Diabetic Amyotrophy

Light and Electron Microscopic Investigation

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SUMMARY

An elderly male with recent onset of mild diabetes mellitus developed diabetic amyotrophy. There were muscle atrophy and pain in the thighs without sensory changes. Multiple biopsies were made over a fourteen-month period and studied with light and electron microscopy. The changes of individual muscle fiber atrophy, typical of this disease, are described. DIABETES *16*:181-90, March, 1967.

During the past decade there has been an increasing interest in a supposedly rare entity, diabetic amyotrophy.[1-9] Within the past two years, editorial comment has underscored the fact that amyotrophy is sufficiently characteristic in its clinical features to be regarded as a nosologic entity.[10,11] More careful evaluation of the morphologic changes in the extremities of diabetics indicates that this lesion may be more prevalent than was previously supposed.[12,13] It seems justifiable, therefore, to present a case of diabetic amyotrophy with a summary of the clinical and pathological findings, including the first report of electron microscopy of muscle in this condition.

CASE REPORT

P.W. (UWH No. 386919), a sixty-year-old white man, was referred to University Hospitals for evaluation of pain in the right leg and thigh of four months' duration. Four years prior to admission, he experienced the onset of polydypsia and frequent nocturia. However, diabetes mellitus was not documented until two months prior to admission, at which time glycosuria was detected. Tolbutamide therapy was instituted with satisfactory control of the glycosuria. Subsequently, he developed cramping pain in his right thigh and weakness of both lower extremities, more severe on the right. The patient resided in a county nursing home and since there was no history of nutritional deficiency or alcoholism, these conditions were considered unlikely causes of his amyotrophy. No history of previous insulin administration or steroid therapy was obtained. One brother was said to have adult-onset diabetes.

Physical examination on his first admission revealed a tall (seventy-one inches), thin individual weighing 140 pounds. The blood pressure was 110/90 mm. Hg; the pulse rate was 76 per minute. Funduscopic examination revealed drusen of both macular areas but no manifestations of diabetic retinopathy. The heart was not enlarged and peripheral pulses were normal. Coordination and muscle tone of the upper extremities were normal. Both thighs were atrophied with the girth of the right thigh measuring 32.5 cm. and the left thigh 34 cm. at a level 15 cm. above the patella. Profound weakness of the right glutei, thigh adductors and quadriceps muscles was present; the left thigh muscles were affected to a lesser extent. Gross muscle fasciculation and tenderness were absent, and all the deep tendon reflexes were intact. All sensory modalities were normal.

Routine laboratory examinations revealed a normal blood count, urinalysis, BUN and chest X ray. Fasting blood sugars of 104 mg. per 100 ml., 120 mg. per 100 ml., and 128 mg. per 100 ml., and two-hour postprandial sugars of 166 mg. per 100 ml., 167 mg. per 100 ml., 184 mg. per 100 ml., and 215 mg. per 100 ml. were obtained with the patient on an 1800-calorie diabetic diet. Urine samples were examined four times daily for the presence of glucose, and trace glycosuria was present on three occasions. The protein-bound iodine was 7.1 mcg. per cent and free acid was present in the gastric juice after stimulation with 50 mg. Histalog (betazole hydrochloride). X rays of the entire spine, including oblique views, revealed minimal degenerative disease with preservation of the disc spaces. Myelography of the spinal canal from the cul-de-sac to the foramen magnum, utilizing 12 cc. of Pantopaque (ethyl iodophenylundecylate), revealed no evidence of disc protrusion or herniation. The spinal fluid contained no cells; spinal fluid sugar was 108 mg. per 100 ml., protein 60 mg. per 100 ml., the VDRL unreactive, and the gold sol curve was flat.

Electromyographic studies demonstrated a normal conduction time of the right peroneal nerve (49 m. per second). Needle examination revealed fibrillation of the right adductor femoris and quadriceps muscles. The motor units of the anterior tibial, vastus lateralis, biceps femoris and gluteus maximus muscles were polyphasic and markedly decreased in number. During this admission two biopsies were obtained from the right quadriceps femoris muscle, the first shortly after admission and the second four months later.

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The patient was discharged on an 1800-calorie diabetic diet, tolbutamide 500 mg., b.i.d., and analgesics as needed for the leg pain. He discontinued his tolbutamide three months after discharge. However, his diabetes remained under adequate control on diet alone.

One year after the initial evaluation, the patient was restudied. Not only were there fewer symptoms referable to the right thigh, especially a striking reduction in pain, but less objective atrophy, with the girth measuring 39 cm. at a level 17 cm. above the patella. The left thigh, however, was more severely affected, measuring 38 cm. in girth at the same distance above the patella. Marked pain was present in the area of the left quadriceps femoris. Fasciculations of the involved muscles were absent. Repeat electromyographic studies again demonstrated bilateral polyphasic potentials and fibrillation, more marked on the left, especially in the adductor femoris group. Repeat peroneal nerve conduction velocities were found to be normal (52 meters per second). Blood pressure on this admission was 155/80 mm. Hg. The fundi revealed "hard" exudates in the retinae but no microaneurysms. There was no indication of renal decompensation or diabetic nephropathy by chemical studies. Fasting blood sugars were 113, 112, and 120 mg. per 100 ml. A twenty-four-hour urine assay of corticosteroids revealed 11 mg. of 17-ketosteroids and 5.9 mg. of 17-hydroxysteroids. In view of the reported association of occult malignancies presenting initially as polymyositis and myopathy, a complete evaluation of the patient for malignancy was undertaken. All studies, including radiographic examination of the entire gastrointestinal tract, proved to be negative. Muscle biopsies were obtained from the right and left quadriceps femoris muscle during this admission.

The patient was discharged to his personal physician on a diabetic diet. He received no insulin or oral hypoglycemic agents.

MATERIALS AND METHODS

The first muscle biopsy was obtained from the right mid-quadriceps muscle shortly after his first admission, at which time the patient was sixty years old. Tissue was fixed in buffered neutral formalin, embedded in paraffin, and stained with hematoxylin and eosin, followed by light microscope examination. A second biopsy was taken from another area of the right quadriceps muscle four months later. Portions of the tissue were immediately diced into 1 × 1 × 1 mm. fragments and fixed in cold, buffered, isotonic osmium tetroxide containing sucrose.[14] After embedding the tissue in epon, sections were cut at 300 to 500 Å on a Porter-Blum MT-2 ultratome, mounted on Formvar-covered copper grids, stained with uranyl acetate, and examined in an RCA EMU 3-G electron microscope.[15] Additional tissue from the second biopsy was immediately placed in cold, buffered, neutral formalin, embedded in paraffin, sections prepared in the usual manner, and stained by hematoxylin and eosin or the PAS technic. Other tissue was fixed immediately in cold, 95 per cent alcohol, embedded in paraffin, sectioned in the usual manner, and serial sections stained with the PAS technic, either with or without prior malt diastase digestion in order to study glycogen content. Both routine paraffin sections and paraffin sections from alcohol-fixed tissues were stained with Best's carmine technic for glycogen. One year later a third biopsy of another area of the right quadriceps femoris muscle and a biopsy of the left quadriceps femoris muscle were performed and the tissues processed for light and electron microscopy as described above. Muscle biopsies from nondiabetic patients and diabetic patients without evidence of amyotrophy were prepared in a similar manner and examined by light and electron microscopy.

RESULTS

The first biopsy was examined only by light microscopy, utilizing hematoxylin and eosin stains. A moderate variation in size of the muscle fibers was present; approximately 20 per cent of the fibers were appreciably reduced in size. The sarcolemmal nuclei of the atrophic fibers were increased in number and were hyperchromatic. Normal nerve elements were present.

Light microscopy of the paraffin-embedded, formalin-fixed tissues from the second biopsy revealed numerous atrophic muscle fibers varying from one-half to one-tenth normal size. With hematoxylin and eosin, some muscle fibers were pale pink and appeared to have persistent myofibrils with occasional cross striations, while other atrophic fibers were dark red and completely amorphous, or granular, without any visible cross striations. Generally, a single atrophic muscle fiber was interspersed between many essentially normal fibers although in some instances three to four contiguous atrophic fibers alternated with a like number of essentially normal fibers (figures 1 and 2). Atrophic fibers were usually, but not always, accompanied by an increase in the number of sarcolemmal nuclei, many of which were enlarged, vesicular and hyperchromatic (figure 3). It is always difficult to ascertain with certainty whether such apparent increases in sarcolemmal nuclei are due to shrinkage of muscle fibers with concentration of nuclei, or whether they represent a true increase due to proliferation.[16] In this case we believe that there was actual proliferation of sarcolemmal nuclei because of the large number of nuclei lined up along short stretches of the sarcolemma. Many of the atrophic muscle fibers had collections of vacuoles along their edges, just beneath the sarcolemmal sheath (figure 3). Cross sections showed a modest separation of the

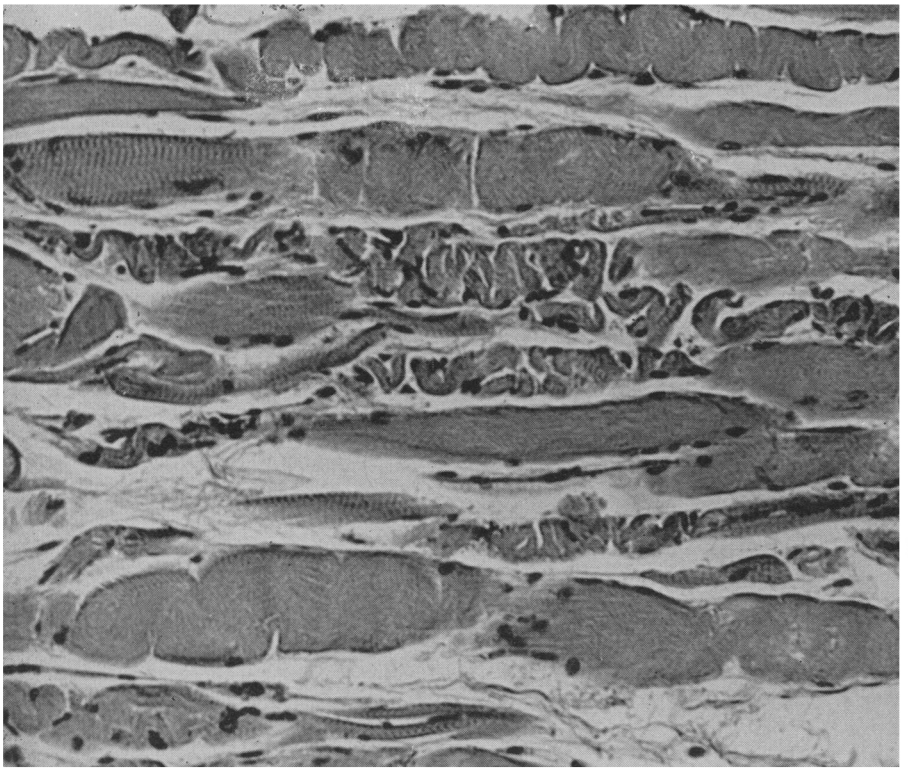

FIG. 1. Light micrograph of longitudinal section of right quadriceps femoris muscle (second biopsy). The muscle fibers show varying degrees of individual fiber atrophy typical of diabetic amyotrophy. Most cross striations have disappeared. Inflammation is absent (H & E, X 240).

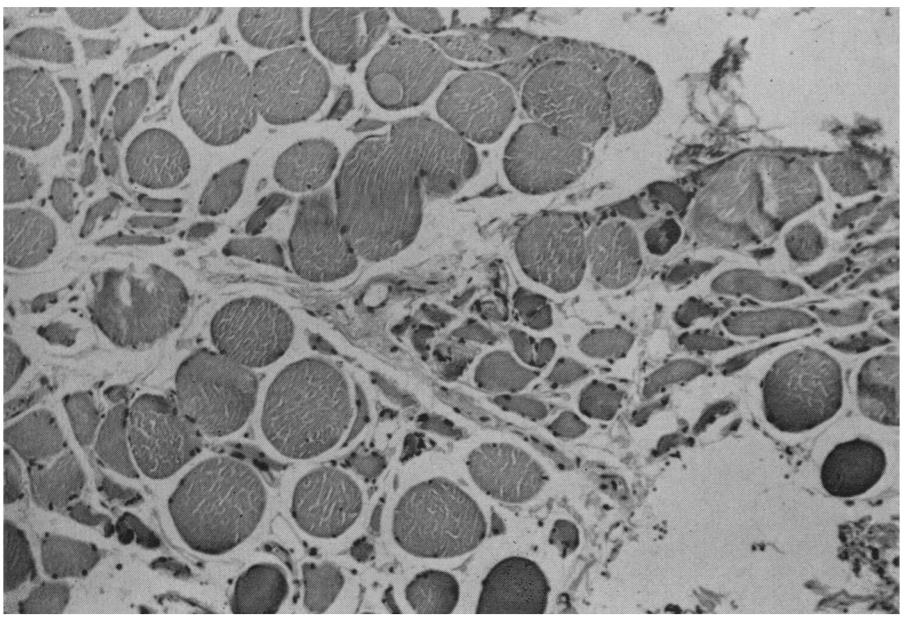

FIG. 2. Light micrograph of cross section of right quadriceps femoris muscle (second biopsy). Atrophic fibers occur individually and in small groups (H & E, X 130).

muscle fibers, especially in areas where atrophic fibers were present. Although such separation could result from an artifact, in this instance a true widening of the interfiber space secondary to fluid accumulation was present, as confirmed by electron microscopy.

Besides the obviously atypical small muscle fibers, there were many pale, striated muscle fibers of normal width which contained fewer myofilaments than usual. Light microscopy revealed occasional nerve elements which were morphologically normal.

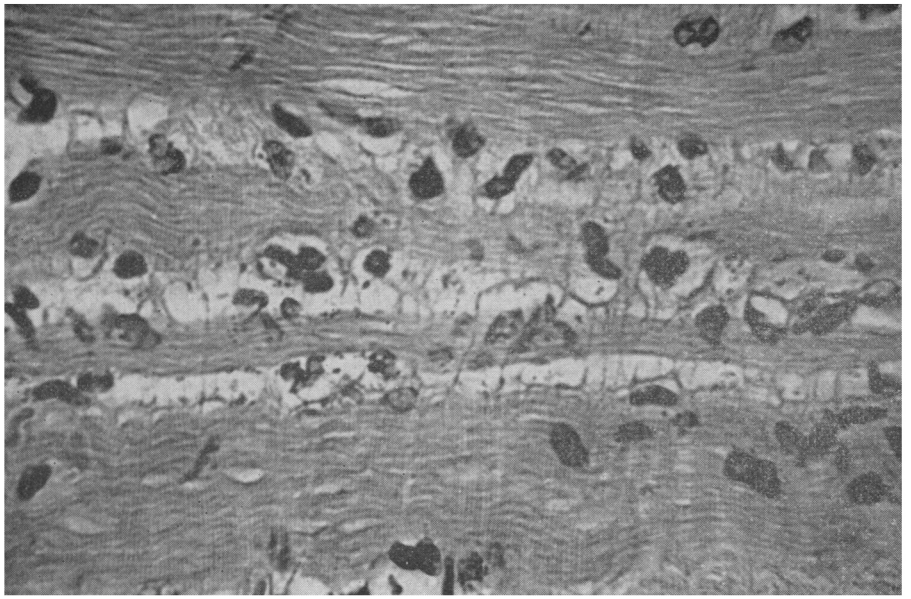

FIG. 3. Light micrograph of longitudinal section of right quadriceps femoris muscle (second biopsy). The two central muscle fibers are markedly atrophic and have developed clear spaces just under the sarcolemmal sheath. Sarcolemmal nuclei are increased; there is no inflammation. Cross striations are irregularly absent (H & E, X 450).

The muscle glycogen content was quite variable. Normal muscle fibers showed a diffuse, moderate glycogen infiltration, as expected. Abnormal pale fibers with a reduced number of myofibrils contained very little glycogen. Atrophic fibers were almost completely devoid of glycogen. Electron microscopy confirmed these findings.

Almost no infiltration by inflammatory cells was encountered within the muscle fiber bundles. A few widely dispersed foci of adipose tissue were found between muscle fibers. Periarteritis was absent. Fibroadipose tissue immediately adjacent to the muscle occasionally contained small numbers of chronic inflammatory cells.

Electron microscopy of the second biopsy revealed a wide spectrum of changes. The majority of muscle fibers appeared normal. Approximately 30 per cent of the fibers showed mild atrophy and degenerative changes and 10 per cent were severely degenerated. The first, and mildest, abnormality which could be identified was a reduction in the number of myofilaments present in each myofibril (figure 5). At this stage, the myofibrils were more widely separated than in normal muscle (figure 4). Cross striations were maintained, but were less distinct than in normal muscle. The sarcoplasmic reticulum and mitochondria appeared to be intact and of average number. Nuclei were completely normal and the periphery of each fiber was free of vacuoles which appeared in more severe stages of degeneration.

The second stage of degeneration revealed loss of striations and a haphazard disorientation or fragmentation and sometimes disappearance of the myofilaments (figure 6). The mitochondria and sarcoplasmic reticulum were often distorted. Peripherally, small spaces of clear fluid or fluid with occasional particulate matter appeared beneath the sarcolemmal sheath (figure 7). Irregular masses of very dark electron dense material were present among the distorted myofibrils (figure 8). These dark masses could not be positively identified but closely resembled the dark material of the "Z" bands. The electron dense (dark) material of the "Z" band, or membrane of Krause, is thought to represent neither actin nor myosin.[17] Its exact nature is unknown, but recent work indicates tropomyosin is a major constituent.[18,19] High magnification of this material (figure 9) showed no resemblance to the nemaline bodies as described by Gonatas et al.,[20] Price et al.,[21] and others.

The fibers showing the most severe stage of degeneration were markedly vacuolated with most of the stainable material composed of structureless debris and localized to the center of the fiber. This material was a conglomerate of finely granular, osmiophilic material with an occasional very fine linear structure compatible with a myofilament. In these severely damaged fibers, nuclei were distinctly abnormal with loss of nucleoli and with pale staining. The mitochondria and endoplasmic reticulum could not be identified.

Capillaries in normal as well as disintegrating muscle of this patient had a markedly thickened basement membrane, from two to five times normal thickness (figure 10). Such changes have been previously report-

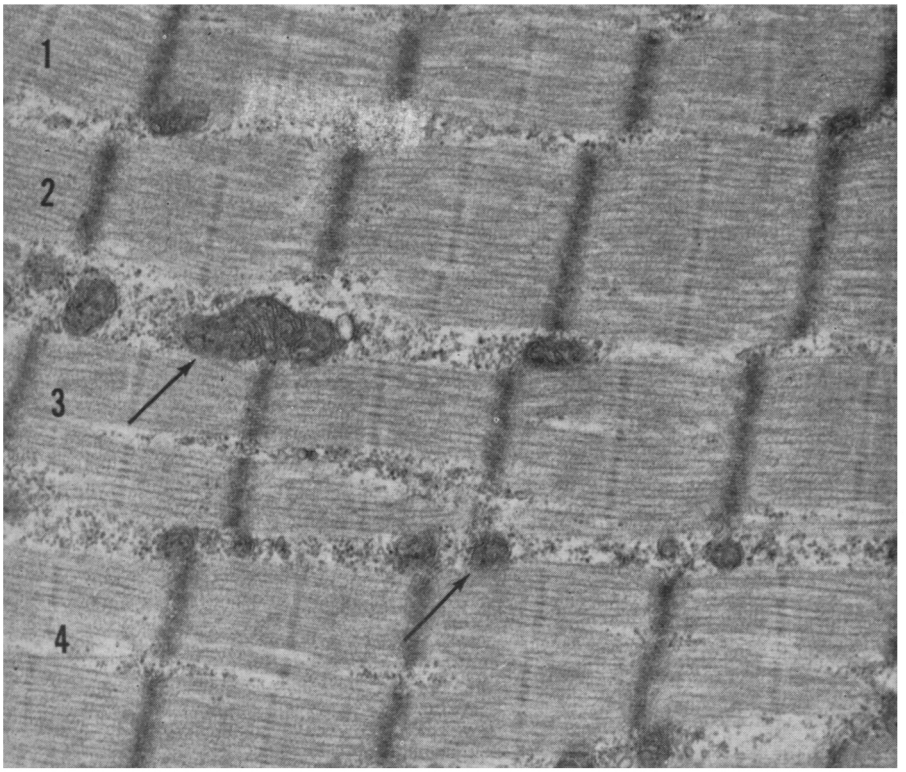

FIG. 4. Electron micrograph of four myofibrils (Nos. 1, 2, 3 and 4) from a muscle fiber of a nondiabetic patient. Each myofibril is subdivided into many myofilaments. Striations cross each fibril. Between each fibril is a space containing several mitochondria (arrows) and a fine granular osmiophilic material which has a morphologic appearance of glycogen (X 20,400).

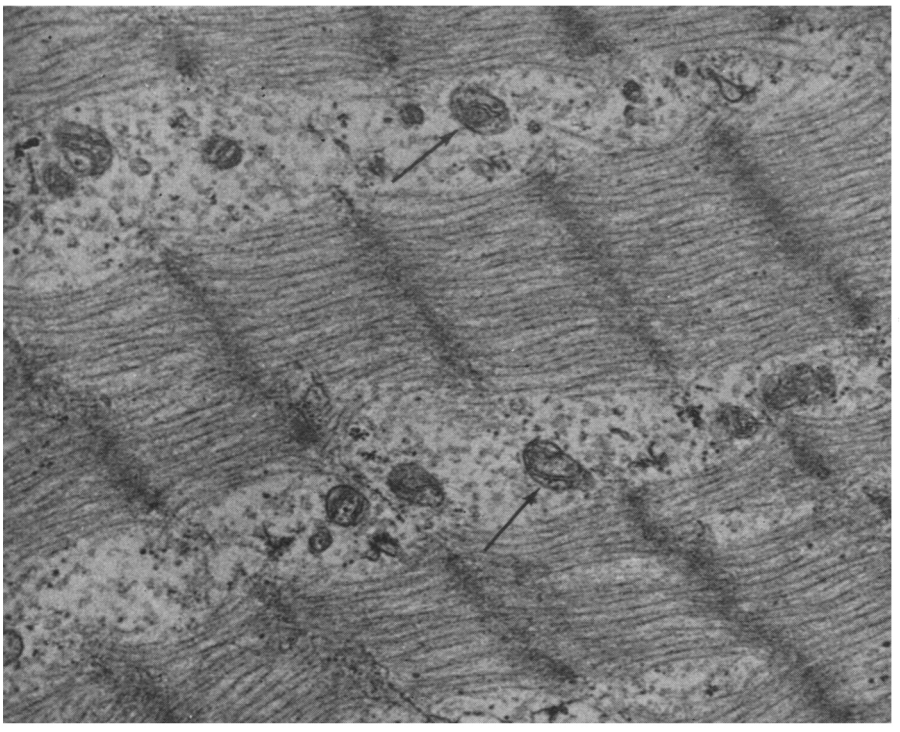

FIG. 5. Electron micrograph of portion of a muscle fiber from the right quadriceps femoris muscle (third biopsy). The mildest indication of atrophy is a widening of the interfibrillar spaces and a reduction in the number of myofilaments in each myofibril. Mitochondria (arrows) are still present and appear to be functional. Striations are less distinct (X 19,800).

ed in many capillaries of the diabetic patient.[22,23]

Examination of the third biopsy from the right thigh revealed light and electron microscopic changes similar to those found in the second biopsy, although

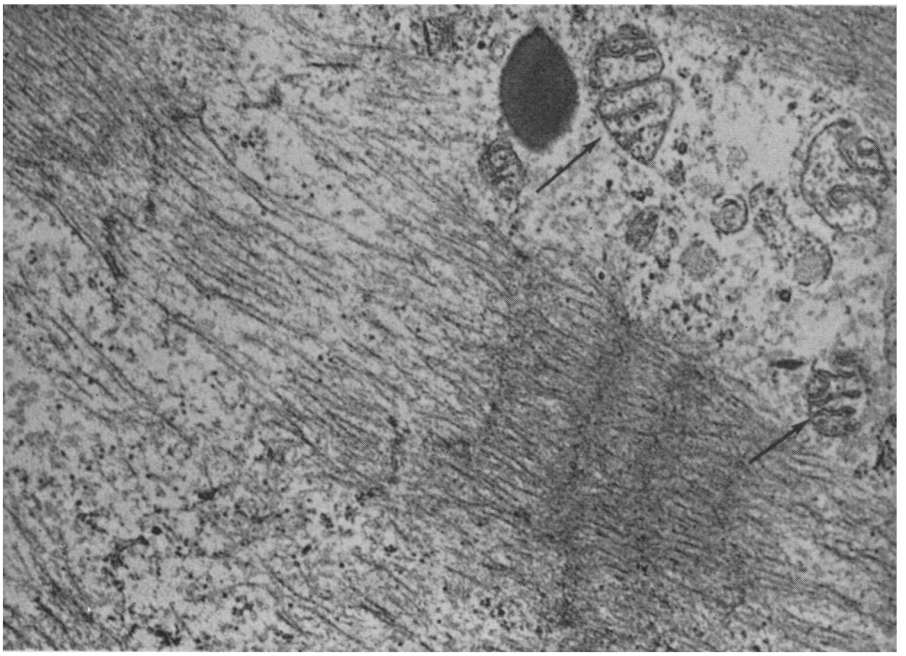

FIG. 6. Electron micrograph of portion of muscle fiber from the right quadriceps femoris muscle (third biopsy). This micrograph demonstrates severe atrophy with almost complete loss of the usual architecture of the muscle. Note that several mitochondria are present (arrows) and appear to be functional. The black "body" probably represents an "autophagic vacuole" (X 19,200).

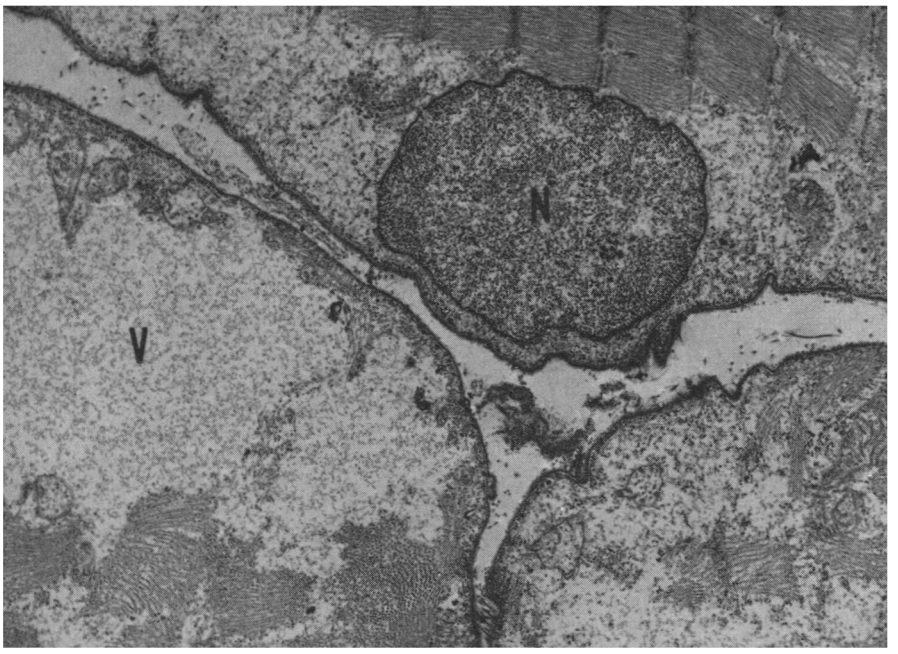

FIG. 7. Electron micrograph of peripheral portions of three muscle fibers from right quadriceps femoris (second biopsy) showing varying degrees of atrophy and degeneration. Note the large vacuole (V) in one fiber under the sarcolemma, and the intact nucleus (N) in another fiber (X 7,650).

the authors gained the impression that they were less frequent. In several areas the electron micrographs revealed essentially normal myofibrils widely separated by large quantities of granular, osmiophilic material resembling glycogen (figure 11). The biopsy from the left thigh revealed extensive involvement by the same spectrum of changes by light and electron microscopy in the second biopsy of the right thigh.

DISCUSSION

This patient's disease conforms to the clinical syndrome of diabetic amyotrophy, reintroduced into medicine twelve years ago by Garland,[1,2,7] and subsequently reported with ever-increasing frequency in both American[3,8,9,24] and European[4-6,12,25-27] literature. Diabetic

FIG. 8. Electron micrograph of area of muscle degeneration and deposition of osmiophilic debris from second biopsy of right quadriceps femoris muscle. The osmiophilic material has not been identified but resembles the material of the "Z" band, several of which can be seen in the lower left of the micrograph (X 10,000).

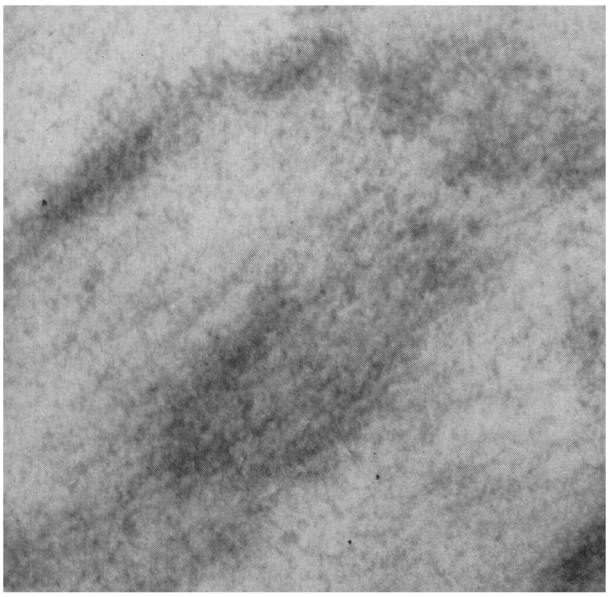

FIG. 9. Higher magnification electron micrograph of the osmiophilic debris demonstrated in an area of muscle degeneration in figure 8. Note the nonspecific nature of this material. There is no crystalline nature demonstrated and it does not bear a relationship to the nemaline bodies previously reported in muscular degeneration. (X 100,000).

amyotrophy consists of bilateral asymmetric wasting and weakness of the proximal lower girdle musculature with concomitant pain. This disorder most frequently appears in an older diabetic male. The degree of amyotrophy correlates poorly with the derivation, severity or treatment of the diabetes.[24] Not infrequently amyotrophy may appear shortly after the diagnosis of diabetes, as exemplified by this case. On the other hand, some investigators report that it is more common in long-standing diabetes.[27] The cerebrospinal fluid protein is frequently elevated.[8,24] Deep tendon reflexes and sensory responses have been reported to be both normal or diminished.[8,24] The condition appears to be self-limited with recovery within a few months or years.[8,24]

In contradistinction to classic diabetic neuropathy, diabetic amyotrophy is usually asymmetrical and often lacks the tendon areflexia, ataxia, trophic changes, and sensory impairment found with neuropathy.

Other clinical entities which were considered, and excluded from the reported case on the basis of history and laboratory determinations, were dermatomyositis and related collagen diseases, the myopathies and myasthenic syndromes associated with occult malignancies, the metabolic myopathies, pernicious anemia, nerve root disease and the girdle muscle myopathies associated with steroid therapy. Local muscle atrophy at the site of previous Protamine Zinc Insulin administration has been reported,[28] but our patient had never received insulin for the regulation of his diabetes.

Despite the successful clinical characterization of this syndrome, and its acceptance as a clinical entity as attested to by its inclusion in a number of recent reviews on the neurologic complications of diabe-

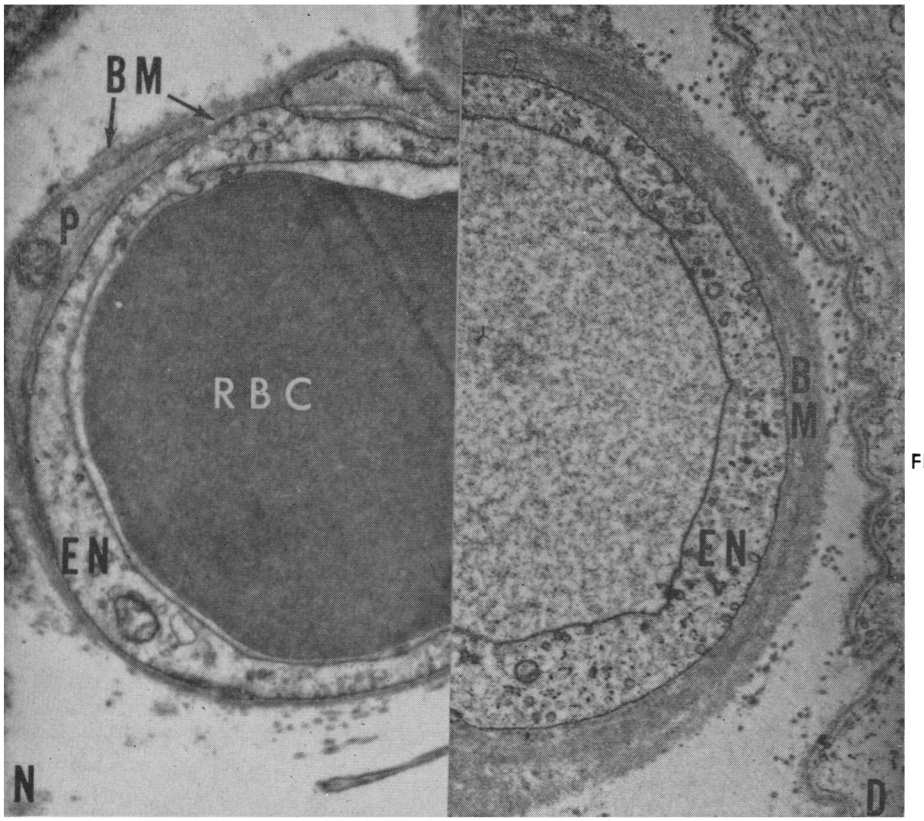

FIG. 10. Composite electron micrograph of portions of two muscle capillaries, printed at the same magnification. The lumen of the capillary from a nondiabetic (N) contains a red blood cell (RBC). The endothelial cell (EN) is surrounded by a thin basement membrane (BM). A pericyte process (P) is also present. The basement membrane of the muscle capillary from the diabetic patient reported here (D) is markedly thickened (X 17,700).

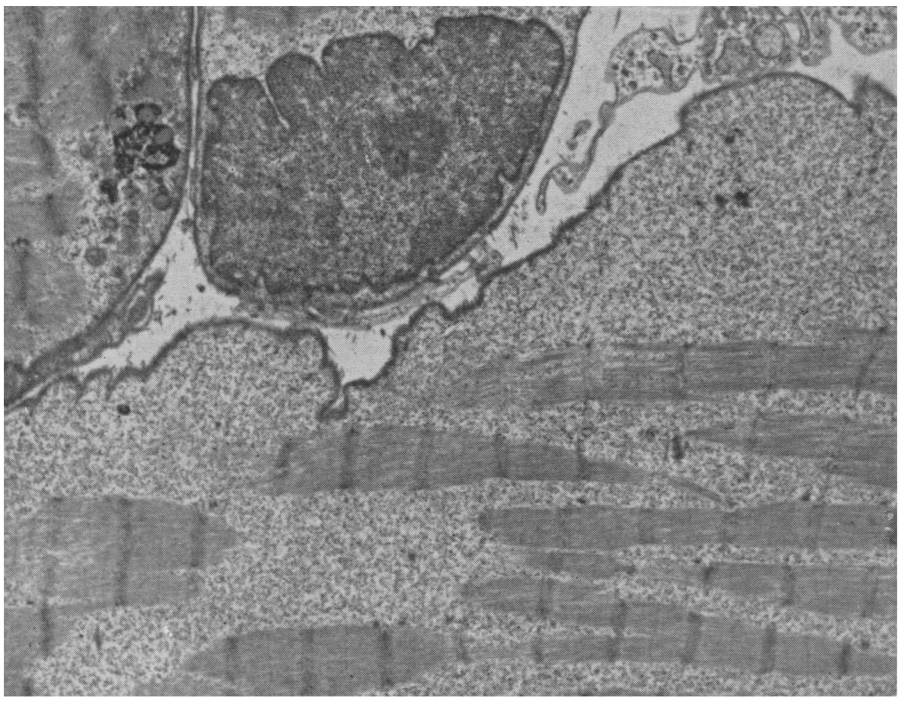

FIG. 11. Electron micrograph of portions of three muscle fibers from right quadriceps femoris muscle (third biopsy). There is a marked increase in the granular osmiophilic material between myofibrils in the lower fiber which is thought to be glycogen. This may represent a recovery phase since the atrophy of the leg was improving clinically and similar storage of glycogen may occur in normal muscle (X 6,600).

tes,[29-32] clinicopathologic correlation has been poor. The interested reader experiences difficulty in finding consistent or characteristic reports of pathologic changes in the biopsied muscles of patients with diabetic amyo-

trophy. Garland[2] reported the results of muscle biopsy in only one of his twelve original patients, and the biopsy was taken from the rectus abdominus muscle rather than from the affected extremity muscle. Bischoff[4] obtained muscle biopsies from three of four affected patients and described a histologic pattern characterized by focal atrophy of individual muscle fibers lying in proximity to normal muscle fibers. The atrophied fibers "lacked striations" and had "cloudy degeneration of the sarcoplasm," with nuclei that were occasionally enlarged or hypochromic. Locke et al.[8] were the first to attempt a comprehensive investigation of the histopathology of diabetic amyotrophy by light microscopy. They reported a total of seventeen muscle biopsies upon affected muscles in thirteen patients. The vastus medialis was the most frequently examined muscle. A distinctive pattern of individual muscle fiber atrophy emerged. In contradistinction to the findings of Bischoff,[4] Locke did not detect loss of cross striations and he reported the sarcolemmal sheath nuclei to be "hyperchromatic" rather than hypochromatic as previously reported.[8]

The muscle lesion usually has been described as atrophy of individual muscle fibers without inflammation as reported in this case. We have demonstrated by electron microscopy that the changes are more severe than can be described as "atrophy" and we suggest that "degeneration" is a better term. Authors do not agree as to loss of striations but our photographs demonstrate quite clearly that striations disappear as the degeneration becomes severe.

The frequent finding of individual muscle fiber atrophy suggests a lesion of the individual muscle fiber or its nerve ending. The finding of atypical motor end plates and nerve endings suggests the possibility of abnormal metabolism of the nerve ending due to the diabetic state. Woolfe and Malins[33] have previously described a soap-bubble appearance of motor end plates in diabetic patients. Heller and Hess[34] have demonstrated that insulin is necessary for the proper respiration of peripheral nerve. Additional studies are needed to confirm this etiology. On the other hand, many investigators have failed to find pathologic changes in spinal cords or peripheral nerves.[7,8]

Many investigators have suggested that diabetic microangiopathy with involvement of capillaries within the peripheral nerve may be responsible for classic diabetic neuropathy and, by inference, for diabetic amyotrophy.[13] However, we are skeptical of a relationship between the diffuse basement membrane thickening of muscle and nerve capillaries found in most diabetics and the focal lesions of amyotrophy. Further, it should be noted that the same basement membrane thickening is present in areas of muscle which are not atrophic. Spontaneous recovery from the amyotrophy without changes in the capillary basement membrane thickening also mitigates against microangiopathy as the etiologic factor.[24]

Although similarities between the gradual degeneration of the muscle fibers which we have described in diabetic amyotrophy, and the denervation atrophy of skeletal muscle exist,[35] similar changes do occur in other conditions such as cold injury[36] and may, therefore, be nonspecific.

Despite disagreement as to the nature of some clinical and pathologic aspects of diabetic amyotrophy, this condition appears established as an entity. Careful search for signs and symptoms of amyotrophy, especially in older male diabetics, is indicated.

ACKNOWLEDGMENT

This project was supported in part by research grant No. AM-07118 from the National Institutes of Health, the Wisconsin Heart Association, and research funds of the Veterans Administration.

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