Striated Muscle in Four Categories of Leprosy. II. Fine Structural Changes[1]

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Within 20 years of the discovery of the leprosy bacillus by Hansen, weakness and wasting of muscle in leprous patients were described by Hansen and Looft ([12]). The first systematic study of the histopathologic changes in muscle, correlated with the clinical features, was reported only about 20 years ago ([3,4]). Muscle involvement was found to be very frequent and to be a disabling change in all types of leprosy. In 1960, WHO published the conservative figure that over 20% of all leprosy patients, from a global point of view, have some form of motor deficit or paralysis ([27]). The number of leprosy patients throughout the world is growing. There were said to be at least 15 million patients in the world (with a fifth of them in India alone) in 1977 ([28]). Thus, the number of patients with muscle involvement is also growing. According to the American Leprosy Missions, leprosy produces more paralysis and deformity of the hand than all other diseases combined.

There are only 2 reports on the ultrastructural changes of muscle in human leprosy in the literature. Job, *et al.* ([17]) have described the electronmicroscopic appearance of leprous myositis in striated and smooth muscle; of the 6 striated muscles examined, solid forms of *M. leprae* were absent in all but two. In contrast, large clusters of bacilli were present inside smooth muscle cells. The smooth muscle of the iris in lepromatous leprosy has been described by Hashizume and Shionuma ([13]). In the accompanying paper ([11]), the histochemistry of muscle in leprosy as well as histologic changes seen in paraffin sections were reported. The fine structural changes being reported here for the first time have been described in a thesis by one of us ([10]) and in abstract form elsewhere ([5]).

MATERIALS AND METHODS

Clinical material. There were 21 patients in all, and they were divided into 4 groups. Group I included patients with early nonlepromatous leprosy; group II—patients with established tuberculoid leprosy; group III—those with untreated lepromatous leprosy; and group IV—treated lepromatous leprosy patients. The history, clinical findings, and relevant laboratory investigations for each of these 4 groups have been given in the accompanying paper ([11]).

Laboratory methods. The essential steps for ultramicrotomy and electronmicroscopy (EM) were the collection and immediate fixation of small longitudinally oriented pieces in cold 4% glutaraldehyde in Millonig's phosphate buffer, post-fixation in OsO_4, dehydration in a graded series of ethyl alcohol, and blocking in araldite. The blocks were sectioned by glass knives; semithin (1 μm) sections were obtained and stained with toluidine blue for survey. Silver-grey thin sections were collected on copper grids for examination on a Philips EM200 electronmicroscope.

The serum creatine phosphokinase (CPK) was estimated by Hughes' method ([16]), and the immunoglobulin, IgG, was measured by a radial diffusion method ([19]).

RESULTS

Light microscopy. Light microscope examination of the araldite embedded sections further confirmed the normal histological appearance seen in paraffin and frozen sections of group I and the diffuse smallness and atrophic groups of group II, as described in the accompanying paper ([11]).

Such 1 μm thick sections of specimens of group III showed the atrophic fascicles to be made up of small degenerating fibers embedded in a collagenous matrix (Fig. 1a).

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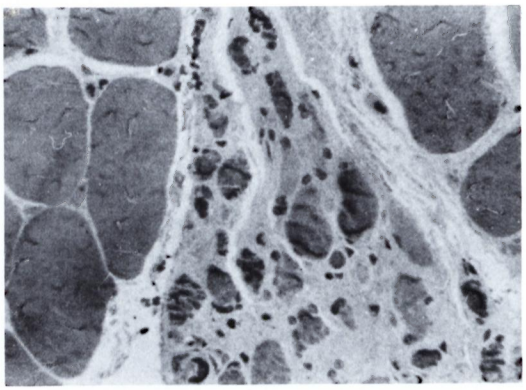

FIG. 1a. (NP/K/31): Weak muscle from an untreated lepromatous patient, showing parts of 3 fascicles, those on the sides with normal sized fibers and the fascicle in the center with atrophied irregular fibers with increased muscle nuclei in a fibrous matrix. (1 μm thick osmicated araldite section stained with toluidine blue, ×300)

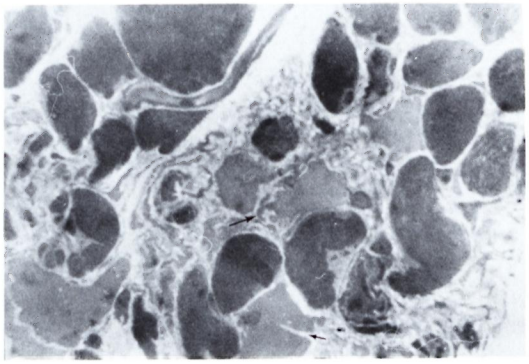

FIG. 1b. (NP/J/45): Weak muscle from a treated lepromatous patient; part of a fascicle showing fibers of varying size, generally smaller, the only normal sized fibers being the one above the capillary (on the left side of the upper border). Note also the several pale degenerating fibers most of which show clear atrophy in the form of crumpled irregular border (long arrow) or splitting (short arrow). (1 μm thick osmicated araldite section stained with toluidine blue, ×850)

These fibers appeared to be pale with loosened myofibrils or dark with homogenous cytoplasm. Two of the 5 specimens of this group showed severe degenerative changes.

Two specimens of group IV, which showed normal histology on paraffin sections, now disclosed stray degenerating fibers. In addition, the muscle of NP/J/45 (which at operation had shown gross adhesions to the overlying skin of the hand) revealed the atrophied fibers to be pale, irregular, and degenerating (Fig. 1b). The blood vessels appeared unremarkable except in the more affected muscle (NP/J/45) where many of the small blood vessels showed proliferation of basement membrane in concentric layers.

Ultrathin sections. The fine structural examination of all muscles of group I (early non-lepromatous leprosy patients) showed essentially normal features. There was no disruption of the normal parallel alignment of the myofibrils, and the A and I bands and Z lines were maintained. These compactly arranged myofibrils were ensheathed by an intact basement membrane (b.m.) and a closely opposed plasma membrane (p.m.). The nuclei were peripherally placed, and normal appearing mitochondria were found between the myofibrils and, rarely, accumulated beneath the p.m.

The muscle biopsy specimens of this group thus appeared to be unaffected as seen by light microscopy, enzyme histochemistry, and even eletronmicroscopy. In fact, this group served as a good control for comparison with the mild, moderate, or severe changes seen in the other 3 groups.

In the specimens of group II (established tuberculoid cases) the generalized smallness of the muscle fibers, as seen at light microscopy, was confirmed. Besides the smallness, these fibers appeared quite normal with compact myofibrils and clear b.m. and p.m. In 1 specimen, well-preserved myoneural endings were seen. Among these fibers were smaller angular atrophic fibers or severely atrophied fibers with loose hanging b.m. thrown into folds (Fig. 2).

The blood vessels between the fibers appeared to be only mildly affected with well-preserved tight junctions and pinocytotic vesicles and only minimal thickening of the b.m. of endothelial cells. Two large interfasicular nerves were seen, made up of normal looking myelinated and unmyelinated fibers surrounded by a perineurium which did not appear to be thickened.

In group III (untreated lepromatous patients), while some of the better preserved fibers still had intact myofibrils, the majority of the fibers appeared to be degenerating with disorganized myofibrils. On one of the

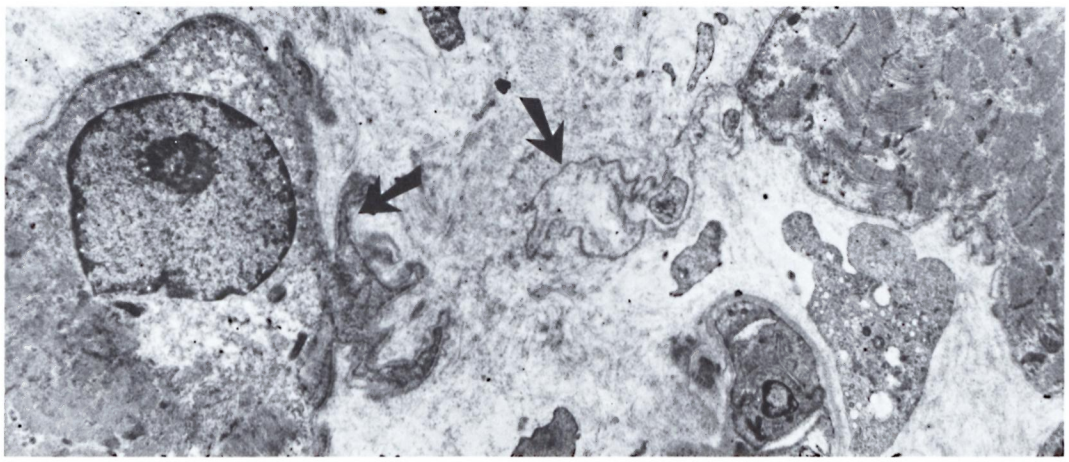

FIG. 2. (NP/J/1000): Established tuberculoid leprosy; parts of 2 muscle fibers with loose hanging and folded basement membrane (arrows) with intact myofibrils. Between them is a blood vessel and a macrophage. (Ultrathin araldite section stained with uranyl acetate and lead citrate, ×4900)

better preserved fibers, there was a myoneural junction with normal looking invaginations and clefts.

Fiber degeneration varied from early myofibrillar disorganization, to myofilament disintegration, to the severest change where the myofilaments were unrecognizable, only homogenous masses being seen. In such degenerating fibers the b.m. was thickened and in places could not be distinguished from the p.m. With severe degeneration, the p.m. had disappeared, and even the b.m. had fragmented. There was minimal to moderate accumulation of lipofusion in such muscle fibers or in the macrophages around such fibers. Rare fibers showed distended sarcoplasmic reticulum or T-tubules, as in the fibers in Fig. 3, which also revealed jumbled myofibrils with myofilaments in ill-defined aggregates and Z-line material in black rosette-like figures (Z in Fig. 3). One specimen showed large numbers of lymphocytes and macrophages in close proximity to blood vessels around remnants of muscle fibers that had totally degenerated. An interesting finding was the presence of a macrophage within the folded b.m. of a degenerating fiber (Fig. 4).

In only 1 specimen, in a distended endothelial cell of an intramuscular blood vessel, 2 bacilli (*M. leprae*), each with a clear cell wall, were seen at electronmicroscopy (Fig. 5). The muscle fibers in close proximity to this blood vessel appeared normal. Many intramuscular blood vessels showed thickened endothelial cells with intact tight junctions and increased pinocytotic vesicles. The basement membrane was thickened or proliferated.

Thus, this group (untreated lepromatous patients of group III) showed the maximum pathology in muscle specimens: a) large

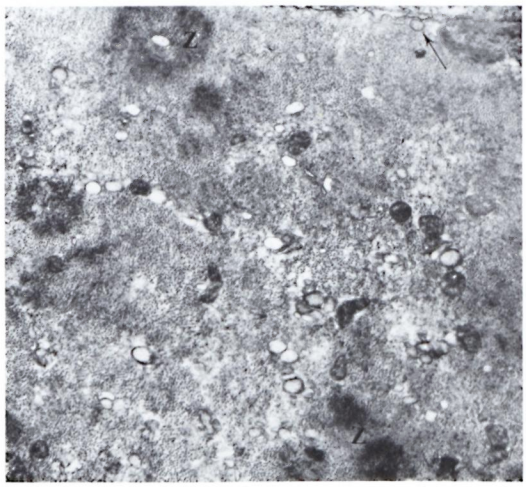

FIG. 3. (NP/J/746): Untreated lepromatous patient; part of a degenerating fiber with its basement membrane at upper right corner and showing clumps of A-band and Z-line material (Z), distended T-tubules including the one beneath the plasma membrane (arrow), and clustered myofilaments representing myofibrils cut transversely. (As in Fig. 2, ×18,200)

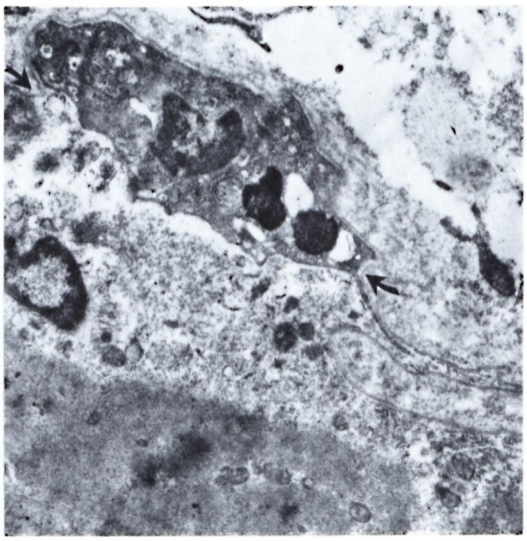

FIG. 4. (NP/K/31): Untreated lepromatous patient; a degenerating fiber with homogenous mass of myofibrillar material (in the lower part of the figure) and a macrophage bearing lipofuscin and its own nucleus just beneath the basement membrane of the fiber (arrows). (As in Fig. 2, ×14,000)

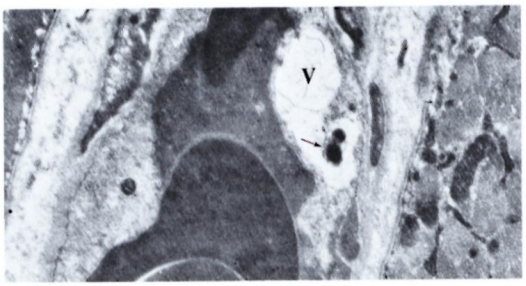

FIG. 5. (NP/J/746): Patient from group III (untreated lepromatous case); part of an intramuscular blood vessel (RBCs in lumen). Two bacilli (*M. leprae*) with clear cell wall (arrow) in a large vacuole (v) distending the endothelial cell which is bulging into the lumen. (As in Fig. 2, ×12,000)

group atrophy; b) type grouping; c) type atrophy; d) considerable degenerative changes at the EM level; e) vascular changes; f) presence of *M. leprae* in an endothelial cell of a blood vessel; and g) intramuscular inflammatory cells.

Six of the 7 specimens of group IV (treated lepromatous cases) showed several to many normal fibers with occasional atrophic fibers being encountered in all. Subsurface loss of myofibrils below scalloped b.m. was frequently seen. This space was usually filled with mitochondria. The remaining myofibrils were invariably well preserved. The most advanced atrophy was represented by a fiber with only a few myofibrils remaining, the sarcoplasm being filled with mitochondria and the b.m. completely collapsed to give an accordion-like appearance (Fig. 6).

The muscle of NP/J/45 showed a striking combination of atrophy and degeneration, the small fibers being devoid of almost all sarcoplasmic and contractile elements and the remaining myofibrils forming a homogenous mass (Fig. 7a). The nuclei were seen to be central and appeared to be large and sometimes indented. Accumulation of lipofuscin material of varying osmiophilia within lysosomal bodies at the sites of myofibrillar loss was common. These were found in increasing amounts in the more degenerating fibers, entire fibers being filled up in 1 instance (Fig. 7b).

The blood vessels showed proliferation or thickening of the b.m. of the endothelial cells and of the proliferated pericytes. The other changes took the form of thickening of the endothelial cells with narrowing of the lumen, luminal protrusions of the endothelial cells, and prominence of pinocytotic vesicles.

Nerve twigs were encountered in the interfiber space, and these showed apparently normal myelinated and unmyelinated fibers and an intact perineurium.

Only 2 of 7 cases showed 1 bacillus each; one was seen in the pericyte of a blood vessel (Fig. 8). Only 1 *M. leprae* was detected actually in the center of a muscle fiber (Fig. 9) with well-preserved myofilaments around it.

Although this group (treated lepromatous cases) revealed atrophy of muscle fibers at light microscopy and degeneration at electronmicroscopy, these changes were less severe than those observed in group III (untreated lepromatous patients).

Serum CPK and IgG. It was possible to collect blood from only 12 patients distributed in the 4 groups; all these showed normal CPK values (Table). In 4 patients of the untreated lepromatous group, serum IgG levels were estimated. All showed elevated IgG values, and the mean value in this group of patients (group III) was statistically significantly higher ($p < 0.001$)

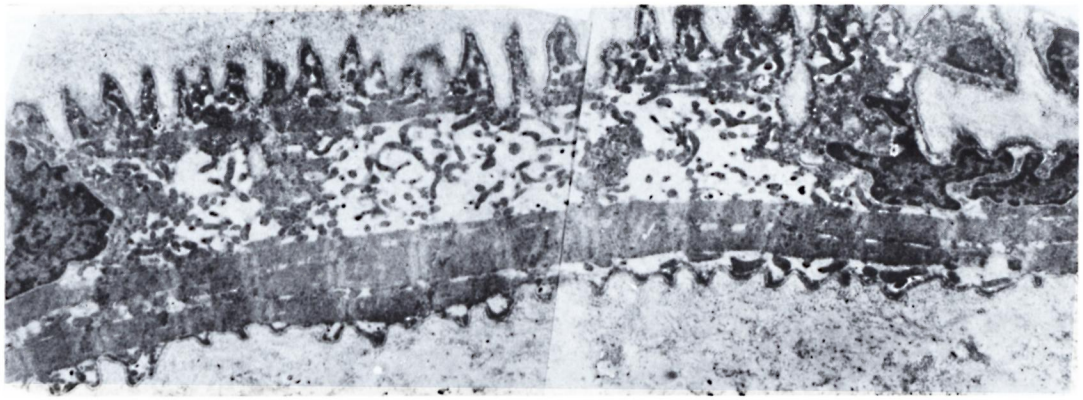

FIG. 6. (NP/J/146): Clinically normal muscle from a treated lepromatous patient, showing a severely atrophic fiber with regular infoldings of basement membrane, many mitochondria, only 2–3 myofibrils remaining, and 2 prominent nuclei. (montage of 2 prints, as in Fig. 2, ×6130)

compared to 11 normal control subjects (Table). In tuberculoid patients the serum IgG remained within normal limits.

DISCUSSION

The main findings of this investigation involving the fine structural examination of muscle from these 4 categories of leprosy have been the confirmation of group atrophy representing denervation in all patients where there was some nerve involvement (groups II, III, and IV) and of muscle fiber degeneration as well in patients with untreated or treated lepromatous leprosy (groups III and IV). The arresting change, though it involved only a few fibers in most of the specimens, was degeneration. Starting with the earliest change of disorganization of myofibrils without actual loss of myofilaments, there was almost total loss of myofibrillar and sarcoplasmic constituents during severe degeneration. This was noticed in 4 of the 12 patients who made up these 2 groups. Such a destructive change was usually afflicted on fibers which were atrophic but also on fibers which were not and was accompanied only infrequently by phagocytic cell reaction or by accumulations

TABLE. *Serum CPK, total serum protein, and IgG levels.*

Group no.	NP no.	Serum CPK (I.U.)	Total serum proteins (gm%)	IgG[a] (gm%)
I. Very early non-lepromatous cases	K-32	20.0	6.4	1.13
II. Established tuberculoid	J-982	40.3	7.0	1.41
	J-1000	14.2	7.0	1.37
III. Untreated lepromatous	J-993	44.6	6.8	1.82
	K-6	14.2	—	1.77
	K-31	5.5	7.3	1.76
	K-39	10.6	6.5	1.70
IV. Treated lepromatous	J-95	5.4	—	—
	J-107	—	—	1.30
	J-146	3.5	—	—
	J-201	5.7	6.3	1.41
Normal control subjects (mean values, N = 11)		20.3	7.2	1.20

[a] Statistically significant difference (by the t test) at a level of $p < 0.001$ between the mean values for IgG for group III (1.76 ± 0.05, S.D., N = 4) and controls (1.20 ± 0.19, S.D., N = 11).

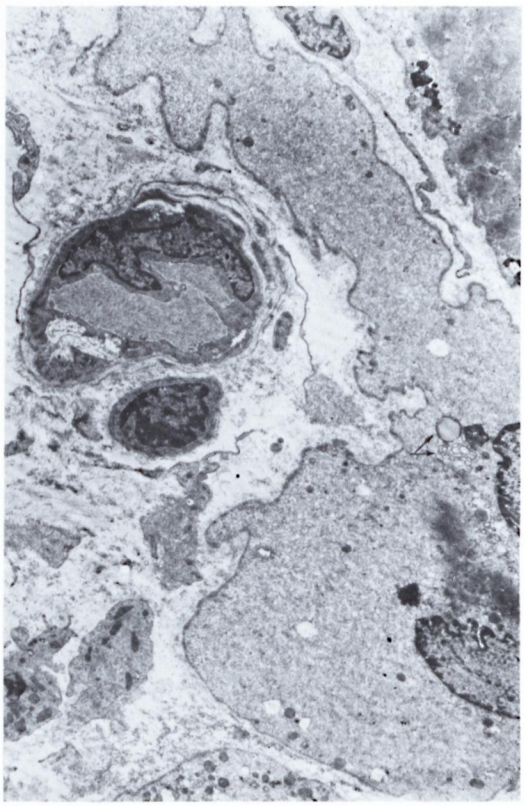

FIG. 7a. (NP/J/45): Weak and wasted muscle from a treated lepromatous patient, showing a degenerated fiber with loss of all structural constituents, only a small homogenous clump of myofibrils between 2 nuclei remaining. The split arrow shows a small lipofuscin body near an aggregate of T-tubules. A fiber with preserved myofibrils and some accumulation of lipofuscin is seen at the right upper corner. A blood vessel with clear lumen, vacuoles in its endothelial cell, and proliferated basement membrane and fibroblast processes around it is also seen. (As in Fig. 2, ×3050)

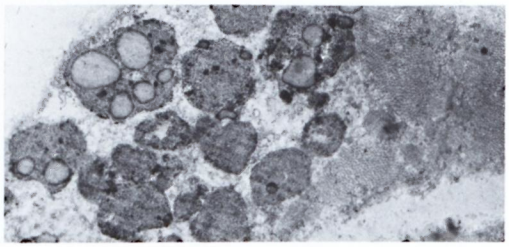

FIG. 7b. Same muscle as above (Fig. 7a), showing part of an atrophic fiber with remaining myofibrils on 1 side; the greater part of the fiber is filled with lipofuscin in lysosome-like bodies which have increased considerably. (As in Fig. 2, ×18,200)

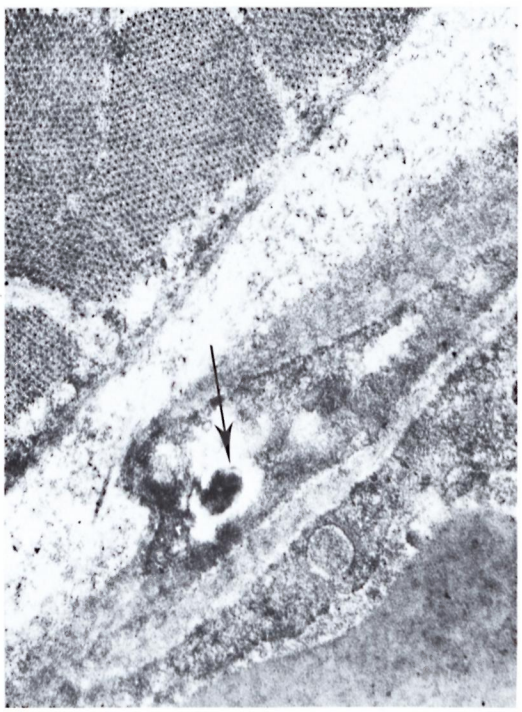

FIG. 8. (NP/J/107): Treated lepromatous leprosy patient; an intact bacillus in its halo (arrow) in the pericyte of a blood vessel. Part of a muscle fiber nearby shows clear myofibrils with well-preserved myofilaments and intact basement and plasma membranes. (As in Fig. 2, ×39,000)

of lipofuscin and lysosomes. Lipofuscin forms readily in degenerating muscles, and we have noticed this in muscle from osteomalacic patients ([8]) and in disuse atrophy ([7]).

Two of the severest changes noticed in degenerating muscle fibers from patients of groups III and IV were the destruction of the plasma and basement membranes and a reduction of the total contents of the muscle fiber to a clumped, faintly osmiophilic homogeneous mass. These changes per se appear nonspecific and have been observed by us in other unrelated muscle disorders of man such as muscular dystrophies and spinal muscular atrophies ([6]), osteomalacia ([8]), and disuse atrophy of muscle ([7]). There are also earlier reports ([22,23,24]), 2 of them on chronic neurogenic atrophies, on the combination of atrophic and degenerative changes besetting human muscles. Recently, we have been led to postulate that the proximal muscles of man may be particu-

larly susceptible to different kinds of inherited or acquired pathogenic mechanisms ([7]). However, in the present study we have detected similar changes in a distal muscle, i.e., the first dorsal interosseous muscle of the hand. Unlike myopathies and chronic polymyositis, which are characterized by phagocytosis of muscle fibers ([15, 20]) and despite severe degenerative changes, the serum CPK was not elevated in our patients. This was possibly because the muscle fibers, which were generally undergoing degeneration, were already atrophied, and atrophied fibers contain smaller amounts of muscle enzymes ([14]).

Another point worth discussing here is the possible pathogenesis of the degenerative changes seen in the muscle fibers in these lepromatous patients. There was a paucity of *M. leprae* in these muscles, only 1 bacillus each being seen in 3 patients on electronmicroscopic examination. Even with these 3 bacilli, 2 were in the wall of an interfascicular blood vessel, and only 1 was within a muscle fiber. In appropriately stained paraffin sections ([11]), a few acid-fast bacilli were seen in or around the blood vessels between muscle fibers in 4 of these cases (6 patients in all showing bacilli in muscles) while there were large numbers of bacilli in the nerves in all 12 of these lepromatous patients. A possible explanation for the severe degeneration in lepromatous muscle can emerge from 2 other apparently unrelated findings of our study, namely the changes in intramuscular blood vessels and the elevation of serum immunoglobulins.

Our observations on blood vessels in the muscles in lepromatous leprosy, namely luminal and adluminal endothelial protrusions with a narrowed lumen and considerable multilayering of the basement membrane, were similar to the fine structural changes in the blood vessels in lepromatous nerves described by Dastur, *et al.* ([9]) and Boddingius ([1]). Perivascular zones of intraneural blood vessels were thought to initiate or aggravate neuropathological changes by impairment of diffusion of oxygen and nutriments or metabolites ([1]). In a comprehensive arteriographic study of blood vessels in the wrist, palm, and fingers of 35 patients with leprosy (the majority of them lepromatous), Kaur, *et al.* ([18]) found occlusion, narrowing, tortuosity

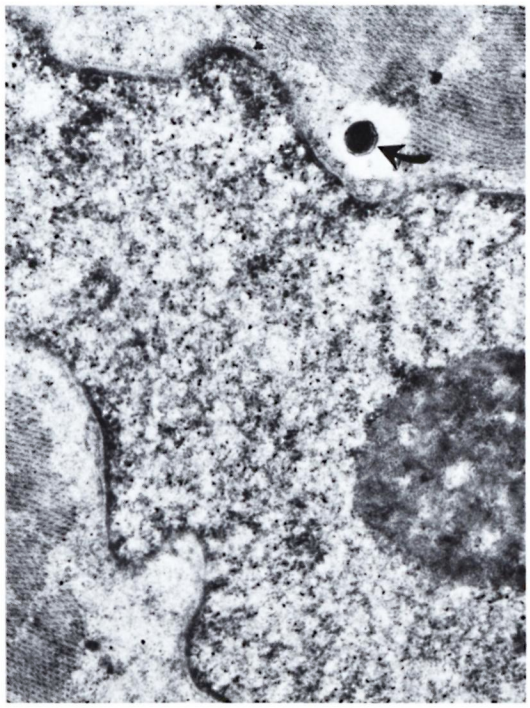

FIG. 9. (NP/J/95): Another patient from group IV (treated lepromatous cases); small part of an otherwise well-preserved fiber, showing a single bacillus (*M. leprae*) with a clear cell wall (arrow) in a halo near a large central nucleolated nucleus. (As in Fig. 2, ×27,000)

or irregularity, post-stenotic dilation, and incomplete filling of the lumen by radioopaque material in the wrist and palm region in over 50% of the arteriograms and in the digital vessels in over 75%. Boddingius, *et al.* ([2]) tested the permeability of endoneurial blood vessels in mice infected with *M. leprae*. They found the increased permeability, i.e., the defect in the blood-nerve barrier, to be proportional to the severity of infection. If this is the case with the intramuscular blood vessels, the leakage of certain plasma proteins may be an important cause of the severe degeneration of muscle fibers seen in our lepromatous cases.

Increases in IgG and other immunoglobulins have been reported in lepromatous cases by Talwar, *et al.* ([26]) and Saha, *et al.* ([25]). Literature on leprosy also records the frequent development of autoantibodies, especially in patients with lepromatous leprosy, against several autoantigens including those from nucleus and smooth muscle.

This has been well reviewed by Masala, *et al.* ([21]). One wonders if the marked elevation of serum IgG noticed by us in the lepromatous patients may be related to the degeneration without denervation of muscle in these patients. On the other hand, the muscles of patients with tuberculoid leprosy showed far fewer degenerative and vascular changes, and these patients had consistently normal serum IgG levels.

SUMMARY

Fine structural changes are described in 21 muscle biopsy specimens from patients with early and established tuberculoid or treated and untreated lepromatous leprosy. The predominant light microscopic finding on paraffin sections of atrophy of the muscle fiber was confirmed in semithin araldite sections, which also revealed considerable degenerative changes in a few or more fibers. These were more clearly seen in ultrathin sections, especially in the lepromatous muscle specimens. The degeneration was in the form of severe loss or disorganization of the myofibrillar elements and in the most affected muscle fiber, a loss of all sarcoplasmic constituents, with occasional accumulation of lipofuscin in lysosome-like bodies. Only 3 of the lepromatous specimens showed 1 or 2 bacilli each, only 1 being within a muscle fiber. There were also mural changes in the small intramuscular blood vessels with proliferation of their basement membrane and pericytes. The mean serum IgG level in lepromatous patients was significantly elevated. This, together with the vascular change, might perhaps be responsible for the degenerative change in the muscle fiber despite a paucity of *M. leprae* in the muscle.

RESÚMEN

Se describen los cambios estructurales finos en 21 biopsias de músculo obtenidas de pacientes con lepra tuberculoide temprana o ya bien establecida y de pacientes con lepra lepromatosa tratada o sin tratar. El hallazgo predominante por microscopía de luz en los cortes en parafina fue la atrófia de las fibras musculares. El daño fue confirmado por microscopía electrónica en cortes semidelgados en araldita en los cuales, además, se observaron considerables cambios degenerativos en algunas o en varias fibras. Estos cambios fueron más aparentes en los cortes ultradelgados de las biopsias musculares provinientes de los pacientes lepromatosos. La degeneración se observó como pérdida severa o desorganización de los elementos miofibrilares y, en las fibras musculares más afectadas, como pérdida de todos los componentes sarcoplásmicos, con acumulación ocasional de lípofuscina y de cuerpos con apariencia lisosomal. Sólo tres de los especímenes de músculo de los pacientes lepromatosos mostraron uno o dos bacilos cada uno, con sólo uno dentro de una fibra muscular. También hubieron cambios en la pared de los pequeños vasos sanguíneos intramusculares con proliferación de su membrana basal y pericitos. El nivel promedio de la IgG sérica en los pacientes lepromatosos estuvo elevado de manera significante. Esto, junto con el cambio vascular, podría quizá ser responsable del cambio degenerativo en las fibras musculares a pesar de la virtual ausencia de *M. leprae* en el músculo.

RÉSUMÉ

Chez des malades souffrant de lèpre tuberculoïde précoce et caractérisée, ou bien de lèpre lépromateuse soit traitée soit non traitée, on a decrit des modifications de structure de détails présentes dans les échantillons de 21 biopsies musculaires. Les observations les plus caractéristiques faites au microscope optique, sur des coupes enrobées à la paraffine de fibres musculaires atrophiques, ont été confirmées dans des coupes semi-minces d'araldite. Ces dernières coupes ont également révélé des modifications dégénératives prononcées dans quelques fibres ou davantage. Ces modifications étaient plus clairement observées dans des sections ultra-minces, et ceci particulièrement dans des échantillons de muscles lépromateux. La dégénérescence se manifestait sous la forme d'une perte grave des éléments myofibrillaire ou sous forme d'une désorganisation de ces éléments, ainsi que, dans les fibres musculaires les plus atteintes, une perte de tous les constituants sarcoplasmiques, avec une accumulation occasionnelle de lipofuchsine dans des corps ressemblant aux lysosomes. Dans trois échantillons lépromateux seulement, on a pu observer un ou deux bacilles, et un seul était dans la fibre musculaire. On a également observé des modifications de la paroi des petits vaisseaux sanguins intramusculaires, avec une prolifération de la membrane basilaire et des pericytes. Le taux moyen des IgG du serum chez des malades lépromateux était significativement augmenté. Ceci, joint aux modifications vasculaires, pourrait peut-être être à l'origine des modifications dégénératives des fibres musculaires, qui se produisent malgré la rareté de *M. leprae* dans les muscles.

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REFERENCES

1. Boddingius, J. Ultrastructural changes in blood vessels of peripheral nerves in leprosy neuropathy. III. Borderline, borderline-lepromatous and lepromatous leprosy patients. Acta Neuropathol. (Berl.) **40** (1977) 21–39.
2. Boddingius, J., Rees, R. J. W. and Weddell, A. G. M. Defects in the blood-nerve barrier in mice with leprosy neuropathy. Nature (New Biol.) **237** (1972) 190–191.
3. Dastur, D. K. Leprosy—An infectious and immunological disorder of the nervous system. *In: Handbook of Clinical Neurology*. Vol. 33. Vinken, P. J. and Bruyn, G. W., eds. New York: North Holland Publishing Co., 1978, pp. 421–468.
4. Dastur, D. K. The motor unit in leprous neuritis: A clinicopathological study. Neurol. India **4** (1956) 1–27.
5. Dastur, D. K. and Daver, S. M. Long treated lepromatous leprosy: Histochemistry and electronmicroscopy of muscle. IV International Congress on Neuromuscular Diseases, Abst. No. 93, Montreal, 1978.
6. Dastur, D. K. and Razzak, Z. A. Electronmicroscopic comparison of muscular dystrophies and atrophies. *In: Proceedings of the III International Congress on Muscle Diseases*. Newcastle-upon-Tyne: Excerpta Medica Int. Cong. Ser. 334, 1974, p. 93.
7. Dastur, D. K., Gagrat, B. M. and Manghani, D. K. Fine structures of muscle in human disuse atrophy: Significance of proximal muscle involvement in muscle disorders. Neuropathol. Appl. Neurobiol. **5** (1979) 85–101.
8. Dastur, D. K., Gagrat, B. M., Wadia, N. H., Desai, M. N. and Bharucha, E. P. The nature of muscular change in osteomalacia—light and electronmicroscopic observations. J. Pathol. **117** (1975) 211–228.
9. Dastur, D. K., Ramamohan, Y. and Shah, J. S. Ultrastruture of lepromatous nerves. Neural pathogenesis in leprosy. Int. J. Lepr. **41** (1973) 47–80.
10. Daver, S. M. Striated muscle in different types of leprosy—Fine structure. Histochemistry and histology. M.Sc. thesis, Bombay University, Bombay, India, 1979.
11. Daver, S. M., Dastur, D. K., Shah, J. S. and Revankar, C. R. Striated muscle in four categories of leprosy. I. Histology and histochemistry. Int. J. Lepr. **48** (1980) 140–148.
12. Hansen, G. A. and Looft, C. *Leprosy in its Clinical and Pathological Aspects*. Walker, N., tr. London: Simkin, Marshall, Hamilton, Kent & Co. Ltd., 1895, pp. 61–72.
13. Hashizume, H. and Shionuma, E. Electronmicroscope study of lepromatous changes in the iris. Int. J. Lepr. **33** (1965) 61–82.
14. Heyck, H. and Laudahn, G. Muscle and serum enzymes in muscular dystrophy and neurogenic muscular atrophy. A comparative study. *In: Exploratory Concepts in Muscular Dystrophy and Related Disorders*. Milhorat, A. T., ed. New York: Excerpta Medica Foundation, 1967, pp. 232–244.
15. Hudgson, P. and Mastaglia, F. L. Ultrastructural studies of diseased muscle. *In: Disorders of Voluntary Muscle*. Walton, J. N., ed. London: Churchill Livingstone, 1974, pp. 360–416.
16. Hughes, B. P. A method for the estimation of serum creatine kinase and its use in comparing creatine kinase and adolase activity in normal and pathological sera. Clin. Chim. Acta. **7** (1962) 597–603.
17. Job, C. K., Karat, A. B. A., Karat, S. and Mathan, M. Leprous myositis—A histopathological and electronmicroscopic study. Lepr. Rev. **40** (1969) 9–16.
18. Kaur, S., Wahi, P. L., Chakravarti, R. N., Sodhi, J. S., Vadhwa, M. B. and Khera, A. S. Peripheral vascular deficit in leprosy. Int. J. Lepr. **44** (1976) 332–339.
19. Mancini, G., Carbonara, A. O. and Heremans, J. F. Immunochemical quantitation of antigens by single radial immunodiffusion. Int. J. Immunochem. **2** (1965) 235–254.
20. Manghani, D. K. and Dastur, D. K. Fine structural changes in muscle in chronic polymyositis. IV International Congress on Neuromuscular Diseases, Abst. No. 220, Montreal, 1978.
21. Masala, C., Amendolea, M. A., Nuti, M., Riccarducci, R., Tarabini, C. G. L. and Tarabini, C. G. Autoantibodies in leprosy. Int. J. Lepr. **47** (1979) 171–175.
22. Milhorat, A. T., Shafiq, S. A. and Goldstone, L. Changes in muscle structure in dystrophic patients, carriers and normal siblings seen by electronmicroscopy; correlation with levels of serum creatine phosphokinase (CPK). Ann. N. Y. Acad. Sci. **138** (1966) 246–292.
23. Roth, R. G., Graziani, L. J., Terry, R. D. and Scheinberg, L. C. Muscle fine structure in the Kugelberg-Welander syndrome. J. Neuropathol. Exp. Neurol. **24** (1965) 444–454.
24. Roy, S., Dubowitz, V. and Wolman, L. Ultra-

structure of muscle in infantile spinal muscular atrophy. J. Neurol. Sci. **12** (1971) 219–232.

25. SAHA, K., DUTTA, R. N. and DASGUPTA, A. Immunologic aspects of leprosy with special reference to the study of immunoglobulin. Int. J. Lepr. **43** (1975) 314–319.
26. TALWAR, G. P., KRISHNAN, A. D. and GUPTA, P. D. Quantitative evaluation of the progress of intracellular infection *in vitro*: Incorporation of ^{3}H-thymidine into deoxyribonucleic acid by *Mycobacterium leprae* in cultivated blood monocytes. Infect. Immun. **9** (1974) 187–191.
27. WORLD HEALTH ORGANIZATION. *Second Report of the Expert Committee on Leprosy*. Tech. Rep. Ser. No. 189. Geneva: 1960.
28. WORLD HEALTH ORGANIZATION. *Fifth Report of the WHO Expert Committee on Leprosy*. Sect. 1.1. Tech. Rep. Ser. No. 607. Geneva: 1977.