The monkeys were sacrificed at 4 days and 68 days postinjection. The monkeys were deeply anesthetized with Ketalar and perfused through the heart with 4% paraformaldehyde in phosphate buffer (pH 7.4). The lower lids were dissected out and postfixed overnight in the same fixative. The lower lids were embedded in paraffin, sectioned and stained with hematoxylin/eosin or Masson trichrome stains. The latter is especially good for visualizing necrosis or hyalinization of muscle fibers. Pathologic changes in the skin, surrounding connective tissue and muscles were assessed with the light microscope.

Results
The chronic monkey showed evidence of skin ulceration and necrosis by 7 days after the doxorubicin injection (Fig. 1). The ulceration was healed completely by 3 weeks, although there was some loss of skin pigment and hair. After healing there was no change in the lower eyelid position or strength to blink stimulation under light ketamine anesthesia. The upper eyelid function and position was normal when the monkey was awake, but a slight relative ptosis was seen under ketamine anesthesia. This was unexplained.

Compared to the normal lid (Fig. 2), histological examination of the lower eyelid in the acute monkey 4 days after the doxorubicin injection showed that the entire lid was edematous (Fig. 3A). There was some evidence of a mild inflammatory mononuclear cell interstitial infiltrate in the preseptal portion of the lower lid, although this was very localized. The epithelium was markedly thickened in the area over the injection site, although the conjunctival epithelium and meibomian glands appeared normal. At 4 days, the injected orbicularis oculi showed an increasing gradient of muscle injury at decreasing distance from the injection site. There was evidence of extensive degeneration in the preseptal regions of the muscle, where the doxorubicin had been injected, but little evidence of pathologic change in the pretarsal regions of the muscle distant from the injection site (Fig. 3B, C). The preseptal orbicularis oculi muscle fibers were characterized by cytoplasmic vacuolization, edema and myofibrillar disorganization. Many necrotic muscle cells could be seen. In the Masson trichrome stained sections, dark fibers, the so-called hyaline fibers, were present. They were round, enlarged in diameter and individual myofibers were no longer visible. There was marked fragmentation and dissolution of the muscle fibers, typical of muscle pathology in severe forms of toxic damage.

In the monkey sacrificed 68 days after doxorubicin injection, the orbicularis oculi was greatly reduced in muscle mass. Very few muscle fascicles or fibers were present (Fig. 4A-C). There was an increased variation in fiber size, with an increase in connective tissue between fascicles. Again, the pretarsal region of the muscle appeared normal (Fig. 4B). The myofibers had a relatively normal appearance, with visible myofibrillar material within them. In the preseptal region of the orbicularis oculi, very little muscle remained (Fig. 4C). Most fibers showed architectural changes including whorled fibers and floccular changes. There was a small number of fibers present with visible myofibrillar material within them. This could either be the result of regeneration of a few of the injured muscle fibers, or a manifestation of the focal sparing of some fibers within injured regions of muscles treated with doxorubicin.

Discussion
Injection of doxorubicin into the orbicularis oculi of the lower eyelid resulted in a gradient of injury to
Fig. 3. Photomicrographs of a cross-section of the lower eyelid of a cynomolgus monkey 4 days after injection with 2 mg doxorubicin in the preseptal portion of the muscle. (A) Low-power photomicrograph of the lower lids. Arrowhead indicates the pretarsal muscle. Arrow indicates the preseptal muscle. Note extensive edema distorting the outline of the skin anterior to the muscle. (B) A high-power photomicrograph of the pretarsal portion of the injected lid. Note the normal muscle histology in this region. (C) A high-power photomicrograph of the preseptal portion of the injected lid. No normal fibers remain. The muscle fibers (arrowheads) are vacuolated and lack myofibrillar organization. Bar indicates 50 μm.

doxorubicin into the pretarsal region of the lower eye lid in rabbits that resulted in a decrease in total muscle mass with loss or vacuolization of almost all the pretarsal muscle fibers, complete loss of preseptal muscle fibers and sparing of muscle fibers in the orbital portion of the orbicularis oculi (unpublished). This feature of doxorubicin myotoxicity makes its particularly attractive as a potential treatment for blepharospasm, since manipulation of the dose administered would effect the quantity of muscle removed by the drug.

The characteristics of the muscle fiber necrosis are similar to those described for a number of types of trauma and toxins. These include vacuolization and the appearance of hyaline fibers. While a number of investigators have described the chemodenervation effects of doxorubicin, it appears from the present study that the chemomyotoxic effect of doxorubicin...
Fig. 4. Photomicrographs of a cross section of the lower eyelid of a monkey 68 days after doxorubicin injection. (A) Low-power photomicrograph. Arrowhead indicates pretarsal muscle. Arrow indicates preseptal muscle. The skin surface is at the top of the micrograph. (B) High-power photomicrograph of the pretarsal portion of the orbicularis oculi. The muscle fibers in this region appear normal. (C) High-power photomicrograph of the preseptal portion of the muscle. There is an increased variation in fiber size, with an increase in connective tissue between fascicles. Most fibers show architectural changes, including whorled fibers (arrowhead) and floccular changes. Bar indicates 50 μm.

previously described may be more important than its chemonedervation effect when it is used as an agent for the selective removal of a particular muscle. The effect of doxorubicin injection on the facial neurons projecting to the orbicularis oculi is under investigation.

Skin ulceration and necrosis have been described as a result of extravasation of doxorubicin during intravenous administration of the drug. Even after the high dose administered in the present study, the skin was fully healed by 3 weeks postinjection. In rabbits, lower doses of doxorubicin, from 0.4 to 1 mg, injected into the antecubital and popliteal spaces also resulted in skin ulceration and necrosis, while identical doses injected into the eyelids resulted in little evidence of alteration in the appearance of the eyelid skin, both at the gross level and at histological examination. There appear to be regional differences in the reaction of skin to doxorubicin injection. It may be that the greater amount of vasculature and the presence of muscle tissue in the skin of the eyelid make it less susceptible to injury by doxorubicin.

The possible use of doxorubicin in effecting a permanent treatment for blepharospasm and other related muscle diseases needs to be explored further. Its ability to selectively destroy a given population of muscle fibers with minimal side effects at the injection site makes it a prime candidate for use in these patients.

Key words: myopathy, myotoxin, orbicularis oculi, monkey, blepharospasm
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