DISCUSSION

W. KING ENGEL and DALE E. MCFARLIN *(The Medical Neurology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Md.)*: In discussing Dr. Fenichel's paper, we will compare and contrast his biopsy findings in paraffin-embedded material with our own preliminary observations of 30 myasthenia gravis patients whose muscle biopsies were analysed with histochemical techniques applied to fresh-frozen sections.[1-3] The biopsies were reviewed independently by the two investigators without knowledge of the patients' clinical condition. (Full details of this investigation will be recorded elsewhere.)

In Dr. Fenichel's series, 12 of 37 patients had inflammatory cells in the biopsy. In some instances, the inflammatory cells, particularly macrophages, were around acutely necrotic fibers, this being the type I change of Russell.[4] Such fibers were found in two of our patients (FIGURE 1). In other instances of Dr. Fenichel's series, a number of the cellular collections were considered to be perivascular. In our material, no such perivascular collections were observed which, on serial section, could not be found to be surrounding a degenerating muscle fiber (FIGURE 2).

Not noted in Dr. Fenichel's series was the first stage of Russell's type II change:[4] namely, fibers undergoing what she called "progressive atrophy," and we tentatively call "chronic necrosis." Such fibers (FIGURE 3a) were found in 30 per cent of our patients. In the next stage of Russell's type II change, these abnormal fibers are surrounded by inflammatory cell collections consisting mainly of lymphocytes (FIGURES 3b and 3c). Such cellular collections were present in 23 per cent of our patients. These collections of lymphocytes have been called "lymphorrhages."[4,5] However, the definition of a lymphorrhage varies among current authors. For example, Adams and coauthors[6] state that it is a "focal collection of small lymphocytes . . . surrounding small venules or capillaries," while Greenfield and coauthors[7] state that it is "a compact group of mononuclear leucocytes, predominantly lymphocytes, about blood vessels or adjacent to undamaged muscle fibers." Dr. Fenichel, though, has suggested it is probable that a lymphorrhage is always related to "an abnormal fiber which is either out of plane of section or is fully degenerated." In our material, histochemical reactions revealed that, indeed, there was always one or more abnormal muscle fibers undergoing chronic necrosis in the midst of every lymphorrhage (FIGURE 4). The lymphorrhages, defined by us as collections of inflammatory cells consisting mainly of lymphocytes (with very few, if any, polymorphonuclear leucocytes and macrophages, and no epitheloid or giant cells), were present in 23 per cent of our patients.

In 11 of 37 patients, Dr. Fenichel found small angulated fibers in groups, termed the change one of "denervation," and considered it comparable to the type III change of Russell,[4] which she called "simple fiber atrophy." Russell

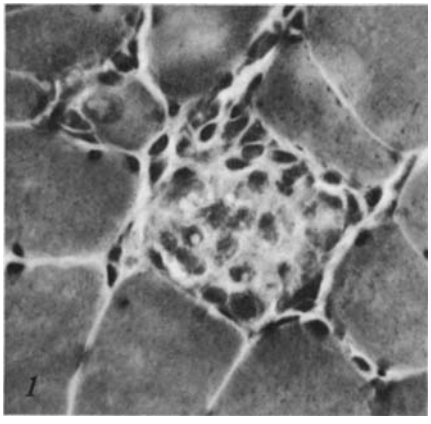


FIGURE 1. Phagocytosis of an acutely necrotic muscle fiber. Modified trichrome[6] (X 540). (All figures are cross-sections of unfixed frozen sections of skeletal muscle from myasthenia gravis patients, unless otherwise noted.)

did not place much importance on this change, perhaps because all her material was from autopsies. However, Dr. Fenichel's material was all of biopsy origin. Our biopsies were from muscles of nearly normal strength, in which effects of disuse and bedrest were thought, on clinical judgment, not to be of importance. In the general category of simple fiber atrophy, our histochemical studies revealed that (a) there were two subtypes of this abnormality and (b) every patient of our series had at least one form of this change. The first type was called "denervation atrophy," because it was like

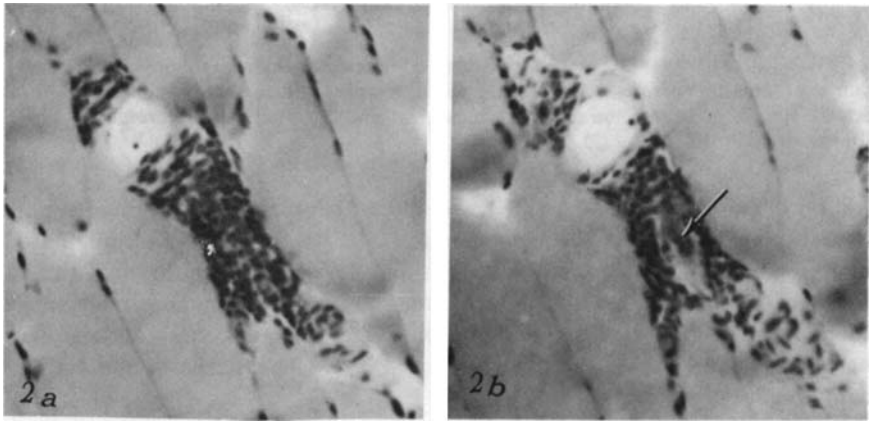
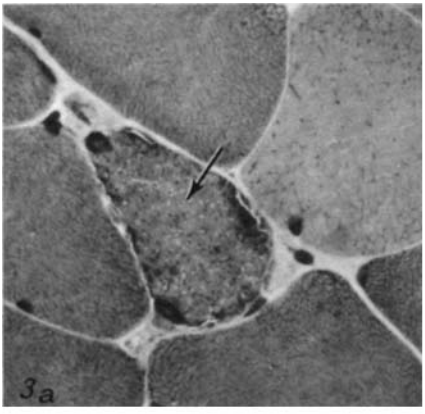
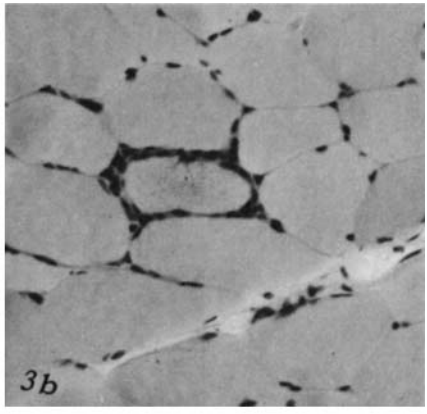


FIGURE 2. A collection of lymphocytes appears to be primarily perivascular in (a), but on serial section (b) a "chronically necrotic" fiber is found in the midst of the lymphocyte collection. Hematoxylin-eosin (X 225).





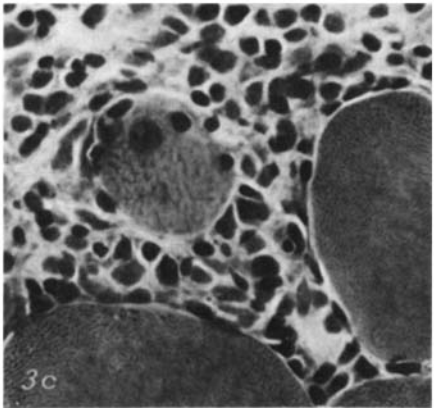


FIGURE 3. Three stages of Russell's type II change include (a) mild architectural changes ("chronic necrosis"), (b) then a slight lymphocytic reaction, and (c) finally a prolific lymphocytic reaction (lymphorrhage). (a) and (c) modified trichrome (X 560); (b) hematoxylin-eosin (X 225).

what we see in diseases of the lower motor neuron, such as amyotrophic lateral sclerosis. Fibers with this change (FIGURE 5) were small, angulated in cross-sectional appearance, both light and dark (within the normal range) with the myosin ATPase reaction, lighter than normal (or more mahogany-colored than normal) with the phosphorylase reaction, and darker than normal with the lactate dehydrogenase, TPNH dehydrogenase, and DPNH dehydrogenase reactions.*

*Histochemical activities are described as the degree of end-product deposition after incubation in appropriate media, without interpretation of possible false localization, enzyme diffusion, nonspecific activity and other factors.

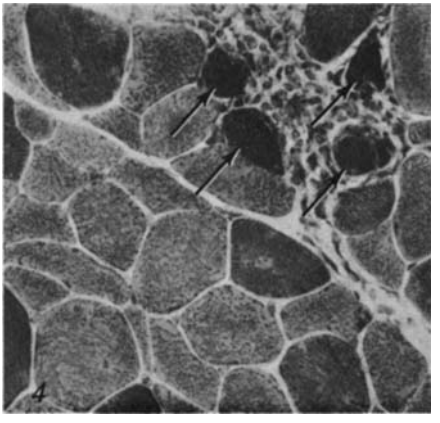


FIGURE 4. Four darkly stained abnormal fibers showing "chronic necrosis" in the midst of a lymphocyte collection (lymphorrhage). TPNH dehydrogenase (X 225).

The second variety was called "type II fiber atrophy," because it appeared as reduced diameter of only the fibers whose histochemical activities were in the normal range and characteristic of normal type II fibers (viz., high myosin ATPase, high phosphorylase, high menadione α-glycerophosphate dehydrogenase, low lactate dehydrogenase, low TPNH dehydrogenase and low DPNH dehydrogenase activities). The cross-sectional contour of these fibers was less rounded than normal but more rounded than the "denervated" fibers (FIGURES 6, 7, 8). The significance of type II fiber atrophy is not known. We have also observed it in patients taking corticosteroids for collagen-vascular disease and acute leukemia.[3] Changes of at least one of these types were found in each of our 30 patients, "denervation atrophy" being present in 63 per cent and "type II fiber atrophy" in 50 per cent.

In 15 of 37 patients, Dr. Fenichel found no abnormality in the paraffin sections. With the histochemical techniques disclosing more subtle abnormalities, none of our 30 patients was free of abnormality, though some of the abnormalities were slight (FIGURE 9).

It has now become important for investigators to correlate the different histochemical changes with abnormalities disclosed by the methylene blue, cholinesterase and immunofluorescent reactions, by performing two or more reactions on the same or serial sections. Further, it is important to correlate these histochemical changes with physiologic abnormalities discovered by study of whole muscle preparations as well as of single fibers.

Preliminary survey of our material has shown no obvious clinical correlation of age, sex, duration of disease, severity of disease, serum antibodies or thymoma with the muscle pathology, in contrast to the correlation with disease duration noted by Dr. Fenichel. A possibility not yet analyzed by us is that some of the histochemical changes in the muscle fibers are produced

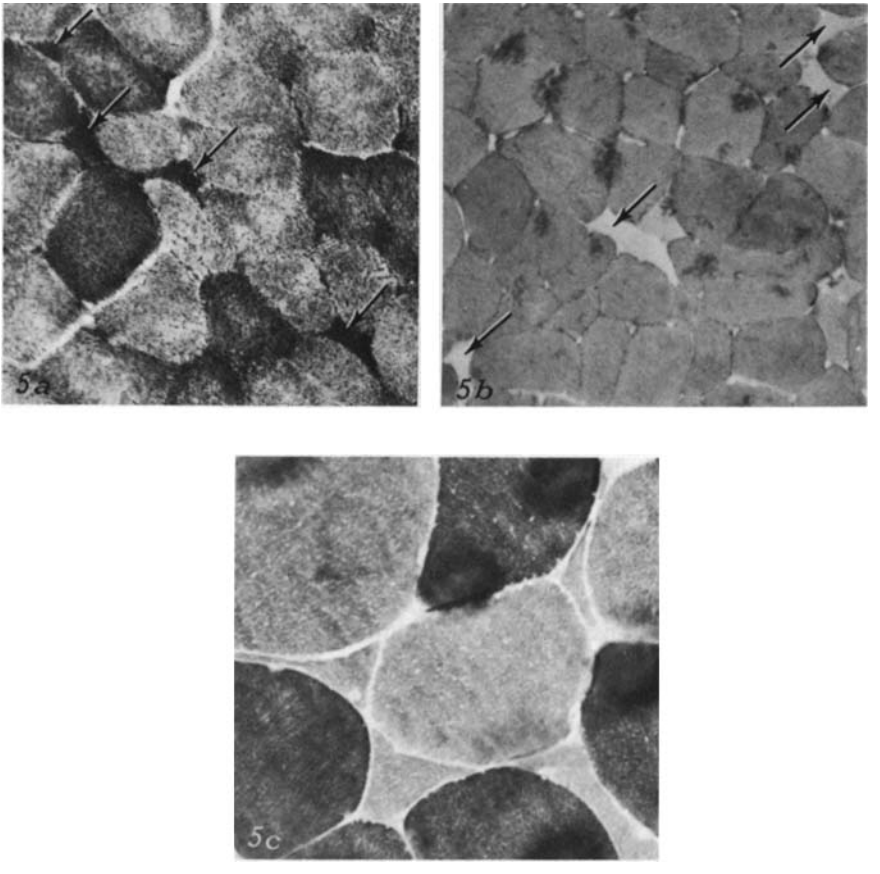


FIGURE 5. Fibers showing "denervation atrophy" are small and angulated, (a) very dark with the lactate dehydrogenase reaction, (b) very light with the phosphorylase reaction, and (c) both light and dark (in the normal range) with the myosin ATPase reaction (though only light ones are in this field). (a) and (b) are X 225, (c) is X 560.

by anticholinesterase drugs. Also, the possibility that some of the histochemically delineated abnormalities explain why certain myasthenic patients respond poorly or incompletely to medication has not been studied.

Though myasthenia gravis, in the final analysis, may well be a symptom complex of various etiologies, it seems unwise to attempt, as yet, any subgrouping on the basis of pathology in the small sample of tissue provided in a muscle biopsy. Moreover, possible variations in the muscle abnormalities from different regions and even different parts of the same muscle preclude the use of muscle biopsy to follow the course of the disease and the effect of treatment (FIGURES 10 and 11).[10]

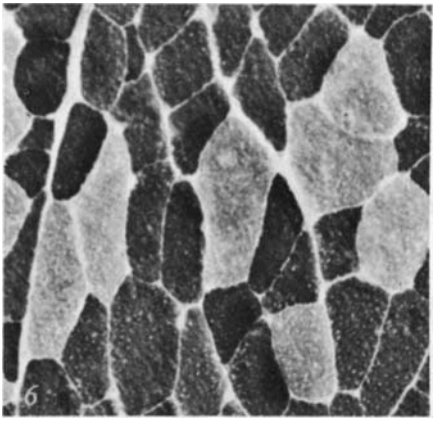


FIGURE 6. All the fibers of reduced diameter are normally dark with the myosin ATPase reaction in "type II fiber atrophy." (X 225).

Now let us turn from comments on the observations to ones on the interpretations of muscle histochemistry. Actually, the interpretations are still not certain. For example, in a 50-year-old lady who had myasthenia gravis for 47 years, the muscle fibers showed histochemical evidence of long-standing "denervation" (FIGURE 11) in her very weak and tensilon-unresponsive tibialis anterior, while there was minimal evidence of the same type of change in her nearly normal and tensilon-responsive biceps brachialis.[8] However, even though her muscle fibers responded as if their nerve supply had been damaged, the same histochemical picture could theoretically

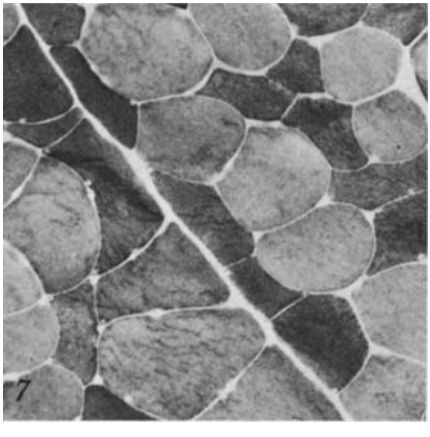


FIGURE 7. In type II fiber atrophy, the small fibers retain high phosphorylase activity. (X 225).

also have resulted if the lack of a trophic factor from the nerve fiber to the muscle fibers was through a defect on the muscle side of the neuromuscular junction.

Finally, in regard to speculating on the etiology from the histochemical changes of the muscle, I will defer to the numerous experts who will in the

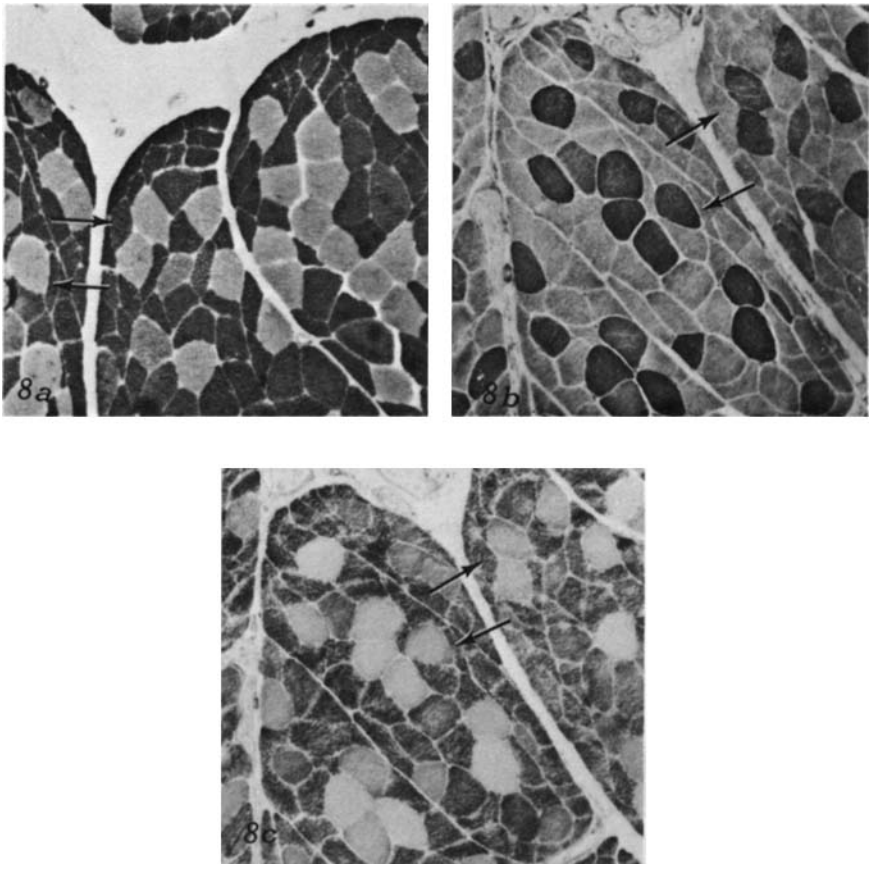


FIGURE 8. Type II fiber atrophy in nearly serial sections. The small fibers retain the histochemical characteristics of normal type II fibers: e.g., (a) high myosin ATPase, (b) low DPNH dehydrogenase, and (c) high menadione-linked α-glycerophosphate dehydrogenase activities (X 89).

succeeding papers speculate on the autoimmune and even the psychiatric aspects of the etiology and pathogenesis of myasthenia gravis. However, it appears that there is no paper on the possible viral origin of myasthenia gravis. If the evidence available on myasthenia gravis were assembled and emphasized differently, one might be able to make an equally reasonable

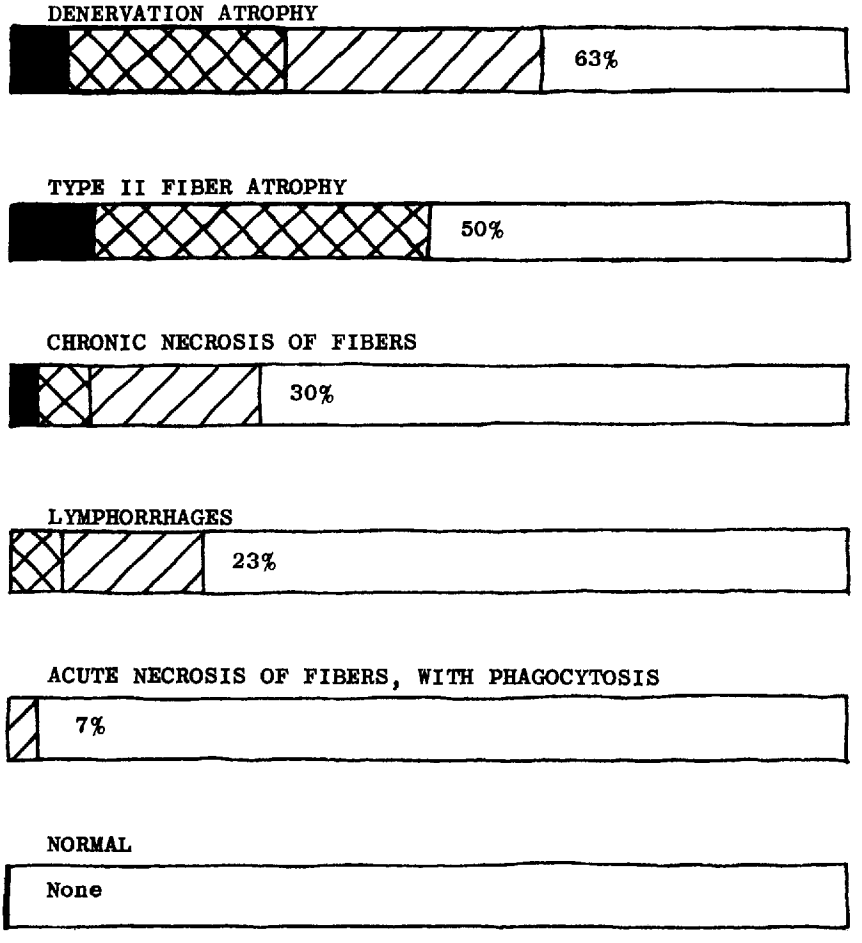


FIGURE 9. Graph of histochemical changes occurring in 30 myasthenic patients. Amount of involvement of each abnormality in each biopsy is denoted: solid—abundant, double hatched—moderate, single hatched—slight.

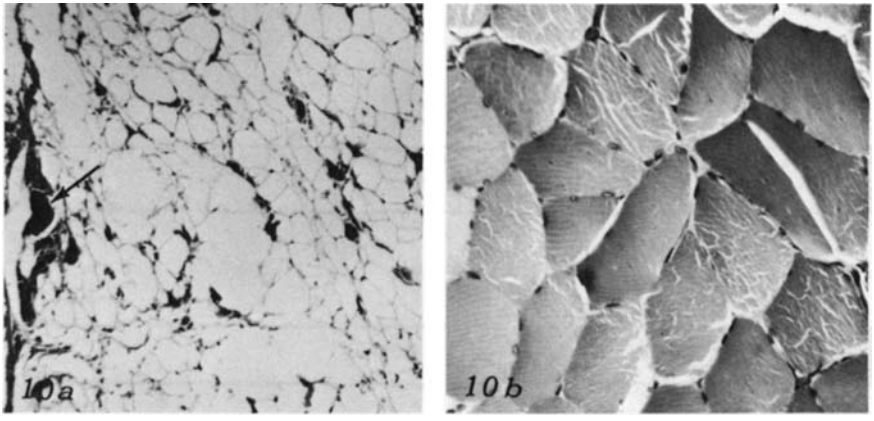


FIGURE 10. Two biopsies from the same patient (with facio-scapulohumeral dystrophy) taken the same week. (a) In the quadriceps, only one muscle fiber was found in a sea of fat (X 225) while (b) the gastrocnemius appeared normal (X 560). Paraffin section, Gomori trichrome, (X 225).

case for this possibility, especially considering that there is no evidence of an immunologic abnormality related to the neuromuscular junction.[9] Should anyone like to support the virus etiology, we can say that the muscle histopathology is not too unlike that of certain viral or viral-suspect diseases, and we can even provide him with the evidence of cytoplasmic "inclusion bodies" (FIGURE 12a) found in muscle fibers in 10 per cent of our patients. In fact, we can even provide an "inclusion body" in an intramuscular nerve fiber (FIGURE 12b) in one of our patients. The point is that the relation of such

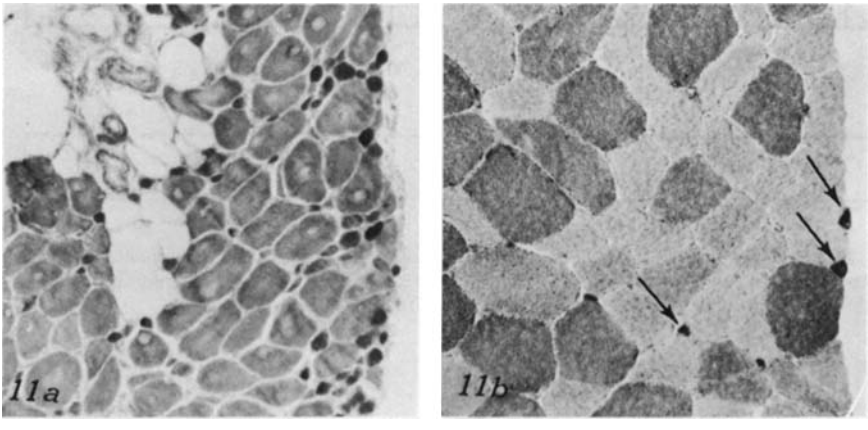


FIGURE 11. (a) Severe denervation with numerous target fibers and scattered small very dark fibers in a fascicle of tibialis anterior (X 225); (b) rare small, very dark fibers in otherwise normal biceps brachials (X 560). DPNH dehydrogenase.

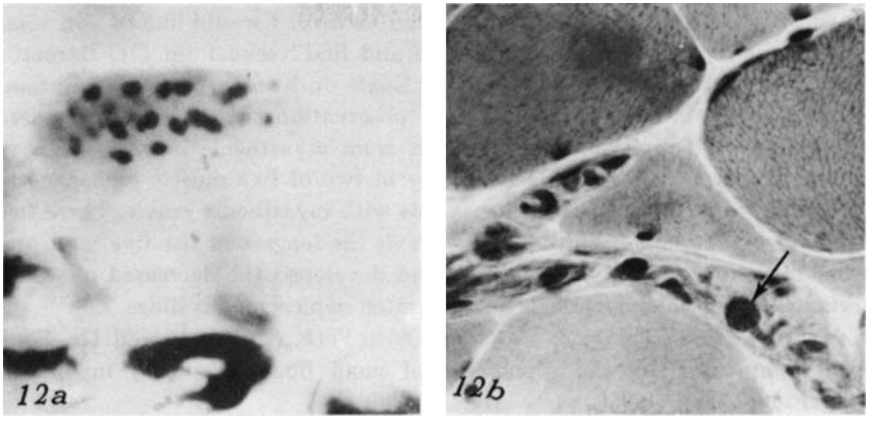


FIGURE 12. Cytoplasmic "inclusion bodies," stained red by the modified trichrome method, are in (a) a skeletal muscle fiber (X 1100), and (b) an intramuscular nerve fiber (X 560).

"inclusion bodies" to a possible viral etiology is no more fanciful than the relevance of certain evidence used to support the possible autoimmune origin of myasthenia gravis.

References

1. ENGEL, W. K. 1962. The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology **12:** 778–94.
2. ENGEL, W. K. & G. G. CUNNINGHAM. 1963. Rapid examination of muscle tissue. An improved trichrome method for fresh frozen biopsy sections. Neurology **13:** 919–23.
3. ENGEL, W. K. 1965. Diseases of the neuromuscular junction and muscle. *In* Histochemistry and Cytochemistry of the Nervous System. C. Adams, Ed. Elsvier Press, Amsterdam, Netherlands. (in press).
4. RUSSELL, D. S. 1953. Histological changes in the striped muscles in myasthenia gravis. J. Path. Bact. **65:** 279–89.
5. BUZZARD, E. F. 1905. The clinical history and postmortem examination of 5 cases of myasthenia gravis. Brain **28:** 438–83.
6. ADAMS, R. D., D. DENNY-BROWN & C. M. PEARSON. 1962. Diseases of muscle. *In* A Study in Pathology. 2nd Edit. : 632. Harper and Row, New York, N. Y.
7. GREENFIELD, J. G., G. M. SHY, E. C. ALVORD, JR. & L. BERG. 1957. An Atlas of Muscle Pathology in Neuromuscular Disease. : 95. Williams & Wilkins Co., Baltimore, Md.
8. BRODY, I. A. & W. K. ENGEL. 1964. Denervation of muscle in myasthenia gravis. Arch. Neur. **11:** 350–54.
9. MCFARLIN, D. E., W. K. ENGEL & A. J. L. STRAUSS. 1965. Does myasthenic serum bind to the neuromuscular junction? This Annal.
10. ENGEL, W. K. 1965. Current concepts of the myopathies. *In* Clinical Orthopaedics. Chap. 7. : 80–105. Lippincott, Philadelphia, Pa.

J. W. HESS *(Wayne State Univ., Detroit, Mich.)*: I would like to add some corroborative evidence from the "grind and find" school (in Dr. Barnett's terminology) to the observations of Dr. Engle on histochemical preparations of myasthenic muscle. In line with his observation that there is decreased phosphorylase activity in certain fibers from myasthenic muscle, we have found subnormal phosphorylase activity in two of five muscle homogenates prepared from biopsies from five patients with myasthenia gravis. These two patients had had clinical myasthenia gravis the longest of the five, and presumably more of their muscle fibers had developed the decreased phosphorylase activity which Dr. Engle demonstrated so nicely in his slides.

L. ROWLAND *(Columbia University, New York, N. Y.)*: Would Dr. Fenichel comment about the appearance of small fibers in purely myopathic conditions?

DR. FENICHEL: I have used Dr. Shy's criteria that three small fibers in contiguity are evidence of a "group lesion." These are not seen in myopath. The elongation and angulation of fibers are especially evidence for denervation.

F. A. HOEFER *(Columbia University, New York, N. Y.)*: Are group lesions found in myasthenia gravis?

DR. FENICHEL: The small fibers comprising the "group lesion" are considered to be members of the same motor unit in other neuropathics, and I have only assumed that the same is true in myasthenia.