have been identified. That this is a different type of alkaline phosphatase from that seen in capillary wall endothelium seems likely because of differing sensitivity to pH and environmental conditions.

BETTY PINNER
JOHN F. DAVISON
JAMES B. CAMPBELL

Departments of Neurological Surgery and Physical Medicine and Rehabilitation, New York University School of Medicine, New York

References and Notes

1. G. Gomöri, *J. Cellular Comp. Physiol.* **17**, 71 (1941); H. Landow, E. A. Kabat, W. Newman, *Arch. Neurol. Psychiat. Chicago*, **48**, 518 (1942); G. Bourne, *Quart. J. Exptl. Physiol.* **32**, 1 (1943); J. Marchant, *J. Anat.* **83**, 227 (1949); W. Newman, I. Feigin, A. Wolf, E. A. Kabat, *Am. J. Pathol.* **26**, 257 (1950); T. Samorajski, *J. Histochem. Cytochem.* **5**, 15 (1957); F. Wolfgram and A. S. Rose, *Neurology* **10**, 365 (1960); H. B. Tewari, R. Quinton-Cox, G. H. Bourne, *Exptl. Cell Res.* **28**, 576 (1962); E. R. Fisher and B. S. Turano, *Arch. Pathol.* **75**, 517 (1963).
2. M. S. Burstone, *Enzyme Histochemistry* (Academic Press, New York, 1962).
3. T. Barka and P. Anderson, *Histochemistry* (Harper and Row, New York, 1963).
4. H. D. Schmidt, *Monthly Microscop. J.* **11**, 200 (1874).
5. A. J. Lanterman, *Arch. Mikroskop. Anat.* **13**, 1 (1877).
6. S. Ramon y Cajal, *Degeneration and Regeneration of the Nervous System* (Oxford Univ. Press, Oxford, 1928).
7. J. D. Robertson, *J. Physiol.* **135**, 56 (1956).
8. ——, *J. Biophys. Biochem. Cytol.* **4**, 39 (1958).
9. ——, in *Ultrastructure and Metabolism of the Nervous System*, S. R. Korey, Ed. (Williams and Wilkins, Baltimore, 1962), pp. 94–158.
10. M. Luxoro, "Studies on the ultrastructure of myelinated nerve fibers," dissertation, Massachusetts Institute of Technology, Cambridge (1956).
11. D. C. Tosteson, R. H. Moulton, M. P. Blaustein, *Federation Proc.* **20**, 138 (1961).
12. J. F. Danielli, *Symp. Soc. Exptl. Biol.* **6**, 1 (1952).
13. This study was carried on in the research laboratory of the Institue for the Crippled and Disabled with the following support: Grants from the NIH, NB 04257-02; United Cerebral Palsy Association, Inc., Research and Educational Fund R-601-62C; Association for the Aid of Crippled Children; and Army Medical Service contract DA-49-193-MD-2232.

26 June 1964

"Red" Skeletal Muscle Fibers: Relative Independence of Neural Control

Abstract. *Studies on skeletal muscle of rats and mice indicate significant differences between the behaviors of the two basic types of muscle fibers during the development of denervation atrophy. While the diameter of fibers possessing sarcoplasmic granularity ("red" fibers) is preserved for a long time after complete severance of motor and sensory nerves, fiber with agranular cytoplasm ("white" fibers) undergo rapid atrophy. It is concluded that, at least in this respect, the "white" fibers are more dependent on neural control than are the "red" fibers.*

It has been known for some time that, even in the same species, the rate of atrophy following severance of motor nerves is significantly different in the various skeletal muscles and that some fibers atrophy much more rapidly than do others within the same muscle (*1*). No satisfactory hypothesis has been offered to explain this peculiar phenomenon in denervated muscle and, in fact, the problems associated with the genesis and prevention of neural atrophies are still largely unsolved (*2*).

It is now well established that the terms "red" and "white," as applied to a muscle, do not imply homogeneity of the component fibers, but that most of the mammalian skeletal muscles are composed of two general categories of fibers—that is, "red" and "white" fibers, that differ from each other both in certain structural characteristics and biochemical activities (*3*). In this report it is shown that evaluation of the progression of denervation atrophy, from this point of view, not only offers an explanation for the difference in reactivity of the various muscle groups subsequent to nerve lesions, but also suggests that the "white" fibers are more dependent on neural control than are the "red" ones.

The object of this study was to analyze comparatively the behaviors of the two basic types of muscle fibers during the development of three forms of muscle atrophy: (i) denervation atrophy, (ii) disuse atrophy, and (iii) dystrophy that develops spontaneously in strain-129 mice suffering from a hereditary form of myopathy. Male and female rats and mice of various ages were used and the investigations were centered on groups of muscles in the hind limbs. Total denervation was effected through a suprapubic incision; the left obturator, femoral, and sciatic nerves were sectioned intrapelvically, and a portion of the axons, several millimeters in length, was then removed to prevent regeneration. Disuse atrophy was induced either by tenotomy or by application of a plaster cast (*4*); the tenotomized triceps surae was isolated in a Millipore tube to prevent adhesions or regeneration of the sectioned tendon. The strain-129 mice were selected from our breeder colony (*5*). The animals were killed by decapitation at various intervals ranging from 1 day to several months after surgical intervention, after the initiation of immobilization by a plaster cast, or, in the case of strain-129 mice, after the presence of dystrophy could be clinically diagnosed. At autopsy, the muscle specimens were frozen rapidly in liquid air, stored at −70°C, and sectioned in a cryostat in 10-μ thicknesses. Succinic dehydrogenase was demonstrated with 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl-tetrazolium bromide (MTT) (*6*); for routine morphologic studies the frozen tissue was fixed after sectioning with formalin and stained with hematoxylin-phloxine-saffron (HPS).

The distribution pattern of oxidative enzymes is different in the "red" and "white" fibers (*7*) and fibers showing significantly greater succinic dehydrogenase activity are usually smaller in diameter than those showing less activity (Fig. 1). The technique for detecting succinic dehydrogenase activity was not suitable for analyzing the two categories of fibers because soon after denervation a qualitative alteration in the normal distribution of succinic dehydrogenase ensued; gradually, all denervated fibers reacted uniformly in the test for this enzyme (Fig. 2).

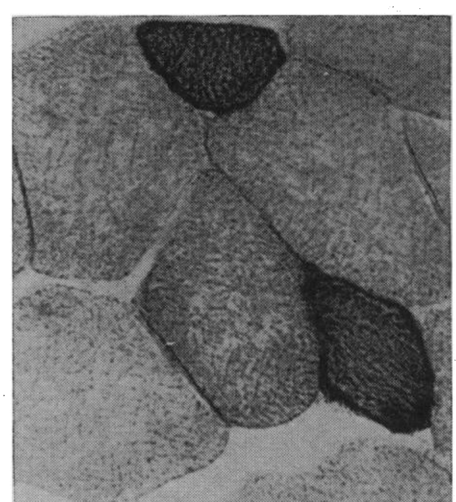

Fig. 1. Distribution of succinic dehydrogenase in muscle fibers (triceps surae of normal mouse).

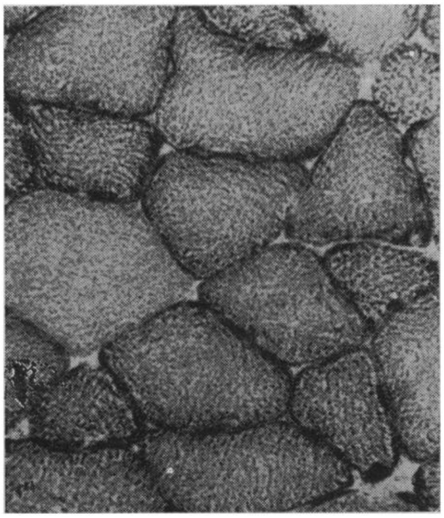

Fig. 2. Succinic dehydrogenase in denervated muscle 4 days after severance of motor and sensory nerves (triceps surae of mouse). Fibers reacting much more strongly than others can no longer be easily distinguished.

Moreover, later studies (*8*) have indicated that the nervous system exerts a decisive influence upon the distribution and functional activity of oxidative enzymes of prime importance in respiratory processes of muscle cells.

It was possible to distinguish between two types of fibers in frozen sections stained with HPS: those showing a granular cytoplasm and those lacking such sarcoplasmic granules. No attempt was made to establish the nature of the sarcoplasmic granularity, although paraffin-embedded sections stained in the same way did not show this granularity. In frozen sections, the fibers showing granular cytoplasm were, as a rule, smaller in diameter than those possessing relatively agranular cytoplasm (Fig. 3), suggesting that the former correspond to the "red" fibers, which are believed to be rich in myoglobin and functionally concerned with tonic (postural) contraction (*9*). This conclusion is supported by the pattern of distribution of the sarcoplasmic granules (especially their denser concentration in regions just underneath the sarcolemma), and by the fact that the granular fibers proved to be identical with those showing high succinic dehydrogenase activity. These findings also suggest that the increased basophilic staining of the fibers of small diameter is sarcosomal in nature.

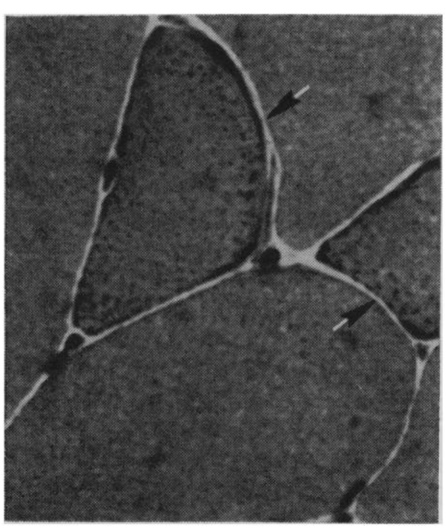

Fig. 3. Distinction between muscle fibers with granular (arrows) and agranular cytoplasm (triceps surae of normal mouse; HPS).

The granular ("red") and agranular ("white") fibers behaved differently during the course of denervation atrophy (Fig. 4). Within a few days after section of the motor (and sensory) nerves, both types of fibers lost their normal polygonal shape, but only the "white" fibers underwent progressive atrophy. The diameters of the "red" fibers remained approximately the same for a long time; consequently, during advanced stages of atrophy the cross-sectioned denervated muscle was characterized by some large-diameter fibers (preserved granular, or "red" fibers) surrounded by small-diameter fibers (atrophic agranular, or "white" fibers).

In muscle rendered atrophic by disuse (either by tenotomy or immobilization in a plaster cast), both the granular and agranular fibers underwent slow but progressive atrophy; there were no significant differences in the behavior of the two types of fibers. In the paralyzed dystrophic muscles of strain-129 mice, both granular and agranular fibers were observed in various degrees of atrophy or in different stages of degeneration. The obviously hypertrophic fibers were, however, constantly those of the agranular type.

Our observations suggest that the great variation in fiber diameter characteristic of the histogram of a denervated muscle is possibly the consequence of the relatively greater dependence of the "white" than the "red" fibers on neuromuscular integrity. Earlier investigators have repeatedly proposed that at least one of the reasons for the difference between "red" and "white" fibers in speed and duration of contraction lies in the type of innervation present (*10*). Although a number of criticisms have been directed against this assumption (*11*), the observations reported herein may be taken as indirect evidence to support the theory that the quality, extent, and significance of nerve impulses are not the same for the two basic types of muscle fibers.

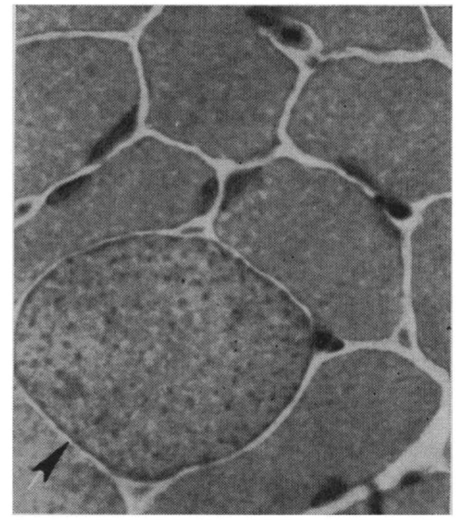

Fig. 4. Fourteen days after denervation the agranular "white" muscle fibers are in a stage of advanced atrophy; these surround a large, granulated "red" fiber that has retained a normal average diameter although it has become rounded in shape (triceps surae of mouse; HPS).

EÖRS BAJUSZ

Laboratory of Experimental Pathology, Department of Pathology, University of Montreal, Montreal, Quebec

References and Notes

1. For a review of the problem see E. Gutmann and J. Zelená, in *The Denervated Muscle*, E. Gutmann, Ed. (Czechoslovak Academy of Science, Prague, 1962), pp. 57–98.
2. E. Bajusz and G. Jasmin, *Can. Med. Assoc. J.* **89**, 555 (1963).
3. See, for example, V. T. Nachmias and H. A. Padykula, *J. Biophys. Biochem. Cytol.* **4**, 47 (1958); E. B. Beckett, *Rev. Can. Biol.* **21**, 391 (1962).
4. E. Bajusz, *Can. J. Biochem. Physiol.* **36**, 825 (1958).
5. The colony originated in the Jackson Memorial Laboratories, Bar Harbor, Maine.
6. A. G. E. Pearse, *Histochemistry, Theoretical and Applied* (Churchill, London, 1960).
7. H. A. Padykula, *Am. J. Anat.* **91**, 107 (1952); V. Thimann and H. A. Padykula, *Anat. Rec.* **121**, 419 (1955); E. Bajusz and G. Jasmin, *Rev. Can. Biol.* **21**, 409 (1962).
8. E. Bajusz, in *Muscle Structure and Function*, G. Monckton and E. E. Daniel, Eds. (Pergamon, Oxford, in press).
9. D. Needham, *Physiol. Rev.* **6**, 1 (1926).
10. W. Graf, *Acta Psychiat. Neurol. Scand.* **22**, 21 (1944); P. Krüger, *Acta Anat.* **40**, 186 (1960); G. Häggqvist and J. Lindberg, *Rev. Can. Biol.* **21**, 235 (1962).
11. A. Hess, *Rev. Can. Biol.* **21**, 241 (1962).
12. Supported by the Muscular Dystrophy Association of Canada and the Medical Research Council of Canada.
* Present address: Bio-Research Institute, 9 Commercial Ave., Cambridge 41, Mass.

29 June 1964