mology, University Eye Clinic, Lund, Sweden. We did not find any effect of topical imidazole on the response to arachidonic acid either. The effect of topical imidazole on the α-MSH response was striking, however. The AFB to α-MSH was greatly facilitated and an aqueous flare could also provoke in otherwise unresponsive animals. Furthermore, the contralateral eye, which had not been pretreated with imidazole was made sensitive to α-MSH. From earlier studies it is well known that repeated daily injections of only α-MSH cause a successive decrease of the aqueous flare response. If the injections are continued long enough the animals become completely refractory. A rest period of about two weeks restores the previous ability to give a positive response.

The time relationships of the engagement of the contralateral eye by topical imidazole are of considerable interest. Two hours (the amount of time between administration of imidazole and injection of α-MSH) is obviously not enough for facilitation of the α-MSH response in the contralateral eye. After 24 hours the facilitating effect of imidazole is, however, equally pronounced in both eyes. This tells against the hypothesis that the imidazole effect is transmitted to the contralateral eye by humoral routes. This result, in conjunction with the experiment involving a small dose (10 mg. per kilogram of body weight) of intraperitoneal imidazole, also contradicts the idea that varying concentrations of imidazole in the eye are responsible for its divergent effects. The long-lasting effect of topical imidazole (10 weeks or more) is confusing and at present defies explanation. Zink, Podos, and Becker found no effect of intraperitoneal imidazole after 24 hours, a result that we have been able to confirm in our study. The way in which the effect of topical imidazole reaches the contralateral eye is also completely obscure and will be the subject of further studies.

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Key words: blood-aqueous barrier, aqueous flare, intraocular pressure, imidazole, prostaglandin, arachidonic acid, infrared irradiation, endotoxin, α-MSH.

REFERENCES


Congenital total external ophthalmoplegia associated with infantile spinal muscular atrophy. Fine structure of extraocular muscle. BRUCE R. PACHTER, JOHN PAISON, JACOB DAVIDOWITZ, RICHARD REUBEN, DINKAR BOAL, RONALD CARHI, AND GOODWIN M. BREININ.

A case of total congenital external ophthalmoplegia associated with infantile spinal muscular atrophy is presented. In the first 29 months of life, ophthalmoplegia has remained complete. Ultrastructure of lateral rectus extraocular muscle indicates a neurogenic process as the basis of the ophthalmoplegia. Light microscopy alone is insufficient to distinguish primary "myopathic" from "neurogenic" disease in external eye muscles.

Bilateral congenital ophthalmoplegia associated with neurogenic atrophy of skeletal muscle is rare.1-2 Reports of eye muscle pathology have frequently been insufficient to distinguish primary "myopathic" from "neurogenic" processes. The case presented here appears to be unique both
in the totality of the congenital external ophthalmoplegia and in presenting ultrastructural evidence of neurogenic atrophy in eye muscle which, at light microscopic level, would most likely have been described in the past as myopathic.

**Clinical findings.** A girl, born of a full-term normal delivery to healthy parents, was markedly hypotonic, with poor cry, poor sucking reflex, and constant drooling. A similarly affected sister had died at the age of seven weeks from aspiration pneumonia. A two-year-old sister was in good health. Initially nasogastric feeding and suction were required. By three months milk and soft foods could be swallowed. At three months there were no active eye movements but the eyes could be moved passively. Eyelid movements were normal. Corneal reflex, pupil morphology, light reflex, and retina were all normal. There were symmetrical facial and generalized body weakness. Tendon jerks were absent. Plantar reflexes were flexor. Sensation to pinprick was normal.

Skeletal muscles remained hypotonic but function gradually improved. The child sat at six months, spoke at 12 months, crawled at 19 months, stood at 21 months, and could walk by 29 months. Bilateral partial facial paresis persisted. Ophthalmoplegia remained total. Cog reflex and hearing were normal, as were other sensory functions. Cerebellar and cerebral functions were normal.

Serum enzyme levels were: creatine phosphokinase 72 u. per milliliter (normal less than 145), lactic dehydrogenase 262 mu. per milliliter (normal less than 225), and glutamate oxalate transaminase 51 mu. per milliliter (normal less than 35).

Median, ulnar, and peroneal nerves had normal conduction velocities. Latency in the facial nerve was normal. Fibrillation, positive waves, long duration polyphasies, and incomplete interference patterns were found in many of the skeletal muscles. Using high gain a few motor units were detected in the right and left inferior oblique extraocular muscles. The small amplitude and short duration (< 3 msec.) of the potentials did not permit differentiation between spontaneous activity and voluntary motor unit potentials. No interference patterns could be obtained. Orbicularis oris was electrically normal. The electroencephalogram (EEC) was normal.

During biopsy of the right lateral rectus muscle, it was found that the eye could no longer be moved passively. The right quadriceps femoris was also sampled.

**Biopsy methods.**

Eye muscle. The belly region of the right lateral rectus muscle was biopsied. Fixation: 1 per cent paraformaldehyde + 1 per cent glutaraldehyde in phosphate buffer for four hours, transferred to 4 per cent glutaraldehyde overnight. Postfixation: 1 per cent osmium tetroxide for one and one-half hours. The muscle was then dehydrated in graded alcohols, and embedded in Epon 812. The entire Epon embedded biopsy was serially sectioned at 15 microns. These thick sections were cleared for light microscopy by curing a layer of Epon onto them within a sandwich of polystyrene film; the entire biopsy could thus be surveyed by phase contrast. Sections of interest were remounted for further one micron and ultrathin sectioning.

Skeletal muscle. A left quadriceps femoris biopsy was removed in a Price clamp and processed for histochemistry. A small sample in a second clamp was treated for fine structure and electron microscopy as above.

**Pathologic findings.**

Skeletal muscle. The left quadriceps femoris was grossly normal. The fascicular pattern was well preserved. There was slight increase in connective tissue. Necrosis, regeneration, fiber splitting, "ragged-red fibers," internalization of nuclei, and inflammation were absent. Amylophosphorylase was normal.

There was an abnormal range of myofiber diameters (Type I: 8 to 53 microns, mean 22 microns; Type II: 9 to 72 microns, mean 30 microns). Randomly scattered atrophic fibers were of both major histochemical types. There were a few clusters of atrophic Type I fibers. In some regions a normal mosaic distribution of fiber types persisted but in others there were distinct "type groups." There were no target fibers on light microscopy but two were found in the electron microscopy sample.

**Fig. 1.** Lateral rectus muscle, methylene blue stain. Severe reduction in number of myofibers with a marked increase in connective tissue. ×520.
Fig. 2. An axonal terminal with partial axolemma and apparent absence of synaptic vesicles overlies a junctional region which shows marked widening of synaptic clefts. Often associated with such endings were profuse elaborations of secondary synaptic clefts with associated basement membranes. These appeared as isolated islands well within the junctional sarcoplasm. x12,500.

A diagnosis of chronic, continuing neurogenic atrophy was made.

Eye muscle, gross appearance and light microscopy. The right lateral rectus was highly fibrotic. Microscopically, considerable variation in size and shape of individual fibers, a high degree of atrophy, an increase in fibroelastic tissue, loss of myofibrillar organization, and some vacuolated muscle fibers were seen (Fig. 1). Central nucleation, fiber splitting, inflammatory infiltrates, and lymphocytic collections were not observed. The packing density of axons within small nerve twigs was less than normal.

Electron microscopy. Because marked morphological differences can exist between different end plates and even in different regions of the same end plate, our analysis of abnormal changes was based on a sampling of 50 end plates, many of which were studied in sequential thin sections.

Both pre- and postsynaptic anomalies of junctions were found on abnormal and otherwise normal myofibers. Two types of postsynaptic alterations were observed. A few fibers which normally show postjunctional folding exhibited marked retraction or virtual absence of postjunctional folds. Overdeveloped postsynaptic areas were seen in other fibers. The primary and secondary synaptic clefts were often widened and distended and elaborate branching of secondary synaptic clefts was frequent (Fig. 2). Overdeveloped junctional regions were occasionally seen as exaggerated cellular outgrowths which were shown, by serial section, to be derived from the "mother fiber." Myelin figures were observed in Schwann cells, underlying axonal terminals (Fig. 3, A), as well as in the postsynaptic sarcoplasm. Some axonal terminals evidenced advanced degenerative states, appearing virtually empty with but few axoplasmic remnants (Fig. 3, B). Many of the presynaptic regions were shown, by serial section, to contain no terminal axon beneath the overlying Schwann cell (Fig. 3, C).

Of the 50 end plates sampled, only two appeared ultrastructurally normal, as did their associated muscle fibers.

Alteration of the myofibers was evidenced by myofibrillar disorganization and breakdown, disruption of Z bands, glycogen accumulations, honeycomb structures, myelin figures, vacuolization, mitochondrial disorganization and intracristae breakdown, pyknotic nuclei, and total cell necrosis.

Discussion. The histologic evidence clearly indicates that this young girl has neurogenic atrophy of skeletal muscles. Compensatory re-innervation has occurred in skeletal muscles, as evidenced by type grouping, and has led to clinical improvement. The presence of clustered and randomly scattered atrophic fibers indicate that the basic disease process is continuing. The diseased eye muscles have been functionless since birth and show severe histologic changes. There are no widespread abnormalities of mitochondria nor clinical evidence to support the diagnosis of Kearns-Sayre syndrome of congenital ophthalmoplegia with "ragged-red" fibers. Widespread skeletal muscle involvement and both the severity and congenital nature of the eye weakness exclude the diagnosis of progressive dystrophic ophthalmoplegia. The early onset and neurogenic electromyogram (EMG) features exclude oculopharyngeal dystrophy. This clinical course excludes facioscapulohumeral dystrophy. Congenital facial diplegia (Moebius syndrome) is excluded by the severity of the external ophthalmoplegia, the relatively mild nature of involvement of facial muscles, and the widespread skeletal motor dysfunction.

This would appear to be a case of congenital infantile spinal muscular atrophy with unusually severe neurogenic involvement of external ocular muscles. In rare cases spinal muscular atrophy in children may be associated with cranial nerve disease but inclusion of ophthalmoplegia in the clinical syndrome is very rare. A fifteen-year-old
A boy with Kugelberg-Welander disease developed incomplete ophthalmoplegia over a two-year period. A sixteen-year-old girl who may have had spinal muscular atrophy had progressive ophthalmoplegia beginning at the age of four years. In a Japanese family with neurogenic atrophy and progressive ophthalmoplegia, eye muscles had light microscopic characteristics of "ocular myopathy." Nevertheless, the authors concluded that the oculopharyngeal involvement was of neurogenic origin. In none of the above cases has the ophthalmoplegia been congenital and total. In no report has there been electron microscopic description of eye muscles.

Most of the ultrastructural alterations that were seen to affect the eye muscle fibers themselves, have been previously observed in various neuromuscular disorders, as well as in experimentally denervated extracocular muscle. Thus none of the ultrastructural changes within myofibers would themselves permit differentiation of intrinsic myopathy from a neurogenic process.

Ultrastructurally, the eye muscle shows many abnormalities of nerve terminals and postjunctional regions. In many instances, the remainder of the myofiber is normal suggesting that changes in the neural apparatus are the initial pathologic process. One type of end plate anomaly is the overdevelopment and ramification of the postjunctional folding. Another type of remodeling of the end plate seen in this present case is the development of pseudopod-like elaborations of junctional sarcoplasm. These changes may provide an increased synaptic surface.

Experimental denervation of extracocular muscle leads to the absence of the nerve terminal from the motor end plate. Similar morphological changes are observed in the present case. Both "neurogenic" and "myopathic" etiologies have been proposed as the basis of ophthalmoplegia but no prior reports have contained adequate ultrastructural analysis of the innervation of external eye muscles. Daroff and co-workers have emphasized that the origin of ocular muscle atrophy can only be established if both muscle and its innervation are studied.

Experimentally denervated ocular muscles present a light microscopic picture mimicking that of a primary myopathy. If taken alone, the light microscopic picture of the ocular muscle observed in the present study might also be suggestive of
a myopathy, whereas, electron microscopic examination, would imply a progressing neurogenic process. It would thus seem that in extraocular muscle, electron microscopy is required for the distinction of primary myopathic processes from those which are neurogenic in origin. Conclusions from previous studies on pathologic extraocular muscle relying solely on light microscopy, may require re-evaluation.

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Key words: extraocular muscle, congenital external ophthalmoplegia, infantile spinal muscular atrophy, motor end plate.

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Quantitation of pilocarpine delivery across isolated rabbit cornea by noncross-linked high viscosity polymer gel. DAVID L. KROHN AND JULIANNA M. BREITFELLER.

The effect on pilocarpine flux across rabbit cornea in vitro by a noncross-linked polymeric gel vehicle was measured. A closed system transport chamber was used. Its design featured continuous flow of a tear analog but excluded variables of the internal eye. Results were compared to previously determined data in the same chamber system for cross-linked hydrogel buttons and for free pilocarpine fluid. Gel-mediated flux was equal to that with lens buttons to 90 minutes in the case of a 30 per cent gel (viscosity $\sim 70,000$ centipoises). Elution by the tear analog system limited flux duration of gels relative to lenses. Greater viscosity of 30 per cent gel relative to 25 per cent gel ($\sim 15,000$ centipoises) was associated with prolonged transcorneal drug flux. The congruence of flux slopes for 30 per cent gel and lens button vehicles despite the difference in available dose suggests saturable mediation of pilocarpine transport across the cornea, but a greater "flux efficiency" through 90 minutes for 30 per cent gel.

The pressure-lowering effect of pilocarpine has been shown in clinical studies to be significantly greater, and also significantly prolonged by transient use of drug-soaked soft lenses, as result of an enhanced rate of transport of the drug into the internal eye.\textsuperscript{1,2} Quantitation of this lens-mediated flux augmentation relative to an equal dose administered as a fluid has been reported.\textsuperscript{4} Use of the same polymer without cross-linking, and therefore in "ointment" or gel form, might be expected to have a similar effect to that of the cross-linked lenses but without the many inconveniences and expense for patients. Quantitation of the effect on transcorneal flux of pilocarpine in a noncross-linked gel was therefore carried out under experimental conditions identical to those used in previous studies on rabbit cornea to compare free fluid and lens-mediated pilocarpine administration.