STRUCTURAL IDENTIFICATION OF TWITCH AND SLOW STRIATED MUSCLE FIBERS OF THE FROG

LEE D. PEACHEY and ANDREW F. HUXLEY. From the Department of Zoology, Columbia University, New York, and the Physiological Laboratory, Cambridge, England

An unusual histological appearance was found by Krüger (1929) and by Fürlinger (1930) (see also Krüger et al., 1933, and Krüger, 1952) to be characteristic of a proportion of the muscle fibers in those muscles of the frog that are able (Sommerkamp, 1928) to give long lasting, slow contractions. This unusual appearance was named "Felderstruktur" to distinguish it from the usual "Fibrillenstruktur" associated with twitch fibers (see Fig. 1). These authors made a strong case for believing that it is only the Felderstruktur fibers that can give slow contractions, but the single attempt that has been made to check this idea more directly failed to support it (Brecht and Feneis, 1950). Hess (1960), combining light and electron microscopy, showed differences in fine structure and innervation corresponding to Krüger's results, but gave no new evidence for supposing that the fibers of Felderstruktur type are solely responsible for the slow contractions.

Following the pioneer discovery by Tasaki and Mizutani (1943) that some toad muscles are partially innervated by small diameter nerves that produce only slow contractions, Kuffler and Vaughan Williams (1953) conclusively demonstrated that special nerve and muscle fibers, completely separate from the twitch-producing fibers, are present in certain muscles of the frog and are responsible for the slow contractions. Kuffler and Vaughan Williams further showed that these slow muscle fibers do not conduct propagated responses, and on this basis and with visual judgment of the speed of contraction we have been able to distinguish between slow and fast isolated muscle fibers. By examining these isolated fibers by electron microscopy, we have confirmed directly the correlation between structure and function suggested earlier. We are presenting these results in only a preliminary form because of suggestions that a third type of fiber is present which may or may not have been included in this series.

1 The rather extensive literature behind this problem has recently been reviewed (Peachey, 1961).

MATERIALS AND METHODS

Twitch fibers were isolated from semitendinosus muscles and both twitch and slow fibers were isolated from the tonus region of iliofibularis muscles of the frog, *Rana temporaria*, by dissection in Ringer's solution with the addition of 10⁻⁴ gm/ml d-tubocurarine. Each isolated fiber was tested for contractile type with direct electrical shocks from external electrodes. Fibers responding to single shocks with propagated twitches (visual observation) were classed as "twitch fibers." Fibers that did not twitch, but which responded to repetitive shocks (10 per sec. or greater) with local, slow contractions were classed as "slow fibers." Ten twitch and twelve slow fibers were dissected and classified in this way.

There was no possibility of confusion between slow fibers and damaged twitch fibers since the latter, after ceasing to give propagated responses, regularly give, opposite the cathode, contractions with time course similar to that of a normal twitch or tetanus when stimulated with single or repeated shocks, respectively. The contractions which we took as characteristic of slow fibers were at least ten times slower than a twitch fiber tetanus and resembled the contractions recorded by Tasaki and Mizutani and others when stimulating through the small nerve fibers.

Each fiber was held at constant length and fixed in a solution containing 1 per cent osmium tetroxide. Thin transverse and longitudinal sections were prepared using standard techniques and examined in an RCA EMU-3F electron microscope. All electron micrographs were taken under identical optical conditions, at 11,200 X, and enlarged photographically to 25,000 X for analysis.

All twenty-two fibers were examined in transverse sections, and five of each type were examined in longitudinal sections.

RESULTS

Several clear differences between twitch and slow fibers were observed in the electron micrographs. Transverse section of twitch fibers always showed myofibrils less than 1 μ across and well delineated by sarcoplasmic elements. In contrast, slow fibers in transverse sections always had larger ribbon-like fibrils less regular in shape and fused together, forming a more or less continuous mass of myo-
Light micrograph of a transverse section through the neural region (Sommerkamp's tonus bundle) of the iliofibularis of *R. temporaria*. This muscle was fixed in Susa's fluid, and the paraffin section shown was stained with iron alum and iron haematoxylin. The appearance of fibers with “Felderstruktur” (*Fl*) and of those with “Fibrillenstruktur” (*Fb*) is as described by Krüger and his co-workers, with larger, less regularly shaped fibrils in the Felderstruktur fibers. X 940.

**FIGURE 1**

Electron micrographs of isolated muscle fibers. X 25,000.

**FIGURE 2**
Transverse section of a twitch fiber (iliofibularis).

**FIGURE 3**
Transverse section of a slow fiber (iliofibularis).

**FIGURE 4**
Longitudinal section of a twitch fiber (semitendinosus).

**FIGURE 5**
Longitudinal section of a slow fiber (iliofibularis).
filaments in which isolated areas of sarcoplasm were interspersed. Representative micrographs of transverse sections of each type are shown in Figs. 2 and 3.

Longitudinal sections showed further differences between the two types. Slow fibers (Fig. 5) lack M bands which are always seen in the myofibrils of twitch fibers. And “triads” of the sarcoplasmic reticulum (Porter and Palade, 1957) were not found in any of the slow fibers, although they were present in each of the twitch fibers (see Figs. 4 and 5). The Z discs and the boundaries of the various bands of the slow fibers appeared, to a varying degree, to be less straight than in twitch fibers. The Z discs of the slow fibers are thicker than those of the twitch fibers and often extend between adjacent fibrils through the intervening sarcoplasm. Mitochondria are more numerous in twitch fibers than in slow fibers.

Each of the fibers examined showed all the characteristics of its type, as described above, and none showed features of the other type.

DISCUSSION

The shapes of the myofibrils as seen here in electron micrographs seem to provide a basis for the large ribbon-like fibrils of Krüger’s “Felderstruktur” and the finer, more circular fibrils of his “Fibrillenstruktur.” Nevertheless, the complete demonstration of an exact correlation between Krüger’s two histological patterns and physiological type requires showing that the fibers with the large myofibrils seen in the electron microscope, and only these fibers, would have Felderstruktur if prepared by the techniques used by Krüger for the light microscope. We have, in fact, shown that this is the case by preparing bundles of fibers with different fixatives applied to the two ends of the bundles and examining identical fibers in one end with light microscopy and in the other end with electron microscopy. These results will be presented in a later report.

The difference that we have seen in transverse sections clearly agrees with that described by Hess (1960) as distinguishing his two fiber types. On the other hand, the zigzag appearance of the Z discs which he describes in longitudinal sections was not seen so prominently in these isolated fibers and was not a consistent feature in preparations of tonus bundles.

When this work was nearly completed, our attention was drawn by Dr. Shamarina to evidence that a proportion of the slow fibers of the frog’s iliofibularis are capable of propagating action potentials (Shamarina, 1956). Also, Burke and Ginsborg (1956) saw one such fiber in a series of fifty slow fibers, also from the iliofibularis of the frog. Two slow fibers isolated early in the present study gave extensive slow contractions on strong stimulation. We discarded these fibers because we thought the responses to be due to damage of the membrane, and in subsequent dissections we limited the stimulus strength to the minimum that would cause a visible response. It now seems possible, however, that these two widespread slow responses were due to propagated action potentials, and that some of the slow fibers isolated later in our work and studied with the electron microscope may also have been capable of propagated activity but were not tested with strong enough stimuli to elicit it. We are continuing this work in an attempt to isolate fibers of this third type and, if they exist, to establish their electrical, contractile, and structural features.

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BIBLIOGRAPHY

BURKE, W., and GINSBORG, B. L., 1956, J. Physiol. (London), 132, 586.
KRÜGER, P., DUSPIVA, F., and FÜRLINGER, F., 1933, Arch. ges. Physiol., 231, 750.