Mechanisms of Ocular Toxicity Using the in Vitro Bovine Lens and Sodium Dodecyl Sulfate as a Chemical Model

Vladimir Bantseev,* David McCanna,*† Alice Banh,* Winnie W. Wong,* Kelley L. Moran,* D. George Dixon,* John R. Trevithick,*‡ and Jacob G. Sivak*†

*School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 Canada; †Bausch and Lomb, Rochester, New York 14603-0450; and ‡Faculty of Medicine and Dentistry, Department of Biochemistry, University of Western Ontario, London, Ontario, N6A 5C1 Canada

Received December 4, 2002; accepted February 8, 2003

Previous work using the in vitro bovine lens as a model has shown a correlation between toxicity and lens optical function and showed much higher sensitivity in detecting irritancy of several surfactants at much lower concentrations than the Draize score. In the current study, cultured bovine lenses were used to study the effects of the surfactant sodium dodecyl sulfate (SDS) on lens optical properties and mitochondrial integrity. Bovine lenses were exposed to SDS (0.1 to 0.00625%) for 30 min and cultured for 24 h. Compared to controls (n = 17), loss of sharp focus was evident immediately following exposure to 0.1% SDS (n = 14, p < 0.0001). At 24 h loss of sharp focus became evident in all groups. Loss of lens transparency, significant increase in lens wet weight, and axial length were seen 24 h postexposure in lenses treated with 0.1 to 0.025% SDS. Confocal analysis 24 h postexposure showed SDS concentration-dependent decrease in number and length of the mitochondria in lens epithelial and superficial cortical fiber cells. The results of this study show a correlation between lens optical properties and metabolic function and together provide a sensitive in vitro model of ocular chemical toxicity. Results of confocal analysis suggest that the mitochondrial integrity of the superficial cortical fiber cells is most sensitive to damage caused by SDS. The results further suggest that recovery of lens metabolic function is necessary for the recovery of lens optical properties.

Key Words: mitochondria; bovine lens optical properties; in vitro toxicology; ocular toxicity; mild irritation and recovery; sodium dodecyl sulfate.

Damage of cultured intact lenses has been quantitatively evaluated by measuring changes in focal quality (focal length variability) using an automated scanning laser system (Herbert et al., 1998; Sivak and Herbert, 1997; Sivak et al., 1992, 1994, 1995). The effects of known ocular irritants are important to consider regarding the specific mechanisms of the damage they cause. The negatively charged anionic surfactant sodium dodecyl sulfate (SDS) was chosen as a model chemical in this study because rabbit eyes exposed to SDS during Draize test-

1 To whom correspondence should be addressed at School of Optometry, University of Waterloo, Waterloo, Ontario, N2L 3G1 Canada. Fax: (519) 725-0784. E-mail: jsivak@uwaterloo.ca.
of sharpness of focus over time using an automated scanning laser monitor was used to assess lens optical properties. Confocal microscopy and a mitochondria-specific fluorescent dye, Rhodamine 123, were used to assess lens mitochondrial distribution and morphology.

MATERIALS AND METHODS

Chemicals and reagents. Culture medium (M199), sodium bicarbonate, agarose, L-glutamine, NaCl, SDS, and NaOH were purchased from Sigma Chemical Co., (St. Louis, MO). HEPES, penicillin, streptomycin, and dialyzed fetal bovine serum were obtained from Gibco-BRL (Burlington, ON, Canada). Rhodamine 123 was obtained from Molecular Probes (Eugene, OR).

Eye dissection. Bovine eyes obtained from a local abattoir were opened under sterile conditions and the lenses were removed. To minimize physical handling of lenses (i.e., transfer from the culture plate and then to the chamber for scanning) they were immediately placed into a three-part chamber (see Analysis of the Lens Optical Properties below) containing 25 ml of culture medium (M199) supplemented with 21 mM HEPES, 26 mM sodium bicarbonate, 0.7 mM L-glutamine, 7 mM of NaOH, 100,000 units penicillin and 100 mg streptomycin, and 3% dialyzed fetal bovine serum and incubated at 37°C with 4–5% CO₂. After 24 h lenses exhibiting mechanical damage during dissection, as evaluated by the visible opacities, were discarded.

Lens treatment. Lenses were exposed to SDS (0.1 to 0.00625%) for 30 min, rinsed with saline (0.9% NaCl), placed in fresh M199 and incubated at 37°C and 4–5% CO₂. A minimum of 11 lenses (total n = 84) were used for each treatment group.

Analysis of the lens optical properties. Lens optical quality (the average back vertex distance, BVD) and sharpness of focus (BVD variability) were assessed using the Scantox In Vitro Lens Assay System (Harvard Apparatus, Holliston, MA) before exposure, immediately, 4, 8, and 24 h after the treatment. The Scantox In Vitro Lens Assay System consists of a collimated laser source that projects a laser beam onto a plain mirror mounted at 45° on a carriage assembly. This mirror reflects the laser beam directly up through the scanner table surface and through the lens under examination. The mirror carriage is connected via a drive screw to a positioning motor. This positioning motor turns the drive screw and thereby moves the laser in user-defined steps across the lens in an automated fashion. A digital camera captures the actual position and slope of the laser beam at each step. When all steps have been taken, the captured data for each step position is used to calculate the BVD for each position and the difference in that measurement between beams. Lenses were placed in 25 ml of M199 into specially designed three-part chamber made from 70 mm tall glass, silicone rubber insert, and a metal base (modified from Weerheim and Sivak, 1992) and suspended within the chamber on a 14-mm inner diameter beveled washer designed to support the lens at the equatorial rim. A series of 22 laser beams were passed at specified increments of 0.5 mm for a total range of 11 mm. Thus, the results for this part of the study involved 9240 objective optical measurements (84 lenses, 5 scan points, 22 beams).

Confocal analysis of mitochondrial integrity. For confocal analysis lenses were transferred into 10 ml serum-free M199 in Wheaton-33 sample glass vials (VWR, Mississauga, ON, Canada). A minimum of nine lenses for each treatment group were used for confocal analysis (total n = 60). These lenses were stained for mitochondria using 20 µM Rhodamine 123 for 45 min at 37°C. Rhodamine 123 is a lipophilic cell-permeable, cationic nontoxic fluorescent dye that is readily sequestered specifically by active mitochondria (Johnson et al., 1980). Lenses were immobilized on cover glasses (no. 1, 18 mm², Corning Labware & Equipment, Corning, NY), attached over 10 mm holes drilled in the bottom of each well of a six well plate using 1% agarose, previously melted in M199 and cooled to 35°C. A Zeiss confocal laser scanning microscope (CLSM) 410 system attached to an Axiovert 100 microscope with a 40× water-immersion C-Apochromat objective (numeric aperture 1.2) was used. The combination of an argon/krypton laser with a 488 nm excitation laser line, and a 590 nm long pass emission filter, were used to visualize Rhodamine 123 fluorescence. To minimize laser bleaching of Rhodamine 123, a T = 0.01 neutral density filter for laser attenuation was applied to a 488 nm excitation laser line.

RESULTS

Lens Anatomy and Optical Properties after In Vitro SDS treatments

The untreated control lenses remained transparent at the end of the experiment, as evaluated visually under the dissection microscope for the presence of opacities (Fig. 1, control). The severity of opacities 24 h postexposure were SDS concentration-dependent. While exposure to concentrations of SDS at 0.0125 and 0.00625% caused moderate opacities, concentrations of SDS at 0.025–0.1% caused severe opacities (Fig. 1).

These concentrations caused opacities around the equator and the anterior and posterior lens surfaces. Lenses axial swelling also took place as a function of SDS concentration (Fig. 1). While the 0.025–0.1% SDS treated lenses showed general cloudiness of the anterior and posterior surfaces, opacities localized at the posterior suture of the 0.0125 and 0.00625% SDS treated lenses were seen (Fig. 1, 0.0125 and 0.00625% SDS, posterior).
No significant increase in lens wet weight as compared to controls was seen in the 0.00625 and 0.0125% SDS treated lenses (Table 1, \( p = 0.99 \) and \( p = 0.83 \), respectively). Compared to controls (1.90 ± 0.04 g), lens wet weight significantly increased in the 0.025 (2.14 ± 0.04 g, \( p = 0.0006 \)), 0.05 (2.10 ± 0.04 g, \( p = 0.013 \)), and 0.1% (2.09 ± 0.05 g, \( p = 0.02 \)) SDS treated lenses (Table 1). The treatment with SDS did not cause significant changes in lens equatorial diameter (Table 1). Significant increase as compared to controls in lens axial diameter (an indication of lens swelling) was seen in all SDS treatments, except 0.00625% SDS treated lenses (Table 1). Lens axial diameter increased in a dose-dependent manner with increasing SDS concentration (Table 1).

Measurements in BVD did not vary systematically from the controls for lenses of various treatment groups. On average BVD amounted to about 37.0 mm in controls, ranging in different treatment groups from a high of 39.6 to a low of 34.7 mm. Since BVD is a function both of lens curvature and lens refractive index, change in one of these factors may be neutralized by change in the other. Thus BVD is not a sensitive indicator of lens optical damage, as shown previously (Herbert et al., 1999; Sivak et al., 1992).

Figure 2 shows representative scan plots obtained using an automated Scantox™ In Vitro Lens Assay System. These show little change in back vertex distance variability (BVD variability) in controls (Fig. 2A) and a significant increase in the BVD variability associated with SDS treatment (Fig. 2B, showing an example for 0.1% SDS). A 2.5-fold increase in BVD variability (an indication of loss of sharp focus) was evident in the 0.1% SDS group lenses as early as the 0 h scan point (0.85 ± 0.16 mm, \( p < 0.0001 \)) as compared to controls (0.34 ± 0.04 mm, Table 2). By the 4 h scan point significant increases in BVD variability were seen in the 0.0125% (0.61 ± 0.05 mm, \( p = 0.004 \)) and 0.05% SDS treated group lenses (0.51 ± 0.05 mm, \( p = 0.05 \); Table 2). While at the 4 h scan no difference in BVD variability was seen in the 0.00625% SDS group lenses, a borderline increase in BVD variability was seen in the 0.025% SDS group lenses (0.48 ± 0.05 mm, \( p = 0.086 \)). At the 8 h scan point a significant increase in BVD variability was seen in the 0.0125% (0.53 ± 0.05 mm, \( p = 0.0283 \)), 0.0125% (0.60 ± 0.05 mm, \( p = 0.0012 \)), and the 0.1% (0.95 ± 0.13 mm, \( p < 0.0001 \)) SDS group lenses as compared to controls (0.33 ± 0.02 mm, Table 2). At the 24 h scan point, significant increases in BVD variability were seen in the

### TABLE 1
Comparison of Lens Wet Weight (g), Equatorial Diameter, and Axial Length (mm) ± SEM of Control and SDS Treated Bovine Lenses 24 h Postexposure

<table>
<thead>
<tr>
<th>Dimension</th>
<th>0.1 (n = 9)</th>
<th>0.05 (n = 10)</th>
<th>0.025 (n = 11)</th>
<th>0.0125 (n = 11)</th>
<th>0.00625 (n = 10)</th>
<th>Control (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens wet weight (g)</td>
<td>2.09 ± 0.05*</td>
<td>2.10 ± 0.04*</td>
<td>2.14 ± 0.04**</td>
<td>1.97 ± 0.03</td>
<td>1.88 ± 0.04</td>
<td>1.90 ± 0.04</td>
</tr>
<tr>
<td>Equatorial diameter (mm)</td>
<td>16.29 ± 0.17</td>
<td>16.70 ± 0.17</td>
<td>16.78 ± 0.12</td>
<td>16.38 ± 0.12</td>
<td>16.22 ± 0.14</td>
<td>15.92 ± 0.54</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>13.55 ± 0.11**</td>
<td>13.69 ± 0.07**</td>
<td>13.66 ± 0.08**</td>
<td>13.06 ± 0.09*</td>
<td>12.31 ± 0.12</td>
<td>12.31 ± 0.36</td>
</tr>
</tbody>
</table>

*Indicates significant difference from controls at \( p = 0.05 \).

**Indicates significant difference from controls at \( p < 0.001 \).
0.00625% SDS group lenses (0.66 ± 0.06 mm, \( p = 0.001 \)) as compared to controls (0.34 ± 0.02 mm, Table 2).

**Mitochondrial Distribution and Rhodamine 123 Fluorescence in Lens Epithelial and Superficial Cortical Fiber Cells**

**Lens epithelial cells.** Figure 3 is a typical confocal micrograph showing the distribution of the mitochondria in different anatomical zones of control lens epithelial cells: central (Fig. 3A), intermediate (Fig. 3B), and equator (Fig. 3C). Numerous mitochondria can be seen surrounding individual lens epithelial nuclei (Figs. 3A–3C). No difference could be seen in the average length of the mitochondria between different zones of epithelial cells of control lenses. Average length amounted to 7.01 ± 0.41 \( \mu \)m in the central, 7.03 ± 0.24 \( \mu \)m in the intermediate, and 7.04 ± 0.25 \( \mu \)m in the equatorial epithelial cells (Table 3).

The amount of damage seen 24 h after exposure was so severe in lenses treated with 0.025–0.1% SDS that no mitochondria in the epithelial cells could be seen (Fig. 4). Moreover, fewer epithelial cells with increased intracellular spacing were seen (Fig. 4). Changes in the mitochondrial morphology in different epithelial zones (central, intermediate, and equatorial) appeared to be SDS concentration-dependent. While only a few mitochondria could be seen in the central zone, the mitochondria seen in the intermediate and equatorial epithelial cells in the 0.0125% SDS treated group lenses appeared swollen (Fig. 4). The average length of mitochondria in central

### TABLE 2

List of Back Vertex Distance Variability (Loss of Sharp Focus, BVD Variability, mm) ± SEM in Bovine Lenses Treated with Different SDS Concentrations over Time

<table>
<thead>
<tr>
<th>SDS treatment (%)</th>
<th>0.1 (n = 14)</th>
<th>0.05 (n = 15)</th>
<th>0.025 (n = 16)</th>
<th>0.0125 (n = 11)</th>
<th>0.00625 (n = 11)</th>
<th>Control (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.42 ± 0.05</td>
<td>0.30 ± 0.02</td>
<td>0.39 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.40 ± 0.05</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>0 h</td>
<td>0.85 ± 0.16**</td>
<td>0.42 ± 0.04</td>
<td>0.50 ± 0.05</td>
<td>0.35 ± 0.03</td>
<td>0.45 ± 0.05</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>4 h</td>
<td>0.93 ± 0.23**</td>
<td>0.51 ± 0.05*</td>
<td>0.48 ± 0.05</td>
<td>0.61 ± 0.05*</td>
<td>0.43 ± 0.03</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>8 h</td>
<td>0.947 ± 0.13**</td>
<td>0.47 ± 0.03</td>
<td>0.60 ± 0.05*</td>
<td>0.53 ± 0.05*</td>
<td>0.43 ± 0.05</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>24 h</td>
<td>0.952 ± 0.14**</td>
<td>0.64 ± 0.04*</td>
<td>0.75 ± 0.10**</td>
<td>0.92 ± 0.09**</td>
<td>0.66 ± 0.06*</td>
<td>0.34 ± 0.02</td>
</tr>
</tbody>
</table>

*Indicates significant loss of sharp focus at \( p \leq 0.05 \) as compared to controls.

**Indicates significant loss of sharp focus at \( p < 0.0001 \) as compared to controls.
epithelial cells of lenses treated with 0.0125% SDS were significantly shorter (2.95 ± 0.72 μm, p < 0.0001) than those of controls (7.01 ± 0.41 μm, Table 3). In the intermediate epithelial cells of lenses treated with 0.0125% SDS mitochondrial lengths were also significantly shorter (3.98 ± 0.31 μm, p < 0.0001) than those of controls (7.03 ± 0.24 μm, Table 3). While the average length of the mitochondria in the equatorial epithelial cells in lenses treated with 0.0125% SDS was longer than in central or intermediate epithelial zones, it was significantly shorter (4.55 ± 0.47 μm, p < 0.0001) than those of controls (7.04 ± 0.25 μm, Table 3). The distribution, morphology, and average length of mitochondria in the different zones of epithelial cells in lenses treated with 0.00625% SDS was similar to that of controls (Table 3).

Since the mitochondria were totally absent in the epithelial cells in the 0.025–0.1% SDS treated lenses, relative fluorescence of Rhodamine 123, based on mitochondrial electron transport chain potential (∆V_m) could not be measured. A greater than threefold decrease in the relative Rhodamine 123 fluorescence was seen in the central epithelial cells of the 0.0125% SDS-treated lenses (12,815 ± 1657, p < 0.0001) as compared with the same region in controls (Table 3, 40,216 ± 766). A similar significant decrease in the Rhodamine 123 fluorescence (about threefold) was seen in the intermediate

![Representative confocal laser scanning microscopy micrographs showing the distribution of the mitochondria in different regions of epithelial and superficial cortical fiber cells of control lenses. Lenses were stained with 20 μM Rhodamine 123 for metabolically active mitochondria and analyzed with confocal microscope as described in Material and Methods. In the lens epithelial cells cover only the anterior surface. These epithelial cells are divided into three general zones, based on mitotic activity and their location: central, intermediate, and equatorial. (A) Distribution and morphology of the mitochondria in the central epithelial cells overlying the anterior suture that under normal conditions do not undergo mitosis. (B) Morphology and distribution of mitochondria in the intermediate epithelial cells that under normal condition have a slow rate of division. (C) Morphology and distribution of mitochondria in epithelial cells at equator. Epithelial cells at equator undergo mitosis and give rise to the superficial cortical fiber cells to support an ever continuous growth of the lens. (D) Distribution and morphology of mitochondria in anterior superficial cortical fiber cells; (E) superficial cortical fiber cells at equator; and (F) posterior superficial cortical fiber cells. Mitochondria of the superficial cortical fiber cells were not as dense as seen in the epithelium, were longer and were aligned along the long axis of the superficial cortical fiber cells. No difference in distribution and morphology of the mitochondria was seen between epithelial cells of different zones (A–C). Denser and longer mitochondria were seen in the superficial cortical fiber cells at equator (E) as compared to the mitochondria seen in the anterior (D) and posterior (F) superficial cortical fiber cells. Images of epithelial cells were taken immediately below the lens capsule, whereas images of the superficial cortical fiber cells they were taken 20 μm below the surface. Bar = 10 μm.]
TABLE 3
List of average mitochondrial (MT) length (µm) ± SEM and Relative Rhodamine 123 Fluorescence in Epithelial and Superficial Cortical Fiber Cells of Bovine Lenses Treated with Different SDS Concentrations 24 h Postexposure and Controls

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SDS treatment (%)</th>
<th>SDS treatment (%)</th>
<th>SDS treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0125 (n = 8)</td>
<td>0.00625 (n = 8)</td>
<td>Control (n = 8)</td>
</tr>
<tr>
<td>Average MT length in the central epi.</td>
<td>2.95 ± 0.72*</td>
<td>7.19 ± 0.29</td>
<td>7.01 ± 0.41</td>
</tr>
<tr>
<td>Average MT length in the intermediate epi.</td>
<td>3.98 ± 0.31*</td>
<td>7.37 ± 0.41</td>
<td>7.03 ± 0.24</td>
</tr>
<tr>
<td>Average MT length in the epi. at equator</td>
<td>4.55 ± 0.47*</td>
<td>7.49 ± 0.44</td>
<td>7.04 ± 0.25</td>
</tr>
<tr>
<td>The ∆Ψm in the central epi. ± SEM</td>
<td>12.816 ± 1657*</td>
<td>35.485 ± 665*</td>
<td>40.216 ± 766</td>
</tr>
<tr>
<td>The ∆Ψm in the intermediate epi. ± SEM</td>
<td>11.487 ± 601*</td>
<td>26.978 ± 381*</td>
<td>40.072 ± 1046</td>
</tr>
<tr>
<td>The ∆Ψm in the epi. at equator ± SEM</td>
<td>14,168 ± 1131*</td>
<td>28,182 ± 1374*</td>
<td>40,976 ± 1457</td>
</tr>
</tbody>
</table>

**Note.** Relative Rhodamine 123 fluorescence indicates changes in mitochondrial electron transport chain potential (ΔΨm).

*Indicates significantly lower ΔΨm at p < 0.0001 as compared to controls.

Figure 4: Representative confocal laser scanning microscopy micrographs showing the effect of SDS treatment on morphology of the epithelial cells in different zones and distribution of the mitochondria (stained with 20 µM Rhodamine 123). Decreased number and increased spacing between the epithelial cells was seen in lenses treated with 0.1 to 0.025% SDS. Such an effect appeared to be the same in different epithelial zones: the central, intermediate, and epithelial cells at the equator. No mitochondria were seen in epithelial cells of lenses treated with 0.1 to 0.025% SDS. Increased spacing between the central epithelial cells with few mitochondria was seen in lenses treated with 0.0125% SDS. Morphology of epithelial cells was similar to that of controls (Fig. 1) in the intermediate and epithelial cells at equator in lenses treated with 0.0125% SDS, while mitochondria appeared short and swollen in comparison to the same relative regions of control lenses (Fig. 1). Morphology of epithelial cells, length and distribution of mitochondria in epithelial cells of lenses treated with 0.00625% SDS appeared similar to the same relative regions of control lenses seen in Figure 1. Bar = 10 µm.
higher (35,485 ± 665, p < 0.0001) that in the 0.0125% SDS-treated lenses, this measure was significantly lower (p < 0.0003) when compared to the same region of control lenses (Table 3). Similarly, while the Rhodamine 123 fluorescence in the intermediate (26,977 ± 381) and equatorial (28,181 ± 1374) epithelial zones of the 0.00625% SDS-treated lenses was significantly higher (p < 0.0001 and p < 0.0001, respectively) when compared with the same relative region in the 0.0125% SDS-treated lenses, this measure was significantly lower (p < 0.0001 and p < 0.0001, respectively) when compared to controls (Table 3).

Lens superficial cortical fiber cells. Overall, the mitochondria in the superficial cortical fiber cells of control lenses were not as dense as those in the epithelium and were evenly distributed throughout the cytoplasm (Figs. 3D–3F). However much higher numbers and significantly longer average length (41.16 ± 2.24 μm, p < 0.0001) of mitochondria was seen in the superficial cortical fiber cells at the equator (Fig. 3E), in comparison to the mitochondria in the anterior zone (18.37 ± 1.22, Table 4 and Fig. 3D) and posterior zone (17.22 ± 1.22 μm, Table 4 and Fig. 3F) of the superficial cortical fiber cells in controls (Table 4).

The amount of damage seen 24 h postexposure was so severe in lenses treated with the 0.025–0.1% SDS that no mitochondria in the superficial cortical fiber cells could be seen (Fig. 5). Moreover, the normal alignment and equal spacing between the superficial cortical fiber cells seen in the controls (Fig. 3) was disrupted by extensive large vacuoles (Fig. 5). The amount of that damage in different regions (anterior, equatorial, and posterior) of the superficial cortical fiber cells appeared to be SDS concentration-dependent. Fewer shortened and swollen mitochondria could be seen in the superficial cortical fiber cells at the equator in the 0.0125% SDS-treated group lenses (Fig. 5). Their average length was significantly shorter (17.42 ± 2.30 μm, p < 0.0001) than those of controls (41.16 ± 2.24 μm, Table 4). In lenses treated with 0.0125% SDS, the mitochondria were absent in the anterior and posterior superficial cortical fiber cells. Moreover the presence of extensive vacuoles could be seen (Fig. 5). While the distribution, morphology, and average length of the mitochondria in anterior and equatorial superficial cortical fiber cells in lenses treated with the 0.00625% SDS was similar to that of controls (Table 4 and Fig. 5), no mitochondria could be seen in the posterior superficial cortical fiber cells (Fig. 5). Moreover, extensive vacuoles could be seen in the posterior superficial cortical fiber cells in lenses treated with 0.00625% SDS (Fig. 5).

Since mitochondria were totally absent in the superficial cortical fiber cells in the 0.025–0.1% SDS-treated lenses, the relative fluorescence of Rhodamine 123, based on mitochondrial electron transport chain potential (ΔΨₘ) could not be measured. A threefold decrease in Rhodamine 123 fluorescence was seen in the superficial cortical fiber cells at the equator in the 0.0125% SDS treated lenses (151,369 ± 24,424, p < 0.0001), as compared with the same region in controls (Table 3, 446,390 ± 59,100). Due to the absence of mitochondria and the presence of numerous large vacuoles, relative Rhodamine 123 fluorescence could not be measured in the anterior or posterior superficial cortical fiber cells in lenses treated with the 0.0125% SDS (Fig. 5, Table 4). Similarly, due to the absence of mitochondria and the presence of numerous small vacuoles in the posterior superficial cortical fiber cells (Fig. 5) in lenses treated with the 0.00625% SDS, the relative Rhodamine 123 fluorescence could not be measured (Fig. 5, Table 4). While the relative Rhodamine 123 fluorescence in the equatorial superficial cortical fiber cells in lenses treated with 0.00625% SDS was significantly higher (292,835 ± 42,773, p < 0.0001) that in the 0.0125% SDS-treated lenses, this measure was significantly lower (p < 0.0003) as compared with the same region of controls (Table 4). A greater than

**Table 4**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>0.0125 (n = 8)</th>
<th>0.00625 (n = 8)</th>
<th>Control (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average MT length in the central fib.</td>
<td>Unable to measure</td>
<td>19.55 ± 1.41</td>
<td>18.37 ± 1.22</td>
</tr>
<tr>
<td>Average MT length in the fibr at equator</td>
<td>17.42 ± 2.30**</td>
<td>23.00 ± 1.78**</td>
<td>41.16 ± 2.24</td>
</tr>
<tr>
<td>Average MT length in the posterior fibr.</td>
<td>Unable to measure</td>
<td>17.22 ± 1.22</td>
<td>17.22 ± 1.22</td>
</tr>
<tr>
<td>The ΔΨₘ in the fibr at equator ± SEM</td>
<td>151,369 ± 24,424**</td>
<td>292,836 ± 42,774*</td>
<td>446,390 ± 59,100</td>
</tr>
<tr>
<td>The ΔΨₘ in the anterior fibr. ± SEM</td>
<td>Unable to measure</td>
<td>255,575 ± 22,786*</td>
<td>361,329 ± 16,115</td>
</tr>
<tr>
<td>Anterior MFZ ± SEM</td>
<td>142.50 ± 5.70</td>
<td>153.90 ± 17.10</td>
<td>153.90 ± 17.10</td>
</tr>
<tr>
<td>MFZ at equator ± SEM</td>
<td>71.35 ± 2.95**</td>
<td>183.10 ± 34.90</td>
<td>205.20 ± 11.40</td>
</tr>
</tbody>
</table>

Note. Relative Rhodamine 123 fluorescence indicates changes in mitochondrial electron transport chain potential (ΔΨₘ).

**Indicates significant difference from that of control at p ≤ 0.05.

**Indicates significant difference from that of control at p ≤ 0.0001.
onefold decrease was seen in the relative Rhodamine 123 fluorescence in the anterior superficial cortical fiber cells in lenses treated with 0.1 to 0.025% SDS. No mitochondria were seen in these zones of the superficial cortical fiber cells of lenses treated with 0.1 to 0.025% SDS. Large vacuoles and no mitochondria were seen in the anterior and posterior superficial cortical fiber cells of lenses treated with 0.0125% SDS (0.0125% SDS, anterior and posterior). However, mitochondria were seen in the equatorial superficial cortical fiber cells in lenses treated with 0.0125% SDS (0.0125% SDS, equator). While morphology of the anterior and equatorial superficial cortical fiber cells and the distribution of mitochondria were similar to that of controls (Fig. 1) presence of vacuoles and absence of mitochondria were seen in the posterior superficial cortical fiber cells in lenses treated with 0.00625% SDS (0.00625% SDS, posterior). Bar = 10 μm.

FIG. 5. Representative confocal laser scanning microscopy micrographs showing the effect of SDS treatment on morphology of the superficial cortical fiber cells in different zones and the distribution of the mitochondria (stained with 20 μM Rhodamine 123). Large vacuoles were seen in anterior, equatorial, and posterior superficial cortical fiber cells in lenses treated with 0.1 to 0.025% SDS. No mitochondria were seen in these zones of the superficial cortical fiber cells of lenses treated with 0.1 to 0.025% SDS. Large vacuoles and no mitochondria were seen in the anterior and posterior superficial cortical fiber cells of lenses treated with 0.0125% SDS (0.0125% SDS, anterior and posterior). However, mitochondria were seen in the equatorial superficial cortical fiber cells in lenses treated with 0.0125% SDS (0.0125% SDS, equator). While morphology of the anterior and equatorial superficial cortical fiber cells and the distribution of mitochondria were similar to that of controls (Fig. 1) presence of vacuoles and absence of mitochondria were seen in the posterior superficial cortical fiber cells in lenses treated with 0.00625% SDS (0.00625% SDS, posterior). Bar = 10 μm.

The mitochondrial distribution from the surface of the lens to the depth at which the MFZ starts was measured in the anterior, equatorial, and posterior superficial cortical fiber cells and compared with the same relative regions in controls (Table 4). Due to the absence of the mitochondria in lenses treated with 0.025–0.1% SDS, the MFZ could not be measured. Similarly the MFZ could not be measured in the posterior superficial cortical fiber cells in the 0.0125 and 0.00625% SDS-treated lenses or the anterior superficial cortical fiber cells of the 0.0125% SDS-treated lenses. The MFZ measured at the equator in the superficial cortical fiber cells started at a significantly reduced depth in the 0.0125% SDS-treated lenses (71.35 ± 2.95 μm, p < 0.0001) as compared with controls (205.20 ± 11.10 μm, Table 4). While the MFZ in the equatorial superficial cortical fiber cells in lenses treated with the 0.00625% SDS started significantly deeper (183.10 ± 34.90, p < 0.0001) than that in the 0.0125% SDS-treated lenses, this measure was similar the same region of controls (Table 4). Similarly the MFZ in the anterior superficial cortical fiber cells in lenses treated with 0.00625% SDS started at a depth (142.50 ± 5.70 μm, Table 4) similar to same relative region in controls (153.90 ± 17.10 μm, Table 4).

**DISCUSSION**

Widely used in many household and cosmetic products (Bruner, 1992; Grant and Acosta, 1996), the surfactant SDS bears a negative anionic polar head group and a hydrophobic portion. In an aqueous environment the SDS molecules organize themselves in such way that the hydrophobic portion is sequestered from the highly polar aqueous medium by a surrounding, approximately spherical shell formed by the anionic head groups (Schreier et al., 2000). Thus when biological tissues, such as the lens of the eye, are exposed to SDS, a series of events including lysis, extraction of proteins, and, ult-
mately, cell membrane disruption, takes place (Helenius and Simons, 1975). Once the cell membrane is disrupted, the SDS will accumulate inside the cell, continuing to dissolve intracellular proteins even after the chemical is rinsed off. Together these events lead to the observed ocular toxicity and delayed recovery after exposure to SDS at higher concentrations (Schreier et al., 2000).

Previous work using the cultured bovine ocular lens model has shown a correlation between toxicity and lens optical function (Sivak et al., 1990, 1995). In another example this in vitro model showed its effectiveness in measuring the ocular irritancy potential of several surfactants (Sivak et al., 1994). While the irritancy ranking of tested surfactants were similar to that of Draize scores, the bovine lens in vitro model showed much higher sensitivity in detecting irritancy at much lower concentrations than the Draize score (Sivak et al., 1994). The results of the current study showed an SDS concentration dependent change in both lens optical properties and a decrease in number and length of the mitochondria in epithelial and superficial cortical fiber cells. Exposure to higher concentrations of SDS (0.025 to 0.1%) for 30 min leads to loss of lens optical function over time that was sustained 24 h after treatment. Likewise, 24 h following treatment with higher concentrations of SDS, no mitochondria were seen in lens epithelial and superficial cortical fiber cells. Overall, loss of lens transparency, erosion of the epithelium, vacuoles in the superficial cortical fiber cells, increases in lens wet weight, and axial swelling seen 24 h after exposure indicate that the lenses sustained damage after exposure to SDS at these concentrations.

The morphology and distribution of mitochondria in lens epithelial cells seen 24 h after exposure of lenses treated with 0.0125 and 0.00625% SDS was similar to that of control lenses. However, mitochondrial morphology and distribution in lens superficial cortical fiber cells looked much different. In anterior and superficial cortical fiber cells at equator fewer and shorter mitochondria were seen. However, the absence of mitochondria in the posterior superficial cortical fiber cells and the presence of vacuoles indicate that the mitochondria of the superficial cortical fiber cells are most sensitive to SDS induced changes, even at concentrations as low as 0.00625%. Therefore, loss of mitochondria and presumably reduced metabolic function in the posterior superficial cortical fiber cells may be responsible for the opacities around the posterior sutures and also the loss of sharp focus seen at the 24 h scan point in lenses treated with the 0.00625% SDS.

In lenses treated with SDS the significant morphological changes in the mitochondria of lens epithelial and superficial cortical fiber cells studied by confocal microscopy are similar to the changes in the morphology of the mitochondria of Hep-2 neoplastic cells treated with staphylococcal α-toxin (Paradisi et al., 1976). Besides some nuclear modification seen in those cells associated with treatment, the most significant biphasic morphological change over time was observed in the mitochondrial structure studied by electron microscopy. A decrease in length of the mitochondria (from elongated to shorter) was outlined as an early structural change in cells treated for 30 min whereas swelling and change from shorter to round morphology of the mitochondria in cells treated for 3 and 6 h was outlined as a later phase of the cytotoxicity response. However, the confocal analysis of mitochondrial morphology was done using a mitochondria-specific fluorescent dye, Rhodamine 123, that emits fluorescence from metabolically active mitochondria. Therefore, a third stage was noted in lenses treated with higher concentrations of SDS where Rhodamine 123 fluorescence was not seen, indicating the absence of metabolically active mitochondria in both lens epithelial and superficial cortical fiber cells.

The Draize test (Draize et al., 1944), adopted widely for the assessment of ocular irritancy and toxicity, is currently used to evaluate the safety of chemical substances that are foreign to biological systems (xenobiotics). The Draize test is based mainly on scoring of observed macroscopic changes to the rabbit cornea, conjunctiva, and iris after exposure to a test compound. The test has been criticized because of its subjectivity, the high doses used (Chambers et al., 1993; Freeberg et al., 1986; Griffith et al., 1980; Lambert et al., 1993; Williams, 1985), their intra- and interlaboratory variability (McDonald et al., 1977; Weil and Scala, 1971), and most important, the harmful effect to living animals (Rowan, 1984; Zbinden, 1985). The test has also been criticized because of its inability to distinguish between the irritating effects of chemicals at low concentrations. The in vitro approach used here showed sufficient sensitivity to assess the toxicity caused by low SDS concentrations.

The transparent cornea and the lens of the vertebrate eye are responsible for focusing light on the retina. During embryonic development, the surface ectoderm gives rise to the epithelial cells of the cornea and the lens, making them similar not only functionally but in their gene expression as well (Piatigorsky, 1998). Therefore changes in the lens in response to potentially toxic chemicals can provide a relevant measure of corneal (i.e., ocular), irritancy (Edwards et al., 1970).

The ability to measure changes associated with low concentrations of SDS seen in this study is an important toxicity parameter that has been difficult to measure using other in vitro methods (McCulley and Stephens, 1993). The automated scanning laser monitor provides objective measurements of changes in lens optical function over time. Confocal microscopy analysis of the morphology and distribution of the mitochondria in lens epithelial and superficial cortical fiber cells showed a significant morphological change in the mitochondria. Confocal microscopy permits optical sectioning through a living intact tissue, the lens, with resolution and contrast superior to conventional light microscopy, and without the artifacts induced by the preparation of specimens for electron microscopy (Wilson, 1989; Wright et al., 1993).
ACKNOWLEDGMENTS

This work was supported by a grant from the Natural Science and Engineering Research Council of Canada and by Bausch & Lomb, Rochester, NY (J.G.S.). The support of an Ontario Graduate Scholarship to V.B. is acknowledged.

REFERENCES


