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 photoreceptor outer segment degeneration induced in young rats by a vitamin A–deficient diet and by the occurrence 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 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 Bok 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.


The interaction between retinol-binding protein and normal bovine pigment epithelium has been studied with the use of iodinated retinol-binding protein isolated from the plasma of patients with the recessive form of retinitis pigmentosa and of normal subjects. It is concluded that the capacity of the plasma carrier protein to interact with the retinol-binding protein receptor of bovine pigment epithelium is unimpaired in retinitis pigmentosa with autosomal recessive inheritance.

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We therefore considered it interesting to investigate whether RBP isolated from RP patients demonstrates normal binding capacity to isolated normal PE cells. In the present paper we give some experimental evidence indicating that not only the transport function of RBP but also its capacity to interact with normal bovine PE are unimpaired in RP.

RBP was isolated from the plasma of patients with the recessive form of RP and of normal subjects by the procedure described in detail by Peterson and Berggard. Normal and RP RBP were then further purified on an affinity chromatography column of prealbumin coupled Sepharose 4B, according to the method of Vahlquist, Nilsson, and Peterson.

Iodination of normal and RP RBP was performed as described by Heller. RBP 50 μg in 30 μl of sodium phosphate buffer (0.05 M; pH 7.5) were added to 1 mCi (10 μl) of Na 125I (Sorin, Saluggia, Italy); to these were added 5 μl of chloramine-T (17.5 mM in phosphate buffer) and, after 60 sec. at 22°C, 50 μl of Na2S2O8 (26 mM in phosphate buffer). After 20 sec. 250 μl of phosphate buffer containing 0.1 per cent ovalbumin were added and the sample was applied to a column (1.5 by 15 cm.) of Sephadex G-25 equilibrated with the phosphate buffer containing 0.1 per cent ovalbumin. The column was eluted with the same buffer, fractions of 0.5 ml. were collected, and the radioactivity was measured with a Packard γ-well counter. Iodinated RBP was eluted following the void volume and its radioactivity peak was clearly separated from that of unbound 125I.

PE was obtained from freshly enucleated dark-adapted bovine eyes following the procedure described in detail in a previous paper. After isolation the cells were washed two more times with ice cold Krebs-Ringer-bicarbonate buffer and re-suspended in a final volume of 4.5 ml. of the same buffer containing 0.1 per cent ovalbumin. Examination of PE preparations by light and electron microscopy demonstrated that contamination by rod outer segments or choroidal remnants was negligible. A 0.5 ml. amount of the cell suspension was used for dry weight determination by drying to constant weight in an oven. The remaining 4 ml. of PE cells suspension were utilized for the binding assay. The incubation mixture included PE suspension (2 ml.) and normal or RP 125I-RBP (100 μl) (9 × 10^11 M; about 3 × 10^6 c.p.m.). The incubation was carried out in water bath at 22°C, under slow constant stirring. At fixed time intervals 0.3 ml. aliquots of the incubation mixture were withdrawn and filtered under vacuum through a Whatman glass-fiber paper CF/C filter. The filters had been pre-soaked in Krebs-Ringer-bicarbonate buffer containing 0.1 per cent ovalbumin and after filtration of the PE suspension they were washed with 2 ml. of ice cold buffer. The filters were then placed in vials and counted.

The specificity of the binding of 125I-RBP to bovine PE was assessed by determining the amount of iodinated RBP which was displaced from PE cells in the presence of a 4-fold molar excess of unlabeled native RBP. Heller has shown that competitive binding by unlabeled RBP is about 80 per cent as effective at 1-
2-fold molar excess as that of 600-fold molar excess. The percentage of specific binding was calculated from the difference between the counts in the absence and in the presence of unlabeled RBP. This difference represented 30 per cent of the total counts bound to PE in the absence of unlabeled RBP, for a PE concentration of 8 mg dry weight per milliliter. Results from two typical experiments are reproduced in Fig. 1. From analysis of the figure, it is evident that RBP isolated from the plasma of patients with the recessive form of RP does not demonstrate any appreciable difference from normal human RBP in its capacity to interact with the RBP receptor present on the cell membrane of normal bovine PE.

Present results seem to definitely demonstrate that no impairment of RBP function exists in the most common form of RP. They do not of course rule out the possibility that the RBP receptor on the plasma membrane of PE may be abnormal in this disease.

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REFERENCES


Niemann-Pick disease-like inclusions caused by a hypocholesteremic agent.

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AY9944, an inhibitor of cholesterol biosynthesis, was injected into albino rats and the ocular tissue was studied by light and electron microscopy. Abundant lamellar inclusion bodies accumulated in various cells of the eye, especially in the ganglion cells of the retina and glial cells of the optic nerve. Prolonged administration of this drug resulted in degeneration of retinal ganglion cells and oligodendroglial cells of the optic nerve. Micro-organelles of the inclusion body–laden cells were otherwise normal in their appearance. The electron microscopic appearance of these inclusion bodies and their distribution in the ocular tissues closely resembled those of Niemann-Pick disease.

Trans-1,4-bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY9944) is one of the most effective hypocholesteremic agents. Suzuki and Zagoren3 have extensively reported electron microscopic and biochemical observations on the central and peripheral nervous systems of developing rats following the injection of this drug. Also, several other authors have described membranous inclusion bodies which abundantly accumulate in the affected cells.4 5

The present experiments have revealed that administration of AY9944 to albino rats causes considerable changes in various cells of the eye and that the accumulation of the inclusion bodies strikingly resembles that in Niemann-Pick disease. The purpose of this communication is to emphasize a possible correlation between the effects of this drug and the pathogenesis of certain sphingo-lipidos.

Materials and methods. Sprague-Dawley strain albino rats were used in this study. AY9944 was dissolved in physiologic saline solution (3 mg per milliliter) and sterilized by ultrafiltration. A daily dose of 50 mg per kilogram body weight (about 0.35 mg for a 2-day-old rat) was injected into the peritoneal cavity. Animals were injected commencing on the second postnatal day (Group