is also capable of binding 1 molecule of thyroxine. As shown in Table 1, prealbumin was not necessary for binding of retinol-RBP to pigment epithelium and did not seem to interfere with the specific binding of retinol-RBP.

**DISCUSSION**

Specific cellular receptors with high binding affinities for various polypeptide hormones, steroids, neurotransmitters, and other substances have recently been demonstrated and intensively investigated (12). The lipid chromophore retinol (vitamin A) is transported in plasma as a specific complex with a small protein (1). Thus, it is reasonable to inquire if the target cells possess a receptor for RBP. As the experiments reported in this paper show pigment epithelial cells from bovine eyes have a receptor for retinol-RBP that shows the expected properties of this specific binding site. It possesses a high binding affinity for retinol-RBP, it is a saturable process with respect to retinol-RBP, and it is rapid and temperature dependent. It is a cell surface receptor, as indicated by the observation that unlabeled retinol-RBP rapidly displaces receptor-bound retinol-125I-RBP, even after considerable incubation time. The binding rate in this study was so rapid that, due to experimental limitations, the calculated binding rate constant of 10^8 M⁻¹ s⁻¹ is probably only a fair estimate.

Consequently the calculated dissociation constant of 5 × 10⁻¹⁵ M for the complex was also only an approximation yet it gave an idea about the order of magnitude of this parameter.

Although some of the iodinated retinol-RBP was apparently modified as detected by its inability to bind prealbumin (Fig. 2), this modified fraction was still able to bind specifically to pigment epithelial cells as effectively as the fraction of 125I-RBP that did bind to prealbumin. It is possible that the topographical areas on the RBP molecule which are important in the binding to prealbumin and to pigment epithelium cells are different. We have evidence that it is possible to modify various amino acid groups in RBP in such a way as to prevent its binding to prealbumin without affecting its binding to retinol (10, 11).

The pigment epithelium preparations proved to be a fortunate choice for these binding studies. Isolated intact cells were obtained with minimal tissue injury and without the use of proteolytic enzymes or harsh mechanical means. The binding qualities of the cells were stable for at least several hours and fairly reproducible from day to day. Although the pigment epithelium preparations also contained red blood cells and free pigment granules, we have shown by means of autoradiographic tech-
niques (using $^{125}$I) that the specific binding of retinol-$^{125}$I-RBP was to pigment epithelium cells and that this binding was displaced by excess unlabeled retinol-RBP.*

Perhaps the most intriguing observation to come out of the present study was that apo-RBP was less effective than retinol-RBP in displacing the iodinated RBP from pigment epithelium cells. Because of the importance of this experiment it was repeated several times, and it always gave the same results. Reconstituted retinol-RBP made from the same apo-RBP preparation was indistinguishable from native retinol-RBP in its capacity to displace retinol-$^{125}$I-RBP from the receptor. We observed before that the removal of retinol from RBP leads to a conformational change such that apo-RBP does not bind to plasma prealbumin under the experimental conditions at which retinol-RBP does bind (6). Reconstitution of apo-RBP with retinol generated a retinol-RBP complex that had all the properties of native retinol-RBP and did bind normally to prealbumin (6). It is possible to hypothesize that the pigment epithelium receptor site also distinguishes between the different conformations of apo- and retinol-RBP. This observed difference in binding affinity to the receptor suggests that after the retinol chromophore is delivered to the target cell, apo-RBP at the receptor site is displaced by another retinol-RBP molecule. Because apo-RBP still has a certain affinity for the binding site the process of replacing apo-RBP at the receptor site by retinol-RBP is not an "all-or-none" process but, rather, is controlled by the relative amounts of apo- and retinol-RBP in the plasma. We would then suggest that under normal physiological conditions all the receptor sites are occupied, some by apo-RBP and some by retinol-RBP, and that both are in equilibrium with the nonbound carrier protein in the plasma. Both free apo-RBP and retinol-RBP compete then for the same binding site, although with different binding constants. Thus the final equilibrium is dependent on the interplay between the relative amounts of apo-RBP and retinol-RBP and is probably useful in achieving a precise control over the delivery of retinol to the tissue.

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