Neonatal Myotubular Myopathy: Neuropathy and Failure of Postnatal Maturation of Fetal Muscle

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SUMMARY: *The natural course of the pathologic features in striated muscle was studied in a full-term infant with myotubular myopathy. At 5 days of age a muscle biopsy revealed that more than 90 percent of muscle fibers fulfilled histologic, histochemical and electron microscopic criteria of fetal myotubes. The infant died unexpectedly at 9 months of age from spontaneous rupture of a multifocal cavernous hemangioma of the liver. Postmortem examination revealed that progressive maturation of the fetal muscle had not occurred postnatally, and more than 90 percent of myofibers were still apparent myotubes. This maturational arrest was generalized to all striated muscles. The only changes detected since the neonatal period were hypertrophy of the small population of large fibers, but with minor cytoarchitectural alterations, and loss of the incomplete histochemical differentiation with ATPase stains or dedifferentiation not attributed to postmortem diffusion. Involvement of the gubernaculum testis accounted for the undescended testicles. The brain and spinal cord appeared normal. Evidence of degenerating and regenerating axons in the sciatic nerve suggested that the etiology of this maturational arrest of fetal muscle may be neurogenic.*

RÉSUMÉ: *Nous avons étudié l'évolution naturelle des changements pathologiques dans le muscle strié chez un enfant né à terme avec myopathie myotubulaire. A 5 jours, tous les critères biopsiques en faveur de myotubules faetales furent remplis pour 90% des fibres musculaires. L'enfant est mort à l'âge de 9 mois de la rupture spontanée d'un hémangiome caverneux multifocal du foie. L'examen port-mortem révèle que la maturation progressive du muscle faetal ne s'était pas produite après la naissance, laissant plus de 90% des fébrilles musculaires sous forme de myotubes. Cet arrêt de maturation était généralisé à tous les muscles striés. Les seuls changements à s'être produit au cours de la période post-natale sont une hypertrophie de quelques grosses fibres, toutes cependant partiellement atteintes histochimiquement (ATP ase). Le cerveau et la moelle étaient normaux. L'évidence d'axones dégénérés et régénérés dans le nerf sciatique suggère que l'étiologie de l'arrêt de maturation du muscle foetal peut être neurogénique.*

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INTRODUCTION

The myotubular myopathies of early infancy are clinically severe neuromuscular diseases, characterized pathologically by large numbers of muscle fibers with morphologic, histochemical and ultrastructural characteristics of fetal myotubes normally found at 8 to 15 weeks gestation in man. In neonatal myotonic dystrophy, persistent myotube-like muscle cells are interpreted as a "maturational arrest" of muscle (Sarnat and Silbert, 1976), but other investigators prefer the term "delay" because muscle biopsies of other patients with the same disease, performed after the neonatal period, do not show this severe degree of immaturity (Karpati and Carpenter, 1976). Serial muscle biopsies of the same patients are needed to resolve this question, but few such studies are available to elucidate the natural course and evolution of maturational disorders of fetal muscle.

This report describes an infant with perinatal myotubular myopathy whose muscle was studied histopathologically in the neonatal period and after his death at 9 months of age.

CLINICAL HISTORY

A full-term infant boy weighing 3300 grams was born to a 25-year-old primigravida. The pregnancy was complicated by polyhydramnios. Vertex vaginal delivery was performed. The mother had noted only minimal fetal movement during the third trimester. Apgar score was 7 at 1 and 5 minutes. The infant was noted to have dimin-

ished muscle bulk, generalized hypotonia, and weakness of severe degree. He did not require respiratory assistance, but his cry and suck were very weak and he had difficulty swallowing. Vocal cords were observed to move well. Orogastric tube feedings were given, and he required frequent suctioning of oropharyngeal secretions. Tendon reflexes could not be elicited. Facial weakness was present and the palate was high-arched. Thin temporalis muscles were associated with a dolichocephalic-shaped head measuring 46.5 cm in circumference (75th percentile). Extra-ocular movements were full and conjugate. The tongue was thin but did not exhibit fasciculations. The testes were undescended. Examination of the heart, lungs, and abdomen were normal. A muscle biopsy was performed at 5 days of age.

During the ensuing 9 months the infant continued to be severely weak, hypotonic, and areflexic. Orogastric or nasogastric tube feedings were required because he could not swallow, although the gag reflex was active. The caloric and nutritional content of the gavage mixture was adequate, and supplementary multivitamins were given. He had frequent respiratory infections. Head control was poor and he did not sit or roll over. He moved his extremities, but was barely able to overcome gravity. Despite his weakness, his development proceeded, and by seven months of age he was transferring objects from hand-to-hand and playing with his toes. Several determinations of serum CPK and LDH were normal. Electrocardiograms were interpreted as showing mild biventricular hypertrophy, but he had no cardiac symptoms. The abdominal viscera were not palpably enlarged.

Necroscopy was performed after he died unexpectedly in the office of his pediatrician at nine months of age.

Family history of neuromuscular disease was lacking. Both parents were examined clinically and no abnormalities found. At their request, muscle biopsies were performed on both parents for evidence of the X-linked recessive myotubular myopathy or myotonic muscular dystrophy.

PATHOLOGIC FINDINGS

Quadriceps femoris muscle biopsy at 5 days of age:

Histology: Connective tissue was not increased. More than 90 percent of muscle fibers in all fascicles were between 5 and 10 μm in cross-sectional diameter (normal for age 15 μm) and had either a single central nucleus or a pale central zone devoid of myofibrils (Fig. 1). In longitudinal section, each muscle fiber had a row of vesicular central nuclei evenly spaced about two to three nuclear diameters apart. Cross-striations were visible only at the periphery of the fiber and central zones between nuclei were amorphous and pale in sections stained by hematoxylin-eosin (H&E) or modified Gomori trichrome stain. About ten percent of scattered muscle were larger and normal in size, had peripheral sarcolemmal nuclei, and exhibited a mature reticular pattern of intermyofibrillar sarcoplasm. Degenerative changes, necrosis, and inclusion-like

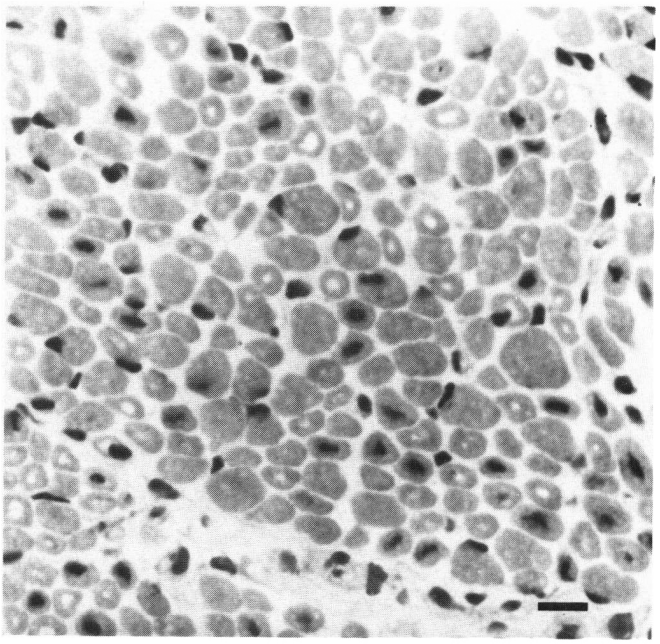

Figure 1 — Quadriceps femoris biopsy at 5 days of age. Ninety percent of muscle fibers are small and have central nuclei or central pale zones in cross-section. Scattered fibers of normal size have peripheral sarcolemmal nuclei. H&E. Bar = 10 μm

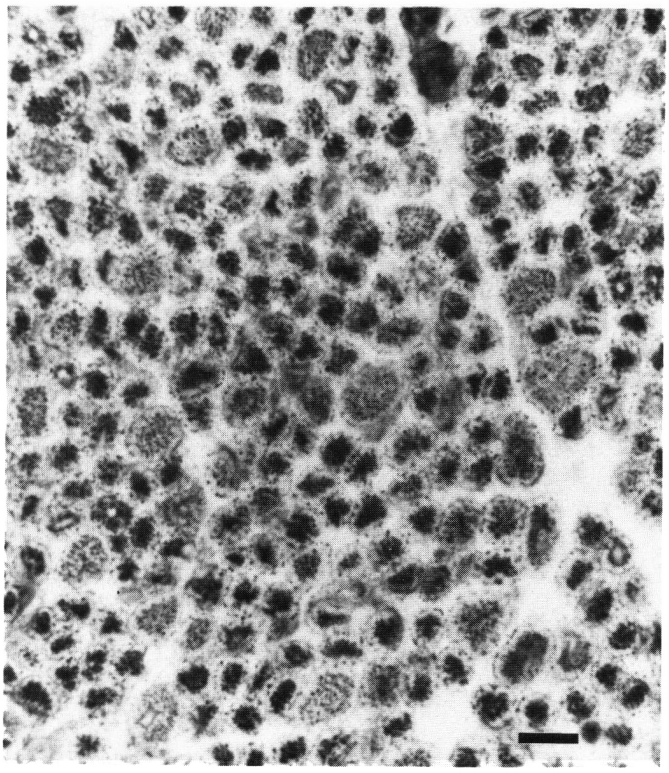

Figure 2 — Same biopsy as Fig. 1. Oxidative enzymatic activity is concentrated in the centers of fibers at levels between nuclei, and in the subsarcolemmal regions, as in fetal myotubes. Large fibers have uniform distribution or a small unstained peripheral rim. Fiber types are indistinguishable. NADH-TR Bar = 10 μm

structures such as nemaline rods were not found. Paraffin-embedded sections stained with H&E and 1 μm epon-embedded sections stained with methylene blue demonstrated similar findings to those seen in frozen sections.

Histochemistry: Nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) stain showed a uniform appearance of the small centronuclear fibers, with a high concentration of enzymatic activity concentrated in the centers of fibers between nuclei and in the subsarcolemmal region (Fig. 2). The distribution of diastase-digested, PAS-positive material was similar. Oil red 0 stain showed increased numbers of large lipid droplets in the centers of many fibers. Myofibrillar adenosine triphosphatase (ATPase) stains, preincubated at pH 9.6, 4.7, and 4.4, showed some differentiation of fiber types among the small centronuclear muscle fibers, but subtypes of II could not be recognized (Fig. 3). A mosaic distribution of equal numbers of types I and II fibers was identified with ATPase stains, but fiber types could not be distinguished with NADH, PAS, or phosphorylase stains. The scattered, mature-appearing, normal size fibers had uniform histochemical characteristics of type I. Intrafusal muscle fibers of two spindles appeared histologically and histochemically normal.

Electron Microscopy: Well organized sarcomeres of myofibrils formed a peripheral tube around the central nuclei (Fig. 4). Central zones of the myofiber between nuclei lacked myofilaments and were occupied by organelles including mitochondria, sarcoplasmic reticulum and poorly formed triads, and glycogen granules. Products of degeneration were not seen. A few focally disoriented myofibrils and isolated myofilaments were found at the edges of these central zones. (Fig. 5).

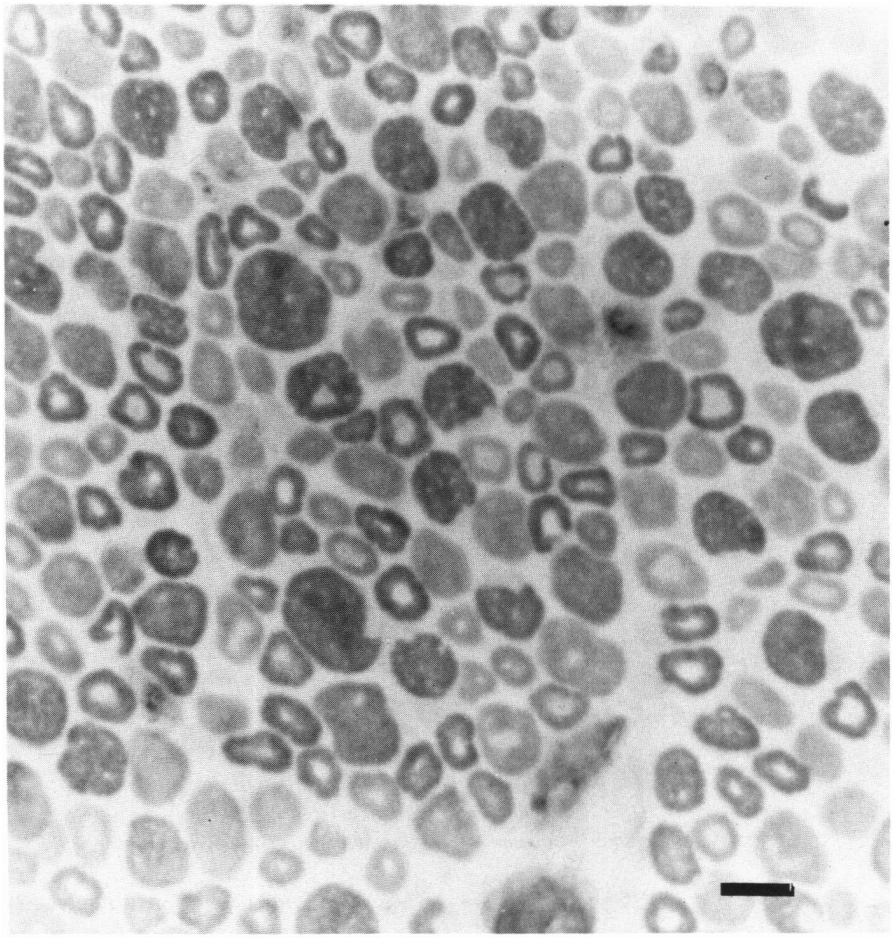

Figure 3—Same biopsy as Fig. 1. Myofibrillar ATPase preincubated at pH 4.7 shows some differentiation of fiber types, but not subtypes, among the small centronuclear fibers. Large fibers are uniformly of type I. Small fibers are of both types, unlike the condition termed "type I hypotrophy with central nuclei". Bar = 10 μm

Postmortem muscle biopsies at 9 months of age: Samples of several muscles were obtained within 45 minutes after death and portions were fixed in formalin, frozen in 2-methylbutane cooled to -160° C in liquid nitrogen for cryostat sections for histology and histochemistry, and fixed in glutaraldehyde for electron microscopy. The following muscles were sampled: right and left quadriceps femoris, gastrocnemius, iliopsoas, intercostal, paraspinal, diaphragm, deltoid, extensor digitorum of forearm, pharyngeal and paralaryngeal muscles, and gubernaculum.

Histology: The findings were similar in all muscles examined and resembled those seen in the neonatal muscle biopsy. More than 90 percent of muscle fibers still had histologic and histochemical characteristics of fetal myotubes. Differences between the quadriceps biopsy in the neonatal period and at 9 months of age were mainly in the size of individual muscle fibers; the centronuclear fiber slightly increased to a mean diameter of 10±2 μm and the larger, mature fibers were 17±2 μm, but the proportion of myotube-like fibers was unchanged. In distal muscles, such as the gastrocnemius and extensor digitorum of the forearm, the percent of total muscle fibers of normal size and having peripheral nuclei was the same as in proximal muscles, but the scattered more mature fibers were hypertrophic and measured 27±3 μm in diameter (Fig. 6). A few of these large muscle fibers also contained internal nuclei.

The diaphragm, intercostal muscles, pharynx, paralaryngeal muscles, and the striated component of the gubernaculum were equally involved as were appendicular muscles.

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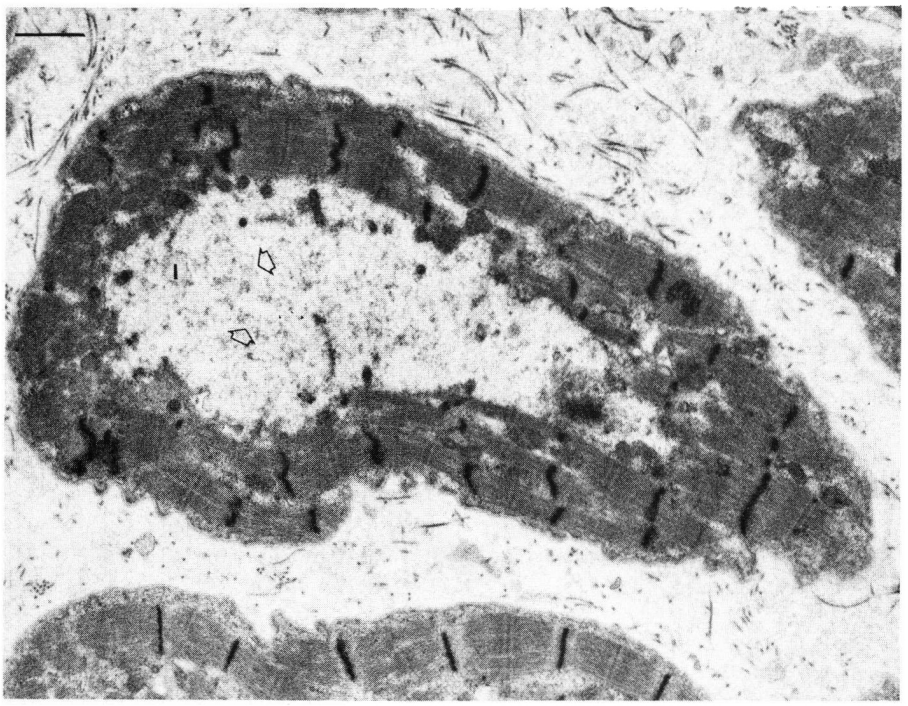

Figure 4 — Angled cross-section of a well developed myotube in the 5-day biopsy. The center is free of myofibrils. Vesicles (arrows), lipid droplet (l) and mitochondria (m) are present. The adjacent myofibrils are well organized. Uranyl acetate and lead citrate. Bar = 1 μm. Original magnification X 13,300.

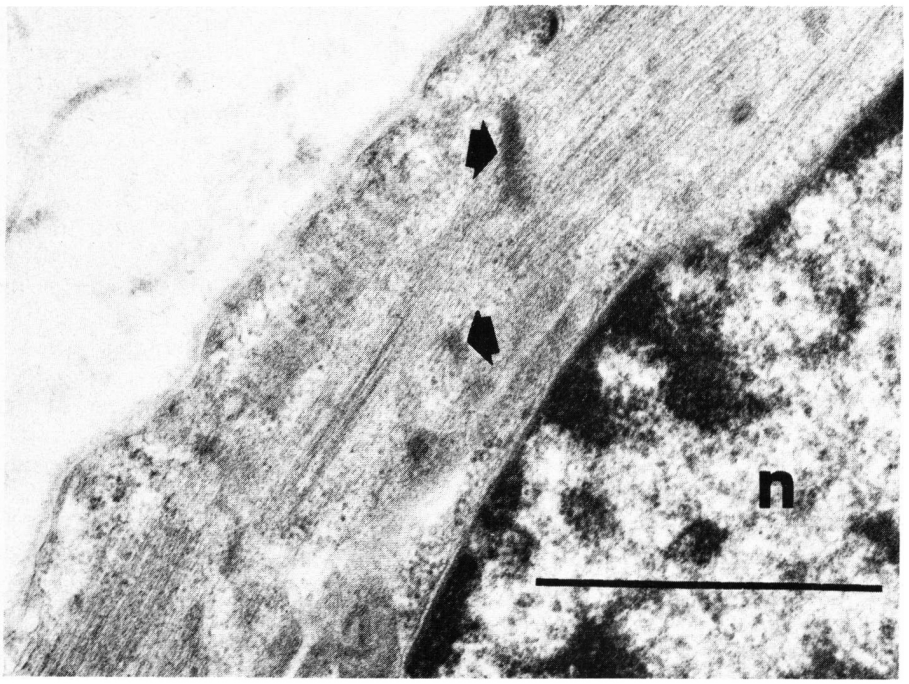

Figure 5 — Edge of a muscle cell seen in the 5 day biopsy showing a central nucleus (n). Between the nucleus and the sarcolemma the myofibrils show disorganization with a poorly formed Z-band (arrow). Uranyl acetate and lead citrate. Bar = 1 μm. Original magnification X 34,300.

Histochemistry: Changes were similar to those described in the neonatal biopsy. The tendency of the small centronuclear fibers to exhibit a degree of differentiation with ATPase stains at birth was lost, and all fibers, including hypertrophic ones, appeared uniformly of type I at all pH ranges. Most large fibers had a uniform, reticular distribution of NADH-TR activity in the center of the fiber, and an unstained peripheral rim beneath the sarcolemma (Fig. 7). This rim had less prominent intermyofibrillar sarcoplasm seen with Gomori trichrome stain (Fig. 7). The rim was indistinguishable from the central myofibrils with ATPase stains.

Electron Microscopy: Alterations resembling fetal myotubes again were identified, similar to the neonatal biopsy (Figs. 8 and 9). Intramuscular nerve twigs showed mild changes of the same character as those described in the sciatic nerve.

Postmortem Examination of nervous system: The brain weighed 1057 grams after formalin fixation. The lateral ventricles were minimally dilated symmetrically, but the brain was otherwise grossly and histologically normal. Sections of representative regions stained with luxol fast blue showed normal myelination of tracts. The spinal cord was grossly and histologically normal at all levels. In particular, the motor neurons of the ventral horns appeared intact and qualitatively normal in number.

Postmortem examination of sciatic nerve: Histologically, numerous myelin sheaths were devoid of axons or contained small, eccentric axons; regenerating axons also were seen (Fig. 10). Electron microscopy revealed disorganization, with beading, swelling, and fragmentation of the myelin bundles (Figs. 11 and 12). Several axons had lost their myelin sheath, and disordered myelin bundles were seen at the edge of a regenerating axon (Fig.

11). Other axons had lipid and poorly formed myelin figures, but had contrastingly normal myelin sheaths (Fig. 11). These changes could not be attributed to postmortem or crush artifacts.

General necropsy findings: The peritoneal cavity contained 250 ml of blood. The liver weighed 340 grams (mean for age 264 grams). A ruptured subcapsular hematoma was demonstrated on the anterosuperior aspect of the right hepatic lobe, and the cut surface of the liver exhibited multiple islands of hemorrhage involving most of the right and left lobes. Microscopically, the liver contained zones of multiple tortuous and dilated veins with blood but no thrombi. At the periphery of these cavernous hemangiomas was neocapillary proliferation and extensive areas of recent hemorrhage. Fibromyxoid tissue surrounded and obliterated some of the neovascularity in the subcapsular region. No calcification, necrosis, or anaplasia were seen. Other visceral organs appeared normal. The cause of death was ascribed to hypovolemic shock secondary to spontaneous rupture of the multifocal hepatic cavernous hemangioma.

Muscle biopsy of patents: Quadriceps femoris muscle biopsies of both parents revealed normal histologic and histochemical findings.

DISCUSSION

The centronuclear muscle fibers of this infant had several morphologic features abnormal for his gestational age. Myofibers contained chains of large, vesicular nuclei. Zones between nuclei contained sarcoplasm with mitochondria, sarcolemmal tubules, glycogen, and lipid, but not myofibrils. The distribution of oxidative enzymatic activity was concentrated in the centre of the fiber and in the subsarcolemmal region, but not in the intermyofibrillar sarcoplasm. The sarcotubular system was dilated and triad formation was incomplete. Histochemical differentiation was lacking,

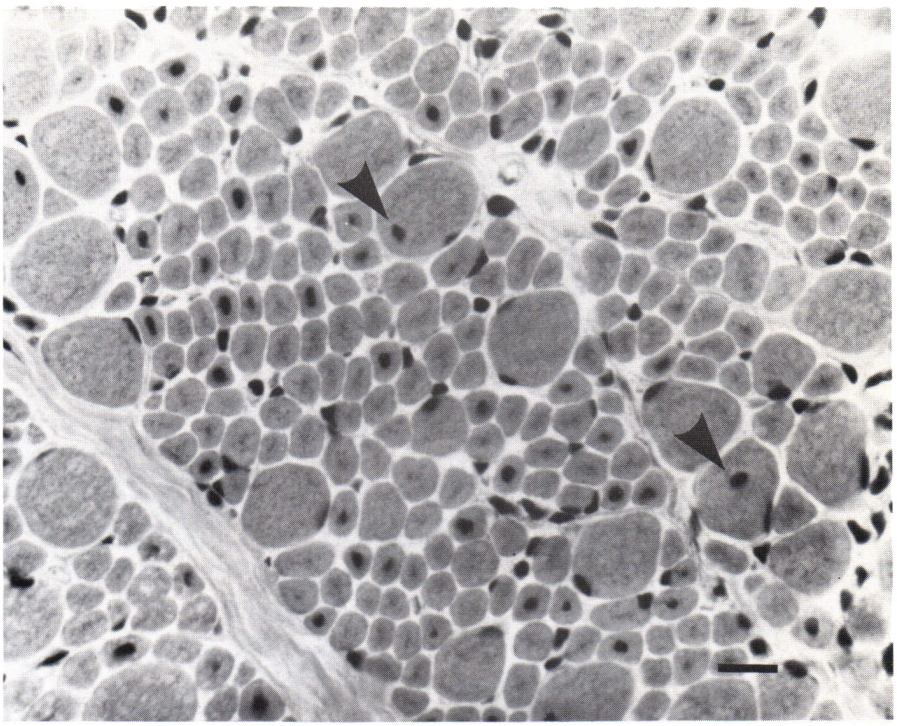

Figure 6 — Extensor digitorum muscle at 9 months of age. More than 90 percent of muscle cells still are small centronuclear fibers, and the larger fibers with peripheral nuclei are now hypertrophic. A few of these large fibers also have internal nuclei (arrowheads). The intermyofibrillar sarcoplasm is less prominent in the peripheral rim of the large fibers in cross-section Modified Gomori trichrome stain. Bar = 10 μm

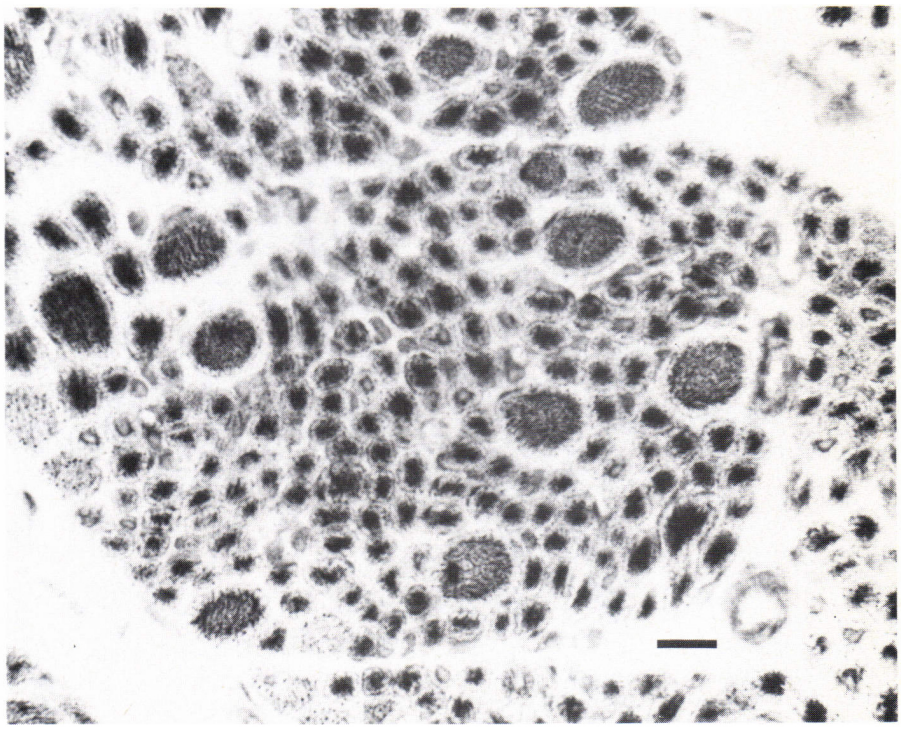

Figure 7 — Same biopsy as Fig. 5. The characteristic distribution of NADH-TR activity of fetal myotubes persists at 9 months. Even the hypertrophic fibers have this peripheral unstained zone beneath the sarcolemma. Bar = 10 μm

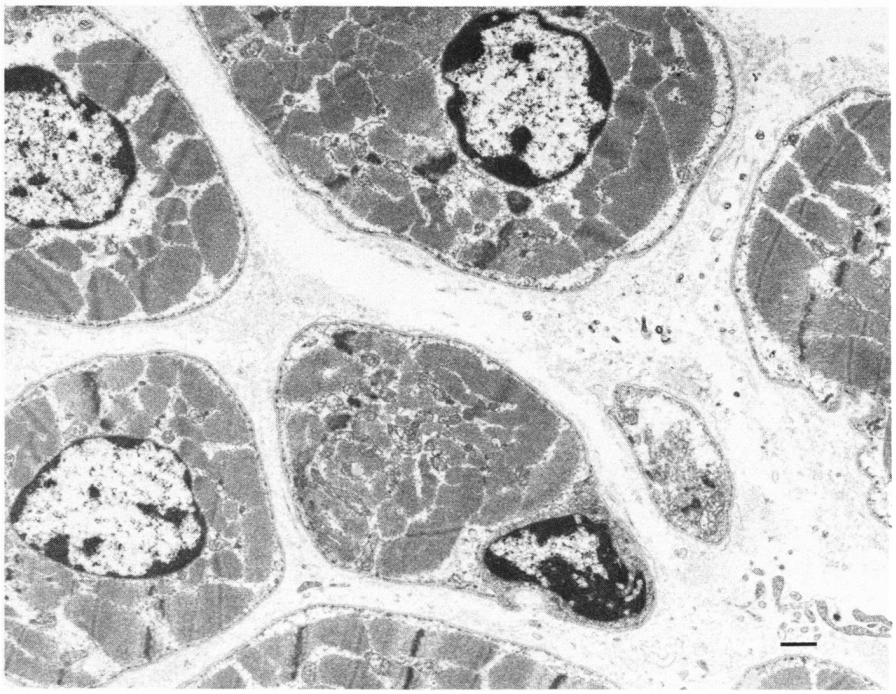

Figure 8—Cross-sections of multiple myotubules in a postmortem muscle. At the periphery beneath the sarcolemma many of the myofibers show intracellular edema, possibly a postmortem artifact. Uranyl acetate and lead citrate. Bar = 1 μm. Original magnification X 7400.

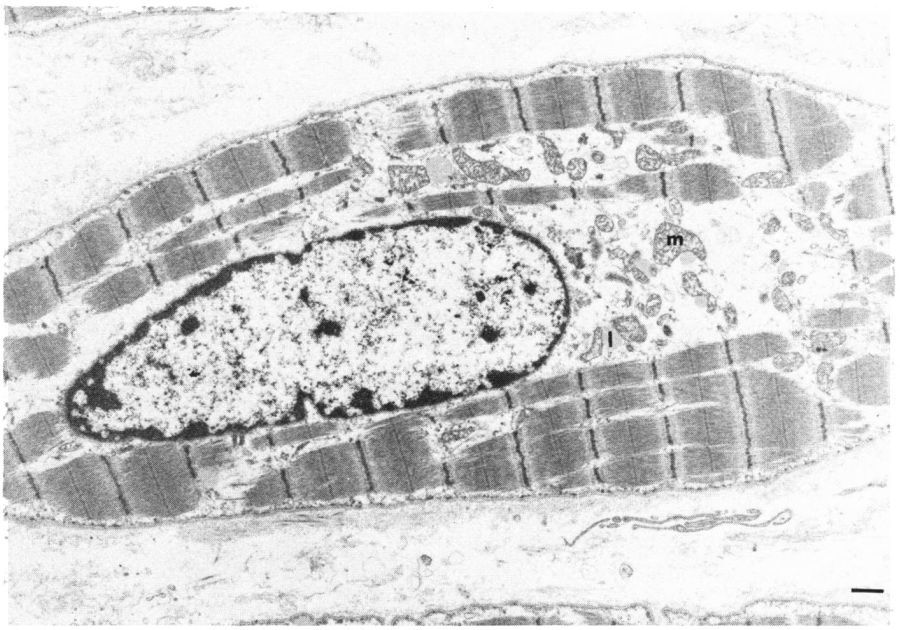

Figure 9—Angled longitudinal section through a myotubule in a postmortem muscle. The central nucleus is surrounded laterally by well-ordered myofibrils. The central perinuclear zone contains lipid droplets (l), swollen mitochondria (m) and clear areas where glycogen has been lost in processing. Small tubular remnants are also seen. The presence of these various cellular components in the central zones is a fetal feature and is unlike "central cores" consisting of focal degeneration and necrosis. Uranyl acetate and lead citrate. Bar = 1 μm. Original magnification X 6400.

another characteristic described in other infantile cases of this disease. Fiber types were partially developed with ATPase stains; this feature is one difference from true fetal muscle in which fiber types are not recognized until 20 to 28 weeks gestation. It is unlikely that the failure to demonstrate fiber types with ATPase stains at 9 months was due to postmortem changes because the tissue was removed and frozen within an hour after death, and was incubated with control tissue of other patients showing good results. This short postmortem interval does not generally alter ATPase results. The change from the neonatal muscle biopsy therefore may be interpreted as dedifferentiation, not necessarily indicating progressive disease, but perhaps the result of chronic abnormal neural stimulation.

After surviving nine months postnatally, the necropsy revealed that the myotubular immaturity was generalized to all striated muscles, and equally severe as that demonstrated previously in the neonatal biopsy. Clinically, the patient also had failed to improve in the postnatal period. The hypertrophy of the more mature scattered muscle fibers in the interval suggest a trophic response of these muscle cells to innervation, not shared by the majority population of small fibers. Involvement of the gubernaculum explains the failure of the testicles to descend into the scrotum (Sarnat and Sarnat, 1982).

Some investigators object to use of the term "myotube" in any reference other than to the myofiber normally seen at 8 to 15 weeks gestation in the human embryo. Despite the lack of semantic purity, it is useful to designate muscle fibers as "myotubes" in some pathologic conditions of early infancy, if sufficient morphologic similarity to true fetal myotubes suggests that maturational delay is the likely mechanism. Use of the term myotube requires some features detected only by histochemistry and electron microscopy, and the term should not be applied indiscriminately to any muscle fiber with internal nuclei; the secondary migration of peripheral sarcolemmal nuclei to an internal position between myofibrils is a common nonspecific myopathic feature of many

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myopathies including polymyositis and muscular dystrophy. In some congenital centronuclear neuromuscular diseases, the zones adjacent to central nuclei are devoid of myofibrils and contain sarcoplasm with organelles; the relation of these "pericentronuclear myopathies" to perinatal "myotubular myopathy" is uncertain (Campbell et al, 1969). In the same regard it is not known whether the condition termed "type I hypotrophy with central nuclei", (Bethlem et al, 1969; Engel et al, 1968; Zimmerman and Weber, 1979) is a milder form of myotubular myopathy or whether it is more closely related to congenital muscle fiber type disproportion. Until the mechanisms of these developmental disorders of muscle are elucidated, we prefer to restrict the name "myotubular myopathy" to these disorders of the perinatal period.

Denervation of fetal muscle in the myotubular stage of development interferes with the maturational process (Engel and Karpati, 1968). Direct anatomic evidence of neuropathy has not previously been presented in myotubular myopathy, but smallness and simplification of motor endings suggestive of impaired neural maturation are described (Coërs et al, 1976), and electromyography revealed profuse fibrillation activity in a neonate with a centronuclear myopathy (Elder et al, 1981). In neonatal myotonic dystrophy, evidence of a neurogenic mechanism is meager, and no increase is demonstrated in extrajunctional acetylcholine receptor sites on the muscle fiber membrane (Drachman and Farbrough, 1976). An hypothesis for maturational impairment in the severe neonatal form of myotonic dystrophy is lack of responsiveness of an abnormal sarcolemma to normal trophic influences of the motor neuron (Sarnat and Silbert, 1976). An important difference from myotubular myopathy in the muscle biopsy of neonatal myotonic dystrophy is that the myotubes are less uniform and many myofibers in various other stages of development are scattered throughout the muscle fascicles.

Among the myotubular myopathies symptomatic at birth, an X-linked recessive form is well documented

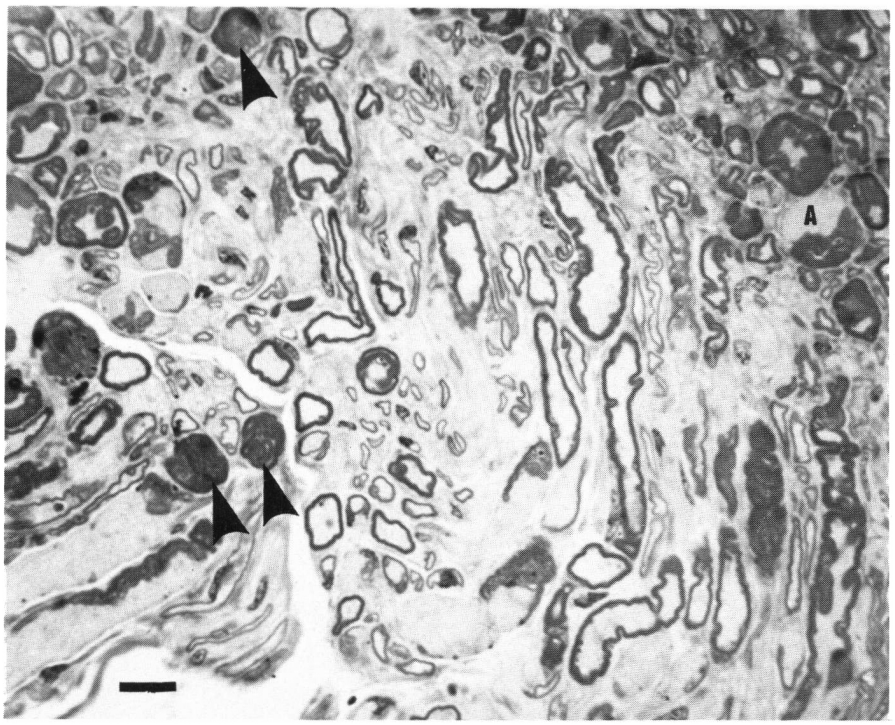

Figure 10—Light micrograph of 1 μm section of nerve taken at autopsy. There is extensive variation in axonal size and amount of myelin in both longitudinal and cross-sections. Numerous myelin sheaths are devoid of axons (arrowheads). Others contain small, often eccentric, axons. A regenerating axon (A) shows a large bundle of myelin at the periphery without appreciable myelin surrounding the axon itself. Toluidine blue. Bar = 10μm. Original magnification X 490.

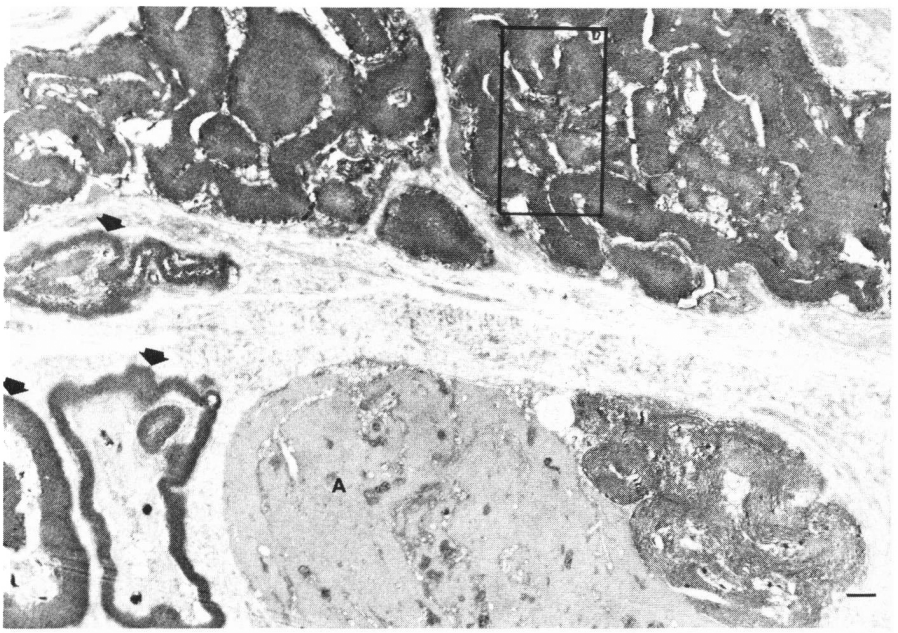

Figure 11—Section of nerve taken postmortem. In the lower part of the field is a regenerating axon (A) without myelin. Adjacent to the axon is a bundle of myelin with swelling, fragmentation and beading. At the top is a mass of myelin showing similar alterations. The myelinated nerves (arrows) show evidence of axonal degeneration with vacuolization and myelin-like figures. The area outlined in the box is shown at higher magnification in Figure 12. Uranyl acetate and lead citrate. Bar = 1μm. Original magnification X 6350.

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(Barth et al, 1975; Ilina et al, 1979), and an autosomal recessive form also is likely. The normal muscle biopsy of the mother in the present case does not exclude an X-linked disease, but the maternal muscle biopsy is usually abnormal in our experience and in the studies of others (Barth et al, 1975; Sarnat and Sarnat, 1982).

Severely involved neonates with myotubular myopathy nearly always die soon after birth. Ironically, the neuromuscular disease was not the cause of death in our case. No previous association of myotubular myopathy with vascular malformations of the viscera is known, and this combination probably was coincidental.

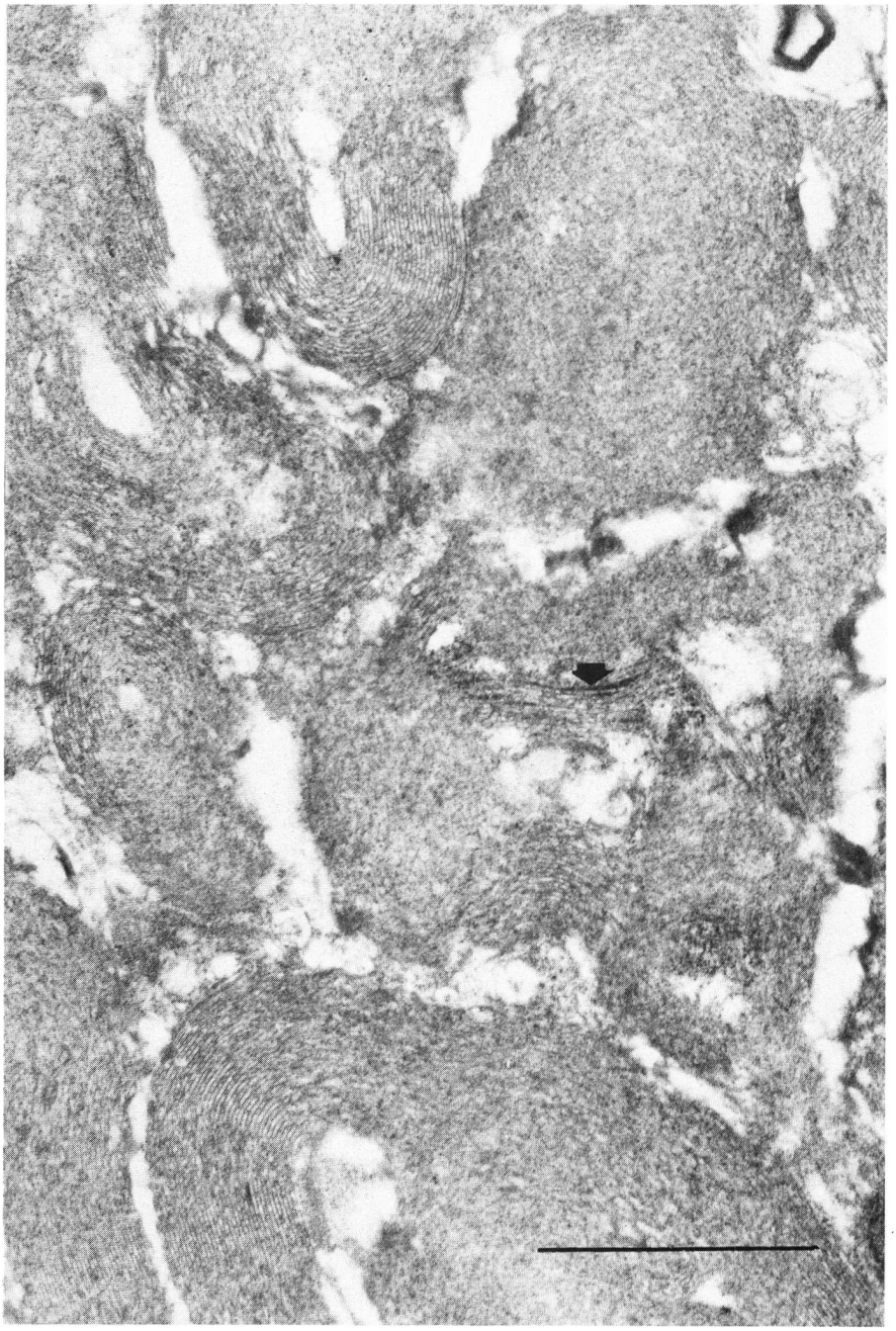

Figure 12—High magnification of the area of myelin beading, fragmentation and swelling outlined in Figure 10. Some zones of the myelin lamellae remain well-ordered, while others show pathologic alterations. Portions of a remnant of a node of Ranvier (arrow) are noted. Uranyl acetate and lead citrate. Bar = 1 μm. Original magnification X 39,450.

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