Ultraviolet Photography of the In Vivo Human Cornea Unmasks the Hudson-Stähli Line and Physiologic Vortex Patterns

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PURPOSE. To perform ultraviolet (UV) macrophotography of the normal in vivo human cornea, establishing biometric data of the major component of UV absorption for comparison with the Hudson-Stähli (HS) line, the distribution of iron demonstrated by Perl stain, and cases of typical amiodarone keratopathy.

METHODS. Nonrandomized comparative case series of UV photographs of 76 normal corneas (group 1) and 16 corneas with typical amiodarone keratopathy (group 2). Image-analysis software was used to grade the major component of UV absorption for slope and the coordinates of its points of intersection with the vertical corneal meridian and inflection.

RESULTS. In group 1 the major component had a mean slope of 5.8°, sloping down from nasal to temporal cornea. The mean coordinates of points of intersection with the vertical corneal meridian and inflection were (0, 0.30) and (0.02, 0.31), respectively. No significant differences between groups 1 and 2 were found for slope ($P = 0.155$), intersection with the vertical corneal meridian ($P = 0.517$), and point of inflection ($P = 0.544$). The major component of UV absorption was consistent with published characteristics of the HS line, and coincidence of UV absorption and Perl-stained iron was demonstrated in one corneal button. A vortex pattern of UV absorption was observed in all corneas.

CONCLUSIONS. UV photography demonstrates subclinical corneal iron, confirming its deposition in an integrated HS line/vortex pattern. Coincident iron and amiodarone deposition occurs in amiodarone keratopathy. (Invest Ophthalmol Vis Sci. 2005;46:3616–3622) DOI:10.1167/iovs.04-1455

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Ultraviolet (UV) photography of human tissue reveals features that are not seen in visible light. There are few reports of human UV photography aside from applications of the Wood’s lamp in dermatology, forensic pathology, and reports of contact lens fitting with fluorescein dye.

The requirements for UV photography of the ocular surface and the camera system used in this study have been reported elsewhere. Generic UV photography requires: (1) controlled studio illumination or appropriate filters so that only UV radiation exposes the film; (2) a UV radiation source; (3) a UV-transmitting lens system; (4) a focus system; and (5) UV-sensitive film.

UV irradiation of the in vivo eye requires consideration of the spectral properties of ocular tissues and radiometric dosimetry. The ocular media act as a series of spectral filters characterized in studies of the rabbit and human eye, documenting the spectral properties of tears, whole cornea and its component layers, aqueous humor, and the lens. Kinsey established the threshold wavelength for corneal transmission at 290 nm, increasing to 78% transmission at 320 nm, and a threshold for lenticular transmission at 320 nm (the bandwidth 290–320 nm being absorbed by the lens). The action spectrum of UV keratitis and cataract has the lowest threshold for damage at 290 nm, whereas UV-induced free radical formation in the cornea and lens is negligible above 320 nm. The bandwidth of the light used to irradiate ocular tissues in the current study was 318 to 328 nm, to maximize corneal absorption and minimize light hazard. The International Commission on Nonionizing Radiation Protection has recommended an exposure limit of 1 J/cm² over an 8-hour period for UV wavelengths incident on unprotected eyes.

Preliminary UV photographs taken of the human in vivo cornea revealed a UV-absorption pattern suggestive of an integrated Hudson-Stähli (HS) line and vortex pattern. Our impression was that corneal ferritin is unmasked in UV light for the following reasons: the occurrence of maximum UV absorption at the typical location of the HS line, the similarity between corneal absorption patterns in UV light with the HS line photographed in cobalt blue light, and spectral absorption characteristics of ferritin in the UV region.

The physiologic HS line composed of ferritin has an obscure origin that is increasingly attributed to epithelial cell migration. On slit lamp examination (SLE) with cobalt blue light, Bron described the HS line as consisting of a horizontal major component, and radial minor components which converge to the inflection of the major component, and his terminology is used in this study (Fig. 1). Bron comments that “when the [HS] line is very strongly developed, the figure produced strongly resembles the framework of a vortex pattern.” Pathologic vortex patterns that occur in various inherited metabolic diseases, treatment with amphiphilic cationic drugs (most commonly amiodarone) and local corneal disease, are due to an opaque pigment depositing in otherwise transparent corneal epithelium unmasking a physiologic cellular arrangement. The purpose of this study was to investigate whether UV photography of the cornea would add new information to this field.

METHODS

The UV source was a broad-band xenon arc lamp with output optics of a 25-mm diameter aperture; a 5-D quartz condensing lens, to give
Hudson-Stähli Line and Physiologic Vortex Patterns

Subjects were recruited at Dunedin Hospital between June 2002 and June 2003, after consultation with an ophthalmologist or in response to recruitment posters. Inclusion criteria for group 1 (normal cornea) consisted of a normal ocular surface determined by SLE, including subjects with isolated posterior segment disease or prior cataract extraction. Exclusion criteria consisted of an abnormal surface (Nikon 0/4.5; Nikon) confocal for visible and UV wavelengths.6 Two black-and-white films sensitive to UV wavelengths were used (initially; model XPF, Ilford, Basildon, UK; and, subsequently, T-Max 400; Eastman Kodak, Rochester, NY) which has UV sensitivity down to 250 nm.6 Spectral scans of the light source with a spectroradiometer (model II700: International Light, Newburyport, MA) and a double monochrometer/photomultiplier (GM200; International Light, Newburyport, MA) determined total power in the corneal plane to be 0.1 mW/cm². As photographs were taken with a 1-second exposure, the total exposure dose per photograph in this study was 0.1 mJ/cm², which is one-ten thousandth of the 8-hour exposure limit for humans.31 Local ethics committee approval was granted, and the study adhered to the tenets of the Declaration of Helsinki.

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Initial SLE of the cornea used both white and cobalt blue light—the latter increasing the ability to observe corneal iron.53 The presence of the HS line was recorded if any typically located ferritin was observed, regardless of length or intensity. Topical fluorescein was avoided before UV photography. Strict vertical positioning of the subject’s head ensured there was no head tilt that would affect orientation of the UV photographs. The lens was focused in ambient light before UV photography. Films were developed using standard processors, and subsequent Perl staining duplicating the method published by Barraquer-Somers et al.40 Using SLE in white light, we made a sketch of the pattern of amiodarone keratopathy in one subject to allow subsequent comparison with UV photographs. This was achieved by replacing the standard slit lamp eye piece with one containing a grid graticule, which was centered on the pupillary axis, enabling a sketch of the amiodarone keratopathy on grid paper.

RESULTS

Group 1 included 76 normal corneas (38 subjects), comprising 20 women and 18 men (mean age, 47 years; range 20–82). Photographs of four subjects are shown in Figure 2. Fifteen subjects (40%) had an HS line visible on SLE (13 bilateral, 2 unilateral) compared with 38 subjects (100%) who had a major component consistent with the HS line on UV photography. The major component, with or without contributions from minor components, was traceable across the cornea from limbus to limbus in 66 (87%) of 76 cases.

Group 2 consisted of 16 corneas (eight subjects; mean age, 82 years; range 73–96). The UV photograph and pattern of amiodarone deposition sketched in white light for one subject is shown in Figure 3.

The minor components that collectively give rise to a vortex pattern were evident in all subjects in groups 1 and 2 and are self-evident in Figures 2 and 3. The mean slope of the major component in groups 1 and 2 was 5.8 ± 3.2° (SD) and 6.7 ± 1.2°, respectively, with no significant difference (P = 0.155). Subgroup analysis of group 1 showed no significant difference in slope when analyzed for age less than or more than 45 years (P = 0.864), sex (P = 0.675), or laterality (P = 0.107).

The mean coordinates of the intersection of the major component with the vertical meridian in groups 1 and 2 were (0, 0.30) and (0, 0.31) respectively, with no significant difference found (P = 0.517). Subgroup analysis of group 1 showed no significant difference when analyzed for age less than or more than 45 years (P = 0.309), sex (P = 0.382), or laterality (P = 0.687).

The coordinates of the point of inflection in groups 1 and 2 were (0.02, 0.31) and (0.02, 0.33) respectively, with no significant difference on univariate or multivariate analysis (P = 0.344). Subgroup analysis of group 1 showed no significant difference in location of point of inflection when analyzed for age less than or more than 45 years (P = 0.184), sex (P = 0.197), and laterality (P = 0.152).
Data representing the slope and coordinates of intersection with the vertical meridian and inflection for paired right and left eyes from group 1 are plotted in Figure 4. Figure 5 shows UV photography and Perl staining of the same donor corneal button.

**DISCUSSION**

The major component of corneal UV absorption was evident in UV photographs and was consistent with published data on the HS line observed in white light. The coincidence in one corneal button of UV absorption lines and corneal iron staining established that UV photography unmasked the distribution of corneal ferritin which was predominantly subclinical on SLE with white light. The HS line remains an age-related phenomenon in white light; however, it was a constant phenomenon in UV light in the population photographed in this study.

The appearance of the cornea photographed in UV light was due to absorption of UV light by ferritin highlighted against a background illuminated by unknown components of reflection, backscatter, and protein fluorescence from the cornea, iris, and lens. The contribution of lens fluorescence is illustrated in Figure 2D which shows a phakic left eye and a pseudophakic right eye (Implant: SI40NB, Allergan, Irvine, CA), with left corneal UV absorption highlighted by lens fluorescence. The UV absorbance of modern intraocular lenses has been described by Laube et al.

In group 1, the prevalence of the HS line was 100% (78/78) in UV photographs compared with 40% (30/78) detected with SLE. Prevalence studies of the HS line on SLE reported by Norn and Rose and Lavin involved the use of white light alone. Norn excluded faint HS lines and reported prevalence rates of 29% and 18% in consecutive studies, whereas Rose and Lavin included them, reporting rates of 69% bilaterally and 4% unilaterally. These rates are lower than the 100% prevalence of the HS line in UV photographs reported herein. However, the results of histologic studies staining for corneal iron are consistent with our findings. Gass reported iron in 95% of stained corneas including 10 from subjects younger than 21 years, and Barraquer-Somers et al. demonstrated the HS line in 90% of stained corneas.
In group 1 the mean slope of the major component was 5.8° ± 3.2° sloping down from nasal to temporal cornea. We do not consider it possible to determine accurately the gradient of the HS line in a mobile eye with SLE, and previous studies have generally emphasized its horizontal orientation. Norn describes the HS line as horizontal in 61% of 406 eyes, stating that in subjects with bilateral HS lines, the lines usually sloped from nasal down to temporal cornea with the opposite slope observed in only 2 of 406 eyes. Rose and Lavin describe the HS line as generally horizontal in the temporal cornea, rising in the nasal cornea. Bron described the HS line as horizontal; however, his illustration of a right eye with a well-developed HS line photographed in cobalt blue light, shows it sloping down from nasal to temporal cornea.

In group 1 the coordinates of the intersection of the major component with the vertical meridian were calculated at (0, 0.29) and are consistent with published data. Norn reports the mean height of the HS line to be 3.7 mm above the inferior limbus in 168 eyes. Given that the mean vertical corneal diameter in the adult population is 11.7 mm, the coordinates are (0, 0.32), and they have been usefully approximated to the junction of the middle and inferior thirds of the cornea.

The coordinates of the inflection of the major component in group 1 were (0.02, 0.31) reflecting its location in the vertical meridian in 52 (68.4%) of 76 corneas, more nasal in 22 (28.9%) of 76 corneas and temporal in only 2 (2.7%) of 76 corneas. The data for subgroup analysis of right and left corneas is plotted in Figure 4. Bron describes the minor components converging to the inflection point of the major component, and this is evident in Figures 2 and 3.

In group 1, 87% (66/76) demonstrated a major component with or without minor component contributions, which could be traced horizontally across the cornea from limbus to limbus. Published lengths of the HS are significantly shorter; however,
our impression is that the length of the HS line is largely a function of detection, and improved contrast provided by UV photographs results in increased visible lengths. Rose and Lavin\textsuperscript{49} report a length ratio of the HS line greater than half the maximum horizontal corneal diameter in only 35% of subjects bilaterally and 7% unilaterally. Gass\textsuperscript{50} and Duke-Elder\textsuperscript{51} state that the HS line does not reach the limbus, and Norn\textsuperscript{57} calculated its average length as 1.5 mm.

This study had several potential sources of error that may bias the biometric data of the major component, including errors of head or ocular alignment and measurement errors in the software (Photoshop; Adobe Systems). We attempted to control alignment errors by vigilant head positioning and encouraging the subject to fixate the axis of the camera lens, facilitated by prompt photography when studio lights were extinguished for UV photography. With the software, calculation of coordinates was very accurate, but estimation of the gradient was interpolative, requiring superposition of a line of best fit over the major component and systematic bias may have occurred.

Comparison of groups 1 and 2 showed no statistically significant differences for any graded feature of the major component, and Figure 3 showed coincidence of the pattern of amiodarone sketched on SLE with the lines of maximum UV absorption. This suggests that amiodarone deposition is superimposed on the physiologic pattern of ferritin deposition and that these corneal pigments are not the mutually exclusive corneal deposits they appear to be on SLE with white light. A potential confounder was the unknown UV-absorption characteristics of the corneal amiodarone lysosomal complex. It is possible that corneal amiodarone completely displaces ferritin, and if it absorbed the irradiating bandwidth 318 to 328-nm, absorption lines in UV photographs would mimic the presence of ferritin. Clarification of this would require UV spectral studies of corneal amiodarone lysosomal complex or Perl-staining corneal buttons with amiodarone keratopathy. The only UV absorption spectrophotometry of amiodarone in the literature is that of amiodarone in solution.\textsuperscript{55}

The horizontal orientation of the HS line and its location at the middle and inferior thirds of the cornea are attributed by Gass\textsuperscript{50} and Lemp and Mathers\textsuperscript{56} to the line of lid closure and by Barraquer-Somers et al.\textsuperscript{40} to the inferior tear meniscus. However, there is no strong evidence that line of lid closure correlates with the location of the HS line. Spontaneous blink dynamics recorded on high speed video by Doane\textsuperscript{58} describe the lower lid primarily undergoing horizontal translation nasally on closure and temporally on opening, sometimes accompanied by a downward movement of 1 to 2 mm. This suggests that the line of lid closure is well below the location of the HS line. Bron\textsuperscript{53} suggests that the HS line occurs at the junctional fronts of centripetally migrating epithelial cells, and Rose and Lavin\textsuperscript{55} postulate that its inferior location is due to greater epithelial population pressure from the superior limbus.

Bron\textsuperscript{53} proposed that “the same growth and repair patterns of the corneal epithelium” result in generic vortex corneal patterns; however, the pathophysiology of this phenomenon remains unclear. Thoft and Friend\textsuperscript{58} synthesized contemporary knowledge regarding epithelial cell population maintenance into the $X,Y,Z$ hypothesis, respectively describing mitotic, centripetal movement and exfoliative components in the corneal profile. Lemp and Mathers\textsuperscript{56} suggested that these components might have regional variations, proposing that centripetal epithelial cell movement ($Y$) in the corneal plane varies as a function of blink force applied to the corneal surface. Lavker and Sun\textsuperscript{59} stated that the limbal stem cells are compartmentalized at the limbus, indicating that movement of transient amplifying cells ($X$) into the corneal plane is discontinuous around the corneolimbus.

Although these observations are consistent with the development of corneal vortex patterns, they do not explain its origin. Dua and Forrester\textsuperscript{60} describe preferential circumferential migration of peripheral epithelial cells, suggesting this may impart a torsional force to epithelial cell migration; however, this was in the context of wound healing and may not be relevant to epithelial cell population homeostasis. Subsequently, Dua et al.\textsuperscript{61} demonstrated the effects of static magnetic fields on cultured corneal epithelium, suggesting that the eye acts as a dipole oriented along its anteroposterior axis, influencing the migration of cells containing magnetic compounds (e.g., ferritin). Nagasaki and Zhao\textsuperscript{62} recorded vortex patterns in a transgenic mouse expressing a green fluorescent protein, writing “the vortex is a natural consequence of many cells converging in a small central area from all directions”—reiterating Bron’s 1973 concept without further elaboration.

The source of iron incorporated into the HS line and vortex pattern is unknown. Gass\textsuperscript{50} proposes the following sources for corneal iron: tears, blood plasma, breakdown of blood in perilimbal tissues, the aqueous, and a breakdown of intracellular cytochrome enzymes. Rose and Lavin\textsuperscript{55} and Assil et al.\textsuperscript{59} presented arguments against the tear film as a source of iron and postulated the deposition of corneal iron in relatively senescent basal epithelial cells. Rose and Lavin\textsuperscript{55} emphasized the confluence of migrating sheets in the formation of iron lines in general. Cai et al.\textsuperscript{63} provided evidence that showed that ferritin in corneal epithelium protects against DNA mutations, and Applegate et al.\textsuperscript{64} show that ferritin is induced by UV irradiation of the skin. It may be that UV radiation induces corneal
epithelial ferritin locally, which would be capable of scavenging free iron and minimizing consequent oxidative damage. Future work with UV photography could include correlation of the HS line with corneal topography and high-speed video recordings of individual blink dynamics. The development of a digital camera system is desirable, and Inoue and Spring\(^5\) discuss increasing the sensitivity of charge coupled diodes to UV wavelengths.

**References**


