EXTRAOCULAR MUSCLE DEGENERATION AND REGENERATION AFTER EXPOSURE OF RATS TO INCANDESCENT RADIANT ENERGY

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SUMMARY

Exposure of albino rats to incandescent radiant energy for a short period of time in an elevated environmental temperature (39 °C) causes degenerative changes in the extraocular muscles. The muscle fibres regenerate and the muscles reorganize if the animals are returned to room lighting and temperature. Extraocular muscles (EOMs) were damaged first near their insertion on the eyeball. All EOMs of both eyes were affected, but the degeneration did not extend the entire length of the muscle. The peripheral fibres of each muscle were damaged before the more central fibres. Mitochondria were swollen and often contained dense bodies. Numerous vesicular profiles, possibly from the sarcotubular system, were present. Myofibrils of the more severely damaged fibres lacked typical Z-disk structures, and I-bands had disappeared by 24 h after the exposure period, a degenerative pattern which seems to be unique for this method of EOM damage.

EOM degeneration appeared to be dependent on the interaction between thermal and radiant energy on the orbital contents. However, EOMs were only rarely and very slightly affected when rats were exposed to elevated temperature in the absence of incandescent radiant energy. When an opaque, black, ocular occluder was placed over one eye and the contralateral eye was left unoccluded, EOMs and retinas of occluded eyes were undamaged, while those tissues were severely damaged in unoccluded eyes. Therefore, the most critical single variable in inducing EOM degeneration appears to be exposure to radiant energy.

INTRODUCTION

The stages of degeneration and regeneration of skeletal muscle after several forms of experimental traumatic injury, such as crushing or transection, have been described in the vertebrate classes, including mammals (Godman, 1957; Field, 1960; Betz, Firket & Reznik, 1966). More recently, Carlson (1973) and Reznik (1973) have reviewed new concepts related to the early cellular changes in skeletal muscle after injury, including mincing, devascularization, denervation and exposure to anaesthetics and to the origin of the new myoblastic cells; the subsequent fusion of the myoblasts and growth of the myotubes along the sarcolemmal sheaths were described from studies of regenerating, mammalian skeletal muscle.

Little attention has been given to the damaging effects of radiant energy on skeletal muscle, a procedure which offers several advantages over the use of conventional methods of traumatic injury in studies of muscle degeneration and regeneration. For example, the use of radiant energy permits muscle injury without severe traumatic damage to tissues, such as the skin, overlying the muscle.
While examining several aspects of light exposure on retinal photoreceptor damage (O'Steen & Anderson, 1972; O'Steen & Karcioglu, 1974; O'Steen, Anderson & Shear, 1974), degenerative changes were observed in the extraocular skeletal muscles (EOM) of albino rats exposed to moderately intense incandescent light sources for short periods of time. The present studies arising from these observations were designed to determine (1) the degree of damage and sequential stages of the 'light-induced' degenerative changes in the extraocular muscles, (2) the destructive influences on these skeletal muscles of radiant energy derived from an ordinary incandescent light source and of conducted heat by elevating the environmental temperature of the experimental animals, and (3) the subsequent regenerative changes leading to the reconstitution of the damaged portion of the extraocular muscles.

MATERIALS AND METHODS

Albino rats (CR Strain, Charles River) were kept in a cyclic light environment (14 h of light: 10 h of darkness; 756 lux, direct illuminance) at 22 °C ± 1 °C prior to incandescent radiant energy exposure to induce skeletal muscle degeneration. When weanling rats were used, they came from litters born in the cyclic photoperiod. Food and water were given ad libitum.

At each exposure period, a clear plastic cage (36 × 30 × 15 cm) containing one or two rats was placed in a chamber illuminated with two 150-W incandescent bulbs (General Electric Co., spectral wavelengths = 350-2500 nm; peak wavelength = 1000 nm) located in the back of the box (O'Steen & Karcioglu, 1974). The energy flux density, which was measured with a Model SR Spectroradiometer (Instrumentation Specialties, Co., Inc., Lincoln, Nebraska) at animal eye level, formed a bell-shaped curve. Maximal energy density at 975-1000 nm was 18.6 μW cm⁻², and, at one-half maximal energy intensity, the wavelength width of the bell-shaped curve was 675—1175 nm, measured 39 cm from the energy source, the cage location most commonly selected by the animals during the exposure period. Maximal energy density in the centre of the cage (24 cm from source) was 39.9 μW cm⁻². At the cage wall closest (9 cm) to the energy source, maximal energy flux density was 87.9 μW cm⁻²; the animals were not seen in this location during exposure. The cage temperature was thermostatically controlled at 39 °C during the exposure period.

The chamber, which measured 508 cm high × 508 cm wide × 635 cm deep, was equipped with a 3-in. (7.6 cm) electric fan for ventilation.

Exposure time was controlled by an electric timer and ranged from 3.5 to 17.5 h, according to the experimental designs described below.

During the exposure period, rectal and orbital temperatures of several rats were monitored with a YSI Tele-Thermometer equipped with a remote small animal probe (Yellow Springs Instruments Co.). The orbital probe was placed among the extraocular muscles immediately adjacent to the eyeball and the optic nerve in ether-anaesthetized animals.

Following exposure, the animals remained in the box in total darkness for a period of 4.5 h. Afterwards, the rats were returned to the control level, low intensity, cyclic photoperiod, where they remained until the experiment was terminated. They then were anaesthetized with ether, and the entire orbital contents were removed, fixed in Bouin's solution, dehydrated, cleared and paraffin embedded. Prior to eye removal, 1 ml of Bouin's solution was injected into the orbit around the eye to fix the muscles in an extended state; after approximately 5 min, the entire orbital contents were removed and immersed in the fixative. A small specimen of peri-orbital skeletal muscle (frontalis muscle) from control and experimental groups was fixed as an example of extraocular muscle; in addition, the levator palpebrae superioris muscles of several rats were examined for degeneration. In early experiments black silk sutures were placed through the anterior nasal and superior aspects of the conjunctiva of extirpated eyes to facilitate orientation during embedding and identification of each specific extraocular muscle in tissue sections. Eyes were cut serially at 7 μm thickness on the anterior–posterior ocular axis, and every twentieth section was mounted on a slide for staining with Harris' haematoxylin and eosin.

For electron microscopy, the rats were anaesthetized with ether, and the orbital contents
were removed and fixed in cold (4 °C) 2-5 % glutaraldehyde fixative buffered to pH 7-2 with 0-1 M phosphate buffer and containing 0-5 % glucose (Shear & Goldspink, 1971). Prior to eye removal, 1 ml of the glutaraldehyde solution was injected into the orbit to fix the muscles in an extended state. After 10 min the entire orbital contents were removed, and the muscles were exposed and dissected free of connective tissue. The muscles were then placed in a larger volume of the same fixative at 4 °C for an additional 2 h. After fixation the muscles were washed briefly in cold (4 °C), 0-1 M phosphate buffer and postfixed for 2 h in 1 % OsO₄ buffered to pH 7-2 with 0-1 M phosphate buffer and containing 0-5 % glucose. The muscles were dehydrated in ethanol and embedded in Araldite, CIBA-CY212 (Glauert, 1965). Ultrathin sections showing a silver-grey interference colour were collected on naked grids and stained with uranyl acetate and lead citrate solutions (Reynolds, 1963). The sections were examined with a Zeiss EM9 S2 electron microscope.

The following experimental procedures were performed:

Degeneration-regeneration sequence. After exposure (17-5 h as described above), rats were anaesthetized at 0 h (n = 6), 24 h (n = 8), 48 h (n = 6), 72 h (n = 4), 96 h (n = 4), 7 days (n = 6), and 14 days (n = 6), to determine the time sequence series of stages of degeneration and regeneration.

Minimum exposure time to cause EOM degeneration. Rats were exposed for 3.5 (n = 4), 7.0 (n = 4), and 24 h (n = 8), and anaesthetized at 0 and 24 h later to assess muscle damage.

Minimum environmental temperature during radiant energy exposure to cause degeneration. Rats were exposed for 17-5 h, as described above, but the environmental (box) temperature was regulated at 25 °C (n = 4), 30 °C (n = 4), and 35 °C (n = 4), instead of the usual environmental temperature of 39 °C. Rats were sacrificed immediately after exposure (0 h) or 24 h after exposure.

Heated (39 °C) environment with no radiant energy exposure. To determine whether environmental heat conducted through the eye in the absence of radiant energy would cause EOM damage, rats (n = 8) were placed in the same, but darkened, exposure box used in the above experiments. The darkened box, kept in a photographic dark room, was thermostatically maintained at 39 °C for 17-5 h with an electric blower-heater placed outside the box, so that the heat coil, and any radiant energy emitted from it, were shielded from the interior of the box. Extraocular muscles were examined 24 (n = 2 rats), 48 (n = 4 rats), and 72 (n = 2 rats) h after heat exposure.

Black contact lenses shielding one eye. The right eye of 4 rats, lightly anaesthetized with ether, was fitted with a thick, opaque, black plastic contact lens which shielded that eye from radiant exposure, but allowed for heat conduction. The left eye (unoccluded) was fully exposed to the incandescent energy source (17-5 h) as described in experiment 1 above. Environmental temperature was 39 °C. The contact occluders, which completely blocked the full energy flux density at all spectral wavelengths as measured with the Model SR Spectroradiometer, were supplied by Drs Irving Zucker and Richard King, University of California, Berkeley.

Lensectomized rats. With the rats (n = 4) under light ether anaesthesia, a small corneal incision was made adjacent to the corneal-scleral junction, and the lens was removed with forceps from the right eye only. After a 24-48 h recuperative period, the rats were exposed to the radiant energy-temperature environment described for experiment 1 above, to determine if incandescent, radiant energy might be focused, or concentrated by the lens on the posterior surface of the eye and intraorbital contents, and thereby contribute to the severity of EOM damage.

Age dependency and EOM damage. To determine if age was a factor in the susceptibility of skeletal muscle to damage by radiant energy exposure, rats at each week of age were divided into control (energy flux density = 2-7 μw cm⁻²; animal room temperature = 22 ± 1 °C) and experimental (energy flux density = 18.6 μw cm⁻²; exposure box temperature = 39 °C) exposure groups: 3 weeks (n = 16); 6 weeks (n = 16); 9 weeks (n = 12); 12 weeks (n = 8); 14 weeks (n = 14); and adult rats (ages 16-24 weeks; n = 18). Equal numbers of male and female rats were used in all groups. Experimental animals were returned to the control photoperiod-temperature environment for 48 h prior to anaesthetization and tissue processing.

Degeneration and regeneration in pigmented rats. Adult Long-Evans, pigmented rats (n = 6) were exposed to radiant energy in the same manner as described in experiment 1 above. After exposure, they were returned to the control environment for 24 or 48 h before anaesthetization and tissue processing.
RESULTS

The exposure of albino rats to incandescent radiant energy (186 μW cm⁻²) at elevated environmental temperature (39 °C) caused degenerative changes in all of the extraocular muscles; the damaged myofibres were replaced by developmental stages of regenerating muscle. Each extraocular muscle was most affected at its insertion area on the surface of the eye and from 2 to 3 mm deeper toward the muscle origin. After exposure for the described time periods, the muscles were never affected along their entire lengths. In addition, the myofibres on the periphery of the muscle were extensively damaged before the more central fibres showed any changes at the light-microscopic level (Fig. 2). Extraocular muscles lying closest to the optic nerve (recti muscles) appeared to be more susceptible to damage than the other muscles of the eye. The muscles were equally affected bilaterally in all animal groups. The frontalis muscle, which was examined as an extraorbital skeletal muscle control tissue for comparison with EOMs, was apparently unaffected by the experimental procedures involving radiant and thermal energy exposure. On the other hand, the levator palpebrae superioris muscles were damaged by radiant energy exposure. More damage appeared to be in the fibres in the body of the muscle, particularly those lying in the orbit, as compared to those in the eyelid.

During the exposure period the rectal temperature of the animals increased from 38° to 42.5 °C, with the environmental temperature adjusted to 39 °C. Orbital temperatures increased from 37.2° to 40 °C in the same exposure period.

Degeneration and regeneration sequence

Extraocular muscles examined immediately after exposure (0 h) had early stages of sarcoplasmic destruction characterized by fragmentation within the sarcolemma. An increase in the nuclear population of the muscle occurred as granular leucocytes appeared among the damaged muscle fibres. By 24 h after exposure the sarcoplasm of many muscle fibres had been replaced by large numbers of mononuclear cells, which resembled monocytes, and polymorphonuclear leucocytes (Fig. 1). Although in some instances this cellular invasion seemed to disrupt the orientation of the degenerating fibres, in most cases, the longitudinal arrangement of the damaged fibres remained unchanged (Figs. 2, 3, 9). Nuclei, which appeared to be the original muscle nuclei and differed in shape and in density from those of the invading mononuclear cells, occurred along the sarcomemmal sheaths. These nuclei were surrounded frequently by distinctly basophilic sarcoplasm which occupied spherical areas measuring 20–25 μm in diameter, and sometimes larger. In some instances, the basophilic sarcoplasm extended up to 100 μm along the sarcomemmal sheaths.

All EOM fibres examined electron microscopically 24 h after the end of the exposure period were more severely damaged near their insertion than more distant from the surface of the eye. In many muscle fibres the damage was limited to dilation or swelling of the sarcoplasmic reticulum with little or no damage to the transverse tubules (Figs. 9–11, 15). In those fibres that showed the least damage, the triads (2 terminal cisternae opposed to an intermediate T-tubule) were seen at the A–I junction.
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of the myofibrils (asterisks, Figs. 11, 15). The contractile filaments comprising the myofibrils remained organized and well-defined. The mitochondria appeared normal and regular Z-disks, I-bands, and A-bands were present (Figs. 9, 11). In many of the more degenerated muscle fibres examined 24 h after exposure, the myofibrils lacked typical Z-disk structures and the I-bands had disappeared (Figs. 9, 10, 12). In those degenerated fibres whose myofibrils lacked Z disks, the contractile filaments often were organized into discrete bundles arranged parallel to the long axis of the cell (Fig. 9). However, in other degenerated fibres, poorly organized myofibrils were arranged perpendicularly or transversely to the long axis of the muscle fibre (Figs. 10, 12). In those myofibrils that lacked Z-disks, the thick and thin filaments remained organized as shortened 'pseudosarcomeres' with each thick (12-nm) filament surrounded by more than the usual 6 thin (5-6 nm) filaments typical of the relaxed sarcomere (Fig. 12). Only occasional thin filaments were seen between A-bands of successive pseudosarcomeres, and all traces of recognizable Z-line structure were absent (Figs. 10, 12). The mitochondria were swollen, vacuolized, and often contained small dense bodies (Fig. 10). When compared to control muscles (Figs. 13, 14), numerous vesicular profiles of varying size and shape, derived possibly from the dilated and fragmented sarcotubular system (i.e., transverse tubules plus sarcoplasmic reticulum), were observed (Figs. 9, 10, 12, 15). Macrophages of undetermined origin, containing phagocytic vacuoles and lysosomes, were also seen beneath the basement membranes of the damaged fibres (Figs. 10). Satellite cells, containing abundant free ribosomes, few mitochondria, little rough endoplasmic reticulum, and poorly developed Golgi complexes, were often observed under the basement membranes of the more degenerated fibres (Fig. 9). Presumptive myoblasts were often observed beneath the basement membranes of the damaged fibres (Figs. 10, 12). The nomenclature applied to satellite and myoblastic cells in this investigation has been adopted from studies by Mauro (1961), Muir, Kanj & Allbrook (1965) and Muir (1970) and recently reviewed by Carlson (1973) (see Discussion).

The most obvious differences between stages at 24 and 48 h after radiation damage were (1) a reduction in the number of mononuclear cells within the sarcolemmal tubes and connective tissue of the muscle, (2) reduction, or sometimes absence, of fragmenting sarcoplasm in the sarcolemmal sheaths, (3) an increased population of basophilic, mononuclear cells, resembling myoblasts, undergoing mitotic divisions, (4) an overall increased basophilia in the entire area of regeneration, and (5) the appearance of long, multinucleated, basophilic myotubes oriented along the original longitudinal axis of the muscles (Figs. 2, 3, 5). Some short, multinucleated myotubes were oriented randomly in the sarcolemmal tubes. At 72 and 96 h after damage, multinucleated myotubes (Fig. 4) and immature myofibres filled much of the originally damaged area, and at the light-microscopic level, the regenerating muscle elements appeared to have reoriented and reorganized into a new extraocular muscle mass within the dimensions of the original connective tissue sheath.

Immature myofibres, with cross-striations and long rows of centrally arranged nuclei, and more mature myofibres with peripheral nuclei occurred typically in the extraocular muscles examined 7 days after injury (Fig. 6). Although a series of
measurements was not made, the cross-sectional diameter of regenerating muscles of
several animals appeared to approach that of normal muscles of control animals. By
14 days after muscle damage, the extraocular muscles still seemed to have a deeper
basophilia, a slightly greater nuclear density, and more central-rowing of nuclei than
found in muscles of control rats (Figs. 7, 8). However, in some eyes it was difficult to
distinguish the exact limits of the original muscle damage because of the high degree
of regeneration, or reconstitution, of the muscle.

By the end of the 2-week recovery period, the connective tissue elements of the
muscles had a normal pattern of distribution in the area of regeneration (Figs. 7, 8).

Minimal exposure time to cause EOM degeneration

Extraocular muscles of rats exposed to incandescent radiant energy for 3.5 h had no
obvious damaged areas when examined with the light microscope immediately after
exposure (0 h). However, when animals were exposed for the same period of time and
returned to the control environment for 24 h before muscle examination, damaged
areas extending up to 1.0 mm from the eyeball into the muscle contained myofibres
filled with polymorphonuclear leucocytes and mononuclear cells. The sarcoplasm in
these fibres was vacuolated and fragmented and seemed to be shrinking away from the
sarcolemmal and endomysial sheaths. Immediately after 7 h of exposure (0 h),
muscles closely adjacent to the eyeball had areas of damaged myofibres containing
numerous mononuclear cells and polymorphonuclear leucocytes and appeared similar
to the 3.5-h exposure muscle 24 h after the end of the exposure period. Polymorpho-
nuclear leucocytes were more prevalent at 0 h than at 24 h after exposure in the latter
group (7-h exposure). When examined 24 h after a 7-h exposure, the pattern and
degree of degeneration was difficult to distinguish from that occurring after 17.5 h
of exposure (Fig. 1).

Minimal environmental temperature during radiant energy exposure to cause degeneration

The pattern of muscle degeneration, that is, fragmentation and vacuolization of
myofibres, followed by leucocytic infiltration, after exposure to 25, 30, and 35 °C, was
similar to that of rats exposed to 39 °C. The extent of damage was significantly
reduced in rats exposed to 25 and 30 °C, as compared to those exposed to 35 and
39 °C, and usually involved only the myofibres immediately adjacent to the eye. The
myofibres on the surface of the recti muscles were more damaged than the central
fibres (Fig. 2), and in rats exposed to 25 °C, only occasional areas of focal damage
were observed. Polymorphonuclear leucocytes seemed to be more prevalent in
damaged muscles examined at 0 h than at 24 h after exposure, when mononuclear
cells were in the majority.

Heated (39 °C) environment with no radiant energy exposure

With one exception, the extraocular muscles of rats exposed to elevated environ-
mental temperature, but in the absence of radiant energy, were undamaged. The
lateral rectus muscle of one eye from a rat examined 72 h after exposure had a few
fibres (3-5 in each section) on the muscle periphery which were surrounded by
mononuclear cells and a few granular leucocytes. The cross-sectional area of one fibre in that muscle was filled with mononuclear cells. Cells with basophilic cytoplasm and nuclei resembling those of myoblasts were not observed in this area.

Black contact lens shielding one eye

The extraocular muscles in the orbit of unshielded eyes had extensive damage involving the entire diameter of the muscles, but only in the area adjacent to the eye and optic nerve, as in the above experiments. Muscles of the eye occluded by the black plastic contact lens appeared undamaged when examined 48 h after the exposure period. The fibres had not fragmented, were not invaded by leucocytes, and did not show sarcoplasmic basophilia.

Lensectomized rats

Muscle injury and regenerative changes occurred in the EOM of intact and lensectomized eyes. However, more extensive muscle damage, involving more myofibres, was present in muscles from intact eyes than from lensectomized eyes. Degenerative and regenerative changes extended from 2·18 to 2·37 mm behind the central sclera in intact eyes, and from 1·09 to 1·80 mm behind the central sclera in lensectomized eyes. Detachment and multiple foldings of the retina occurred after lensectomy in all animals.

Age dependency and EOM damage

Extraocular muscles were damaged, and subsequent regenerative stages occurred in all age groups of rats (3–24 weeks of age), and acute susceptibility could not be restricted to any single age period. Comparisons of muscle damage in male and female rats at different ages did not indicate a significant difference in susceptibility related to gender. No EOM lesions were observed in control animals in any age group.

Degeneration and regeneration in pigmented rats

EOMs and photoreceptors of pigmented, Long-Evans rats apparently were unaffected by the spectral intensities and elevation of environmental temperature used in this experiment, since neither degenerative nor regenerative stages were noted in these tissues at the light-microscopic level.

DISCUSSION

Vertebrate skeletal muscles are comprised of at least 2 kinds of muscle fibres, a fast-contracting, twitch fibre and slow-contracting, tonic fibre. Twitch and tonic fibres differ from one another in both functional and ultrastructural features. Among mammals the occurrence of tonic fibres is limited to the extraocular muscles (Krüger, 1957; Hess, 1961, 1962, 1967, 1970; Pilar, 1967; Hess & Pilar, 1963; Bach-y-Rita & Ito, 1966; Peachey, 1968), and an intrinsic muscle of the ear (Wersäll, 1958; Erulkar, Shelanski, Whitsel & Ogle, 1964; Fernand & Hess, 1969). In the present investigation all of the fibres in all EOMs from albino rats examined 24 h after exposure showed
some degeneration. Therefore, muscle fibre damage seems to be unrelated to the fibre type. Levator palpebrae muscles also were damaged by exposure to radiant energy. Contrary to these observations on intraorbital muscles, fibres of the frontalis muscle appeared to be unaffected by the treatment procedures.

The nomenclature pertaining to myogenic cells has recently been reviewed by Carlson (1973) and discussed by Reznik (1970) and Fischman (1972). In agreement with these authors, only those cells seen to contain myofilaments at the electron-microscopic level are considered myoblasts. Cells with approximately the same shape, size, and complement of cytoplasmic organelles, but lacking recognizable myofilaments are classified as presumptive myoblasts. Zhinkin & Andreeva (1963) found the mitotic cycle (cell generation time) of the myogenic cells of embryonic and regenerating muscle in the rat to be 20 h and the duration of mitosis to be 2.5 h. Therefore, the large number of presumptive myoblasts in the EOMs at 24 h after exposure is not surprising. Interrelationships between satellite cells, presumptive myoblasts, and myoblasts and the origin of regenerated myoelements were not examined in these experiments. However, experiments are currently underway utilizing [3H]thymidine labelling for cells synthesizing DNA and the double injury techniques of Walker (1972), as a means of marking the cell types involved in myofibre regeneration.

The fragmentation of the sarcotubular complex and the absence of Z-lines in the myofibrils of the more severely degenerated fibres are most interesting. Typically, in the relaxed vertebrate muscle fibre, each thick filament is surrounded by 6 thin filaments. More than 6 thin filaments were seen to surround each thick filament in the severely degenerating fibres. The increased thin to thick filament ratio indicates sarcomere shortening with extensive double contractile filament overlap. Such overlap would be expected if the dilated and fragmented sarcoplasmic reticular system released normally bound Ca\(^{2+}\) into the sarcoplasm. Troponin inhibition of myosin ATPase would thus be repressed, allowing actin-myosin interaction and shortening to occur (Bailey, 1948; Huxley & Taylor, 1958; Peachey & Porter, 1959; Ebashi & Endo, 1968; Peachey, 1970).

In a recent study by Busch, Stromer & Goll (1972), a protein fraction was isolated that causes Z-line removal from rabbit skeletal muscle myofibrils in the presence of Ca\(^{2+}\). Evidently, Z-line removal by this protein fraction requires Ca\(^{2+}\) levels higher than 0.1 mM. Damage or degradation of other myofibrillar elements was not observed. These authors suggest that the level of unbound Ca\(^{2+}\) in the sarcoplasm may regulate the activity of this protein fraction in vivo. It is tempting to speculate that the loss of Z-lines observed in the present investigation is the result of selective myofibrillar degradation, catalysed by an endogenous muscle protein, whose activity is triggered or regulated by the high Ca\(^{2+}\) levels resulting from sarcoplasmic reticular fragmentation. Those fibres showing less degeneration, i.e. normal Z-lines, possess a dilated, but not fragmented, sarcoplasmic reticulum and intact terminal cisternae. These less severely damaged fibres apparently cease to degenerate in the absence of additional exposure, since regenerative processes are observed shortly after the rats are removed from the intense radiant energy to the control environment.

During the early stages of these experiments, it was unclear as to whether the EOM
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degeneration was the result of exposure to incandescent radiant energy, to elevated body and environmental temperatures, or to both factors. The accompanying degeneration of photoreceptor cells in these rats has been shown to be related directly to the intensity of the light source (either fluorescent or incandescent), to the length of the exposure time, to elevations in the body and environmental temperature, and to whether the animals were pigmented or albino (Noell, Walker, Kang & Berman, 1966; Gorn & Kuwabara, 1967; O'Steen, 1970; O'Steen & Anderson, 1971, 1972; Anderson & O'Steen, 1972).

Since muscle destruction was significantly less in experiments involving exposure of animals to conducted thermal energy than to radiant energy, extraocular muscles appear to be more susceptible to the damaging effects of radiant energy. The elevations observed in body and orbital temperatures after exposure to radiant energy were comparable to those seen after thermal exposure; therefore, these variables from the control situation do not appear to be important in the induction of muscle degeneration. Also EOMs and retinas were undamaged when eyes were shielded with a black plastic occluder, which blocked transmission of the full spectrum of radiant energy, but allowed for the conduction of thermal energy into the orbit. Exposure of pigmented, Long-Evans rats to the same radiation and temperature regimen did not induce EOM degeneration (or photoreceptor damage). The pigmented iris, retinal epithelium, and choroidal tissue of these rats possibly absorbed the radiant energy entering the orbit from the eyeball and prevented it from reaching the EOMs. The EOMs of pigmented rats would have been exposed to conducted thermal elevations and body and orbital temperatures comparable to those of albino rats.

When albino rats were exposed to intense radiant energy and the environmental temperature for the different groups was adjusted from 25 to 39 °C, muscle damage appeared to be more severe at higher temperatures. On the other hand, muscle damage was observed after short periods (3.5 and 7 h) of radiant energy exposure, and when it was assessed after 24 h, the pattern appeared to be similar to that caused by longer exposure. Therefore, these results indicate that the EOM damage possibly is related to an interaction between exposure to intense radiant energy and to thermal energy. However, the most critical single variable apparently is radiant energy.

In order to determine more accurately the effects of thermal versus radiant energy, a more detailed comparison of the thermal properties of the incandescent and environmental energy sources must be made. A critical experiment related to this interpretation would be the exposure of albino rats to high intensity radiant energy at control environmental temperatures; however, the thermal energy would be shielded from the animal's eyes with heat-filter glass. Such an experiment is currently in progress, as is one to determine the exact spectral wavelength most effective in causing EOM damage.

Additional evidence supporting the suggestion that radiant, rather than thermal, energy is more important in the induction of EOM degeneration comes from the unique form of the damage, involving Z-line destruction in severely affected fibres seen at the electron-microscopic level. This observation has not been previously associated with thermal (heat or cold) damage to skeletal muscle (Adams, Denny-
Brown & Pearson, 1962; Price, Howes & Blumberg, 1964; Reznik, 1969, 1970; Schiller & Mair, 1974; Yanko, Behar & Yarom, 1974). The unusual structural modifications of the extraocular muscles described in these experiments, especially the early destruction of the Z-lines, may be peculiar to the method of damaging skeletal muscle, that is, exposure to intense incandescent radiant energy. However, the pattern of destruction may be a result of the degree of injury, since the damage most likely is much more subtle than that caused by the direct application of extreme heat or cold to the surface of a skeletal muscle, as described by several investigators (Adams, et al. 1962; Price et al. 1964).

EOM damage was observed in male and female rats exposed at all ages to high-intensity light. In a previous study, O'Steen et al. (1974) found that retinal damage, although apparently unrelated to gender, became progressively more severe as the animal aged.

Other experimental variables, which were difficult to control, include: (i) the animals were allowed to move freely in the exposure cages, and (ii) movements of the eyelids were unimpeded during exposure. That these 2 variables affected the results of each experiment seems unlikely, since EOM damage among individual rats within a group did not differ significantly.

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All light micrographs were made from longitudinal sections of damaged extraocular muscles from rats exposed to incandescent radiant energy. Sections were stained with Harris' haematoxylin and eosin. Original magnification is shown.

Fig. 1. Degenerating extraocular muscle prepared immediately after exposure. Leucocytes (granular and agranular) have infiltrated around and into an area of degenerating muscle fibres (arrows). Cross-striations can be seen in some fibres in the area adjacent to the damage. ×400.

Fig. 2. Lateral rectus muscle, 48 h after the end of the exposure period. Muscle damage is more severe peripherally (bottom), than centrally (top). Regenerating area (arrows) contains basophilic myoblasts and myotubes. Numerous leucocytes have invaded the damaged area, as evidenced by the increased nuclear density. ×100.

Fig. 3. The periphery of this medial rectus muscle (arrow) contains leucocytes and basophilic myoblasts and myotubes. O, optic nerve. 48 h after injury. ×100.

Fig. 4. Long, basophilic, multinucleated myotubes in a regenerating extraocular muscle examined 72 h after exposure. Note the prominent nucleoli in the myonuclei. Myotubes are oriented on the longitudinal axis of the muscle. ×400.
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Fig. 5. Long, multinucleated myotubes in extraocular muscles examined 48 h after exposure to radiant energy. Some leucocytes remain among the muscle elements. One multinucleated element (arrow) appears to be a young myofibre beginning to form noticeable cross-striations. × 400.

Fig. 6. Regenerating area of a lateral rectus muscle 7 days after damage by exposure. The sarcoplasmic basophilia and the leucocytic population are greatly reduced as compared with earlier stages of regeneration. Central rowing of nuclei and cross-striations can be seen in 4 of the regenerating myofibres. × 400.

Fig. 7. Regenerating area of a superior rectus muscle as it appears 14 days after injury. Some fibres have central rowing of myonuclei and obvious cross-striations. × 400.

Fig. 8. Regenerating area of a medial rectus muscle prepared 14 days after injury. Cross-striations are well developed. Many myofibres in the originally damaged area now have peripherally arranged nuclei, and the connective tissue pattern and leucocytic distribution are typical of those in control rats. × 400.
Extraocular muscle damage
Fig. 9. A longitudinal section of 2 superior oblique muscle fibres, 24 h after exposure to radiant energy. Note, in the lower fibre, the longitudinal orientation of the myofibrillar material (—m—) and the apparent absence of Z-lines. Well defined triads cannot be identified. Dense bodies are seen in the mitochondria and in the disorganized sarcotubular complex. A satellite cell process lies beneath the basement membrane of the lower fibre (*). Except for a slight dilation of the sarcotubular system, the upper fibre appears normal. A fibroblast cell process lies in the intercellular space between the 2 fibres. \( \times 10000 \).

Fig. 10. A longitudinal section of 2 different fibres from the superior oblique muscle of the same animal as Fig. 9. Note in the lower fibre the vesicular profiles of fragmented sarcotubular system (circle), Z-lines are absent and the myofibrillar material (m) is less well oriented than in Fig. 9. Two macrophages (mac, and mac'), containing phagocytic vacuoles and lysosomes, are seen beneath the basement membrane of the lower fibre. A presumptive myoblast (mb) lies under the basement membrane of the lower fibre and separates the 2 macrophage cells. The sarcoplasmic reticulum of the upper fibre appears dilated (arrows). Triads are seen at the A-I junction of the upper fibre. \( \times 11500 \).
Extraocular muscle damage
Fig. 11. A longitudinal section of a superior oblique muscle fibre, showing early damage similar to the upper fibres seen in Figs. 9 and 10, 24 h after exposure to radiant energy. Elements of the sarcoplasmic reticulum appear swollen or dilated (arrows). Triads are present at the A-I junction (*). The mitochondria and Z-lines appear normal. Note that the terminal cisternae are less dilated than other elements of the sarcoplasmic reticulum. Compare with Figs. 13-15. × 18000.

Fig. 12. A longitudinal section of a single superior oblique muscle fibre (mf) 24 h after exposure. Within the fibre, a myoblast (mb) is seen traversing the field. The myoblast cytoplasm only appears to contain myofibrillar material, when in fact the myofibrillar material bridges the myoblast process while remaining within the sarcolemma of the damaged muscle fibre. Note the absence of Z-lines (*), the perpendicular orientation of some of the contractile filaments (arrows), the vesicular profiles of the fragmented sarcotubular system (circles). Portions of a macrophage cell nucleus and cytoplasm are also seen (mac). × 16500.
Extraocular muscle damage
Fig. 13. A longitudinal section of an unexposed, normal superior oblique muscle fibre, showing triads (*) and other elements of the sarcotubular system. Note the narrow longitudinal tubules (lt). Compare with a similar view of a damaged fibre in Fig. 11. \( \times 30,000 \).

Fig. 14. A longitudinal section of an unexposed, normal superior oblique muscle fibre. Portions of 3 myofibrils are visible; the sarcoplasmic reticulum and T-tubule system are cut in tangential, face-view and show various regions of the sarcoplasmic reticulum. Note that the transverse T-tubules (t), terminal cisternae of the sarcoplasmic reticulum (tc), and longitudinal tubules of the sarcoplasmic reticulum (lt) are not dilated. \( \times 41,000 \).

Fig. 15. A longitudinal section of a damaged superior oblique muscle fibre, 24 h after exposure. The longitudinal tubular elements of the sarcoplasmic reticulum (lt) appear more swollen or dilated than in Fig. 11. The triad, composed of 2 terminal cisternae (tc) opposed to an intermediate T-tubule (t) appears less dilated than the other elements of the sarcotubular complex. Compare with a similar view of an undamaged fibre in Fig. 14. \( \times 46,000 \).
Extraocular muscle damage