UV Absorption by Uric Acid in Diurnal Bird Aqueous Humor

Amund Ringvold,1 Erlend Anderssen,2 and Inge Kjønniksen2

PURPOSE. To analyze the components responsible for the UV absorbance in diurnal bird aqueous humor.

METHODS. The absorbance studies were carried out using a Hitachi spectrophotometer (U 2000). Uric acid was determined by high-performance liquid chromatography (LC-10 system; Shimadzu, Kyoto, Japan). Chicken and turkey eyes were examined.

RESULTS. The UV absorbance in chicken aqueous was largely accounted for by the presence of protein, tryptophan, tyrosine, ascorbic acid, and uric acid. Ascorbic acid was low (23 μmol/l). Uric acid was, on the other hand, remarkably high (151 μmol/l) compared with that in mammals (cattle, 16 μmol/l). Principally the same results were obtained in chicken and turkey.

CONCLUSIONS. Uric acid is a significant UV-absorbing substance in the aqueous humor of diurnal birds with its peak absorbance at 292 nm. The hypothesis that the aqueous humor acts as a UV filter seems to be valid also for the avian eye. However, in these eyes uric acid fulfills the role that ascorbic acid does in mammals. (Invest Ophthalmol Vis Sci. 2000;41:2067–2069)

The threshold energy for damage of the corneal epithelium by UV-radiation is different in mammals and birds.1,2 Antarctic birds, penguins, and skua, which are normally exposed to high UV-radiation for prolonged periods of time, show higher resistance to the radiation than chickens and ducks. On the other hand, there is apparently no systematic correlation between corneal transmission and expected environmental exposure of the respective species.2 Different from mammals, any details concerning mechanisms minimizing UV damage in the avian eye have so far not been presented. It is relevant for our purpose that in an early study on reducing oxidative stress in diurnal species regardless of whether mammals or bird. Because of the unique anatomic structure in this organ, its UV-protective mechanisms may in part be different from those indicated above. This field has been excellently reviewed by Dillon.4

In general, there are a number of compounds that absorb UV-radiation and may induce photochemical damage to the tissue. The most common are chromophores, nucleic acids, and proteins including various types of enzymes. Damage is found at the molecular level and is reduced and modified by the presence of quenchers. The eye is under permanent phototoxic stress in diurnal species regardless of whether mammal or bird. Because of the unique anatomic structure in this organ, its UV-protective mechanisms may in part be different from those indicated above. This field has been excellently reviewed by Dillon.4

In mammals, there is a significantly higher concentration of ascorbate in the aqueous humor of diurnal species than in that of nocturnal species,5–7 and the differences are particularly pronounced in the case of the corneal epithelium.8 Because of the high molar absorptivity of ascorbate for UV-radiation, these observations have been taken as an indication that this substance acts as a UV filter for the eye. If this is correct, a similar mechanism may be assumed in diurnal birds. It has been shown, however, that the ascorbate content in the avian aqueous is low,9 which implies that either the hypothesis presented for mammals is wrong or that the material responsible for the aqueous UV absorption is different between mammals and birds. To evaluate these questions we decided to examine the aqueous humor from diurnal birds spectrophotometrically and to analyze the components responsible for the absorbance.

METHODS

Chicken. Chicken eyes were obtained at the local abattoir. The slaughter process was by electric shock, debleeding, and decapitation before aspiration of the aqueous humor from the anterior chamber with a 30-gauge cannula. Four collections, each of roughly 5 ml volume, were transported on ice to the laboratory and either analyzed immediately or stored at −35°C for up to 2 weeks. In addition to spectrophotometry, the contents of protein, tyrosine, tryptophan, and ascorbate were examined.

Another four specimens, each collected from 6 eyes, were analyzed fresh for UV absorbance and uric acid content. To check for any possible change in absorbance induced by the slaughter process at the abattoir, these animals were killed with a moderate blow to the head before decapitation. Spectrophotometry showed principally the same results in the two groups.

Turkey. Two different collections, each of approximately 5 ml volume, were obtained at the abattoir and processed as above.

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TABLE 1. Chemical Data from Aqueous Humor and Serum

<table>
<thead>
<tr>
<th></th>
<th>Chicken</th>
<th>Turkey</th>
<th>Cattle</th>
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<tbody>
<tr>
<td>Aqueous humor</td>
<td></td>
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<tr>
<td>Total protein, mg/l</td>
<td>273 ± 19</td>
<td>225 (230/220)</td>
<td>* 392</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>97 ± 7</td>
<td>233 (254/212)</td>
<td>* 137</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>19 ± 7</td>
<td>26 (32/20)</td>
<td>* 50</td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>24 ± 1</td>
<td>34 (32/36)</td>
<td>* 1225</td>
</tr>
<tr>
<td>Uric acid</td>
<td>151 ± 30</td>
<td>165 (167/163)</td>
<td>16 (17/15)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>62 (56/68)</td>
<td>98 (91/105)</td>
<td>5 (6/5)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>293 (333/253)</td>
<td>255 (248/262)</td>
<td>20 (15/25)</td>
</tr>
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Mean and standard deviation of chicken aqueous humor are based on four samples. Otherwise, two samples were used, indicated by mean; single figures in parentheses. Values are expressed as μmol l⁻¹, except for protein. *Previous values from cattle aqueous are included for comparison.

Cattle. Individual aqueous samples were collected from bovine eyes for uric acid determination. In addition, blood samples were collected from all three species. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Total protein was determined using a micro protein assay (procedure No. 610; Sigma), and ascorbic and uric acids by the high-performance liquid chromatography (HPLC) technique. The uric acid peak, which was recorded at 299 nm, was identified by spiking, alternatively by uricase-deletion, and spectral characteristics. Injection of allantoin did not generate any peak at this wavelength. The amino acid concentrations were obtained using an automatic amino acid analyzer (Eppendorf/Biotronik LC 3000). In mock aqueous, total protein was added as chicken albumin. In addition to ascorbic acid (Riedel-de Haën AG), Sigma products were used. The absorbance studies were carried out using a Hitachi spectrophotometer (U 2000). Quartz cuvettes with 2 mm light pathway and phosphate buffer (0.1 M, pH 7.3) were used throughout.

RESULTS

The absorption of UV-radiation in the aqueous humor of diurnal mammals is due to its content of protein, tyrosine, tryptophan, and ascorbate. To find out whether this was also the case in birds, four aqueous collections from chicken were analyzed with respect to these components, and the mean values obtained (Table 1) were used to mix a mock aqueous humor on a phosphate buffer basis. The results can be seen in Figure 1A. This shows the absorbance of one native chicken sample compared with its matching mock aqueous sample. The marked difference between the two curves (Fig. 1B) indicates that in addition to protein, amino acids, and ascorbate, there is some other material contributing significantly to the total absorbance in chicken aqueous. Based on the tentative conclusion that the difference might be accounted for by one single component, various options were evaluated using a spectrum collection. The best fit for the UV-B range (320–290 nm) turned out to be uric acid (as illustrated in Fig. 1B), and this finding singled out uric acid as a potential UV-absorbing substance in the chicken eye.

To test this hypothesis, another four aqueous sample collections (each pooled from 6 eyes) were analyzed spectrophotometrically and with respect to their uric acid contents by HPLC (Table 1). The chromatograms revealed a marked peak with spectrum and retention time in accordance with the uric acid standard. Subsequently, four identical mock aqueous samples were created (see Table 1), and each of them was supplemented with one of the four observed uric acid concentrations. The absorbance of these upgraded mock samples was
then compared with the equivalent native ones. All four setups showed principally the same results (exemplified in Fig. 1C). As can be seen in Figures 1A through 1C, uric acid is to a large extent responsible for the photometric peak in the UV-B range of native chicken aqueous.

To test whether this conclusion also applies in another species, a similar setup was run for turkey. The results, exemplified in Figure 1D, confirm the previous observation.

**DISCUSSION**

The capacity for synthesis of ascorbic acid is apparently lost in many birds,\(^1\)\(^2\) (i.e., any function in birds based on this substance would be dependent on external supplies). Uric acid, on the other hand, is the major end product of nitrogen metabolism in these animals. It is present in ample amounts and readily available from blood. In addition, uric acid shows the higher molar absorptivity of the two with \(\lambda_{\text{max}}\) at a longer wavelength (uric acid in 0.067 M glycine buffer, pH 9.4; 12,500 at 292 nm versus ascorbic acid in water or neutral solutions; 7,000 at 265 nm),\(^13\)\(^14\) indicating that it is a powerful UV-B absorber. This fact should be considered when comparing uric and ascorbic acid concentrations in the aqueous humor of birds and cattle (Table 1).

The observation of high ascorbic and low uric acid concentrations in the aqueous of mammals, and vice versa in diurnal birds, brings us to the conclusion that the aqueous humor acts as a UV-B filter in diurnal birds just as it does in diurnal mammals but that the basis for the absorption is different: In the avian eye, uric acid fulfills the role that ascorbic acid does in mammals. Any of these antioxidants is likely to deteriorate at physiologically relevant spectral irradiation of the aqueous.\(^15\) It is noteworthy that evolution has taken different tracks in the environmental adaptation of birds and mammals in meeting their common need for a stable radiation barrier in the anterior eye. In many diurnal birds the spectral range of vision is extended into the UV by specific retinal receptors.\(^16\) However, with a spectral sensitivity maximum of 360 to 370 nm, this vision is uninfluenced by the present observations.

It has previously been stated that in humans uric acid is merely a metabolic waste elaborated only to be eliminated, and quite devoid of any perceptible physiological effects.\(^17\) However, today we know that the substance has antioxidant properties\(^18\) and is being ascribed a number of important functions, including modulating redox reactions and oxidative events in the blood, arterial wall, and lung.\(^19\)\(^20\) Uric acid also accounts for the high reducing agent on the extracellular surface of the corneal epithelium in rabbits.\(^21\) It is, therefore, not surprising that it may also have an important function in the avian eye.

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**References**