HISTOPATHOLOGY REPORT

The positive and negative control corneas were photographed by Dr. John Harbell on November 29, 2005. Therefore, there may appear to be slight differences in staining between the control corneas and test article treated corneas. The slides were used for the evaluation.

Negative control, neat, 10-minute exposure, 2-hour post-exposure

Epithelium: The negative control corneas were treated for 10 minutes with sterile, deionized water (slides B8684-B8686). The negative control-treated epithelium was composed of three layers. The basal cell layer was a well-formed, columnar-cell region directly attached to the Bowman's Layer. The basal cells were always tightly attached to each other. Several layers of wing cells covered the columnar basal layer. In both of these layers, the cell nuclei showed diffuse chromatin without clear nucleoli. Rare mitotic figures were seen in the basal layer. The squamous layer was flattened with limited cytoplasm and highly condensed nuclei.

Stroma: The cross section of a negative control-treated cornea, showing the general thickness of the whole cornea and stroma. The stromal elements showed well-organized collagen matrix fibers with dispersed keratocytes. Keratocyte nuclei showed a range of morphologies from moderate sized (smaller than the epithelial nuclei) with diffuse basophilic staining to narrow, elongated and condensed with dark basophilic staining. Cytoplasmic staining, when it was visible, was moderately basophilic. Rare cells, with eosinophilic cytoplasmic staining, were observed. Collagen bundles were generally parallel and well ordered.

Endothelial: The Descemet's Membrane was prominent. The endothelial layer could be seen in most sections and was reasonably well maintained.

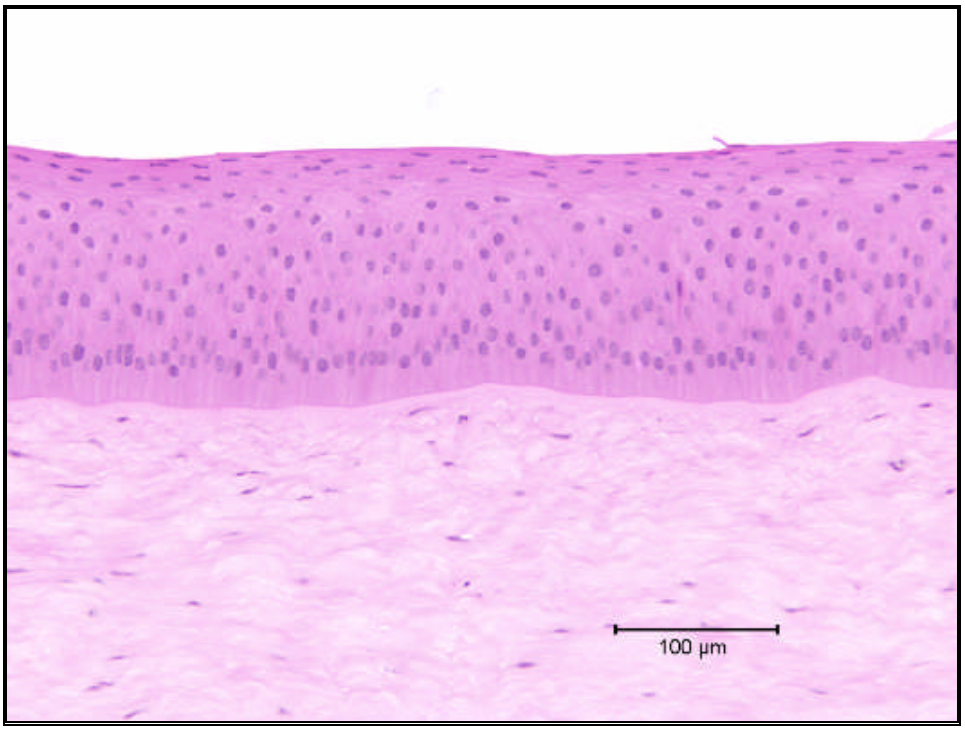

Figure 2. Negative Control (sterile, deionized water), 10-minute exposure, 120-minute post-exposure (10/3/05) - Epithelium (magnification 237x)

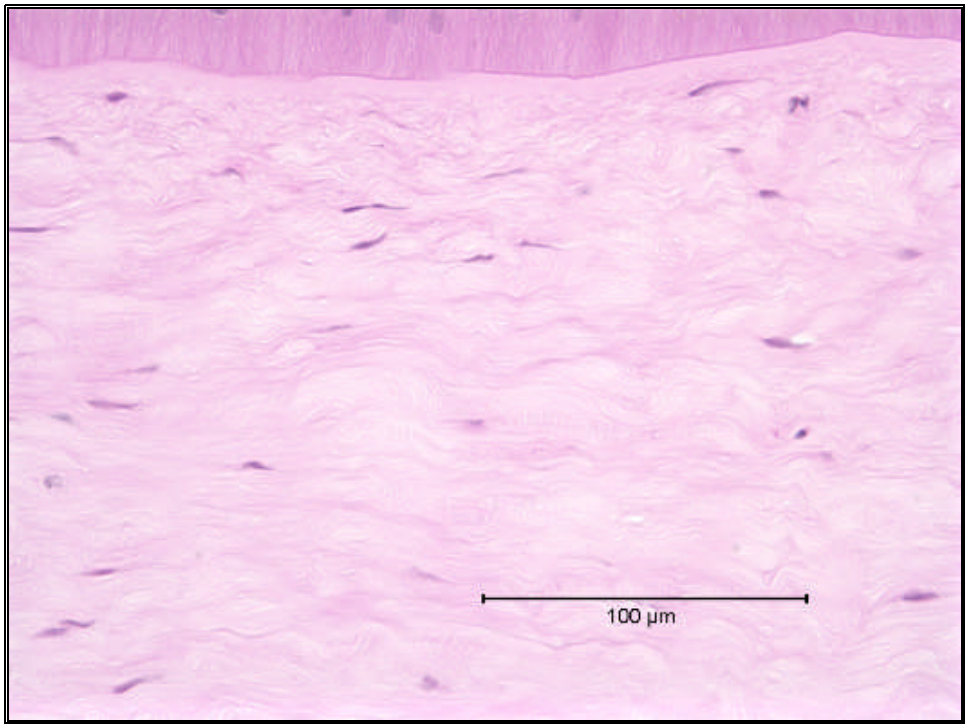

Figure 3. Negative Control (sterile, deionized water), 10-minute exposure, 120-minute post-exposure (10/3/05) - Stroma directly below Bowman's Layer (magnification 475x)

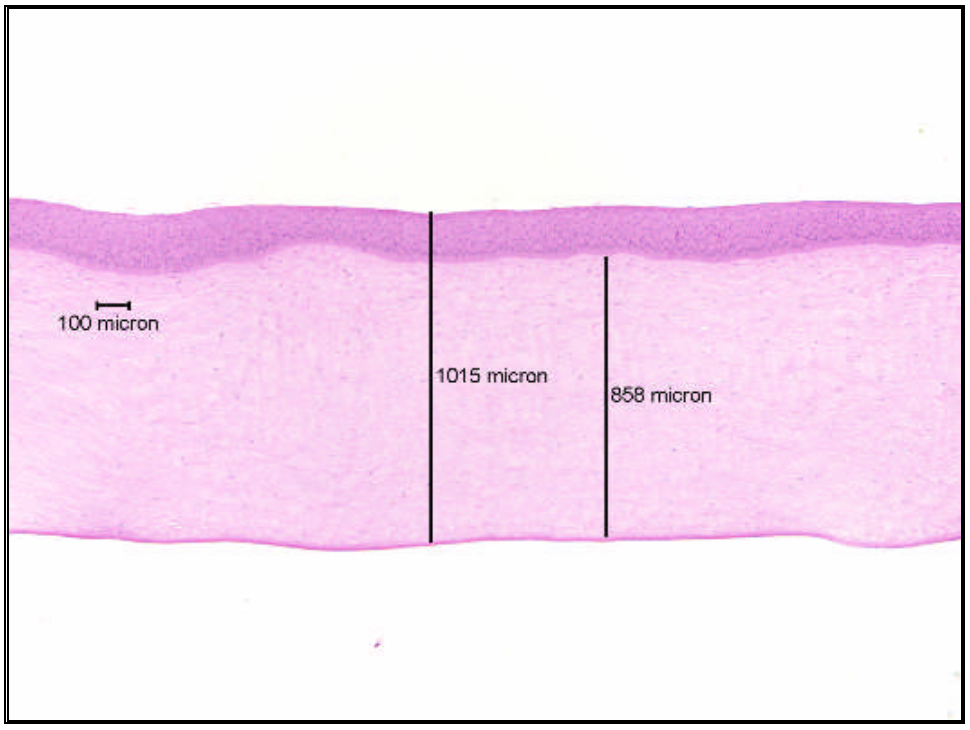

Figure 4. Negative Control (sterile, deionized water), 10-minute exposure, 120-minute post-exposure (10/3/05) - Full thickness (magnification 48x)

Positive control, neat, 10-minute exposure, 2-hour post-exposure

Epithelium: The positive control corneas (slides B8687-B8689), treated for 10 minutes with 100% ethanol, showed the classic pattern of squamous layer coagulation (darkening) and marked vacuolization in the wing and basal cell nuclei. The loss of adhesion between the basal cells (cell to cell) and the basal lamina was marked. The epithelium was probably not viable at the time of fixation.

Stroma: Overall, the positive control-treated corneas were thicker than the negative control-treated corneas. These corneas were not as thick as the positive control-treated corneas from some other studies. In the stroma directly below Bowman's Layer, the collagen matrix showed slight hypereosinophilic staining suggestive of some coagulation. Below this zone, moderate/marked collagen matrix vacuolization extended past 50% depth. In the uppermost stroma, there was a decrease in the density of viable keratocytes as reflected by a marked increase in the frequency of keratocytes showing nuclear vacuolization with a progression towards keratocytes showing pyknotic nuclei deeper into the stroma. In the stroma below mid depth, the keratocytes showed a moderate increase in the frequency of cells with enlarged nuclei and cytoplasmic eosinophilia.

Endothelium: The endothelial cells were generally intact (similar to the negative control-treated corneas).

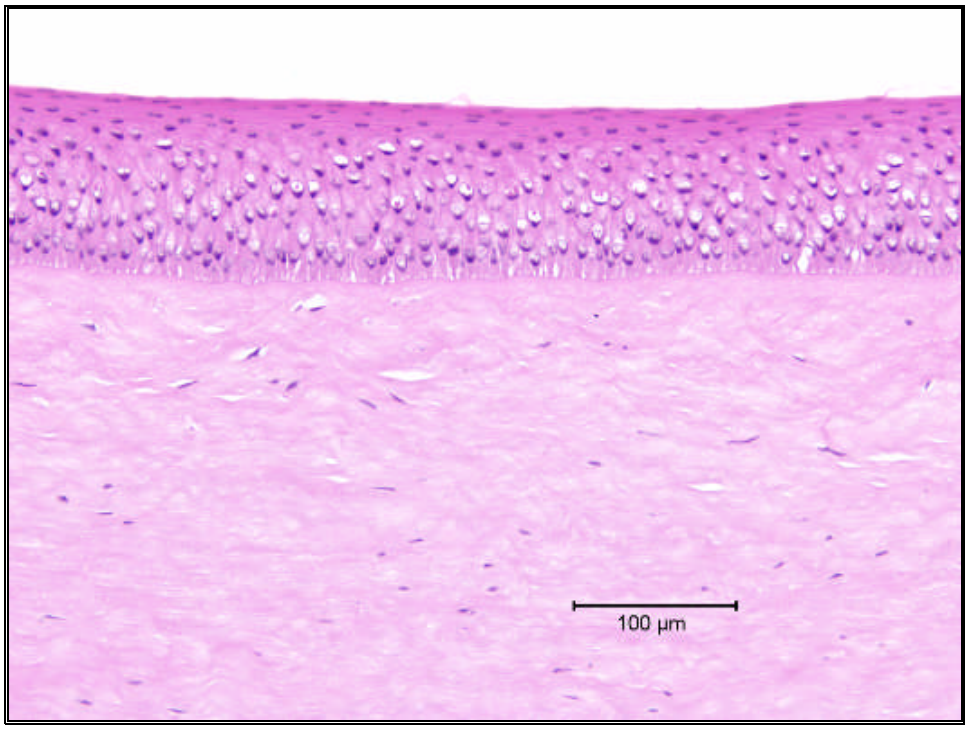

Figure 5. Positive Control (100% ethanol), 10-minute exposure, 120-minute post-exposure (10/3/05) - Epithelium (probably not viable at the time of fixation) (magnification 237x)

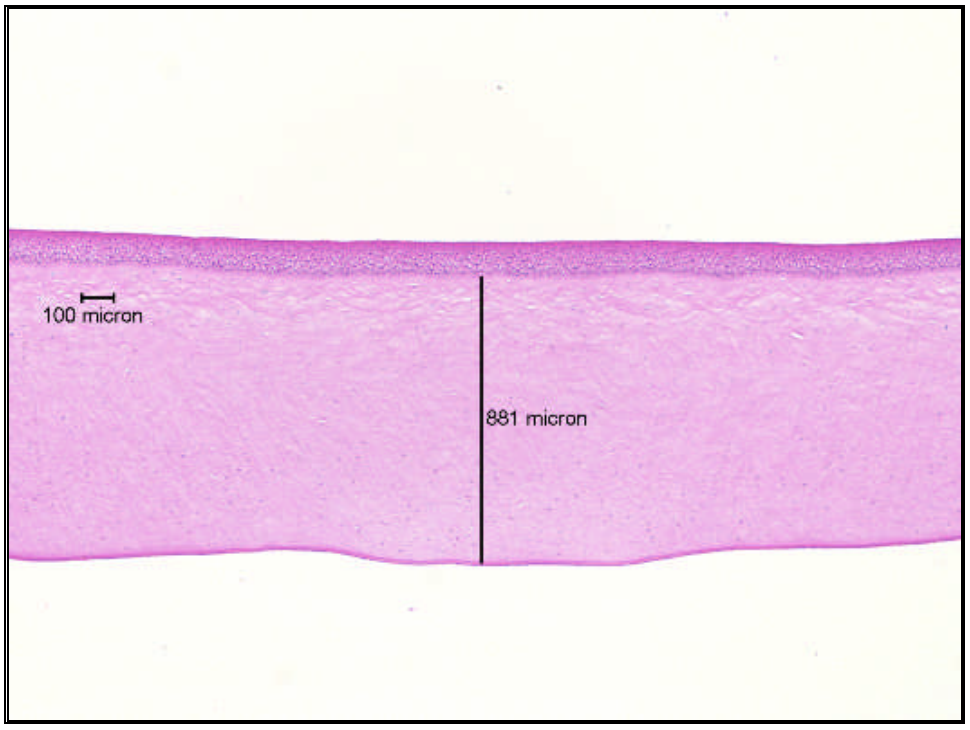

Figure 6. Positive Control (100% ethanol), 10-minute exposure, 120-minute post-exposure (10/3/05) - Full thickness (magnification 48x)

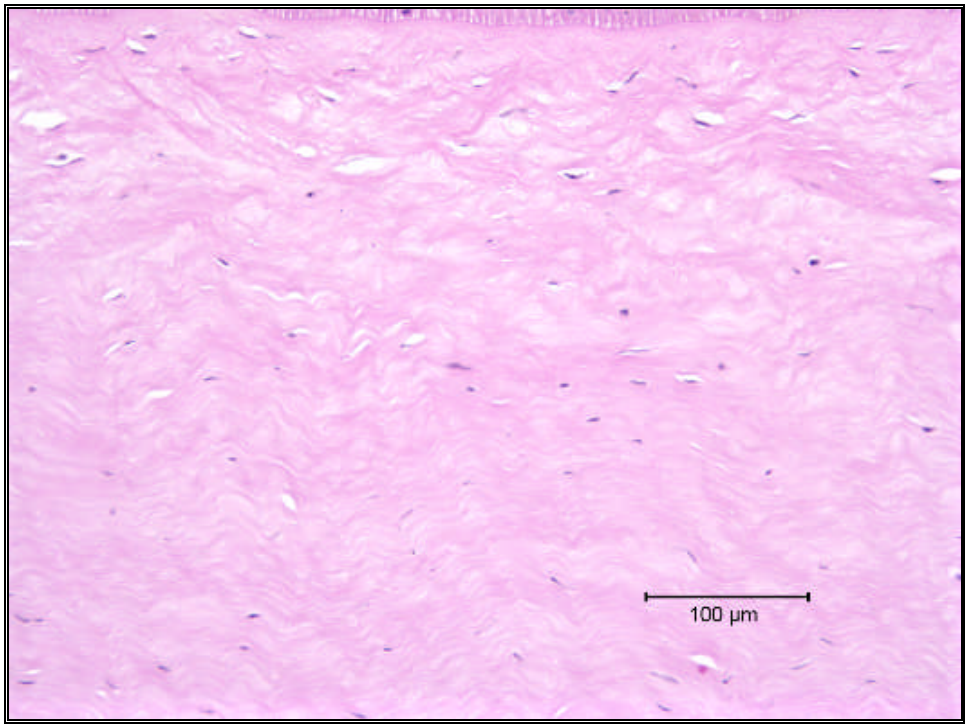

Figure 7. Positive Control (100% ethanol), 10-minute exposure, 120-minute post-exposure (10/3/05) - Upper stroma showing hyperchromic staining in the zone directly below Bowman's Layer and the decrease in the density of viable keratocytes (magnification 237x)

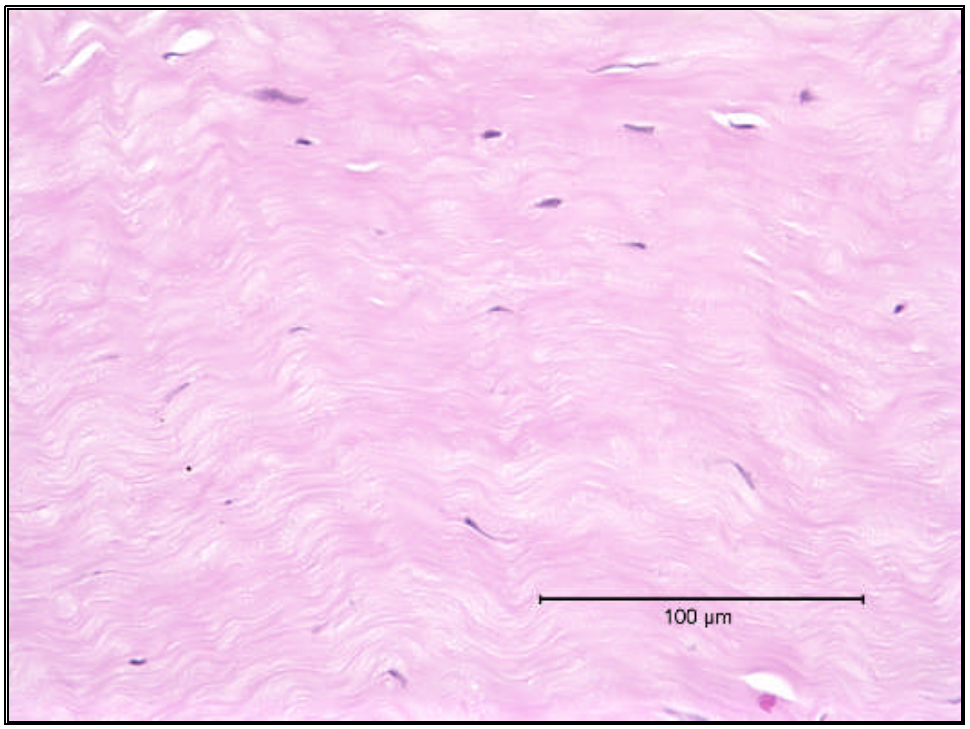

Figure 8. Positive Control (100% ethanol), 10-minute exposure, 120-minute post-exposure (10/3/05) - Stroma at 20% depth showing moderate collagen matrix vacuolization and an increased frequency of keratocytes with abnormal chromatin condensation (magnification 475x)

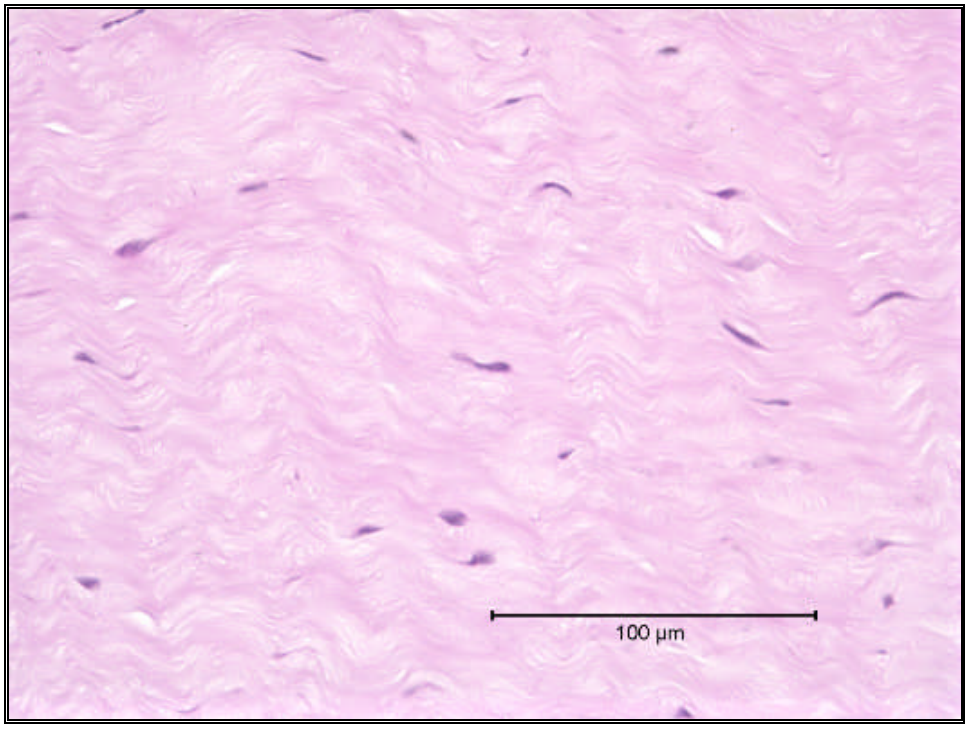

Figure 9. Positive Control (100% ethanol), 10-minute exposure, 120-minute post-exposure (10/3/05) - Stroma near mid depth showing keratocyte nuclear enlargement and cytoplasmic eosinophilia (magnification 475x)

Sample A, neat, 3-minutes, 2 hours

Epithelium: The epithelium of corneas treated with Sample A (slides B8690-B8692) was similar to the positive controls but more severe. The upper squamous is detached with marked vacuolation in the wing and basal cell nuclei. The loss of adhesion between the cells was significant. There was some detachment from basal membrane was noticed.

Stroma: The stroma was thicker than the negative controls. The upper stroma had mild collagen matrix vacuolation with severe cellular vacuolation. There was also a significant amount of pyknotic nuclei. The mid stroma had minimal cellular vacuolation with pyknotic nuclei. The collagen matrix was generally intact. In the stroma below mid depth, there was minimal cellular vacuolation with pyknotic nuclei. The collagen matrix was generally intact.

Endothelium: The endothelium was generally detached from Descement's membrane with vacuolation through the cellular structures.

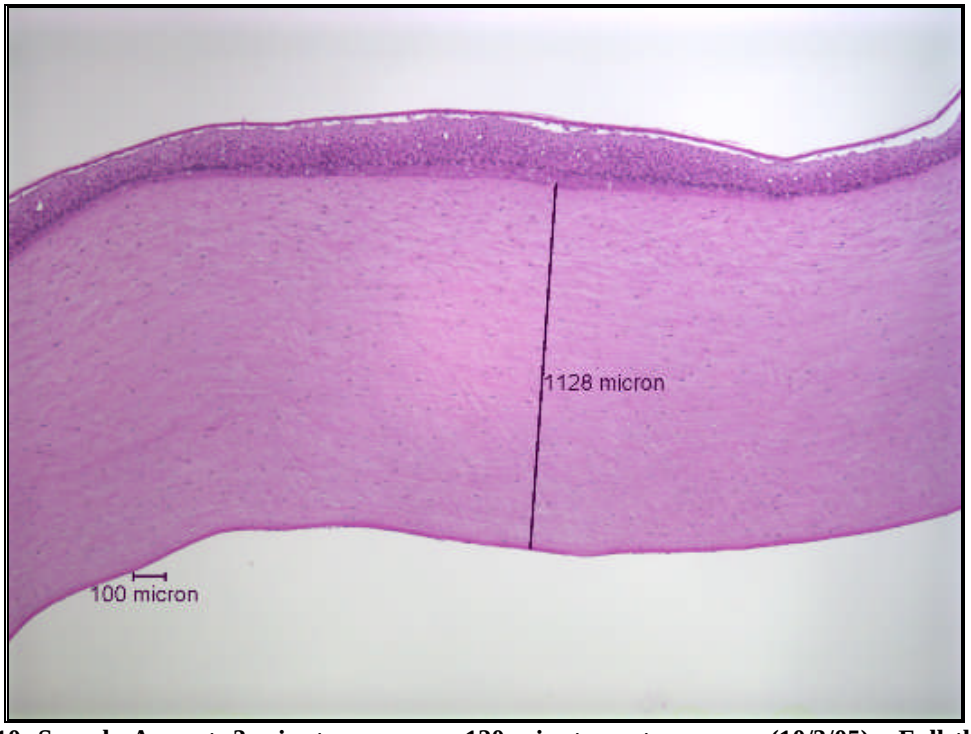

Figure 10. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) - Full thickness (magnification 48x)

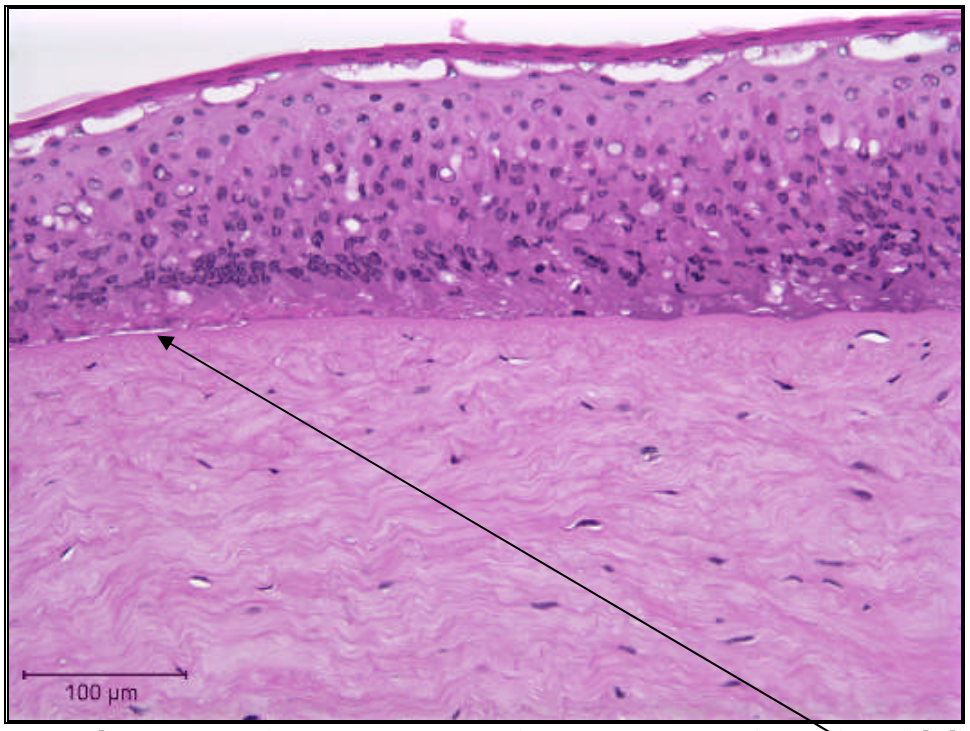

Figure 11. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) - Epithelium with detachment of upper squamous layer. Severe vacuolation of cellular matrix. Detachment from the basal membrane. (magnification 237x)

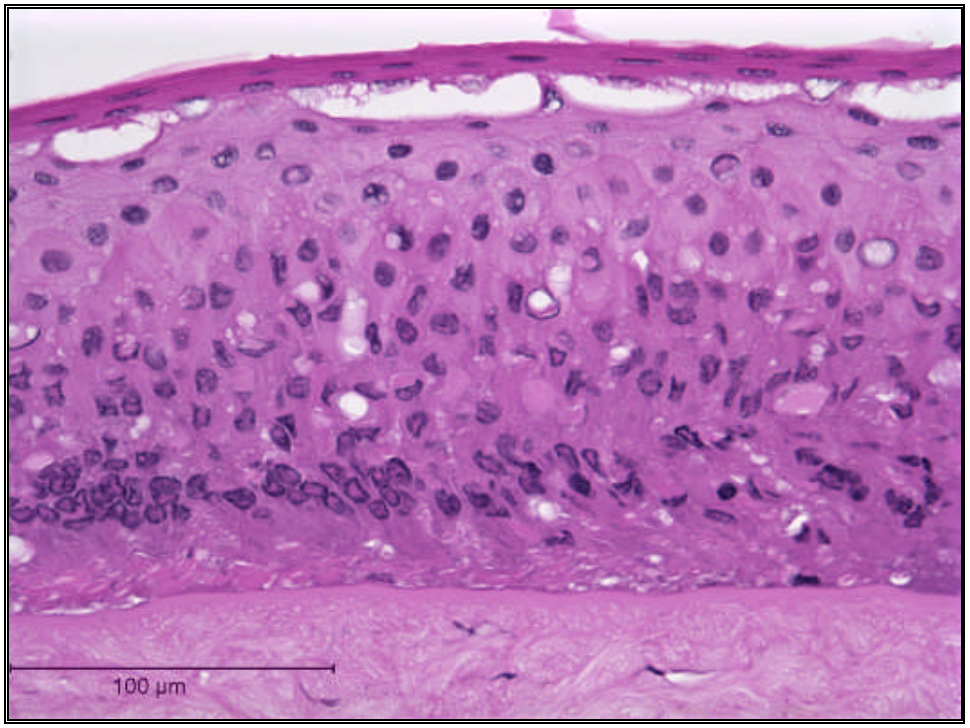

Figure 12. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) - Epithelium with detachment of upper squamous layer. Severe vacuolation of cellular matrix. Detachment from the basal membrane. (magnification 475x)

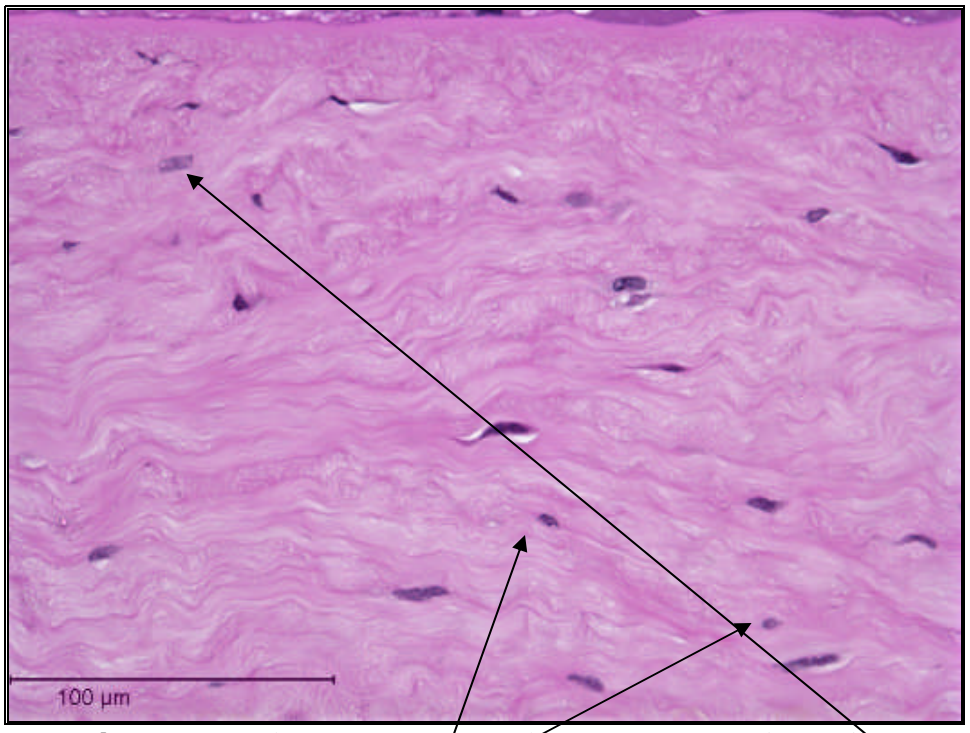

Figure 13. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) - Stroma directly beneath Bowman's layer showing mild collagen matrix vacuolation with severe cellular vacuolation. There was also a significant amount of pyknotic nuclei. (magnification 475x)

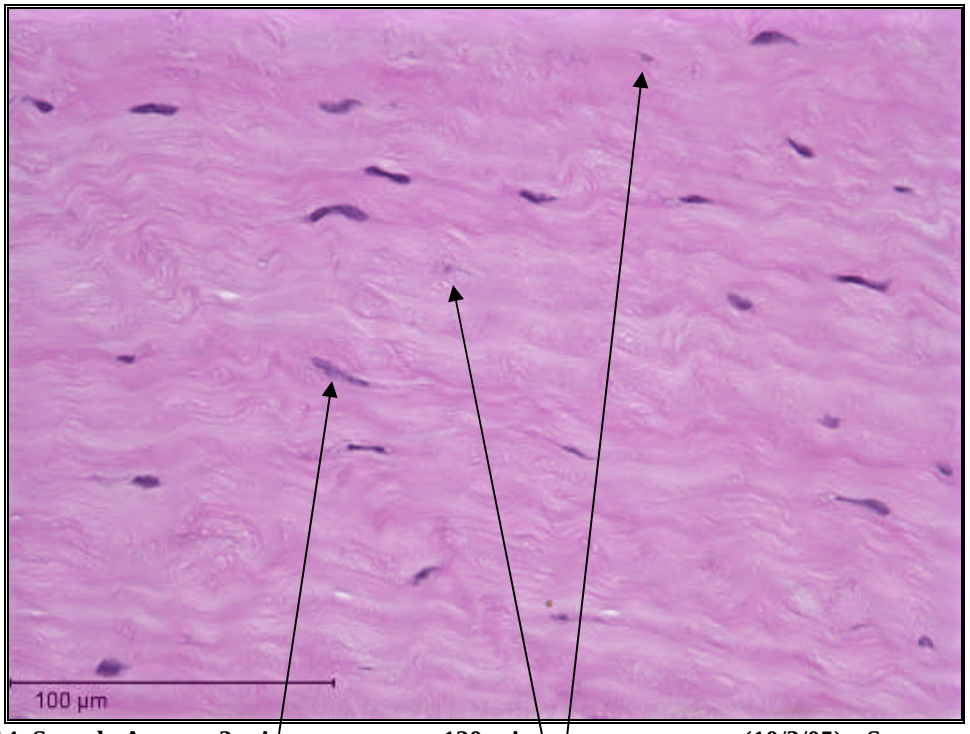

Figure 14. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) - Stroma near mid depth showing minimal cellular vacuolation with pyknotic nuclei. The collagen matrix was generally intact. (magnification 475x)

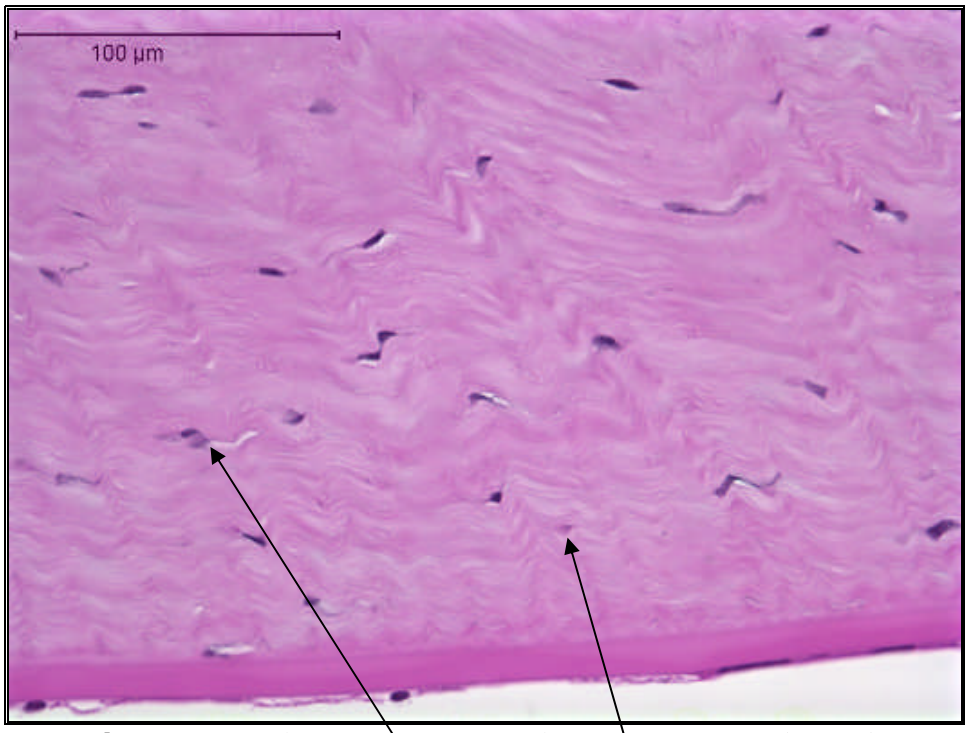

Figure 15. Sample A, neat, 3 minute exposure, 120-minute post-exposure (10/3/05) -Stroma below mid depth there was minimal cellular vacuolation with pyknotic nuclei. The collagen matrix was generally intact. The endothelium was generally detached from Descement's membrane with vacuolation through the cellular structures. (magnification 475x)

Sample A, neat, 10-minutes, 2 hours

Epithelium: The epithelium of corneas treated with Sample A (slides B8693-B8695) was similar to the positive controls but more severe. The upper squamous had minimal detachment with marked vacuolation in the wing and basal cell nuclei. The loss of adhesion between the cells was significant. The epithelium was still attached to the basal membrane.

Stroma: The stroma was thicker than the negative controls. The upper stroma had severe collagen matrix vacuolation with moderate cellular vacuolation. There was also a significant amount of pyknotic nuclei. The mid stroma had cellular vacuolation with significant amounts of pyknotic nuclei. The collagen matrix had moderate vacuolation. In the stroma below mid depth, there was minimal cellular vacuolation with pyknotic nuclei. The collagen matrix had mild vacuolation.

Endothelium: The endothelium was separated from Descement's membrane.

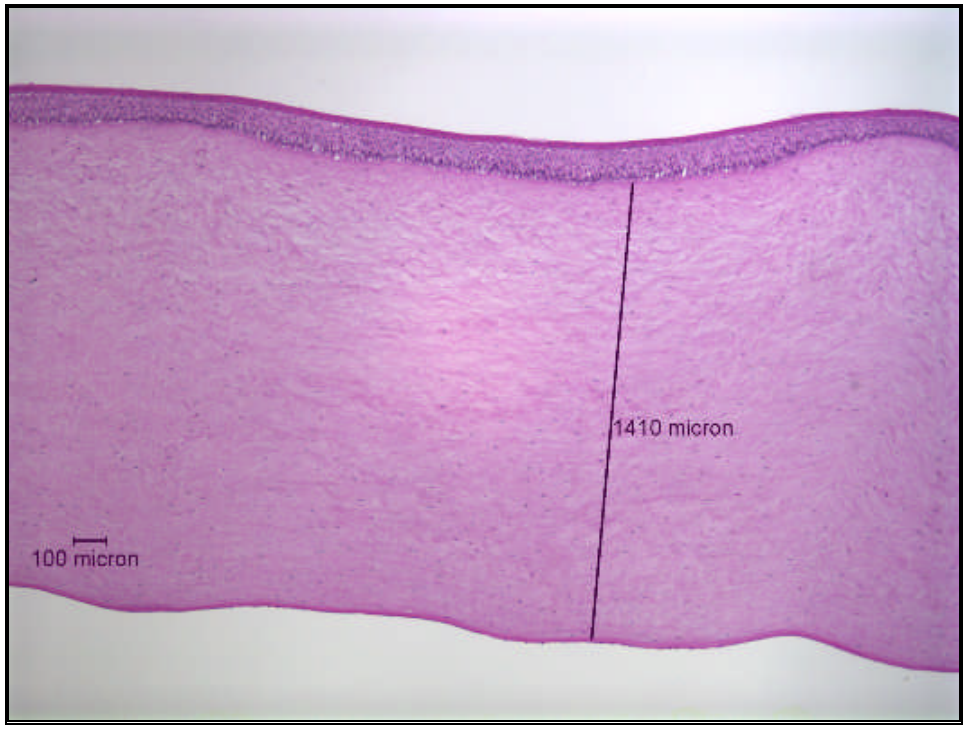

Figure 16. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Full thickness (magnification 48x)

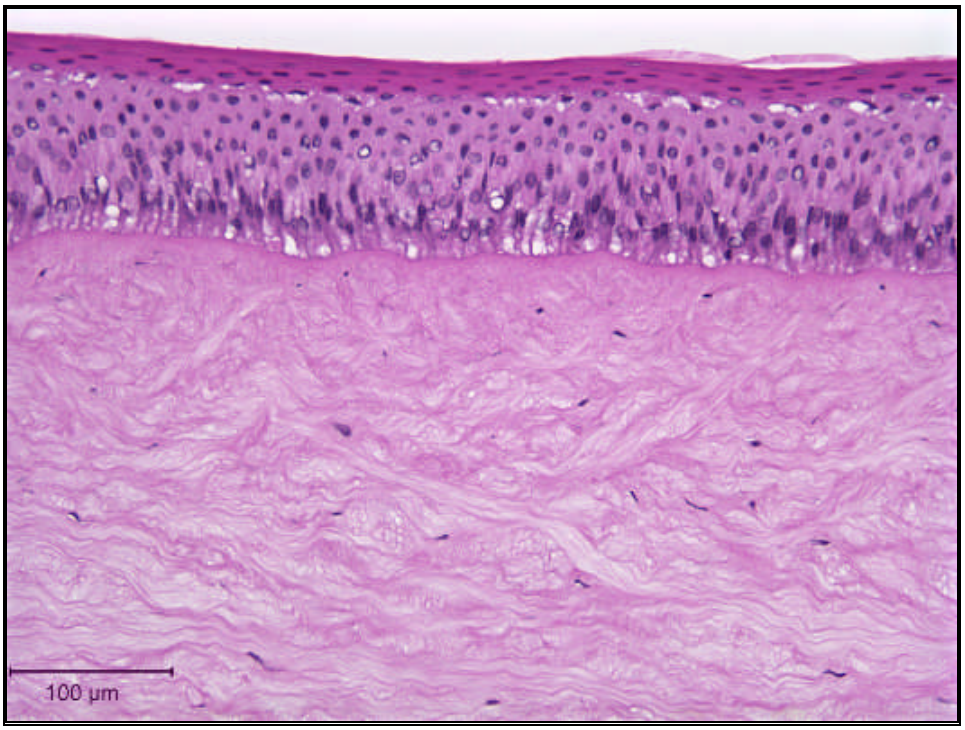

Figure 17. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Epithelium with detachment of upper squamous layer. Severe vacuolation of cellular matrix. Attachment to the basal membrane is weak. (magnification 237x)

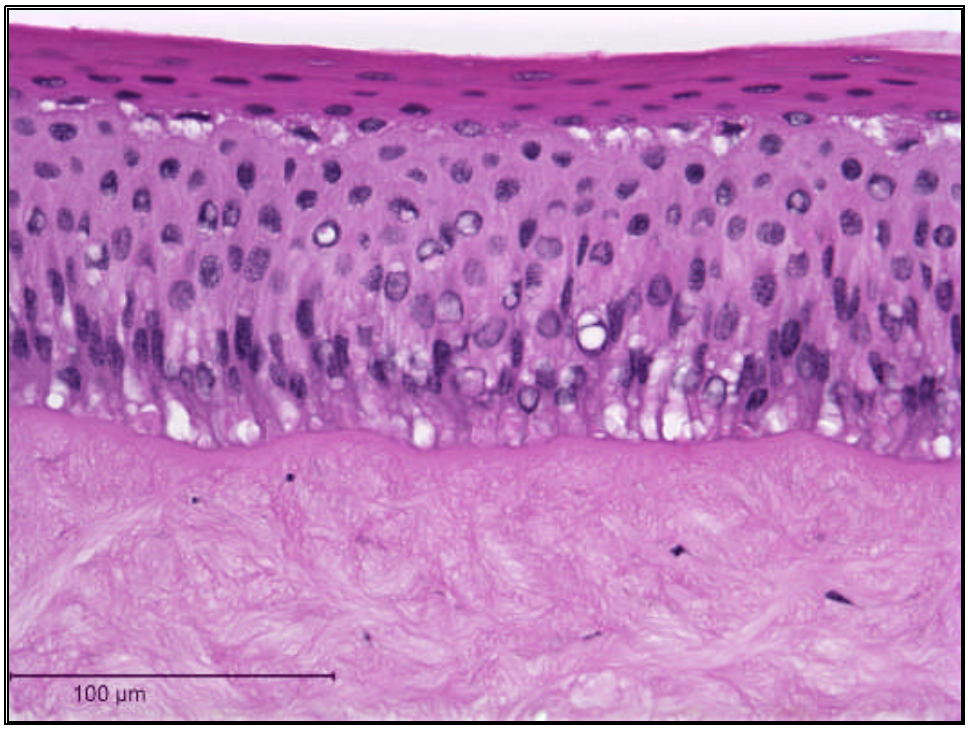

Figure 18. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Epithelium with detachment of upper squamous layer. Severe vacuolation of cellular matrix. Attachment to the basal membrane is weak. (magnification 475x)

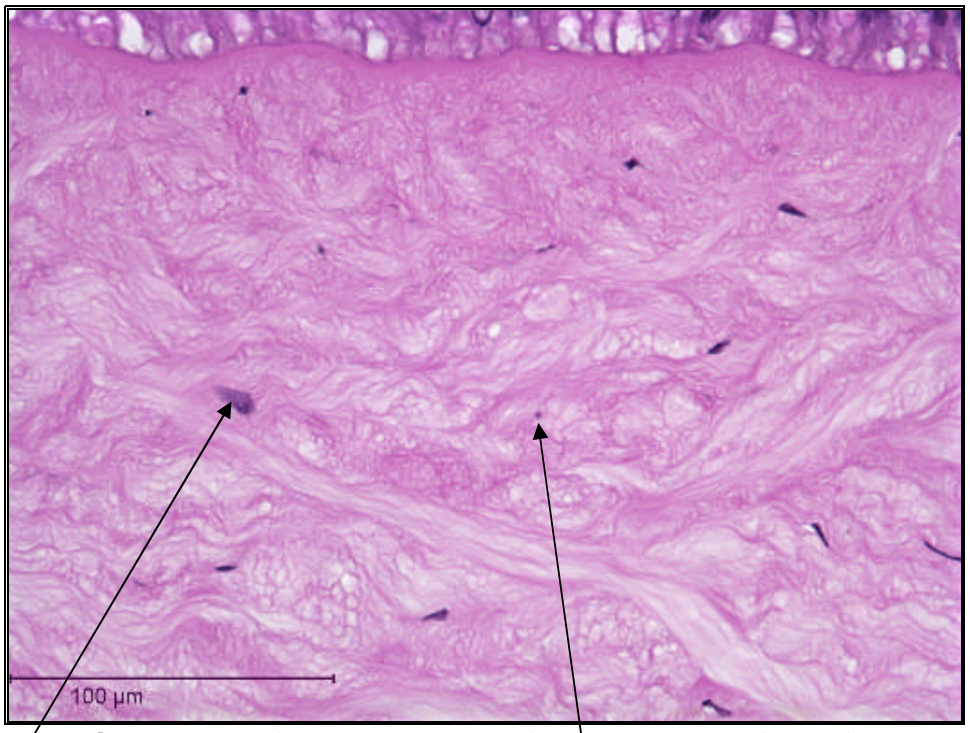

Figure 19. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Stroma directly beneath Bowman's layer showing severe collagen matrix vacuolation with moderate cellular vacuolation. There was also a significant amount of pyknotic nuclei. (magnification 475x)

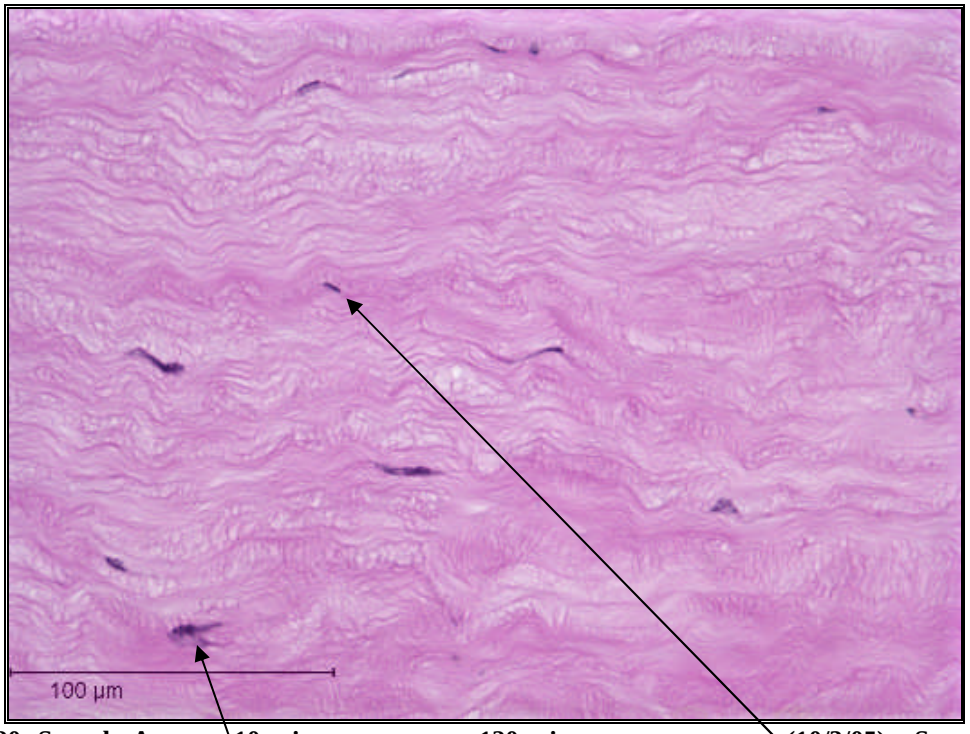

Figure 20. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Stroma near mid depth showing cellular vacuolation with significant amounts of pyknotic nuclei. The collagen matrix had moderate vacuolation. (magnification 475x)

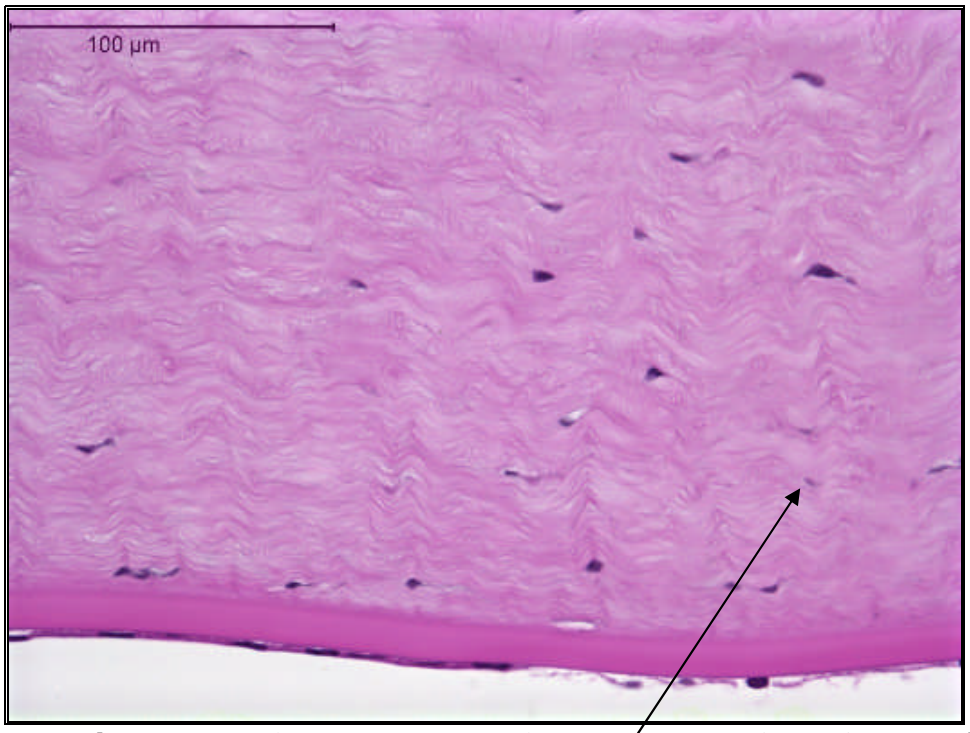

Figure 21. Sample A, neat, 10 minute exposure, 120-minute post-exposure (10/3/05) - Stroma beneath mid depth showing minimal cellular vacuolation with pyknotic nuclei. The collagen matrix had mild vacuolation. The endothelium was separated from Descement's membrane. (magnification 475x)

Histopathology Performed by:

Jennifer R. Nash, M.S. Date