angle glaucoma. They observed cell-mediated immunity, as indicated by leukocyte migration inhibition in only three of 10 patients with open-angle glaucoma and no other ocular diseases.

Evidence which may link open-angle glaucoma to immunologic abnormalities include the observation that such patients have a higher incidence of positive antinuclear antibody reaction (44 per cent), compared with nonglaucomatous controls (7.5 per cent) and gg topical steroid responders (7 per cent). Also, certain HL-A antigens appear in a higher percentage of open-angle glaucoma patients than in the normal population. These studies differ, however, as to which of the HL-A antigens are abnormally increased. Cooperative studies are currently underway to more clearly define this question and to establish its significance in open-angle glaucoma.

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Key words: immunofluorescence, immunoglobulins, antibodies, complement, trabecular meshwork, open-angle glaucoma, choroidal melanoma.

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Retinol- and retinoic acid-binding proteins: occurrence in human retina and absence from human cultured fibroblasts. DAVID SWANSON, SIDNEY FUTTERMAN, AND JOHN C. SAARI.

Low-molecular-weight retinol- and retinoic acid-binding proteins were shown to be present in the
soluble fraction of human retinal tissue but absent from human fibroblasts grown in tissue culture. By the use of gel filtration and comparison with bovine retinal tissue, the human intracellular binding proteins were found to have molecular weights of approximately 17,000 daltons, which are comparable to the molecular weights of bovine intracellular binding proteins. The quantity of retinoic acid bound exceeded that of retinol by about eightfold.

The presence and characterization of low-molecular-weight retinol- and retinoic acid-binding proteins in the supernatant of bovine retina and a variety of other human and animal tissues has been previously reported.\textsuperscript{1-6} We undertook the present study to determine whether human retinal tissue contained these intracellular binding proteins. In addition, because one would like to have available a noninvasive means to obtain human intracellular binding proteins for vitamin A derivatives, human fibroblasts from a skin biopsy and grown in tissue culture were analyzed for the presence of these proteins. Presence of the binding proteins in cultured fibroblasts would permit the use of a skin biopsy as an easily obtained tissue source of these proteins for studies of their possible role in retinal disease.

\textbf{Materials and methods.}

\textit{Human retinal tissue supernatant.} Retinas were grossly dissected from underlying pigment epithelium from 11 human eyes within 48 hours postmortem and stored frozen. Soluble protein was prepared by thawing, sonicating briefly to disperse the tissue, centrifuging at 70,000 \( \times \) g for 1 hour, and drawing off the supernatant. The protein concentration, roughly estimated by \( A_{280} \) measurement using bovine albumin as standard, was adjusted to 12 mg. per milliliter by diluting the supernatant fivefold with buffer solution (0.05M Tris\(\cdot\)Cl, pH 7.5 and containing 0.2M NaCl).

\textit{Human fibroblast supernatant.} Fibroblasts obtained by skin biopsy from a normal human volunteer were grown in Eagle's Minimal Essential Medium with 10 per cent fetal calf serum.\textsuperscript{7} Cells were harvested between the seventh and thirteenth passages by treatment with trypsin, rinsed three times with normal saline, and stored at -67° C. Approximately 0.5 ml. of packed cells suspended in normal saline were thawed, sonicated, and centrifuged at 70,000 \( \times \) g for 1 hour. The fibroblast supernatant was concentrated with an Amicon UM-10 membrane to 2.0 ml and a protein concentration of 6.6 mg./ml.

\textit{Bovine retinal supernatant.} The method of preparation of supernatant from bovine retinal tissue has been described.\textsuperscript{4} Protein concentration was adjusted by diluting with buffer to 12 mg./ml. to equal that of the human retinal supernatant preparation.

\textit{Incubation and analysis.} One-milliliter samples of supernatant were incubated for 20 minutes

![Fig. 1. Gel filtration of the soluble protein from human retina (---) bovine retina (-----) and human fibroblasts (---) following incubation with \(^3\)H-retinol. The low-molecular-weight binding protein for retinol appears in fractions 25 to 30. Mobilities of the human and bovine retinol-binding proteins are comparable with calculated values of the diffusion coefficient, \( K_2^1 \approx 0.50 \) and 0.49, respectively.](https://iovs.arvojournals.org/doi/abs/10.1167/iovs.93-3065)
Fig. 2. Gel filtration of the soluble protein from human retina (—) bovine retina (——) and human fibroblasts (—) following incubation with \(^3\)H-retinoic acid. The low-molecular-weight binding protein for retinoic acid appears in fractions 26 to 33. High-molecular-weight protein capable (like albumin) of binding ligand and aggregated ligand appear in the vicinity of the void volume in fractions 15 to 19.

with either 2 \(\mu\)Ci of \(^3\)H-retinol, specific activity 2.66 Ci per millimole (New England Nuclear, Boston, Mass.); or 1.1 \(\mu\)Ci of \(^3\)H-retinoic acid, specific activity 1.45 Ci per millimole, generously provided by Hoffmann-La Roche. After the addition of 100 mg. of sucrose to increase the density, samples were analyzed by gel filtration with the above buffer through Sephadex G-75 columns of 1.5 by 143 cm. Following gel filtration 0.2 ml. samples of each fraction (5.2 ml.) were counted in 10 ml. of scintillation fluid.

Results and discussion. Analysis of supernatant from human retinal tissue incubated with either \(^3\)H-retinol or \(^3\)H-retinoic acid revealed a peak of radioactivity corresponding to a protein of low molecular weight (Figs. 1 and 2). When compared to similarly analyzed samples from bovine retina, the binding proteins were calculated (see Fig. 1 legend) to migrate at the same rate, indicating the molecular weights of the human intracellular binding proteins to be approximately equal to those of the bovine, previously found to be about 17,000 daltons.4 This molecular weight is lower than the molecular weight of 21,000 daltons obtained for human and bovine serum retinol-binding protein.9,10 The intracellular retinol-binding protein is also distinguished from the serum retinol-binding protein by the observed failure of serum retinol-binding protein to exchange unlabeled retinol for \(^3\)H-retinol during incubation. On the basis of the specific activity of the \(^3\)H-retinol and \(^3\)H-retinoic acid employed and radioactivity recovered in the low-molecular-weight binding proteins, it was estimated that human retinas contain about eight times more binding protein for retinoic acid than for retinol.

When the soluble protein of fibroblasts was analyzed following incubation, no peak of radioactivity was present in the low-molecular-weight protein region (Figs. 1 and 2). We conclude that the binding proteins are absent from human fibroblasts. This finding may represent the biochemical correlate of the observation that vitamin A is not an essential component of fibroblast tissue culture media.8

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The hypothesis that an abnormality in the absorption, transport, or peripheral utilization of retinol might be relevant to the pathogenesis of retinitis pigmentosa (RP) has until now received only indirect support by the observation of a typical RP in a rare disease associated with a defect of vitamin A absorption (abetalipoproteinemia). Vitamin A therapy is ineffective in these patients, who have normal retinol plasma levels.

The discovery of the specific carrier protein for retinol in plasma has provided the opportunity to investigate at a molecular level the metabolism of retinol in RP. The serum level of retinol-binding protein (RBP) was determined by Maraini, Fadda, and Gozzoli in a group of patients with RP carefully classified according to their specific genetic type, and no significant difference from a group of normal controls was found. Working on highly purified samples of RBP isolated from patients with the recessive form of RP, Maraini and Gozzoli failed to demonstrate any appreciable qualitative difference between this protein and normal RBP, no evidence was obtained that RP RBP differs from normal RBP in its capacity to interact with plasma prealbumin or in the ability to act as a carrier of vitamin A alcohol. However these findings, although supporting the view that retinol transport in plasma is normal in RP, do not exclude the possibility of an abnormality in the mechanism of release of retinol from the carrier protein to the retinal pigment epithelium (PE). Very little is known about the intimate mechanism by which vitamin A alcohol is transferred from RBP to the target cells and only recently Heller and Maraini and Gozzoli have presented experimental evidence which suggests that the interaction of plasma RBP with a specific receptor in the cell membrane is involved in the process of release of retinol to PE. By means of autoradiography Heller and Bok have shown that receptors for RBP are present only on the basal plasma membrane of pigment epithelial cells.

The observation that human RBP binds to both human and bovine isolated PE seems to indicate that in spite of immunological difference between human and bovine RBP, the functionally active sites of the molecule involved in the interaction with the membrane receptor are very similar in the two proteins. Since the ability of iodinated RBP to bind to PE has been reported to be independent from its capacity to bind prealbumin, it is possible that the topographical areas on the RBP molecule which are important in the binding to prealbumin and to PE are different.