We have investigated alterations of the metabolic state in vitamin A-deficient (A−) corneas using phosphorus-31 (31P) nuclear magnetic resonance (NMR) spectroscopy. Comparing to the control, A− corneas showed a prominent rise of phosphocreatine (PCr) as well as a total loss of glycerophosphorylcholine (GPC). Further, ATP levels were lower, and sugar phosphates (SP) and inorganic phosphate (Pi) were higher than those of the control. The accumulation of PCr and Pi, and decrease of ATP indicate that the activity of creatine kinase may be altered in vitamin A deficiency. These results suggest that vitamin A may have a role in creatine kinase activation and/or induction and that its deficiency causes a decline of energy metabolism in corneal epithelium. Moreover, disappearance of GPC implies an impaired cellular membrane metabolism. When retinyl acetate was supplied to A− rats for 5 weeks, the 31P profiles of vitamin A-repleted rat corneas recovered to normal. Invest Ophthalmol Vis Sci 30:769–772, 1989

Materials and Methods. All procedures using animals conformed to the ARVO Resolution on the Use of Animals in Research. Male Sprague-Dawley rats, 19 days old, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Seven rats were placed on A− diet for 17 weeks and seven pair-fed control rats were fed the same diet with vitamin A supplement (700 μg/week). The animals were kept until the A− rats had a 20–25% weight loss compared to pair-fed controls and showed ocular signs of vitamin A deficiency, for example, corneal epithelial keratinization, stromal edema, eyelid dermatitis. Another group of eight rats were fed A− diet for 14 weeks until weight loss and ocular signs due to vitamin A deficiency were evident. Thereafter, 700 μg retinyl acetate was administered orally once a week and the rats maintained on the same diet for 5 more weeks. These rats were paired with eight rats fed the A− diet for 19 weeks. Vitamin A-repleted rats recovered to virtually the same weight as pair-fed controls of the same age, and the ocular signs of vitamin A deficiency disappeared. All rats were anesthetized by intramuscular injection of ketamine HCl. Immediately after sedation, eyes were enucleated and rapidly frozen in liquid nitrogen. Then, the rats were sacrificed by overdose of intramuscular injection of ketamin HCl. Frozen corneas and lenses were excised with a Bard-Parker No. 15 blade. Seven or eight pairs of corneas and lenses were used in each sample and all experiments were repeated twice.

Frozen tissues were mixed with equal weights of 10% perchloric acid (PCA) and ground to a fine powder in a liquid nitrogen bath. The mixture was allowed to thaw to 0–4°C and immediately centrifuged at 43,500 g for 15 min at 4°C. The supernatant was then neutralized with concentrated KOH and the pH adjusted to 10. The sample was centrifuged again to remove precipitates. The supernatant was then lyophilized.

Lyophilizates of PCA extracts from the cornea and the lens were reconstituted with 0.5 ml of an NMR solution of 20 mmol/L EDTA and 20% D2O and the pH adjusted to 9.0. The final concentration (tissue weight/ml) was equivalent for each sample. 31P NMR spectroscopy was conducted at 109.3 MHz using a Bruker HX270 spectrometer in the Fourier-transform mode. The NMR parameters included a 45° pulse angle, 4-K data points, 5000 Hz spectral width.
0.5 sec interpulse delay, and proton decoupling. Samples were run at room temperature; 160,000 accumulations were averaged per spectrum. An exponential filter resulting in 2 Hz line-broadening was used. The resonances were identified according to previously established methods.11

Results. Figure 1 shows the $^{31}$P spectra of corneas from pair-fed control and A- rats. The control spectrum shows resonances from α-glycerophosphate (α-GP), sugar phosphates (SP), phosphorylcholine (PCh), glycerophosphorylcholine (GPC), inorganic phosphate (Pi), ADP, ATP and dinucleotides (DN). The A- corneal spectrum, on the other hand, showed a prominent signal from phosphocreatine (PCr), which was undetected in the control. In addition, GPC was lost and ATP levels decreased 53%. Moreover, SP, which overlapped with the signals from AMP, and Pi increased 79% and 102%, respectively.

Figure 2 shows the $^{31}$P profiles of corneas from A- and vitamin A-repleted rats. After 5 weeks of vitamin A supplement, the ocular signs of vitamin A deficiency completely disappeared. PCr reduced to undetectable levels in the corneas of vitamin A-repleted rats. In addition, GPC reappeared and ATP, SP and Pi levels also recovered. The $^{31}$P spectrum of corneas of vitamin A-repleted rats (Fig. 2) was identical to that of the control (Fig. 1).

The $^{31}$P spectra of lenses of pair-fed controls, A-, and vitamin A-repleted rats are presented in Figure 3. All spectra show resonance signals from SP, PCh, phosphorylethanolamine (GPE), GPC, ATP and Pi. ATP and GPC levels of A- lens are 40% and 27%, respectively.

Fig. 1. Phosphorus-31 nuclear magnetic resonance spectra of pair-fed control and vitamin A-deficient rat corneas. Pair-fed control rat cornea: The spectrum shows resonances from α-glycerophosphate (α-GP), sugar phosphates (SP), phosphorylcholine (PCh), glycerophosphorylcholine (GPC), inorganic phosphate (Pi), ADP, ATP and dinucleotides (DN). *Transmitter noise. Vitamin A-deficient rat cornea: The spectrum of A- cornea shows a prominent rise of PCr and loss of GPC.

Fig. 2. Phosphorus-31 nuclear magnetic resonance spectra of vitamin A-deficient and vitamin A-repleted rat corneas. Vitamin A-repleted rat cornea: The spectrum from corneas of vitamin A-repleted rats appeared normal (cf. Fig. 1) Vitamin A-deficient rat cornea: This spectrum is identical to that in Figure 1.
respectively, lower than those of the control. No other changes were found. The $^{31}$P spectrum of lenses of vitamin A-repleted rat was virtually the same as that of the control, indicating a total recovery.

**Discussion.** The current study clearly shows the different metabolic states of A− corneas and the pair-fed control. Since most $^{31}$P signals (>90%) originate from the epithelium, our results reflect the metabolic state in the corneal epithelium. The most striking change in the A− corneas is the appearance of PCr (Figs. 1, 2). This is accompanied by decreased ATP and increased Pi and SP (including the signals of AMP) levels. It is known that PCr serves as an energy reservoir. In the muscle, the high-energy phosphate is transferred from PCr to ADP during exercise (Fig. 4), which permits the muscle to maintain a constant level of ATP. The role of PCr in the cornea is unknown, although it may be analogous to that in the muscle. The change in PCr and ATP levels in the A− corneas is, however, entirely different from the pattern seen in the muscle. A rise in PCr may be related to an altered creatine kinase activity in ADP phosphorylation, or an overall change of the reaction equilibrium. This resulted in a decrease of ATP and increase of ADP, AMP and Pi, as indicated by our data. In addition, GPC, which is a membrane metabolite present in the cytosol, disappeared in A− cornea. It has been demonstrated that vitamin A regulates the stability of the erythrocyte membrane, and its deficiency can result in changes in cell shape. Unfortunately nothing is known regarding cellular membrane metabolism in the cornea.

Both energy and membrane metabolism appeared to be disturbed in the lens; however, the changes were not as dramatic as those in the cornea. The difference may be due to the predominantly anaerobic metabolism in the lens, in which the role of PCr is minimal. Vitamin A has been proposed to be a coenzyme for several enzymes. It is conceivable that vitamin A may also be a coenzyme for creatine kinase, although this is not supported by previous enzymatic studies. Alternatively, it may participate in the induction of creatine kinase. Other indirect factors such as malnutrition may also be contributory. However, after treatment with vitamin A, the $^{31}$P profiles of both the corneas and the lens returned completely to normal (Figs. 2, 3). It is clear therefore that the metabolic changes observed with $^{31}$P NMR are due entirely to vitamin A deficiency.

**Key words:** vitamin A deficiency, corneal epithelium, lens, phosphocreatine, phosphorus metabolism, nuclear magnetic resonance

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References


Ocular Injection of Retinal S Antigen: Suppression of Autoimmune Uveitis

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Uveoretinitis induced in Lewis rats by foot pad injections of retinal soluble (S) antigen is a model system used to study the immunopathogenesis of the autoimmune uveoretinitides in which delayed hypersensitivity plays a dominant role. Since down-regulation of delayed hypersensitivity can be achieved by injection of antigens into the anterior chamber of the eye, we have examined the potential of intracameral S antigen to mitigate experimental autoimmune uveitis. The results of these studies reveal that pretreatment of Lewis rats with S antigen injected into the anterior chamber markedly reduces the incidence and expression of experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 30:772-774, 1989.

Immunologic privilege is the term applied to the fact that histoincompatible tissues can sometimes be grafted successfully into certain sites of the body. Over the past 15 years, the expression of this phenomenon in the anterior chamber (AC) of the eye has been re-explored. It has been learned that intracamerally (IC)-injected antigens have the capacity to induce suppressor T cells that selectively down-regulate the expression of delayed hypersensitivity, but not antibody formation or priming of cytotoxic T cells. This deviant spectrum of immune effectors— anterior chamber associated immune deviation (ACAID)—may account for immunologic privilege and may be important in the success of ocular neoplasms and in chronic ocular HSV-1 infections.  

Autoimmune responses to tissue-specific ocular antigens have been implicated in the pathogenesis of certain diseases of the eye, for example, sympathetic ophthalmia and Harada's disease. We have wondered whether ocular antigens are able to induce ACAID, and therefore, whether prevention or down regulation of these deleterious immune responses might be a physiologic function of ACAID. Experimental autoimmune uveitis (EAU) is a laboratory model of autoimmune eye disease in which the offending antigen is a soluble molecule extracted from the retina, the retinal S antigen (S Ag). Although S Ag is soluble, we have recently demonstrated that ACAID can be induced in mice by soluble antigens. However, S Ag has not been used successfully to induce EAU in normal, unmodified mice. Therefore, to determine whether IC pretreatment with S Ag can suppress the induction and expression of EAU, we decided to use the rat model system. To that end, we have recently reported that S Ag injected alone, or in conjunction with Complete Freund's Adjuvant (CFA) into the AC of Lewis rat eyes evokes typical S Ag-specific ACAID, that is, produces suppressed delayed hypersensitivity to this antigen. 

Materials and Methods. Experimental animals: