Extraocular muscle and Harderian gland degeneration and regeneration after exposure of rats to continuous fluorescent illumination

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Exposure of adult albino rats to continuous cool-white fluorescent illumination caused extensive destruction to the extraocular skeletal muscles (EOM's) and Harderian glands. After 1 day of exposure, leukocytes and macrophages invaded the damaged area and were found among and within the myofibers. After 48 hr of exposure, myoblasts and short myotubes extending from the damaged fibers indicated that regeneration had begun. In spite of constant illumination of the animals, the EOM's continued to reconstitute, and by the seventh day of exposure, regeneration was almost complete. At this time, small loci of degeneration and leucocytic infiltration resembling those seen in the 1-day stage again occurred. Results supported the speculation that differentiated myofibers were susceptible to the damaging effects of continuous fluorescent illumination, but that myoblasts, myotubes, and early undifferentiated myofibers were not. Although the tubular epithelium proliferated in damaged Harderian glands, very few other regenerative changes were observed during the 7-day exposure period. When animals with one eye occluded with a plastic contact lens and the other unoccluded were exposed to continuous illumination, the pattern of tissue destruction in unoccluded eyes was identical to that described in the above series. However, EOM's in occluded eyes were unaffected, and Harderian glands had minimal damage limited to unshielded areas at the conjunctival fornix. Glands apparently were more susceptible to injury than EOM's. Orbital tissue destruction in these animals seemingly was due directly to a radiant energy-dependent mechanism.

Key words: extraocular muscle, degeneration, regeneration, photoperiod, photic damage, Harderian gland

In albino rats exposed to intense, short-term incandescent irradiation, the extraocular mus-
extraocular muscles along with damaged Harderian glands. The purpose of this study was to verify that observation and describe the degenerative and regenerative changes occurring in the orbital tissues of rats exposed to fluorescent illumination.

Methods

Female adult albino rats, CD strain, were kept in a cyclic fluorescent light environment (14 hr of light:10 hr of darkness, 70 foot-candles) prior to the beginning of the experiment. They were housed in clear polyethylene cages with wire tops at 25° ± 1°C. At the beginning of continuous light exposure, 18 rats were placed under cool-white fluorescent light with a spectral range of 397 to 732 nm (maximum at 590 nm) and an approximate intensity of 150 foot-candles. Animals were removed after 1 (n = 2), 2 (n = 4), 3 (n = 4), 4 (n = 4), and 7 (n = 4) days of continuous exposure. In 10 additional rats, a black, opaque plastic contact lens contoured to fit the corneal surface was inserted beneath the eyelid of the left eye while the right eye was unoccluded. These animals were exposed to the same continuous light intensity as the above groups for 3 days. The rats were then overanesthetized with ether, and an autopsy was performed. After removal of the skin from around the orbit, 1 ml of Bouin's solution was injected intraorbitally to fix the extraocular muscles (EOM) in situ. After 15 min, the eyes, along with the Harderian glands and EOM's, were removed and further fixed for 24 hr in Bouin's solution. They were dehydrated in alcohols, embedded in paraplast, and sectioned in 7 μm thicknesses on the anterior-posterior axis of the eye. Sections of the central retina, including the optic nerve, EOM's, and Harderian glands, were stained with Harris' hematoxylin and eosin. Measurements of depth of

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Fig. 1. Longitudinal section of an extraocular muscle from a control rat exposed to cyclic photoperiod. (×400.)
Fig. 2. Longitudinal section of a degenerating extraocular muscle from a rat exposed to 48 hr of continuous fluorescent light. Extensive infiltration of leukocytes occurred during the first 48 hr of exposure. Eyeball is towards the top of photomicrograph. (×100.)

Damage were made with an ocular micrometer from the posterior surface of the eyeball to the approximate boundary between normal and damaged tissue in the EOM's and Harderian glands. The following measurements were made on each retina with an ocular micrometer at ×400 magnification: (1) outer nuclear layer thickness, measured from the outer limiting membrane inward to include all photoreceptor nuclei, and (2) retinal thickness, the distance from the outer limiting membrane to the inner margin of the ganglion cell layer. The two measurements were taken at 12
Fig. 3. Section of a Harderian gland from a control rat exposed to cyclic fluorescent photoperiod. (×100.)

different loci around the circumference of the retinal section, with each locus separated by approximately 540 μm. Mean thickness of the outer nuclear layer and retina was derived by averaging the measurements of the 12 loci.

Results

The extraocular muscles from control, cyclic fluorescent light-exposed rats (Fig. 1) had only occasional pyknotic nuclei and leukocytes scattered among fully differentiated myofibers. After 24 hr of continuous light exposure, large numbers of granular and agranular leukocytes and plasma cells had invaded the perimysium, loose areolar connective tissue, and areas of damaged myofibers (Fig. 2). Depth of damage extended from 2.3 to 2.7 mm into the lateral and medial rectus
Fig. 4. Section of damaged Harderian gland from a rat exposed to 2 days of continuous fluorescent illumination. Tubules nearest the eyeball (top) have disintegrated or are infiltrated extensively with leukocytes. Tubules with a more normal appearance occur away from the eyeball (bottom). Some leukocytes are in the tubular lumina. (×100.)

muscles, the muscles apparently most affected by exposure. The superior oblique muscle did not appear to be extensively damaged after this period of exposure.

When compared with normal Harderian glands (Fig. 3), glands of exposed rats had disrupted tubules, epithelial cell destruction, and extensive leukocytic infiltration in the damaged area, which extended from 1.8 to 2.3 mm behind the eyeball (Fig. 4). Harderian gland destruction after 2 days’ exposure did not appear to differ from that after only 1 day of exposure.

After 2 days of exposure, EOM damage ex-
Fig. 5. Longitudinal section of an extraocular muscle from a rat exposed to continuous illumination for 4 days. Basophilic myotubes with rows of nuclei run diagonally across the photomicrograph. Compared to the 24 and 48 hr degenerative stages, leukocyte number is reduced. (x400.)

tended throughout the circumference of the muscles, but was still more severe nearest to the insertion at the eyeball. Large numbers of granular and agranular leukocytes still filled areas of muscle damage. At this time (48 hr), some fibers had beginning signs of regeneration. Myoblasts with large nuclei, prominent nucleoli, and circumnuclear basophilic cytoplasm occurred within myofibers with fragmenting sarcoplasm. The epimysial and perimysial connective tissue sheaths were continuous from their location in un-
damaged muscle distal to the eye to their insertion on the sclera, where more myofiber damage occurred.

After 3 days of exposure, the damaged muscle area contained a greatly reduced number of leukocytes and evidenced advanced stages of muscle regeneration. From the ends of myofibers, basophilic myotubes with centrally rowed nuclei extended toward the muscle insertion. The pattern and depth (2.4 mm from sclera) of Harderian gland degeneration was similar to that recorded for 1 and 2 days of exposure. However, epithelial cells were proliferating actively, forming new tubules and solid cords of cells among the damaged ones, indicating the possibility of glandular regeneration.

After 96 hr of exposure, EOM regeneration consisted of long, basophilic myotubes containing rows of nuclei with prominent nucleoli (Fig. 5). Longitudinally arranged myofibrils and cross-striations occurred at this time. Although connective tissue cells were still present among the regenerating muscle elements, the number of leukocytes was reduced as compared with the 1- to 3-day stages. Arterioles ranging in diameter from 24 to 32 μm extended lengthwise between the regenerating myofibers. For this exposure time, damage to Harderian glands was measured up to 4.5 mm posterior to the eyeball. Leukocytes and macrophages filled the areas among and within the tubules of the gland. Proliferation of epithelial cells as described earlier continued.

Extraocular muscles of rats exposed to fluorescent light continuously for 7 days had reduced sarcoplasmic basophilia and wider...
myofibers with prominent cross-striations and central rows of nuclei (Fig. 6), as compared with EOM's from rats with shorter exposure period. The leukocytes and other connective tissue cells which formerly populated zones between regenerating muscle fibers were rarely seen in rats with this period of exposure. The extraocular muscles were continuous from the originally undamaged portion through the previously damaged area to insertion on the sclera. An unusual observation after 1 wk of continuous light exposure was new sites of primary degeneration in small loci within regenerating muscles. These sites were filled with granular and agranular leukocytes and were identical to those seen after exposure for 24 hr. The Harderian glands were still extensively damaged, although proliferation of epithelial cells continued and cords and tubules appeared to be reforming at this time. Damage extended up to 4.7 mm into the glands. The pathologic changes described for the extraocular muscles and Harderian glands in the time sequence series mentioned earlier were dependably and consistently found in each eye, with only minimal variation at each time period.

Measurements of the mean outer nuclear layer thickness of the retina in rats exposed to cyclic illumination and after 3 and 7 days of continuous exposure were 48.83 ± 2.46 μm, 29.14 ± 2.76 μm, and 18.23 ± 1.52 μm, respectively, indicating an approximately 63% reduction in photoreceptors after 7 days. Measurements of entire retinal thickness for the same groups were 137.57 ± 6.80, 115.55 ± 10.96, and 101.46 ± 8.35 μm.

Black plastic contact lenses fitted over the cornea of rats prevented the degeneration of extraocular muscles of all occluded eyes. The muscles of the opposite, unoccluded eyes, however, were damaged severely by light exposure and were regenerating in stages identical to those described after 3 days' exposure in the time sequence series described earlier. The pattern of Harderian gland degeneration was unusual in that damage occurred only in superficial tubules of the glands not covered by the contact lens. These tubules were immediately adjacent to the conjunctival fornix between the eyelid and cornea. Average depths of glandular damage were 0.319 mm (range, 0.136 to 0.455 mm) for occluded eyes and 2.350 mm (range, 1.909 to 2.636 mm) for unoccluded eyes. Muscles nearest to the fornical zone were undamaged in all occluded eyes. Retinal thicknesses in these rats were 129.16 ± 8.65 μm and 92.00 ± 5.46 μm (p < 0.05), while outer nuclear layer thicknesses were 47.12 ± 1.86 μm and 31.56 ± 1.23 μm (p < 0.05) for the occluded and unoccluded eyes, respectively.

Discussion

Since previous studies from this laboratory did not reveal any degenerative changes in extraocular muscles of albino rats exposed to fluorescent light, and since exposure to incandescent light did cause extensive EOM damage, it was concluded that the different spectral wavelengths emitted by the two light sources must be responsible for the difference in observations. However, the present experiments, in which rats were exposed to approximately twice the intensity of fluorescent illumination used previously, indicated that cool-white fluorescent light exposure can induce EOM damage. The appearances and time sequence of degeneration and regeneration were identical to those previously described for incandescent damage. In that study, rats were exposed for 17.5 hr and tissues were examined at 0 to 14 days later, while in the present study, they were exposed continuously from 1 to 7 days. This latter experimental design revealed an interesting sequence of events not possible in the former. Exposure to cool-white fluorescent illumination for 1 day was sufficient to damage skeletal muscle, and signs of regeneration were observed during the second day of exposure. Even though the animals remained exposed to continuous illumination during the subsequent day, the degenerative changes apparently ceased, and regeneration progressed rapidly. The developmental myoblastic and myotube stages of regeneration appeared to be resistant to the damaging effect of fluorescent light. However, in tissues examined after 7 days of exposure, loci which
resembled the pattern of primary muscle fiber destruction were observed again. Small localized areas of sarcoplasmic disruption occurred and were filled with leukocytes and other connective tissue cells, as seen previously during the first and second day of exposure. Blood vascular elements which rapidly invaded the extraocular tissues and reactive elements already in the area did not appear to be the causative agents of muscle and glandular destruction. Since these elements were present only for a short period at the beginning of exposure, were then reduced in numbers, and did not reappear until subsequent degeneration at 7 days of continuous exposure, their occurrence seemed to be in response to, and not the cause of, damage. Therefore, it seemed reasonable to speculate that, in EOM’s of adult rats, fully differentiated myofibers were highly susceptible to photic damage, but that myoblasts and myotubes, stages of early myogenesis, were much less susceptible to destruction. As the differentiating myoelements began to lose their sarcoplasmic basophilia and became cross-striated, but while muscle nuclei were still arranged in central rows, EOM’s again became highly susceptible to damage by fluorescent illumination.

Harderian glands were also severely damaged by exposure to incandescent and fluorescent illumination and did not appear to have the capacity to regenerate or repair as rapidly as the EOM’s. Some re-epithelization of the tubules occurred as early as the second and third day of continuous exposure, but extensive damage remained unrepairable during subsequent days of exposure. For animals with one eye occluded with an opaque, black contact lens, Harderian glands were minimally damaged, and only the superficial tubules, which were unshielded at the conjunctival fornix, were affected. Extraocular muscles, even those adjacent to damaged Harderian glands, appeared unchanged at the light microscopic level in all occluded eyes. This observation indicated that glands apparently were more susceptible to photic injury than the EOM’s. Therefore, since severely damaged Harderian glands occurred immediately adjacent to EOM’s during both degeneration and regeneration of muscles in the time sequence series and in the occluded eyes, it seems unlikely that EOM damage is a consequence of gland damage, i.e., resulting from an influence of gland degradation products. Additional evidence for this hypothesis was the observation that muscle damage always occurred first at the muscle insertion on the sclera, while gland destruction extended several millimeters further behind the eyeball, where the EOM’s were undamaged. Related to Harderian tubular destruction, Reiter and Klein⁵ found that Harderian glands from rats exposed to continuous light (70 foot-candles) for 9.5 wk contained tubules whose epithelial lining was sloughed into tubular lumina. The Harderian glands appeared to be under the influence of the pituitary gland, possibly by the influence of thyroid-stimulating and growth hormones,⁶ but exposure to continuous light had little or no effect on synthesis or release of these hormones. Hypophysectomy resulted in atrophy of the gland,⁷ while injection of pituitary extracts produced glandular hyper trophy, and the gland was associated with the experimental development of exophthalmos. Animals in the present experiments developed a transient exophthalmos, which has been shown to be related to Harderian gland enlargement (unpublished results) during the first 24 hr of exposure and is being investigated further at this time.

The possibility that thermal energy might be responsible for EOM damage was considered in a previous experiment which utilized incandescent light sources.¹ In the present experiments, the cool-white fluorescent tubes emitted very little heat or infrared radiation. Also, more thermal energy would be expected to be absorbed by eyes covered with a black plastic occluder than in unoccluded eyes, and in the occluded eyes, injury to EOM’s and Harderian glands, except as noted, was undetectable. Therefore, tissue destruction in light-exposed eyes did not appear to be related directly to the heat-emitting spectrum of the light source.

Results from both the time sequence series of animals and the ocular occlusion experiment strongly suggest that EOM degenera-
tion and Harderian gland destruction were due directly to a photic energy-dependent mechanism.

REFERENCES

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