Identification of a Cytosolic NADP\(^+\)-dependent Isocitrate Dehydrogenase That Is Preferentially Expressed in Bovine Corneal Epithelium

A CORNEAL EPITHELIAL CRYSTALLIN\(^*\)

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Recently, metabolic enzymes have been observed in both the lens and corneal epithelium at levels greatly exceeding what is necessary for normal metabolic functions. These proteins have been termed taxon-specific crystallins and are thought to play a role in maintaining tissue transparency. We report here that cytosolic NADP\(^+\)-dependent isocitrate dehydrogenase (ICDH) represents a new corneal crystallin. Using suppression subtractive hybridization, we identified a gene (with a deduced amino acid sequence that showed 94\% identity to rat cytosolic NADP\(^+\)-dependent ICDH) that is preferentially expressed in bovine corneal epithelium. Northern blots established that its mRNA level in the corneal epithelium was 31-, 39-, 133-, 230-, and 929-fold more than in the liver, bladder epithelium, stomach epithelium, brain, and heart, respectively. This mRNA was detected primarily in corneal epithelial basal cells by in situ hybridization. SDS-polyacrylamide gel electrophoresis, two-dimensional gel analysis, and Western blotting showed that this protein was overexpressed in the corneal epithelium, constituting \(-13\%\) of the total soluble bovine corneal epithelial proteins. Enzyme assays showed a corresponding overabundance of this protein in bovine corneal epithelium. Taken together, these data indicate that bovine cytosolic ICDH fulfills the criteria for a corneal epithelial crystallin and may be involved in maintaining corneal epithelial transparency.

The corneal epithelium is a self-renewing stratified squamous epithelial tissue that protects the underlying delicate structures of the eye, supports a tear film, and maintains transparency so that light can be transmitted to the interior of the eye. This latter quality is essential since the cornea accounts for two-thirds of the refraction of light in the eye. It is unclear how the corneal epithelium satisfies the requirements of transparency. However, the current belief is that high concentrations of metabolic enzymes within the corneal epithelium may be involved in tissue transparency, light absorption, and protection from UV-induced free radicals (1–4). These metabolic enzymes were termed “corneal crystallins” (5) based on their similarity to the situation in the lens (3, 6–8).

“Crystallins” are the major soluble proteins of the crystallin lens (9–12). The \(\alpha\) and \(\beta\)/\(\gamma\)-families of crystallins are ubiquitously found in all vertebrate lenses. The \(\alpha\)-crystallins have sequence similarity to small heat-shock proteins (13) and are thought to be molecular chaperones functioning to protect proteins from heat-induced stresses (14). The \(\beta\)/\(\gamma\)-crystallins are related to microbial oxidative stress proteins (15), and alterations in this family of crystallins have been implicated in lens opacity characterized by cataracts (12). Taxon-specific crystallins are members of a diverse group of metabolic enzymes that are expressed at much lower levels in other tissues compared with the lens (9, 11, 16). These species-specific crystallins represent the recruitment of enzymes to serve the lens in a new structural role, and this phenomenon has been termed “gene sharing” (7, 17).

In this paper, we have identified bovine cytosolic NADP\(^+\)-dependent isocitrate dehydrogenase (ICDH)\(^\dagger\) as an enzyme that fits a gene-sharing profile. Using a combination of Northern and Western blotting as well as enzyme assays, we show that this enzyme is present in unusually high amounts in bovine corneal epithelium. Our data suggest that this enzyme may be a new corneal crystallin (3, 6–8) and thus may play a structural as well as a catalytic role in contributing to corneal transparency.

MATERIALS AND METHODS

Identification of Corneal Epithelium-specific Molecules by Suppression Subtractive Hybridization—Fresh bovine bladder, brain, eye, heart, liver, lung, stomach, and testis were obtained from a local slaughterhouse and immediately placed on ice. The corneal epithelium was removed by scraping the surface of the cornea with a scalpel blade under a dissecting microscope and immediately frozen in liquid nitrogen. The bladder and stomach epithelia were also removed by scraping and frozen in liquid nitrogen. Portions of the other tissues were frozen in liquid nitrogen. Total RNA from these tissues was isolated using TRIzol reagent (Life Technologies, Inc.), and poly(A)\(^+\) mRNA was isolated from total RNA preparations using a QIAGEN Oligotex\textsuperscript{TM} mRNA kit.

Suppression subtractive hybridization was performed using a CLON-TECH PCR-Select\textsuperscript{TM} cDNA Subtraction kit according to the manufacturer’s protocol. Briefly, bovine corneal epithelium was chosen as the tester, and the seven other tissues (bladder and stomach epithelia, brain, heart, liver, lung, and testis) served as the driver. The double-
stranded cDNA synthesized from both tester and driver mRNAs was digested with a four-cutter restriction enzyme (RsaI). The RsaI-digested tester cDNA was ligated to two types of adapters, and hybridization was performed twice among the different adapter-ligated tester cDNAs and driver cDNA. Only the cDNAs with different adapters at both ends were amplified by the polymerase chain reaction (PCR). The PCR products were subsequently cloned into the pCRII vector using a TA Cloning kit (Invitrogen) and transformed into Escherichia coli. For differential screening, the transformed colonies were selected randomly. Colony or dot-blot hybridization was performed with forward- and reverse-subtracted cDNAs as probes using a CLONTECH PCR Select Differential Screening kit. The PCR product was subcloned into the pCRII vector and sequenced on both strands. The resulting sequences were compared with the GenBank data base using a BLAST or FASTA program.

**DNA Sequence Analysis—**Differentially expressed clones obtained from the corneal epithelial subtractive cDNA library were subjected to DNA sequencing and GenBank analysis. To amplify the missing RsaI fragment of bovine ICDH, PCR was conducted using 5'-GCTACGATTAGGCATAG-3' (nucleotides 209–226 on fragment a in Fig. 1) as the 5'-primer and 5'-TGGGCCACCAGCACATCGGCACCGGGAAGGTTTCTAGGCGCAGATGATG-3' (nucleotides 857 to 840 on fragment c in Fig. 1) as the 3'-primer in a thermal cycle (MJ Research, Inc.). The PCR product was subcloned into the pCRII vector and sequenced on both strands. The resulting sequences were compared with the GenBank database using a BLAST or FASTA program.

**Northern Blot Hybridization—**Poly(A)+ RNAs (0.5 μg) from various bovine tissues were used as templates to synthesize double-stranded cDNA using a SMART PCR cDNA Synthesis kit (CLONTECH). The synthesized cDNA was electrophoresed on a 1% agarose gel, transferred onto a positively charged nylon membrane (Roche Molecular Biochemicals), and UV-cross-linked (18). The membrane was prehybridized at 72 °C in ExpressHyb hybridization solution (CLONTECH) for 1 h and hybridized in fresh buffer with denatured random primer-labeled probes at 72 °C overnight. After hybridization, the blot was sequentially washed and then exposed to autoradiography film for 1–11 days. The autoradiogram was quantified using a phosphorimager system (Molecular Dynamics, Sunnyvale, CA).

**Fig. 1. Schematic diagram of bovine NADP⁺-dependent ICDH cDNA.** Fragments a–g represent clones obtained from the bovine subtractive cDNA library; the number of clone obtained is shown in parentheses. Fragment f, labeled with an asterisk, was used as a probe for Northern blot hybridization. Fragment h was amplified by PCR and used as a probe for in situ hybridization. The black box represents the open reading frame (ORF), and R represents the RsaI restriction site. nt, nucleotides.

**Fig. 2. Nucleotide and deduced amino acid sequences of the cDNA encoding bovine ICDH.** Putative protein kinase C phosphorylation sites are underlined, and the putative peroxisomal targeting signal is double-underlined. The isocitrate and isopropylmalate dehydrogenase stop signature is indicated by a dotted line. The stop codon is marked with an asterisk.
washed in 2× SSC and 0.5% SDS for 20 min twice at 68 °C and in 0.2× SSC and 0.5% SDS for 20 min twice at 68 °C and exposed to a PhosphorImager screen overnight. Intensities of signals were quantified by densitometry using the ImageQuant program (Molecular Dynamics, Inc.).

Western Blotting—Fresh corneal epithelium and the other tissues were cut into pieces and homogenized in 4 volumes of cold 0.25 M sucrose solution with a glass homogenizer. The homogenate was first centrifuged at 1000 rpm for 10 min to remove cell debris and then at 14,000 rpm for 10 min. The supernatant was saved as the cytosolic fraction, and the pellet represented the mitochondrial fraction (19). Total soluble lysates were made by sonicating the tissues in 0.25 M sucrose solution for three periods of 20 s on ice and centrifuged at 14,000 rpm for 10 min. Protein concentration was determined in triplicate using a Bio-Rad protein assay (20).

Proteins (10 μg) from cytosolic or total fractions of the various bovine tissues were separated on 12.5% SDS-polyacrylamide gels. Gels were stained with either Coomassie G-250 (Pierce GelCode blue stain reagent) or silver nitrate using a Silver Stain Plus kit (Bio-Rad) to detect proteins, or proteins were transferred to a nitrocellulose membrane using a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad). The membrane was stained with Ponceau S solution. The blot was incubated with a rabbit polyclonal antibody against rat cytosolic NADP<sup>1</sup>-dependent isocitrate dehydrogenase (kindly provided by Drs. Gary T. Jennings and L. McAlister-Henn) and detected with peroxidase-linked second antibody (Amersham Pharmacia Biotech).

Two-dimensional Gel Electrophoresis—Thirty micrograms of total soluble protein isolated from the corneal epithium was loaded on isoelectric focusing tube gels (4% acrylamide, 9.2 M urea, 2% ampholytes (pH 3–10), and 2% Nonidet P-40) and run at 200 V for 20 min, followed by 450 V for 3 h. After focusing, the rod gels were equilibrated for 10 min in a buffer containing 62 mM Tris phosphate (pH 6.8), 10% glycerol, 0.7 μm β-mercaptoethanol, 2% SDS, and 0.005% bromphenol blue. The protein was then resolved in the second dimension by 12.5% SDS-PAGE until the dye reached the end of the gel. The proteins were either silver-stained or transferred to Immobilon-P membrane (Millipore Corp.) by electroblotting (21, 22).

Enzymatic Activity Assays—The activity of NADP<sup>1</sup>-dependent ICDH from the corneal epithelium and other tissues was assayed at 25 °C in a 1-ml reaction mixture composed of 0.1 M Tris (pH 8.0), 3 μM MgCl<sub>2</sub>, 0.5 mM NADP<sup>1</sup>, 1.5 mM isocitrate, and 10 μg/ml cytosolic proteins (19). Glutamate dehydrogenase activity was measured at 25 °C upon addition of 2 mM α-ketoglutarate, 50 μM NH<sub>4</sub>Cl, and 50 μM NADH. α-Ketoglutarate dehydrogenase activity was monitored by adding 2.5 mM NAD<sup>1</sup>, 200 μM thiamine pyrophosphate, 130 μM CoASH, and 2.0 mM α-ketoglutarate (23). The rate of NADPH production was measured SDS-PAGE until the dye reached the end of the gel. The proteins were either silver-stained or transferred to Immobilon-P membrane (Millipore Corp.) by electroblotting (21, 22).
and an antibody against rat cytosolic NADPH was resolved by SDS-PAGE (12.5% acrylamide) and blotted with a body against ICDH. Ten micrograms of bovine total and cytosolic pro-
cytosolic fractions of the corneal epithelium (lanes 2 b and liver, respectively.

The 43-kDa ICDH (marked with asterisks) accumulated in the total and cytosolic corneal epithelial proteins, with
both the total and cytosolic corneal epithelial proteins, with
spects indicated an ALDH3/ICDH ratio of 3.2. This suggests that ICDH constitutes 13% of the total bovine corneal epithelial protein. This concentration of ICDH may be considered
spectrophotometrically at 340 nm. The enzyme activity is expressed in ΔA/min/mg of protein.

In Situ Hybridization—A 649-base pair DNA fragment (fragment h in Fig. 1) of bovine cytosolic NADPH-dependent isocitrate dehydrogenase was amplified by PCR and sub cloned into pCR1. Digestion with Xhol and transcription with Sp6 RNA polymerase were utilized for antisense probes, and digestion with SpeI and transcription with T7 RNA polymerase were used for sense probes. RNA probes were prepared using 35S-labeled UTP. In situ hybridization was conducted as described (24).

RESULTS
To identify genes preferentially expressed in bovine corneal epithelium, we prepared a corneal epithelial subtractive library using the suppression subtractive hybridization technique (25). Common messages of the corneal epithelium were subtracted using driver cDNAs of the bladder epithelium, brain, heart, liver, lung, stomach epithelium, and testis. From the corneal epithelial subtractive cDNA library, 275 clones were picked and probed with the forward-subtracted cDNAs (corneal epithelium-specific) or the reverse-subtracted cDNA (“specific” for the seven tissues) (26). About 40% of the clones gave much stronger signals with forward-subtracted probes, indicating that these clones representing mRNA are cornea-
enriched. Among these cornea-specific clones were those encoding the K3 and K12 cornea-specific keratins (27–37) as well as aldehyde dehydrogenase class 3 (ALDH3), the major water-
soluble protein in mammalian corneas and one of the putative corneal epithelial crystallins (1–6). It should be noted that the number of cornea-enriched clones obtained using suppression subtractive hybridization is not strictly correlated with the frequency of the cDNAs. For example, we found three, two, and two clones for K3 keratin, K12 keratin, and ALDH3, respectively, all major components of the corneal epithelium. Remarkably, 55% of the clones were homologous to various regions of rat cytosolic NADPH-dependent ICDH (Figs. 1 and 3). The bovine cytosolic ICDH cDNA is 1682 nucleotides long and has an open reading frame encoding 414 amino acids (Fig. 2). This protein contains the isocitrate and isopropylmalate dehydrogenase signature: a glycine-rich stretch of residues located in the C-terminal section. The last three amino acids at the C-terminal end, AKL, were found in proteins that are targeted to peroxisomes (38). Comparison of the amino acid sequence of bovine cytosolic ICDH with that of rat ICDH showed 94% identity, and this enzyme is highly homologous to mitochondrial ICDH from other species (Fig. 3). Northern blot analysis showed that the corneal epithelium contains a much higher level of ICDH than other tissues (Fig. 4). Quantification of the Northern blot revealed that the expression level of the mRNA in corneal epithelium is ~31-, 39-, 133-, 230-, and 929-fold more than in the liver, bladder and stomach epithelia, brain, and heart, respectively.

Western blot analysis with a polyclonal antibody against rat cytosolic ICDH revealed an extremely strong 43-kDa bovine corneal epithelial protein, which was minimal in other tissues (Fig. 5a). Analysis of proteins using (extra-long) high resolution SDS-PAGE revealed the presence of an intense 43-kDa band in both the total and cytosolic corneal epithelial proteins, with little or none in the bladder, heart, kidney, or liver (Fig. 5b). Further analysis of total corneal epithelial proteins employing two-dimensional gel electrophoresis (Fig. 6a) in combination with Western blotting (Fig. 6b) revealed only a single protein in the 43-kDa band. An even stronger band was detected in the corneal epithelium at 54 kDa and most likely represented ALDH3, a previously identified major corneal epithelial crystallin that constitutes a maximum of 40% of the total soluble protein (2, 3, 6, 39, 40). Quantitation of the densities of the bands indicated an ALDH3/ICDH ratio of 3.2. This suggests that ICDH constitutes ~13% of the total bovine corneal epithelial protein. This concentration of ICDH may be considered
within the levels that have been reported for corneal crystallins (7).

Similar to our mRNA and protein findings, a much higher ICDH enzymatic activity was detected in bovine corneal epithelium compared with all other tissues (Fig. 7a). However, the activities of the downstream enzymes of ICDH, glutamate dehydrogenase, and α-ketoglutarate dehydrogenase were not significantly higher in the corneal epithelium when compared with the bladder, heart, and liver (Fig. 7, b and c).

To determine whether high levels of ICDH are found in the corneal epithelia from other species, we prepared cytosolic extracts from human, rabbit, rat, and mouse corneal epithelia.
these species, ICDH activity in the cornea was not significantly higher than that in the heart, kidney, liver, pancreas, or skin, indicating that high levels of ICDH may be bovine-specific (Fig. 8).

In situ hybridization showed that ICDH mRNA was present mainly in the basal cells of the corneal epithelium (Fig. 9, c and d) and that ICDH expression stopped abruptly at the corneal/limbal junction (Fig. 9, e and f). No detectable signal was seen over corneal fibroblasts (keratocytes) or endothelial cells (data not shown).

DISCUSSION

High Levels of Corneal Epithelial ICDH Indicate a Dual Role for This Enzyme—The idea that a gene encoding a single protein may acquire and maintain a second function without duplication and without loss of the primary function is known as gene sharing (16, 17). This concept, originally formulated from studies on the lens crystallins (for reviews, see Refs. 7 and 8), appears to apply to many systems. For example, in Tetrahymena, a cytoskeletal 14-nm filament-forming protein involved in oral morphogenesis and in pronuclear behavior during conjugation was also shown to be a mitochondrial enzyme (41). Gene sharing has also been shown to occur in the corneal epithelium, where enzymes such as ALDH3, transketolase, and cyclophilin have been recruited as major corneal proteins that may serve structural as well as catalytic roles (6, 42). In this study, we demonstrate that ICDH is present in bovine corneal epithelium in an amount that far exceeds what would be necessary for its conventional enzymatic requirements. The ICDH family of enzymes is found in a majority of species and has a wide variety of functions. For example, NADP⁺-dependent ICDHs are present in mitochondria, peroxisomes, and the cytoplasm. The mitochondrial enzyme catalyzes the reduction of isocitrate to α-ketoglutarate, which is central to energy production (ATP) in the citric acid cycle. In the ovary, the cytosolic form is thought to supply NADPH for fatty acid synthesis and for fatty acid chain elongation and desaturation reactions (43).

In yeast, the peroxisomal form is thought to function in a reduction/oxidation shuttle during the degradation of polyunsaturated fatty acids (44, 45). This enzyme is also involved in the synthesis of certain amino acids such as glutamate. We reasoned that if the high ICDH levels in the corneal epithelium observed here were solely for metabolic events in glutamate biosynthesis, increases in corneal glutamate dehydrogenase and α-ketoglutarate dehydrogenase activities would be expected, as these are the downstream enzymes in this biosynthetic pathway. This was not the case; and thus, we conclude that corneal epithelial ICDH must perform some other functional role.

Bovine Cytosolic ICDH May be Regarded as a Corneal Epithelial Crystallin—Metabolic enzymes have been observed in corneal epithelia from a wide variety of species in amounts that are in excess of what would be required for a catalytic role and have been termed corneal crystallins (5, 7). The most prevalent

FIG. 6. Analysis of total corneal epithelial proteins by two-dimensional gel electrophoresis (a) in combination with Western blotting (b). Thirty micrograms of total bovine corneal epithelial proteins was resolved in the first dimension by isoelectric focusing (IEF) in ampholytes (pH 3–10) and in the second dimension on a 12.5% SDS-polyacrylamide gel. The protein spot that is circled in the silver-stained gel (a) represents ICDH as determined by Western blotting using an antibody against the rat enzyme (b).

FIG. 7. Enzymatic activities of ICDH (a), glutamate dehydrogenase (b), and α-ketoglutarate dehydrogenase (c) in various bovine tissues. Activities were calculated as ΔA/min/mg of protein, and values were normalized to that of the corneal epithelium. The results are the mean of three separate experiments. Note the marked increase in ICDH activity in the corneal epithelium (a) compared with the other tissues; no differences were noted, however, in glutamate dehydrogenase (b) and α-ketoglutarate dehydrogenase (c) activities among the corneal epithelium, bladder, heart, and liver.
corneal crystallin is ALDH3, found in the corneal epithelia of all mammalian species tested to date (1–4, 6). In addition to ALDH3, human corneal epithelial cells express aldehyde dehydrogenase class 1 (46), whereas bovine corneal epithelial cells express ALDHx (47). Recently, transketolase has been found at high levels in mouse, human, and bovine corneal epithelia (6, 42). All of the proteins that have thus far been classified as corneal crystallins are metabolic enzymes that are abundantly expressed (5–40% of the total soluble protein) and often are taxon-specific (for reviews, see Refs. 7 and 8). Our data suggest that in cattle, ICDH fulfills the enzyme crystallin profile of these aforementioned putative corneal crystallins for several reasons. 1) The mRNA levels of ICDH in the corneal epithelium ranged from 31- to 929-fold higher than those observed in the liver and heart, respectively (Fig. 4). 2) In agreement with the mRNA data, corneal epithelial ICDH protein levels far exceeded those of other tissues as determined by SDS-PAGE and Western blotting (Figs. 5 and 6). 3) Within bovine corneal epithelium, ICDH constituted up to 13% of the total soluble protein (Figs. 5 and 6). 4) The abundance of ICDH greatly exceeded what was required for its enzymatic function (Fig. 7). 5) The abundant levels of ICDH detected in bovine corneal epithelium were not observed in human, mouse, rat, and rabbit corneal epithelia (Fig. 8).

ICDH May Be a Bovine Corneal Epithelial Differentiation Product—It is well established that corneal and limbal basal cells are biochemically distinct. Schermer et al. (32) demonstrated that K3, a major keratin expressed during advanced stages of corneal epithelial differentiation, is expressed only in the suprabasal cells of the limbal epithelium, but uniformly in the central corneal epithelium. This provided the first evidence that limbal basal cells are biochemically more primitive than corneal epithelial basal cells. Our present observation of a strong signal for ICDH mRNA in basal (and wing) cells of bovine corneal epithelium, but not in limbal epithelial basal cells, further distinguishes these two basal cell populations. This ICDH basal cell distribution pattern is analogous to that of the K3/K12 keratin pair and suggests that ICDH may be considered as a bovine corneal epithelial differentiation product. In contrast, α-enolase has been demonstrated to be expressed predominantly in the more primitive limbal basal cells (48). The biochemical differences observed between limbal and corneal epithelial basal cells formed the basis of a model in which corneal epithelial stem cells were postulated to be located in the basal layer of the limbal epithelium (32). This model has received strong support from a series of kinetic (49–51), cell culture (52–55), and wounding/perturbation (49, 51, 56–59) experiments.

**Implications in Maintaining Corneal Epithelial Transparency**—A high concentration of enzyme crystallins in the lens is believed to contribute to the lens’ high refractive index and focusing power (reviewed extensively in Refs. 7, 8, 11, 12, 15, 16, and 60), and a similar role has been proposed for the corneal crystallins (7, 8). In support of this idea, a recent study demonstrated that following wounding or excimer photorefractive keratotomy, rabbit keratocytes assumed a reflective spindle-shaped morphology that contributed to corneal haze (61). Rabbit keratocytes normally have a high expression of proteins homologous to transketolase and ALDH1, which together represent 30% of the total water-soluble cellular proteins and thus may be considered as keratocyte crystallins. A 67% specific decrease in these keratocyte proteins accompanied the change in keratocyte shape following wounding, suggesting that these enzymes might function in regulating cellular refractive index and transparency (61). It is tempting to speculate that in bovine corneal epithelium, ICDH functions in a similar manner.

Presently, it is not clear what regulates the expression of ICDH. Both ALDH3 and transketolase are up-regulated during postnatal development of murine corneal epithelium, coinciding with eye opening (42). This has led to the suggestion that UV light-generated oxidative stress may be one of the inductive factors in corneal crystallin expression. Support for this idea comes from a recent study that demonstrated that higher ALDH3 mRNA levels were detected in corneas of mice exposed to a 12-h light/dark cycle compared with those of age-matched animals raised in the dark (62).

As the first line of defense against environmental insults, the corneal epithelium is continuously exposed to UV radiation. One defense mechanism against UV damage in corneal epithelial cells is nuclear ferritin, which has been shown to protect avian corneal epithelial cells from UV-induced DNA strand breaks (63, 64). Corneal crystallins, acting as UV absorbers, have been proposed as another defense mechanism against UV-induced photodamage (2, 65, 66). In this respect, it is interesting to note that bovine corneal epithelium is thus far unique in that it contains three metabolic enzymes that may serve as corneal crystallins: ALDH3 (2, 3), transketolase (42, 66), and ICDH (this work). The abundant amounts of these three cytosolic enzymes in bovine corneal epithelium are indicative that this tissue has extremely high concentrations of NADPH-producing enzymes. NADPH is an effective scavenger of free radicals and H$_2$O$_2$ resulting from excessive UV radiation. In support of this idea, high levels of ICDH, malic dehydrogenase, and glucose-6-phosphate dehydrogenase were postulated to be involved in defending the lens against oxidative damage (60). Whether UV stimulation regulates ICDH is not
A strong signal for ICDH mRNA was detected in the limbal epithelium (e) and central corneal epithelium. Superficial (a–d) and wing (e) probes to ICDH. A strong signal for ICDH mRNA was detected in basal (B) and wing (W) cells, but not in the superficial (S) cells of the corneal epithelium (c and d). Little if any signal for ICDH mRNA was seen in the limbal epithelium (e) and d. a, c, and e are micrographs taken under bright-field, and b, d, and f were taken under dark-field.

known. Since there is a clear association between development of eye cancer in cattle and increasing levels of radiation (67), ICDH, in addition to ALDH3 and transketolase, may be a protective factor designed to counteract the deleterious effects of UV radiation.

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