Lenticular Levels of Amino Acids and Free UV Filters Differ Significantly between Normals and Cataract Patients

Isla M. Streete,1 Joanne F. Jamie,2 and Roger J. W. Truscott1

PURPOSE. To determine the levels of free UV filters and selected amino acids in cataract lenses compared with normal lenses.

METHODS. Indian cataract lenses (n = 39) and normal lenses (n = 6) were examined by HPLC to quantify levels of UV filter compounds, the UV filter precursor amino acid tryptophan (Trp), as well as tyrosine (Tyr) and uric acid.

RESULTS. The levels of the two major primate UV filters, 3-hydroxykynurenine glucoside (3OHKG) and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside (AHBG), in cataract lenses were markedly decreased compared with levels in normal lenses. By contrast, the levels of Trp were greatly increased. Mean Trp concentrations were an order of magnitude higher than in normal lenses, with 86% of dark-colored cataract lens nuclei having Trp concentrations greater than the mean level in the normal lenses. The concentrations of Tyr were also higher in cataract lenses. The levels of Kyn, however, were unchanged, and the uric acid levels were substantially lower.

CONCLUSIONS. The levels of the free UV filter compounds 3OHKG and AHBG, and also of Trp, Tyr, and uric acid were different in cataract lenses compared to normal lenses. These data suggest that the metabolism of a large proportion of patients with cataract may be substantially different than in persons with normal lenses. Although the mechanism of such metabolic defects are unknown, the authors speculate that an amino acid transporter system may be upregulated in patients with cataract. Because kynurenine levels in cataract were not significantly different from those of normal lenses, there may be a defect in the lenticular UV filter pathway at one, or both, of the steps that convert kynurenine to 3OHKG. (Isla M. Streete, Joanne F. Jamie, and Roger J. W. Truscott; Australian Cataract Research Foundation, Wollongong, Wollongong, New South Wales, Australia; and the Department of Chemistry, Macquarie University, Sydney, New South Wales, Australia)

Cataract is a major cause of blindness, with an estimated 16 million people affected worldwide. In many African and Asian countries, cataract contributes to 50% of blindness. Surgical removal is currently the only treatment available, resulting in a great financial burden on a nation's health system. In 1997, in the United States alone, age-related cataract operations cost $3.4 billion (US). With ageing of populations worldwide, this is set to become even more of a problem. If an understanding of the cause of age-related cataract at a molecular level can be established, then preventative measures can be taken and the development of a therapeutic treatment may be possible.

Age-related nuclear (ARN) cataract is characterized by opacification, tanning, and hardening of the lens. It has been proposed that tryptophan (Trp) metabolites that are present in the lens as UV filters may be involved. Some of these metabolites are indicated in Scheme 1. Of these, two of the major UV filters, kynurenine (Kyn) and 3-hydroxykynurenine (3OHKG), are known to bind in vivo to lens proteins, leading to tanned and fluorescent proteins. This process increases with age, and age is by far the greatest risk factor for cataract.

Studies to determine the concentrations, as a function of age, of the major free UV filters 3OHKG, Kyn, 3-hydroxykynurenine (3OHKyn), 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside (AHBG), and glutathionyl-3-hydroxykynurenine glucoside (GSH-3OHKG), have been undertaken in normal human lenses. It was found that each UV filter, with the exception of GSH-3OHKG, decreases linearly with age. However, no comprehensive study of UV filters present in cataract lenses has been performed, although it has been reported that cataract lenses may contain novel UV filters, such as xanthurenic acid glucoside (XABOG). In research into free amino acid levels in cataract lenses, Barber found that there was a significant elevation of proteogenic amino acid levels above those found in normal lenses.

The purpose of this investigation was to determine the levels of the free UV filters 3OHKG, Kyn, and AHBG; their biosynthetic precursor Trp, in addition to tyrosine (Tyr) and uric acid, in cataract lenses from India compared with normal lenses, to determine whether there were differences and whether there was a correlation with the brunescence of the lens. This approach of profiling the low-molecular-weight compounds present in tissues or fluids of diseased states, particularly in patients with suspected inborn errors of metabolism, has traditionally been used to determine the site of the principal metabolic defect.

MATERIALS AND METHODS

ni-Kynurenine sulfate, 3-hydroxykynurenine, L-tryptophan, L-tyrosine, uric acid, and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (HPLC grade) and ethanol were purchased from Ajax (Auburn, NSW, Australia). Normal human lenses were obtained from donor eyes at The Sydney Eye Hospital Lions Eye Bank and, after removal, placed in sterile vials and stored at −80°C (Human Ethic Clearance, University of Wollongong, HE99/001). Cataract lenses were obtained from India and stored at −20°C. All protocols involving human tissue were conducted in accordance with the Declaration of Helsinki.

Instrumental Conditions

Reversed-phase HPLC was performed on a system (Shimadzu, Kyoto, Japan) with a photodiode array detector and controlled by computer...
(Class-VP software). Standard curves and separations were performed on a 250 × 4.6 mm, 10.0 µm C18 column (Microsorb, Varian, Sunnyvale, CA), fitted with a C18 guard column (Phenomenex, Ltd., Macclesfield, UK). Solvents used were water with 0.05% (vol/vol) TFA (solvent A) and 80% (vol/vol) acetonitrile and water with 0.05% (vol/vol) TFA (solvent B). The percentage of solvent B in the gradient was 0% (5 minutes), 0% to 50% (50 minutes), 50% to 0% (5 minutes), and 0% (15 minutes). The flow rate was 0.5 mL/min. Standard curves for 3OHKyn, DL-kynurenine sulfate, L-tryptophan, L-tyrosine, and uric acid were determined and analysis of the lens extracts were performed in the above conditions. Detection was at 360 nm for the UV filters and 280 nm for the other compounds.

Mass spectra were obtained on a commercial system (Q-TOF2; Micromass, Manchester, UK) equipped with a nanospray source, in positive ion mode.

**UV Filter Extraction**

Each lens was cored (5 mm bore) and the nucleus and cortex separated. Approximately 0.5 mm was cut from each end of the nucleus and added to the cortex fraction. All dissections were performed at 0°C. The average weights of the nucleus and cortex for each lens type were as follows: light-colored cortex, 45 ± 34 mg; light-colored nucleus, 22 ± 4 mg; dark-colored cortex, 50 ± 32 mg; dark-colored nucleus, 24 ± 7 mg; normal cortex, 154 ± 25 mg; and normal nucleus, 49 ± 10 mg.

Immediately after dissection, the lens fractions were weighed and homogenized in 100% (vol/vol) ethanol (300 µL). The homogenate was stored at −20°C for 1 hour and then centrifuged (14,000g, 10°C, 15 minutes). The supernatant was removed and stored at −20°C, and the pellet was re-extracted with 80% (vol/vol) ethanol (300 µL). The homogenate was stored at −20°C for 1 hour and then centrifuged as just described, and the supernatants were combined and dried.17 Each sample was analyzed by HPLC, and the relevant peaks were collected and analyzed by tandem mass spectrometry. The average recovery of compounds from the lenses was measured by adding 250 picomoles Kyn in 100 µL ethanol to one half of both a normal and a cataract nucleus and then extracting and analyzing the lens as just described. The recoveries obtained were 97% and 73%, respectively.

**Amino Acid Analysis**

Amino acid analysis for Trp was performed by the Australian Proteome Analysis Facility (APAF, Sydney), using the a precolumn derivatization method (AccQ-Tag; Waters, Milford, MA).

**Statistical Analysis**

Statistical analysis was performed with a one-way ANOVA t-test, to compare the data points obtained from cataract and normal lenses for significance. P < 0.05 was considered significant.

**RESULTS**

**Lenses**

Cataract lenses were divided into two groups based on color. Because the lenses were frozen, it was not possible to classify them individually into types I to IV according to the Pirie system,18 and so they were assigned to two groups according to whether they were light or dark colored. Light-colored lenses corresponded to types I and II and dark-colored lenses to types III and IV. Nuclei were obtained as described in the Materials and Methods section. The cortex referred to in this study is the material outside the 5 mm core and does not represent the whole of the lens, because the cataract lens material used in this study was the sample removed after extracapsular extraction.

Normal lenses (n = 6) ranged in age from 51 to 64 years with an average age of 56 years. Cataract lenses (n = 39) ranged in age from 26 to 90 years, with an average age of 62 years.

**UV Filters**

Each lens extract was analyzed by HPLC. Representative elution profiles of light- and dark-colored cataract lens ethanol extracts are shown in Figures 1A and 1B. Peak 1 (23 to 24 minutes) corresponded to 3OHKG, peak 2 (27 to 28 minutes) to Kyn, and peak 3 (32 to 34 minutes) to AHBG. The standard curve for 3OHKyn was used to determine the concentration of 3OHKG and AHBG, and the standard curve for Kyn was used to determine the concentration of Kyn, from the HPLC peak area at 360 nm.

As will be discussed in more detail later, the overall appearance of the UV filter profile in cataract lenses was not markedly different from that in normal lenses, in that no new peaks were noted.

**3-Hydroxykynurenine Glucoside.** The concentration of 3OHKG was markedly lower in cataract lenses than in normal lenses (Table 1). This was true of both light- and dark-colored lenses from each group. The concentrations of light-colored lenses were different from those of normal lenses, but not those of dark-colored lenses, which are shown in Table 1. The concentration of 3OHKG was significantly lower in cataract lenses than in normal lenses (t = 4.6, n = 40).
FIGURE 1. UV filters in a light-colored cataract lens (A) and a dark-colored cataract lens (B). UV filters were extracted with ethanol, separated by reversed phase HPLC, and monitored at 360 nm. Peaks: 1, 3OHKG; 2, Kyn; and 3, AHBG.
Table 1. Concentrations of the UV Filters 3OHKG, Kyn, and AHBG in Light- and Dark-Colored Cataract Lenses and Normal Lenses

<table>
<thead>
<tr>
<th></th>
<th>Concentration (pmol/mg)</th>
<th>SEM</th>
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<tbody>
<tr>
<td>3OHKG light (c)</td>
<td>71</td>
<td>23</td>
</tr>
<tr>
<td>3OHKG light (n)</td>
<td>86</td>
<td>27</td>
</tr>
<tr>
<td>3OHKG dark (c)</td>
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<td>40</td>
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<tr>
<td>3OHKG dark (n)</td>
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<td>29</td>
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<tr>
<td>3OHKG normal (c)</td>
<td>396</td>
<td>64</td>
</tr>
<tr>
<td>3OHKG normal (n)</td>
<td>423</td>
<td>36</td>
</tr>
<tr>
<td>Kyn light (c)</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Kyn light (n)</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Kyn dark (c)</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Kyn dark (n)</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Kyn normal (c)</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Kyn normal (n)</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>AHBG light (c)</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>AHBG light (n)</td>
<td>39</td>
<td>11</td>
</tr>
<tr>
<td>AHBG dark (c)</td>
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<tr>
<td>AHBG dark (n)</td>
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<tr>
<td>AHBG normal (c)</td>
<td>105</td>
<td>8</td>
</tr>
<tr>
<td>AHBG normal (n)</td>
<td>203</td>
<td>19</td>
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</table>

Cataract lenses, n = 39; Normal lenses, n = 6. c, cortex; n, nucleus.

Concentrations of the UV Filters 3OHKG, Kyn, and AHBG in Light- and Dark-Colored Cataract Lenses and Normal Lenses. The lenses were analyzed for the low-molecular-weight compounds 3OHKG, Kyn, and AHBG in cataract lenses compared with normal lenses. The concentration of AHBG in cataract lenses was approximately four to five times higher than that in normal lenses. The concentration of Kyn in cataract lenses was slightly higher than that in normal lenses, but the magnitude of the difference was small compared with 3OHKG. The concentration of Trp in cataract lenses varied markedly. Seventy percent of the lenses had higher levels of Trp in the cortical and nuclear regions of the normal lenses compared with the cortex (15 pmol/mg average) and the quantities of Tyr in cataract lenses (data not shown).

### Kynurenine

As shown in Table 1, the average concentration of Kyn in cataract lenses was slightly higher than that in normal lenses, but the magnitude of the difference was small compared with 3OHKG. In normal lenses, there was a slight increase noted in the Kyn levels in the nucleus (15 pmol/mg average) compared with the cortex (10 pmol/mg average). There was less difference between the average levels in the cortical and nuclear regions of the normal lenses, with light-colored (cortex, 13 pmol/mg; nucleus, 17 pmol/mg) and dark-colored (cortex, 15 pmol/mg; nucleus, 17 pmol/mg) lenses having comparable quantities of this UV filter. Statistically, the levels in cataract lenses compared with normal lenses were not significant (dark-colored nucleus, P = 0.7749; dark-colored cortex, P = 0.5148; light-colored nucleus, P = 0.4240; light-colored cortex, P = 0.3834).

There appeared to be no correlation between lenticular age and the level of 3OHKG in cataract lenses (data not shown).

### AHBG

Table 1 demonstrates that the average levels of AHBG in cataract lenses were approximately four to five times lower than those in normal lenses. This difference in levels was similar to its metabolic precursor 3OHKG. As expected in the normal lenses,12 the concentration of AHBG in the nucleus was higher than in the cortex (cortex, 105 pmol/mg average; nucleus, 203 pmol/mg average). In the case of cataract lenses, the average values for cortical and nuclear regions were similar for both light-colored (cortex, 37 pmol/mg; nucleus, 39 pmol/mg) and dark-colored (cortex, 35 pmol/mg; nucleus, 34 pmol/mg) lenses, with the dark-colored lenses having a slightly lower amount than the light-colored lenses. The levels in cataract compared with normal lenses were all lower, and the difference was statistically significant (dark-colored nucleus, P < 0.0001; dark-colored cortex, P = 0.0505; light-colored nucleus, P < 0.0001; light-colored cortex, P = 0.0004).

There appeared to be no relationship between the age of the lens and AHBG levels in cataract lenses (data not shown).

### Tryptophan, Tyrosine, and Uric Acid

The lenses were also analyzed for amino acid analysis (AAA). This confirmed that our value for Trp. The corresponding standard curve for each compound was used to determine the concentration of each from the peak area based on absorbance at 280 nm.

### Uric Acid

The normal lenses had higher concentrations of uric acid than did cataract lenses (Table 2, with slightly elevated levels in the cortex (15 pmol/mg average) compared with the nucleus (35 pmol/mg average). There were slightly higher average concentrations of uric acid in dark-colored lenses (cortex, 16 pmol/mg; nucleus, 15 pmol/mg) compared with their light-colored counterparts (cortex, 9 pmol/mg; nucleus, 10 pmol/mg), but little difference between the cortex and nucleus. Compared with normal lenses, cataract lenses had lower levels of uric acid, and the difference was statistically significant (dark-colored nucleus, P = 0.0005; dark-colored cortex, P = 0.0004).

There was no correlation between uric acid concentration and age in cataract lenses (data not shown).

### Tyrosine

As depicted in Table 2, normal lenses had, on average, a lower concentration of Tyr than cataract lenses, although there was considerable scatter in the data. The average levels in the cortical and nuclear regions of the normal lenses were similar (cortex, 367 pmol/mg; nucleus, 381 pmol/mg). Both light- and dark-colored lenses appeared to have comparable average concentrations, although there was a difference in the light-colored lenses between the cortex and nucleus (cortex, 704 pmol/mg average; nucleus, 711 pmol/mg average). A comparison of cataract lens levels with normal lens levels, showed that the higher Tyr levels in cataract lenses were not statistically significant (dark-colored nucleus, P = 0.3616; dark-colored cortex, P = 0.5151; light-colored nucleus, P = 0.5935; light-colored cortex, P = 0.4136).

Again, no correlation was observed between the age of the lens and the quantities of Tyr in cataract lenses (data not shown).

### Tryptophan

The levels of Trp detected in normal lenses were much lower than those in most cataract lenses (Table 2). The mean levels in cataract lenses were an order of magnitude higher than those in normal lenses. There was remarkably little variation in the concentrations of Trp in normal lenses with the levels detected corresponding to those described previously by Bessem and Hoenders.19 By contrast, the concentrations of Trp in cataract lenses varied markedly. Seventy percent of the dark-colored and 58% of the light-colored cataract lenses contained levels of Trp greater than the mean levels in the normal lenses.

To confirm that in fact only Trp was in the HPLC peak and that other components had not interfered with the assay, the Trp peak from HPLC of one lens extract was collected and sent for amino acid analysis (AAA). This confirmed that our value for
Trp was correct (HPLC, 6.9 nanomoles; AAA, 8.3 nanomoles). Both dark-colored (cortex, 882 pmol/mg; nucleus, 942 pmol/mg) and light-colored (cortex, 1203 pmol/mg; nucleus, 896 pmol/mg) lenses had similar average Trp concentrations. As can be seen in these data, the cortices and nuclei of dark-colored lenses had similar levels of Trp; however, in the light-colored lenses the cortices had slightly increased levels in comparison to the corresponding nuclei. Although the majority of cataract lenses had higher Trp concentrations than normal lenses (cortex, 63 pmol/mg; nucleus, 65 pmol/mg), the differences were not statistically significant (dark-colored nucleus, $P = 0.4492$; dark-colored cortex, $P = 0.4721$; light-colored nucleus, $P = 0.3629$; light-colored cortex, $P = 0.3023$). These probabilities, however, reflect the wide variation of Trp levels throughout cataract lenses, as shown by the SEM. The SEM was very low in normal lenses, indicating that the level of Trp in normal lenses is tightly regulated.

There appeared to be no link between the ages of the lenses and the levels of Trp in the lens (Fig. 3).

Levels of ascorbic acid and glutathione, which are present in normal lenses, were not measured, because the lenses had been stored for an extensive period at –20°C and the instability of these compounds to oxidation may have produced inaccurate results.

GSH-3OHKG concentrations were also examined in all the cataract lenses studied, as this compound is known to be in normal lenses.$^{20}$ Levels were below the detection limit, however.

**DISCUSSION**

The purpose of this investigation was to compare the levels of free UV filters and other low-molecular-weight compounds in cataract lenses (from India) with those in normal lenses (from Australia).

This investigation into the low-molecular-weight compounds present in cataract lenses revealed some remarkable findings. Two major primate UV filters, 3OHKG and AHBG, were found to be present in markedly reduced levels compared with levels in normal lenses. For example, 3OHKG was detected in cataract lenses on average at approximately one sixteenth of the concentration in normal lenses. This factor alone would render the eyes of such patients more susceptible to UV damage. Although UV filter bound proteins also contribute to the absorption of UV light,$^{21}$ recent results in studies by our group show that these bound proteins, when subjected to UV light, are susceptible to photosensitization. This appears to accelerate photooxidative damage of the bound protein, thus reducing protection from UV light.

In normal lenses, 3OHKG is known to be the major free UV filter,$^{2,22-26}$ followed by AHBG and Kyn.$^{17,24}$ These were also the major free UV filter compounds present in cataract lenses, although the relative levels of the individual constituents were

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**FIGURE 2.** Uric acid, Tyr, Trp in a cataract lens. Low-molecular-weight compounds were extracted with ethanol, separated by reversed phase HPLC, and monitored at 280 nm. Peaks: 1, uric acid; 2, Tyr; and 3, Trp.
Altered significantly. Our results with cataract lenses show that whereas the Kyn levels were comparable to those in normal lenses, 3OHKG and AHBG concentrations were markedly decreased. These low levels in the cataract lenses were also lower than those in normal lenses of similar ages in the comprehensive study by Bova et al. Because Trp levels in the cataract lenses are elevated in both nuclear and cortical regions, it is clear that the reason for the markedly lower concentrations of 3OHKG and AHBG is not due to a general loss of small metabolites from the lens.

An increase in protein binding could also be indicated by these findings; however, conjugation to protein represents only a small proportion of the total amount of UV filters that are synthesized by the lens each day. The total amount bound presumably reflects a slow accumulation of UV filters and, on this basis, would not be expected to make a large difference in the measured free UV filter concentrations in the lens. In addition, studies within our group have shown that the levels of protein-bound Kyn in cataract lenses, particularly those that are at an advanced stage, are lower than in normal lenses. Therefore, our finding of a marked disparity between the free levels of Kyn and 3OHKG, which are known to bind to proteins, and also the levels of AHBG, which does not deaminate and cannot therefore bind to lens proteins, were greatly reduced, could indicate that the low levels of UV filters in the cataract lenses in this study are not solely the result of increased protein binding.

Given that Trp is the precursor for UV filter synthesis and indoleamine 2,3-dioxygenase (IDO), the first enzyme in the Trp metabolic pathway, is rate limiting, in a normal lens one may expect that high Trp levels would be associated with correspondingly high concentrations of the downstream UV filters. However, in the cataract lenses there was no direct relationship between the concentrations of Trp and 3OHKG in individual lenses (data not shown). To illustrate this, the eight cataract lenses with Trp concentrations >1000 pmol/mg (approximately 10 times greater than in normal lenses) contained 3OHKG concentrations <50 pmol/mg (approximately eight times less than in normal lenses). The finding of significantly increased Trp, together with slightly elevated Kyn, coupled to markedly reduced 3OHKG and AHBG, points to an impairment in the normal UV filter metabolic pathway in these cataract lenses.

Takikawa et al. showed no noticeable decrease in IDO activity with age in normal lenses. Studies have shown that the IDO enzyme is inhibited by H_2O_2 and, as it is thought that H_2O_2 is elevated in cataract lenses, this inhibition could cause a decrease in the activity of IDO. IDO activity in the cataract lenses, however, was not determined, since the lenses were collected in India and stored frozen for some time. Therefore, it is feasible that IDO activity could be different in these lenses.

Two further enzymatic steps in the pathway that could also be involved are the hydroxylation of Kyn, catalyzed by kynurenine 3-monoxygenase (EC 1.14.13.9), and the glucosylation of 3OHkyn by uridine diphosphate (UDP) glucosyl transferase (EC 2.4.1.15). Because 3OHkyn is present at low levels in lenses and is also highly susceptible to oxidation, we did not think that measurement of 3OHkyn in cataract lenses that had been stored for some time would be meaningful. Both the hydroxylation and the glucosylation steps require cellular organelles. It should be noted that aromatic hydroxylation is a mitochondrial reaction that the glucosylation is thought to involve microsomes, and that these can only occur in the outer regions of the lens where these organelles are present. Therefore, at this stage, we cannot properly differentiate between these two enzymatic steps. As noted, the hydroxylation is a mitochondrial reaction and so impairment at this point in the pathway could be indicative of a more general metabolic dysfunction.

In contrast, if the glucosylation reaction does not operate properly in the presence of cataract, one would expect that the levels of 3OHkyn would be elevated in cataract lenses. This would have major untoward consequences for the lens and would promote oxidation. Oxidation of proteins is the characteristic feature of ARN cataract. Assays of enzyme activities in fresh lenses extracted from patients are the only way to confirm the site(s) of the supposed metabolic problem.

It has been reported that cataract lenses contain a novel UV filter, xanthurenic acid glucoside (XASOG). We therefore looked for the presence of this compound in all the lenses we analyzed. In all but one of the cataract lenses, we did not detect XASOG. It was found in very low levels in one dark-colored lens, and its identity was confirmed by tandem mass spectrometry. It should be recognized that this novel UV filter can form artifactualy quite readily. For example, if a lens extract containing 3OHKG is left to stand, deamination followed by cyclization and oxidation can occur, because of the inherent instability of 3OHKG, thus leading to XASOG.

The other striking finding from this study was that the levels of Trp, and to a lesser extent Tyr, were considerably elevated in most of the cataract lenses. The reason for this is not clear, but it was true of both light- and dark-colored lenses. When we consulted the literature for precedents, we discovered a comprehensive study performed in 1968 by G. Winston Barber. In his investigation of free amino acid concentrations in cataract lenses, however, was not determined, since the lenses were collected in India and stored frozen for some time. Therefore, it is feasible that IDO activity could be different in these lenses.
ract and normal lenses, he reported a dramatic increase in the lens levels of proteogenic amino acids, although he did not measure Trp. In his study, approximately 60% of patients with cataract had lens levels of the proteogenic amino acids greater than normal lenses. Barber proposed that proteolysis of lens proteins could be responsible for the increased amino acid levels. However, the relative amounts of the free proteogenic amino acids do not correspond to those present as lens proteins.

Our results demonstrate (Fig. 4) that those lenses with higher Trp concentrations also had increased Tyr concentrations and those with less Trp also contained a lower concentration of Tyr. These findings could suggest that the same phenomenon occurs with some of the other proteogenic amino acids that were measured by Barber. The increased levels that we found of Trp and Tyr and those found by Barber of other proteogenic amino acids could be caused by an increase in proteolysis in the cataract lens. However, we did not measure levels of the other amino acids. It would be difficult to confirm whether increased proteolysis is indeed the reason for these higher levels; however, we do not believe that increased proteolysis of lens proteins alone can be responsible for our observations. Free Trp levels were one fifth those of Tyr in normal lenses, but in the cataract lenses Trp levels were higher on average than levels of Tyr.

Remarkably, two lenses contained Trp levels nearly 100 times greater than the normal mean level. In the case of Trp, additional information is available that may provide an indicator as to the reason for the unusually high levels of this amino acid within the lens. A study comparing serum Trp and Trp metabolites in patients with cataract, after an oral load of Trp, found that a major subgroup of patients tended to have lower serum Trp levels both at rest and after an oral load of Trp. In contrast, elevated levels of serum Kyn (derived from cellular metabolism) were observed in these patients after the Trp load. This result suggests that, at least in the case of Trp, there may be a greater degree of transport of this amino acid into cells in patients with cataract. Barber’s data suggest that such a defect may not be confined to Trp, but may be a more widespread problem with all proteogenic amino acids.

In our study, we found that although Tyr levels were above normal, uric acid concentrations were markedly reduced in cataract lenses. Uric acid is an antioxidant present in the lens that is more stable than ascorbate or glutathione; thus, the lower levels found in this study may give an indication of the antioxidant status of the cataract lenses. The study by Bessems and Hoenders used HPLC to quantify the levels of Tyr and uric acid, as well as Trp, in cataract and normal lenses. Concentrations of Trp, Tyr, and uric acid in our normal lenses were comparable to those that they found. The level of Tyr found by Barber was also similar. In the case of uric acid, the quantities in the cataract lenses studied by Bessems and Hoenders were higher than those in our study, although the levels of Trp they reported in cataract lenses were lower. The reasons for this are unclear, but may be a reflection of the site of collection or the classification of cataracts used by Bessems and Hoenders.

Our cataract lenses were obtained from India, and the normal lenses from Australia. Although it would have been preferable to look at lenses from the same population, it was not possible because of the surgical procedures used for cataract surgery in Western countries. Because Barber obtained results similar to ours on amino acid contents and he used lenses derived from patients in the United States, it seems unlikely that our elevated amino acid levels were due to genetic or dietary factors related to their Indian origin.

The data reported from our study are an indication that there may be metabolic defects associated with most human cataracts at the level of amino acid and UV filter metabolism. It is tempting to suggest that these defects could be, in part, causative of human ARN cataract.

Acknowledgments

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