Myxovirus-Like Structures in a Case of Human Chronic Polymyositis

Abstract. *Intranuclear and intracytoplasmic aggregates of filaments with tubular structures and transverse striations occurred in muscle tissues biopsied from a patient with chronic polymyositis. The filamentous tubules bear a close resemblance to the incomplete form of myxovirus in which the envelope is missing. Three biopsies from the same patient, taken during a period of 1½ years, all revealed these structures. This finding provides presumptive evidence that a chronic persistent viral infection may be involved in the pathogenesis of chronic polymyositis.*

Polymyositis is a relatively common disease of muscle of unknown etiology manifested as an acute, subacute, or chronic degeneration of muscle fibers characterized by varying degrees of inflammatory reaction. Approximately one-half of the cases are associated with either a collagen disease or a malignant new growth; the other half occurs as isolated "primary" myopathies or in combination with skin lesions (*1*). It is, therefore, generally considered to be a syndrome or symptom complex rather than a specific disease entity. The possible cause of the syndrome that presently receives the greatest attention is an immunologic or allergic mechanism, although the histologic and clinical features are often suggestive of an infectious etiology. The possibility that a virus might be the etiologic agent has long been questioned but no supportive evidence has been demonstrated. Muscle tissues obtained from a patient with a clinical and pathological diagnosis of chronic polymyositis were studied by electron microscopy. Aggregates of tubular filamentous structures resembling those of myxovirus group were demonstrated in both nuclei and cytoplasm. These structures were observed in all three biopsies taken during 18 months.

The patient, a man 66 years old, developed progressive weakness of all muscles, mild dysphagia, and muscle atrophy most pronounced in the shoulder girdle and quadriceps during the past 6 years. The course was recently complicated by a moderate degree of Raynaud's syndrome. The family history was negative. Serum studies (creatine phosphokinase, glutamic oxalopyruvic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, and alkaline phosphatase) revealed no significant elevations in any enzyme fractions. Electromyograms of six muscles suggested myositis. Histological studies on all three muscle biopsies, from the left and right deltoid muscles, revealed lesions of approximately the same extent, characterized by necrosis of muscle fibers, phagocytosis, basophilic regenerating fibers, and diffuse mononuclear cell infiltrate with no relation to blood vessels. Intranuclear or intracytoplasmic inclusions were not observed on histologic examination.

For electron microscopy, the muscle tissues were immediately fixed with 1 percent osmium tetroxide buffered with veronal acetate, to which 4.5 percent sucrose was added. Some tissues were prefixed in 2 percent buffered glutaraldehyde solution for 20 minutes and postfixed in 1 percent buffered osmium tetroxide for comparison. The tissues were dehydrated in an ethanol series and embedded in a mixture of Epon and Araldite. Sections were cut on a Porter-Blum microtome, stained with lead citrate and uranyl acetate, and examined with an RCA EMU-3G electron microscope at 100 kv. A portion of the third biopsy specimen was frozen with dry ice immediately upon removal and retained in a freezer at −70°C for virus study.

The extent of ultrastructural alterations observed in all muscle biopsies was approximately equivalent, although the patient had a short period (about 2 weeks) of steroid therapy after the first biopsy. Most of the muscle fibers appeared to be relatively normal. Fibers in the vicinity of the cellular infiltrate often showed degenerative alterations characterized by disruption, distortion, and disappearance of myofilaments. This was accompanied with numerous membranous bodies that contained electron-dense granules (Fig. 1). In a few muscle fibers that did not show such a degenerative process, many bizarre gigantic mitochondria were observed infrequently but in all biopsies. These mitochondrial abnormalities were considered similar to those described by others (*2*) and their nonspecific nature was presented elsewhere (*3*).

Intracytoplasmic aggregates of filamentous structures were present in the three biopsies and intranuclear clusters of filaments in the last two. The sarcolemmal nuclei that contained interwoven filaments (Fig. 1) were accompanied with or without chromatin margination. The filaments in the nuclei and cytoplasm were approximately the same in morphology and dimension, although those observed in the

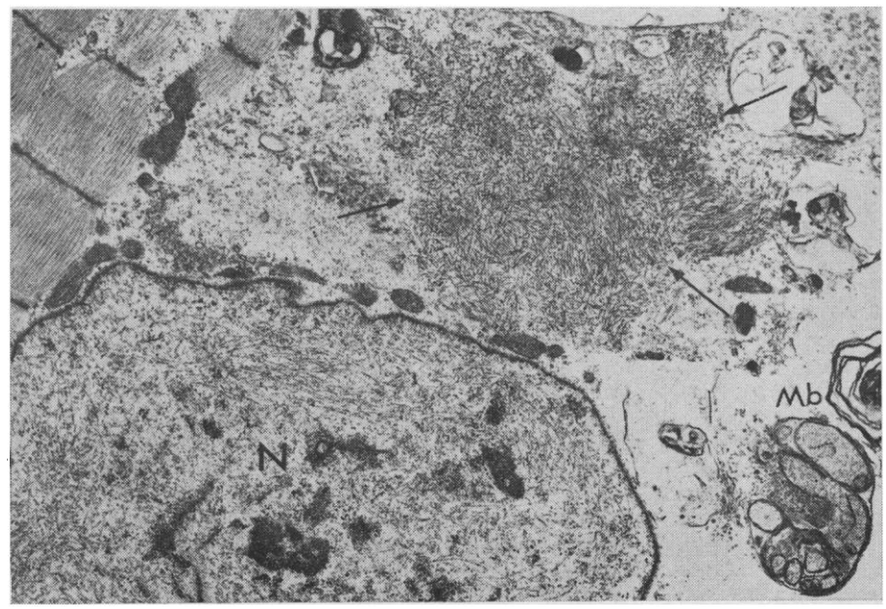

Fig. 1. Aggregates of interwoven filaments in the sarcoplasmic matrix (arrows) and in the nucleus (*N*). Degenerative membranous bodies (*Mb*) are replacing the myofilaments and other sarcoplasmic materials. Myofilaments are present in the upper left-hand corner. (× 8800)

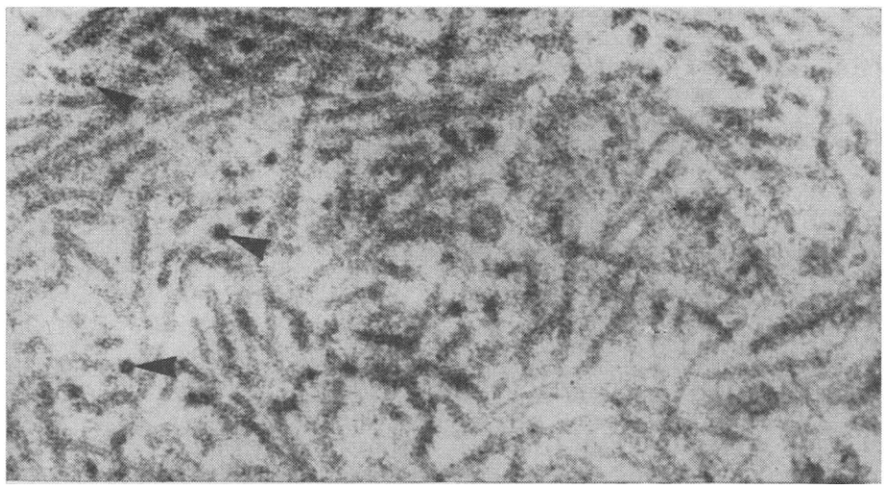

Fig. 2. High magnification of filaments in the nucleus showing tubular structures in both longitudinal and cross (arrowheads) sections. Periodic transverse striations are also recognizable on longitudinal sections. (× 93,000)

nuclei were often smaller in width and were longer. At high magnification (Fig. 2), the filaments, in both nuclei and cytoplasm, revealed distinct tubular structures characterized by circular hollow profiles on cross section and triple density in longitudinal sections. The tubular structures were more readily discernible in glutaraldehyde-fixed tissue. The filaments appeared rather rigid and straight, their length often exceeding 1 μ, especially in the nuclei. The diameter measured 200 to 230 Å (inner diameter, 60 to 74 Å) in the cytoplasm and 100 to 200 Å (inner diameter, 60 to 70 Å) in the nuclei. The outline of both cross and longitudinal sections were rippled, and periodic transverse striations indicative of a helical structure were frequently detected on longitudinal sections. The nuclear envelope that harbored the filamentous aggregates was generally well preserved. Occasionally, the nuclear membranes exhibited reduplication and invagination into the nuclear matrix, forming spherical or oval-shaped vacuoles (Fig. 3). Within, or in the vicinity of, such intranuclear vacuoles were excessive membranous structures resembling annulate lamellae. Infrequently, the nuclei were packed and distended with filaments that disrupted the membranes and extruded into the adjacent cytoplasm. Muscle fibers that contained intranuclear inclusions did not necessarily have intracytoplasmic inclusions; the sarcoplasmic organelles and myofilaments were usually intact (Fig. 3); whereas the muscle fibers that contained intracytoplasmic inclusions constantly displayed signs of degeneration, including numerous multilayered membranous or myelin figures (Fig. 1). In the cytoplasmic inclusion, the tubular filaments were more densely packed and separated from the adjacent myofilaments by an electron-lucid zone (Fig. 1), but occasionally filaments were observed to invade freely into relatively normal-looking myofilaments. Since these filamentous aggregates were never observed concomitantly with the bizarre gigantic mitochondria, a relation between these two structures, if any, could not be established. Sarcolemmal surfaces of muscle fibers showed no significant abnormalities. Inflammatory cells contained no filaments within their cytoplasm or nuclei.

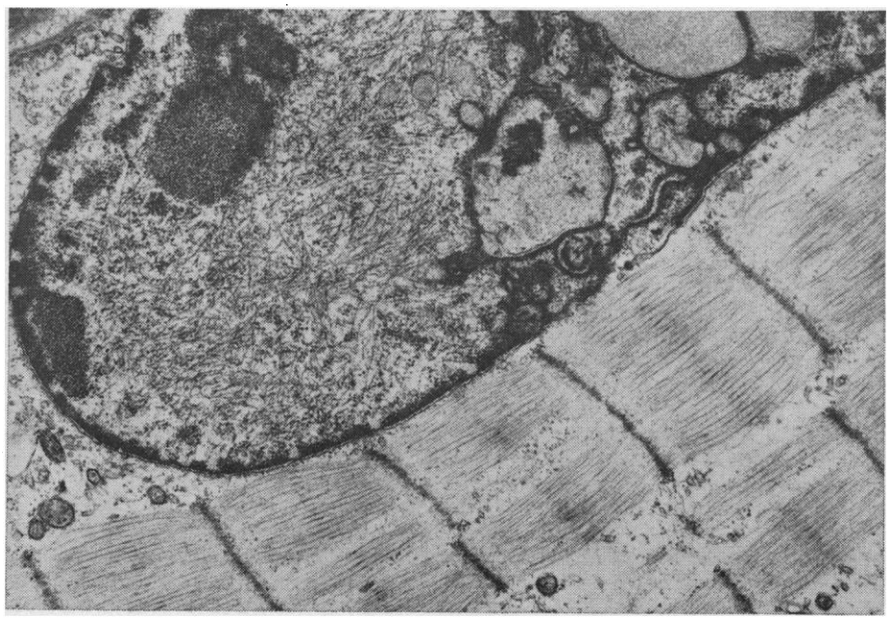

Fig. 3. A sarcolemmal nucleus containing clusters of filaments and prominent nucleoli. Profound changes of the nuclear membranes are characterized by invagination, reduplication, and vacuolar formation. The myofilaments in the vicinity are intact. (× 14,000)

A few members of the picornavirus group (*4*), but none of the myxovirus group, have occasionally been incriminated as a possible cause of some human muscle diseases. The morphology of the filaments described, as well as their intracellular location, are similar to those observed in certain members of the myxovirus group. Filamentous tubules, presumably of helical ribonucleoprotein, are commonly seen in myxovirus-infected cultured cells as an incomplete form of virion without its envelope (*5*). The myxovirus group has been classified into two subgroups on the basis of size, ability to form syncytia, and eosinophilic inclusions in cultured cells. Influenza viruses are distinguished from the paramyxoviruses by their smaller size (with longer, more slender, and rigid internal filaments), their inability to form intracytoplasmic inclusions in cultured cells, and their synthesis of internal antigen in the nucleus. In this study there was considerable variation in size between filaments observed in the nuclei and those in the cytoplasm. Data on the size and morphology of the internal helical filaments of the influenza viruses are based largely on the negative-staining technique; little is documented on the materials fixed in osmic acid. In contradistinction to the paramyxoviruses,

development of discrete foci of filamentous aggregates in infected tissues has never been observed in influenza viruses. Although morphogenesis of the paramyxoviruses has been assumed to occur only in the cell cytoplasm, filamentous tubules located within the nuclei have been observed in cultured cells infected with measles virus (*6*), and intranuclear inclusions have been found in tissues infected with bovine parainfluenza 3 virus (*7*). It is therefore not possible to identify the virus type of myxovirus solely by morphology.

Nevertheless, it seems reasonable at this time to predict that human chronic polymyositis is a disease of virus etiology, most likely caused by a member of the myxovirus group. It is conceivable that the existence of intranuclear and intracytoplasmic clusters of filaments in the three muscle tissues obtained during a period of 1½ years may represent a persistent chronic viral multiplication. Such a chronic viral infection has recently been suggested for herpes simplex virus infection in man (*8*). It is rather surprising to see so little evidence of degeneration of muscle fibers, the nuclei of which harbor filamentous aggregates. Signs of degeneration are seen constantly when intracytoplasmic aggregates exist. It is therefore logical to assume that morphogenesis of these filaments, which presumably are ribonucleoprotein, is initiated within the sarcolemmal nuclei and not in the cytoplasm. This may also indicate a well-balanced host-parasite relation that eventually will culminate in a total upset of the overall metabolism of the host cells. Because chronic polymyositis is commonly associated with such chronic diseases as carcinomatosis or collagen diseases, the possibility of patients' acquiring certain immunological deficits, and hence virus infection, should be considered. Although it is unlikely, it is entirely possible that these filaments, or an incomplete form of virus, represent a latent virus encountered by chance in this particular patient. If this is the case, more electron microscopic studies on muscle tissues from patients with polymyositis should be made to clarify the issue. Virological studies on the muscle tissue are now in progress.

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Transforming Activity of Green Monkey SA7(C8) Adenovirus in Tissue Culture

Abstract. *Simian SA7 (C8) adenovirus can effect transformation of mouse, rat, and hamster cells in tissue culture. The transforming activity of the virus was more pronounced in culture medium containing 0.1 millimolar $CaCl_2$, a lower concentration than that usually used. The transformed hamster cells were tumorigenic in hamsters.*

The transforming activity of oncogenic adenoviruses has not been adequately studied. Only the transforming activity of human type 12 adenovirus in hamster (*1*), rat, and rabbit (*2*) kidney cells and rat embryo (*3*) cells has been described. Freeman *et al.* (*3*) have shown that the transforming activity of this adenovirus is more pronounced when the concentration of calcium ions in the maintaining medium is lowered.

The strain C8 of green monkey SA7 adenovirus (*4*) is oncogenic for hamsters (*5*). We have studied the transforming activity of this agent in cultures of embryonic skin and muscle of hamsters, C3H mice, Wistar rats, and humans, and in cultures of kidney tissue from suckling mice and hamsters.

The C8 adenovirus was supplied by Dr. H. Malherbe. The virus was grown in green monkey kidney tissue maintained in medium No. 199, to which bovine serum (2 percent) had been added. When assayed in the same tissue culture by the plaque method (*6*), the

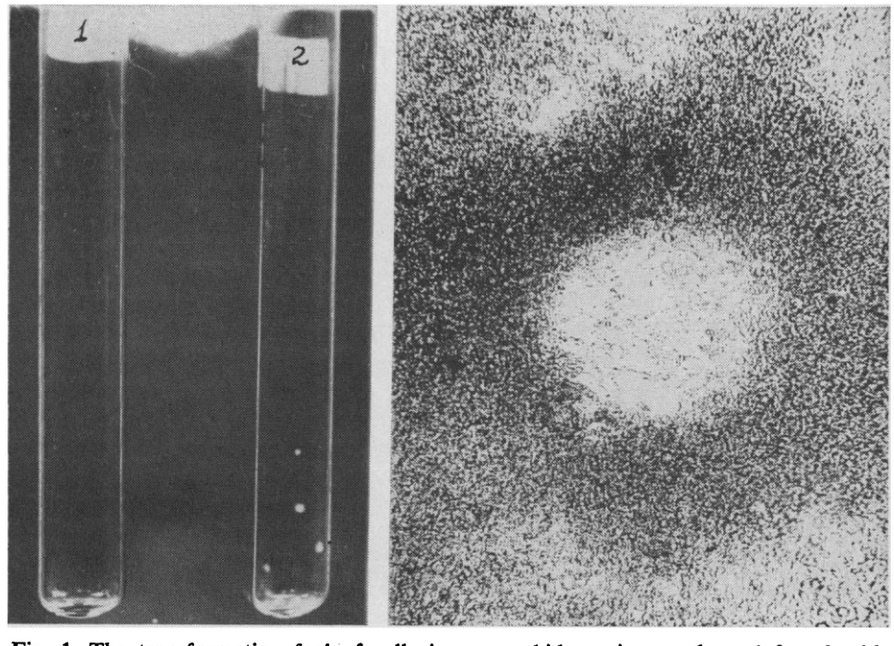

Fig. 1. The transformation foci of cells in mouse kidney tissue culture infected with SA7 (C8) adenovirus. (Left) Noninfected (1) and an infected (2) tissue culture tube (four foci are visible); (right) transformation focus with destruction of the central part. (× 105)