



**Glucose Concentration Profiles
of Normal and Ultraviolet Radiation-Exposed Rabbit Corneas
(Reprint)**

By

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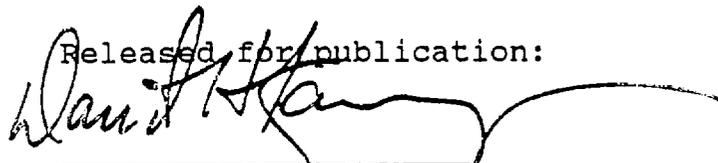


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the presence of an active transport mechanism capable of delivering glucose into the corneal epithelium against a concentration gradient. The presence of a transport system that 'pulls' glucose through the deeper corneal layers thus would make epithelial integrity important for the maintenance of overall corneal viability.

Glucose Concentration Profiles of Normal and Ultraviolet Radiation-Exposed Rabbit Corneas

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The passage of glucose within the cornea has been thought to occur by passive diffusion processes. However, corneal glucose concentration profiles have not been established to support this notion. While microfluorometric methods of metabolite assay typically have been used as a means of assessing regional brain metabolism, this unique methodology of tissue isolation and metabolite determination has not previously been applied to the cornea. Since this technique permits metabolite quantification on microgram-sized tissue samples, a co-ordinated corneal glucose concentration profile can be obtained. Tissue preparation consisted of liquid nitrogen freezing, cryo-sectioning, and freeze-drying, with storage at -20°C . The sections were thawed under vacuum pump, subsectioned, weighed, and assayed for glucose concentration (by dry weight).

This study established a glucose concentration profile of the epithelium, anterior stroma, mid-stroma, posterior stroma, and endothelium for the normal pigmented rabbit cornea. A glucose concentration profile for UV radiation-exposed rabbit corneas also was documented. The UV radiation glucose profile data indicate the presence of an active transport mechanism capable of delivering glucose into the corneal epithelium against a concentration gradient. The presence of a transport system that 'pulls' glucose through the deeper corneal layers thus would make epithelial integrity important for the maintenance of overall corneal viability.

Key words: active transport; cornea; glucose; passive diffusion; UV radiation.

1. Introduction

Glucose nutrition of corneal tissue presents a unique metabolic problem in that the bulk of the tissue is remote from any vasculature. The generally accepted theory of glucose passage into and through the cornea is one of passive diffusion down a concentration gradient from the aqueous humor (Reim, Lax, Lichte, and Turss, 1967; Turss, Friend and Dohlman, 1970). As an adjunct to the passive diffusion theory, some investigators have identified possible active transport mechanisms for glucose passage from the aqueous into the endothelium, with subsequent glucose passage within the cornea occurring by passive diffusion (Hale and Maurice, 1969; DiMaggio, 1984). It appears that other potential sources of corneal glucose have been deemed insignificant since there is very little glucose in the normal tear layer (van Haeringen, 1981), and little glucose is capable of reaching the central cornea for the vessels near the limbus (Davson, 1969).

Early studies attempting to quantify a corneal glucose concentration profile were limited by available assay and tissue separation techniques. Microfluorometric assay methods, using freeze-dried, microgram-sized corneal tissue samples, allow a localized determination of glucose concentration (Lowry and Passonneau, 1972), making it possible to obtain a co-ordinated corneal glucose concentration profile. Since UV radiation (UVR) may act to inhibit corneal tissue metabolism (Lattimore, 1987), the purpose of the UVR portion of the study was to examine changes in laminar corneal glucose concentrations as a relative index of 290-nm UVR damage.

2. Materials and Methods

Experimental animals

Healthy adult pigmented rabbits were used for the experimental animals. All animals were procured from a single source to ensure constant breeding practices. The animals were housed in NIH-approved quarters under controlled, normal lighting conditions. The animals were maintained, and the experiments were conducted, in accordance with procedures outlined in the 'Guide for Laboratory Animal Facilities and Care' of the National Academy of Sciences-National Research Council. Anesthesia was maintained throughout the course of the experiment with i.m. injections of Ketamine (10 mg kg⁻¹) and Rompun (5 mg kg⁻¹). The UVR-exposed animals were killed by cervical dislocation 2 min after the exposure was discontinued. Then, the eyes immediately were removed and immersed in liquid nitrogen in order to prevent significant change in metabolite levels. Control, or normal animals, underwent a mock-exposure period and the same tissue preparation procedures.

Processing procedure

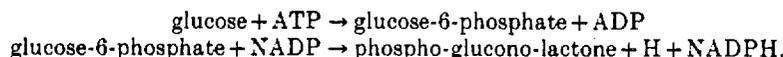
The rabbit eyes were transferred from the liquid nitrogen container into a -80°C freezer for storage until tissue sectioning could be accomplished. The cornea was removed from the globe by dissection under -30°C conditions in a cryostat hood. The isolated cornea was cut into hemispheric halves and mounted on a sectioning button with mounting-paste by immersion in a dry-ice-cooled hexane solution. The corneal button-mount then was transferred to a cryostatic microtome where tissue sectioning was performed. The resulting central corneal cross-sections were approx. 20 μm in thickness. The sectioned tissue samples were placed in a metal tissue-holder, covered with glass slides, and inserted into a vacuum tube. The tube was placed in a -20°C freezer and attached to a vacuum pump. The tissue then was freeze-dried for a 24-hr period. After the freeze-drying process was completed, the tissue was kept at -20°C until assayed.

Samples needed for assay were thawed under vacuum for 1 hr to prevent condensation-stimulated enzyme action. The different layers of the cornea were clearly defined, permitting easy isolation and separation of the corneal laminae under a 3× binocular dissecting microscope. Tissue size was determined by dry weight permitting the analysis of very small and irregularly shaped specimens. The tissue samples were immediately weighed on a quartz-fiber fishpole balance possessing microgram sensitivity. After weighing and recovery, the samples were placed in an oil-well rack for specific metabolite assay.

Underlying principles

The cycling system contains two enzymes which catalyse two interrelated reactions yielding a 'net-reaction'. A byproduct of this two-step reaction is NADPH, which fluoresces at 460-nm wavelength when excited with UVR of 340-nm wavelength. By measuring the amount of reduced pyridine nucleotide fluorescence with a Ratio Fluorometer-2 (Farrand Optical Co., Inc.), the original concentration of the assayed metabolite can be calculated.

The specific net reaction is:



A sterile oil-well rack was used to house the assay system. The rack possessed 60 'cells', each measuring 3 mm in diameter and 5 mm in depth. Each cell, filled with an inert oil preparation, served as the isolation chamber for each tissue sample. Appropriate blanks and standards were employed to monitor the reliability of the assay. The glucose assay required placement of a weighed tissue sample into a 1-μl bubble of 0.02 N HCl held within a cell in the oil-well rack. Initial processing consisted of incubation for 20 min at 60°C. The acid treatment and incubation were sufficient to destroy preformed NADPH as well as tissue enzymes, but too mild to hydrolyse an appreciable amount of glycogen (Lowry and Passonneau, 1972). Subsequent enzymatic cycling ensured that glucose-1-phosphate and glucose-6-phosphate originally present in the tissue sample were driven to an ending before commencement of the glucose assay by the addition of a hexokinase glucose reagent and

incubation at 60°C for 20 min. As a result, glucose was the only substrate in the sample that could lead to NADPH generation under the specific assay conditions. Further enzymatic cycling, by the addition of G6PDH reagent and by incubation at 38°C for 60 min, permitted the final assay step. Reaction activity was terminated by heating the assayed material to 100°C for 3 min.

Exposure instrumentation

The UVR source was a 5000-W xenon-mercury (Xe-Hg) high-pressure arc lamp (Hanovia, Inc.), powered by a 10-kW direct current power supply regulated to $\pm 0.5\%$, and capable of delivering 0–80 A at 25–65 V to the arc electrodes. The lamp housing was cooled by a double blower system. The radiation from the source was focused at a double monochromator entrance slit by the housing optics. A 10-cm quartz-enclosed water chamber was placed between the focusing lenses and the monochromator in order to remove the IR radiation. The exit optical beam was focused by a quartz lens with a beam size of 1.6×1.8 cm at the plane of the experimental animal's cornea.

The desired UVR waveband was obtained with a Czerny-Turner double grating monochromator (Model 25-100; Jarrell Ash Division, Fisher Scientific, Waltham, MA) possessing a double mirror set-up with gratings blazed at 300 nm and grooved with 1180 grooves mm^{-1} , allowing approx. 5.0-nm bandpass. The linear dispersion equates to a value of 0.82 nm mm^{-1} . Entrance, intermediate, and exit slits were set to pass a nominal full bandpass of 6.6 nm. The double monochromator system was aligned with a helium-neon laser and the wavelength counter was calibrated with a mercury source. Exposure durations were set with a Gerbands electronic shutter (Ralph Gerbands Co. Inc., Arlington, MA) controlled by a HP Model 5330B preset counter. The preset counter allowed exposure durations of any desired length with millisecond accuracy.

Source measurement

An Eppley 16 junction thermopile, serial no. 12968 (Eppley Laboratory Inc., Salem, MA), traceable to a National Bureau of Standards (NBS) standard source, was used to characterize the spectral irradiance of the UVR source. When taking the spectral irradiance readings, the thermopile was placed in the same position relative to the monochromator exit port as the rabbit's cornea was to be situated during UVR exposure. The irradiance (E_e), in W cm^{-2} , incident on the thermopile was determined by the relationship $E_e = kVt$. The value k represents the thermopile calibration constant in $\mu\text{W cm}^{-2} \mu\text{V}^{-1}$, while the value Vt represents the thermopile-voltmeter reading in μV . The radiant exposure (H), in J cm^{-2} , was calculated by the formula $H = tE_e$. The value t is simply time in sec; it should be kept in mind that a J is defined as a W-sec. Therefore, for a given irradiance E_e , the exposure duration t can be varied to obtain different values of radiant exposure H as desired. The above means of output characterization and source calibration was estimated to have a $\pm 10\%$ accuracy. The specific source quantification values may be found on Table I.

3. Results

The normal glucose profile assay was performed on 15 different tissue samples in each lamina from three different rabbit eyes. A summary data chart for the control

TABLE I
Quantification of source output

Vt	(thermopile reading)	2.5 μV
k	(thermopile calibration constant)	5.131 $\mu\text{W cm}^{-2} \mu\text{V}^{-1}$
E_e	(irradiance)	12.8 $\mu\text{W cm}^{-2}$
t	(time)	3898 sec
H	(radiant exposure)	0.05 J cm^{-2}

group is provided in Table II. The UVR-exposed glucose profile assay also was performed on 15 different tissue samples in each lamina for three different rabbit eyes. A summary data chart for the 290-nm UVR-exposed group is provided in Table III.

Figure 1 provides a barchart illustration of both the normal and 290-nm UVR-exposed corneal glucose concentrations as a function of the corneal layer. A two-way analysis of variance of the data demonstrated a highly significant between-groups effect ($P < 0.0001$), with specific comparisons revealing each UVR experimental group to be very significantly different from each corresponding normal group ($P < 0.001$).

TABLE II
Normal corneal glucose profile

	Epi	A. Str	M. Str	P. Str	Endo
Mean concentration	5.63	8.20	8.67	8.68	13.48
s.d.	0.68	0.93	1.09	0.97	1.05

The data were gathered from 15 different samples in each lamina, from three different rabbit eyes.

TABLE III
UVR-Exposed corneal glucose profile

	Epi	A. Str	M. Str	P. Str	Endo
Mean concentration	19.16	13.64	12.80	12.79	26.07
s.d.	2.01	2.33	1.60	2.38	4.31

The data were gathered from 15 different tissue samples in each lamina from three different rabbit eyes.

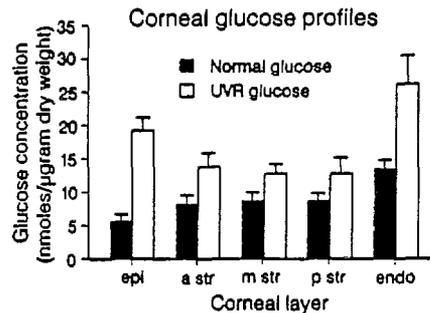


FIG. 1. A comparison bar chart allows the key results to be highlighted: 1. There is an accumulation of glucose in all five corneal laminae. 2. There is an abnormally high accumulation of glucose in the epithelium, above the stromal accumulation. These results indicate the presence of an active glucose delivery mechanism in the epithelium that is capable of acting against a concentration gradient.

4. Discussion

Since the normal glucose profile is roughly linear in nature (Fig. 1), it outwardly appears to illustrate the notion that glucose moves within the cornea in a posterior to anterior direction via passive diffusion. However, the UVR-exposed glucose profile

(Fig. 1) is a U-shaped function, demonstrating a nonuniform accumulation of glucose in all five corneal laminae. The abnormally high accumulation of glucose in the corneal epithelium, above the stromal accumulation, indicates the presence of an active glucose delivery mechanism, at the level of the epithelium, that is capable of acting against a concentration gradient. An epithelial glucose accumulation higher than that of the stroma could not occur as a result of passive processes or as a result of altered permeability; either situation would provide an essentially flat concentration profile.

Corneal epithelium has been shown to possess large amounts of glycogen (Thoft and Friend, 1977) which could be contributing to the experimental glucose assay secondary to UVR exposure. In a related study, epithelial glycogen concentrations were obtained for both the normal and 290-nm UVR-exposed corneas using a similar microfluorometric assay technique. The normal corneas had a mean epithelial glycogen concentration of 38.23 ± 10.30 nmol μg^{-1} dry wt, while the UVR-exposed corneas had a mean epithelial glycogen concentration of 77.42 ± 11.81 nmol μg^{-1} dry wt. A *t* test comparison revealed the data to be significant ($P < 0.0001$). It is clear that the UVR-increased epithelial glucose found in this study is not a result of glycogen breakdown, since glycogen also is shown to accumulate secondary to 290-nm UVR exposure. Since there is a decrease in epithelial glucose utilization without an equal decrease in glucose delivery, the delivery system would appear to be relatively insensitive to immediate effects (i.e. within 2 min after exposure) of UVR.

The generalized glucose accumulation appears to indicate a decrease in metabolic activity throughout the entire cornea as a result of UVR exposure. The increased endothelial glucose is surprising since 290-nm UVR theoretically does not penetrate the cornea beyond the stroma (Boettner and Wolter, 1962; Barker, 1979). In order for endothelial glucose to be increased, 290-nm UVR would have to directly interact with the endothelium, or there would have to be an indirect effect that causes a secondary increase in the aqueous glucose concentration. The latter is certainly possible since UVR has been shown to break down the blood-aqueous barrier (BAB) in the rabbit, thereby allowing protein leakage into the aqueous humor (Peyman, Fishman, Alexander, Woodhouse and Weinreb, 1986). It would be reasonable to assume the smaller glucose molecule could easily pass into the aqueous at a greater than normal rate under BAB-breakdown conditions, thus explaining the highly elevated endothelial glucose concentration following UVR exposure. In any event, regardless of whether the increased endothelial glucose is due to direct or indirect effects, 290-nm UVR must necessarily penetrate to the endothelium or deeper for this phenomenon to occur. It should be stressed that the elevated endothelial glucose could not be responsible for the elevation of epithelial glucose values above that of the stroma. So while there may be some question concerning UVR transmittance, it does not influence the conclusion regarding epithelial glucose transport.

Possible mechanism

What mechanism might accomplish this glucose delivery? A sodium-glucose complex could be capable of moving glucose into the epithelium down a sodium ion concentration gradient from the stroma; once inside the tissue, the combined sodium-glucose molecule would dissociate, with the sodium then actively pumped out of the epithelium into the stroma. Such a system would be capable of establishing the U-shaped glucose concentration profile found in UVR-exposed corneas.

A concentration gradient of sodium ions at the epithelial-stromal border has

previously been demonstrated in the rabbit (Klyce, 1971). In addition, a sodium-glucose complex has been demonstrated to be responsible for carrying glucose in other tissues (Deves and Krupka, 1978; West, 1980; Stryer, 1981; Turner, 1981; Handler, 1983). Therefore, it would not be unreasonable to apply such a glucose transport theory to the cornea. Since the rate and extent of glucose transport would depend on the magnitude of the sodium ion concentration gradient across the epithelial-stromal border, corneal epithelial integrity would thus be critical for the normal rate of glucose passage throughout the entire cornea. As a result, a healthy epithelium may be a prerequisite for a healthy stroma and endothelium.

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