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Glycogen in Crustacean Fast and Slow Muscles

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SYNOPSIS. Ultrastructural examination of crayfish superficial (tonic) and deep (phasic) abdominal extensor muscles reveals a distribution and quantitative difference in glycogen between these muscles. Both superficial and deep fibers have a dense accumulation of glycogen in the interfibrillar sarcoplasm. In addition, the superficial extensors, but not the deep extensors, contain glycogen in the I band region. The glycogen granules are of the β type and can be removed enzymatically. The superficial medial and lateral fibers contain more glycogen than the medial and lateral deep fibers. A possible functional role for this difference is suggested.

INTRODUCTION

In order to fully understand the mechanisms of fast and slow muscle action, it is essential that a correlation be shown between the physiological, ultrastructural, and biochemical properties of each muscle. Vertebrate preparations not only have small fibers, which makes physiological data difficult to obtain, but also their muscles are quite heterogeneous, consisting of populations of fast, slow, and intermediate fiber types. For these reasons, biochemical data is mainly based on an histochemical approach.

The crayfish abdominal extensor muscles provide an excellent preparation for correlated studies of the mechanisms of fast and slow muscle contraction. The extensor muscles are clearly divided into sets of superficial fibers and deep fibers (Fig. 1). Abbott and Parnas (1965) and Parnas and Atwood (1966) found that each of the two sets of fibers behaves quite differently physiologically. The deep set of extensors are phasic fibers, for all give a spike and a fast twitch when stimulated either intracellularly or via the motor nerve. The superficial set of extensors are tonic fibers, for they give small junctional potentials and show long-lasting contractions. Ultrastructural studies show that the deep or fast fibers have short sarcomeres, a smaller actin to myosin filament ratio and more dyadic junctions than the superficial or slow fibers (Jahromi and Atwood, 1967). This difference in dyad numbers could provide for a more efficient inward spread of current in the phasic fibers. Since the

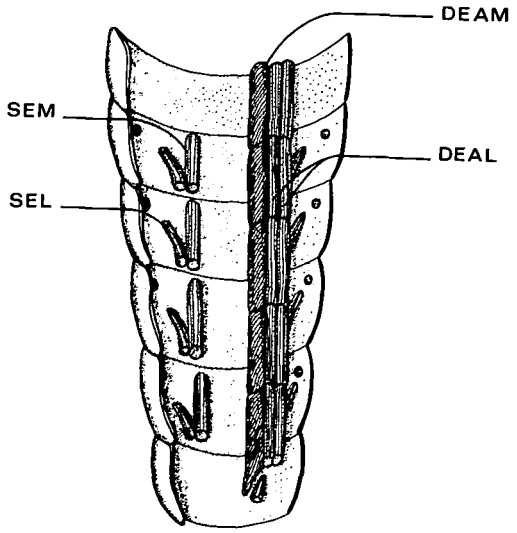

FIG. 1. Diagram of the abdominal extensor muscles of the crayfish showing the superficial fibers on the left and the deep fibers on the right. The superficial fibers are divided into medial (SEM, superficial extensor medial) and lateral (SEL, superficial extensor lateral) bundles. The more ventral deep fibers are composed of deep extensor abdominal medial (DEAM) and deep extensor abdominal lateral (DEAL) fibers.

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For the preparation of the Figures and Tables, we thank Dr. Walter Hewitson of Bridgewater State College.

TABLE 1. *Summary of physiological and morphological differences between crayfish fast and slow abdominal extensor muscles.*

	Crayfish Abdominal Extensors	
	Deep (phasic)	Superficial (tonic)
Physiology	Fast contraction Relaxation time of 30 msec Axons to phasic fibers give mainly spikes or large ejp's Easily fatigued GABA has little or no effect as does inhibitory axon stimulation	Slow contraction Relaxation time of 6–10 sec Axons to tonic fibers give small, facilitating ejp's Fatigue resistant GABA causes inhibition of muscle contraction
Morphology	Punctate arrangement of myofibrils Short sarcomeres (2–3 μ) Actin to Myosin ratio is 3:1 More dyads 5 excitatory and 1 inhibitory axon to muscle	Clumped arrangement of myofibrils Long sarcomeres (9–12 μ) Actin to Myosin ratio is 5:1 Fewer dyads 5 excitatory and 1 inhibitory axon to muscle

sarcomeres are shorter in the phasic fibers, there would be less contractile machinery to engage, thus giving faster contractions than the slow fibers (Jahromi and Atwood, 1967; reviewed by Atwood, 1967).

Table 1 gives a summary of the accumulated data for the deep and superficial fibers, and it is clear that there is a distinct morphological and physiological difference between the phasic deep fibers and the tonic superficial fibers. Since we are dealing with a homogeneous set of muscles, the crayfish abdominal extensors provide good material for studies of differences in biochemical properties thay may exist between the two fiber types. This study of these fast and slow fibers showed that there is not only a difference in the distribution of glycogen but also in the amount of glycogen stored in each of the fiber types.

MATERIALS AND METHODS

For electron microscopic examination, fibers were fixed *in situ* with phosphate buffered 2% glutaraldehyde. The muscles were washed in buffer and post-fixed in buffered 1% osmium tetroxide. The fibers were then dehydrated in acetone, rinsed in propylene oxide and embedded in Epon. Sections were cut with a diamond knife on a Porter-Blum MT-2 microtome, stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmsikop 1A.

For enzymatic digestion of glycogen, the extensor muscles were incubated in a 1% diastase in crayfish solution (Van Harreveld, 1936) for 30 to 60 minutes prior to preparation of the muscles for electron microscopy.

For biochemical assays of glycogen, the abdominal extensors were dissected in eitheir crayfish solution or ethanol, extracted, and examined for glycogen according to the Anthrone colorimetric analysis of Seifter et al. (1950).

RESULTS

One of the first biochemical properties we chose to examine was the glycogen content of each of the fiber types. Earlier light microscopy studies (Morin and Lyons, unpublished) utilizing Periodic Acid Schiff (PAS) staining indicated the superficial fibers contained more glycogen than the deep fibers. Treatment of PAS stained sections with a 0.5% diastase solution removed most of the PAS stain. In both the tonic and phasic fibers there is abundant accumulation of glycogen granules in the interfibrillar sarcoplasm (Figs. 2-7), but there are particularly noticeable differences of glycogen in the myofibrils. The tonic myofibrils have deposits of glycogen located between the myosin and actin filaments and a particularly noticeable accumulation of granules in the I band (Figs. 2, 4, 6).

FIG. 2. Longitudinal section through a crayfish tonic abdominal extensor muscle fiber. Note the dense accumulations of glycogen (G) not only in the interfibrillar area but also in the I band on either side of the Z line (Z). Dyads (D) are evident as well as Z tubules (arrows) between the myofibrils. Note also the long sarcomeres with their dense Z lines.

There are no glycogen deposits along the Z line, but in cross section (Fig. 4) and at high magnification in longitudinal sections (Fig. 6), they appear to be closely associated with the actin filaments in the I band. In direct contrast, the myofibrils of the deep fibers (Figs. 3, 5, 7) are virtually free of any glycogen deposits, and the I band and H zone are particularly clear. Essentially all of the glycogen is located in the interfibrillar sarcoplasm. This aspect is strikingly shown in cross section (Fig. 5).

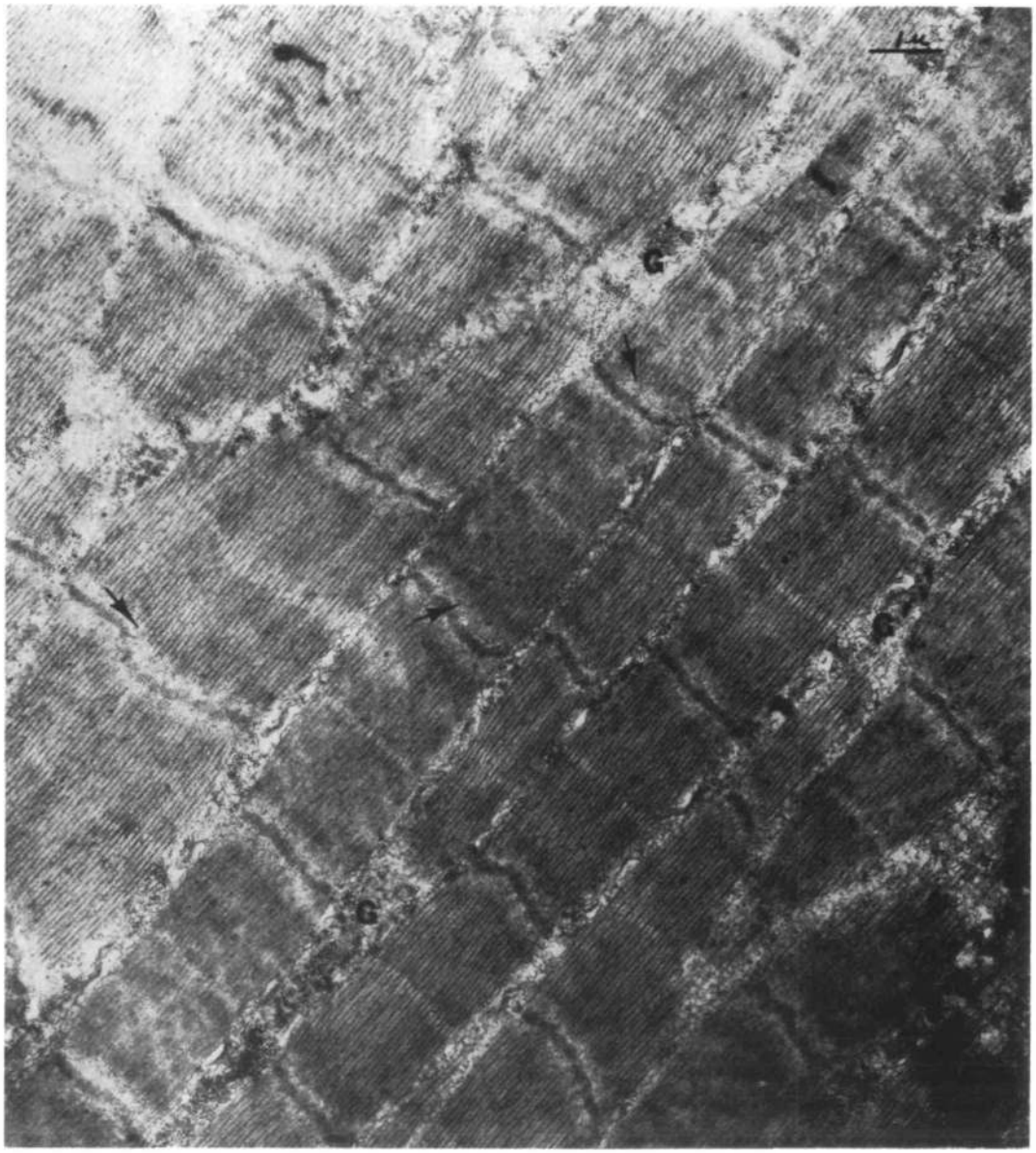

FIG. 3. Longitudinal section through a crayfish phasic abdominal extensor muscle fiber. The glycogen granules (G) lie between the myofibrils and are virtually absent from the I band (arrows).

Under high magnification (Fig. 8), the glycogen granules are approximately 350 Å in diameter and appear to consist of subunits. They correspond to β-glycogen granules characteristic of skeletal muscle as shown by Revel (1964). Even though these granules had all the morphological appearances of glycogen deposits, we attempted a series of experiments to try to remove them by enzyme action. Incubation of the extensor muscles in 1% diastase in crayfish solution (pH 7.65) before preparation for electron microscopy proved to be the best method. Figure 9 shows a longitudinal section of superficial muscle with most of the glycogen granules removed by diastase

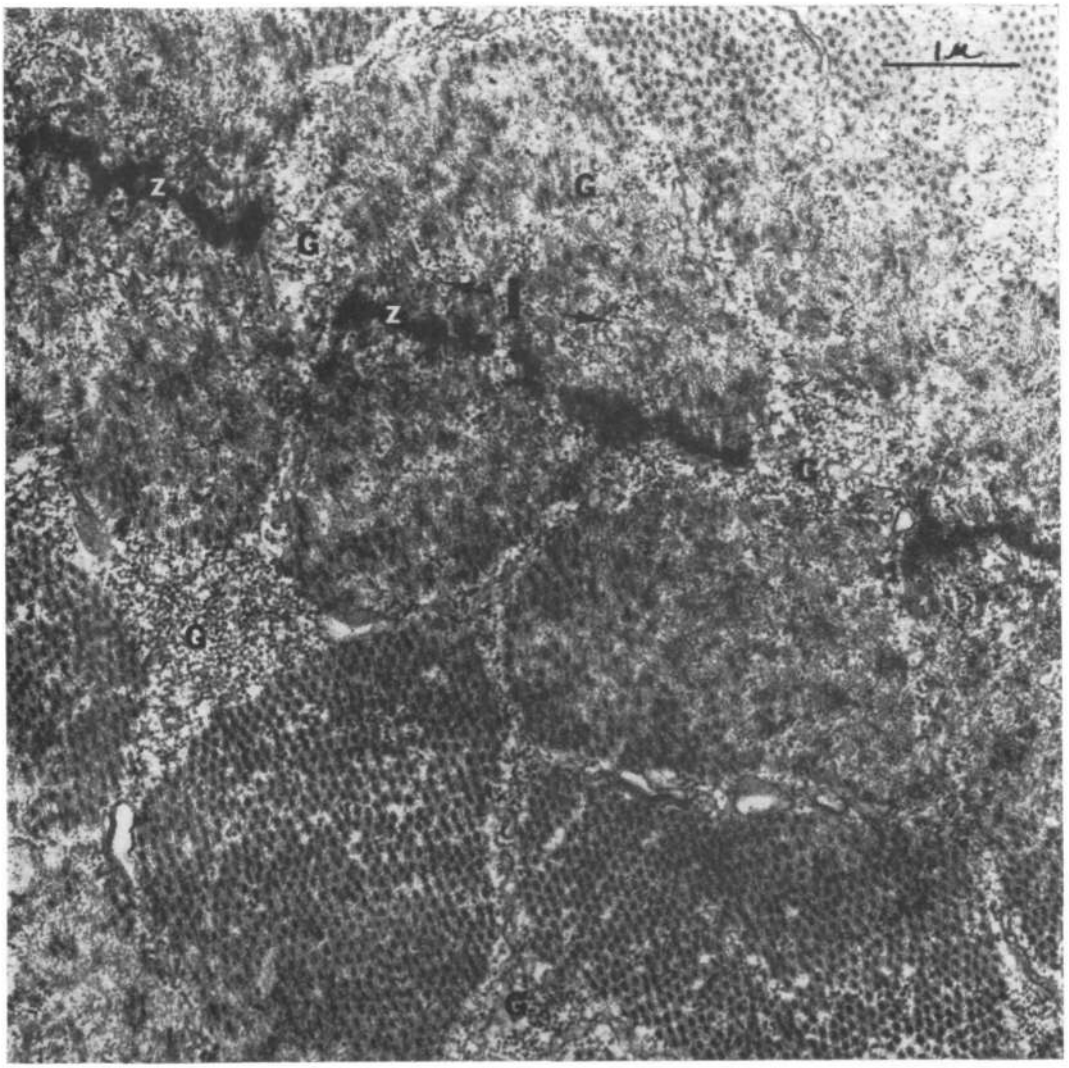

FIG. 4. Cross section through a crayfish tonic abdominal extensor muscle fiber. Note glycogen granules (G) in I band (I).

treatment (compare with Fig. 6). Figure 10 shows both longitudinal and transverse views of deep muscle with glycogen removed (compare with Fig. 5).

There appears, then, to be a distribution difference of glycogen between tonic and phasic fibers, but does this difference also reflect a concentration difference? Since PAS staining indicated a denser concentration of glycogen in the superficials, we extracted each of the muscles for glycogen determinations. At first, the muscles were dissected in crayfish solution and the superficial fibers pooled together since they are smaller than the deep muscle fibers. The results, in Figure 11, show the pooled superficial samples to have almost four times as much glycogen as does each of the deep fibers. The lateral deeps have an average of 0.88 μg/mg, the medial deeps, 0.50 μg/mg and the pooled superficials, 3.8 μg/mg. Much higher yields of glycogen were ob-

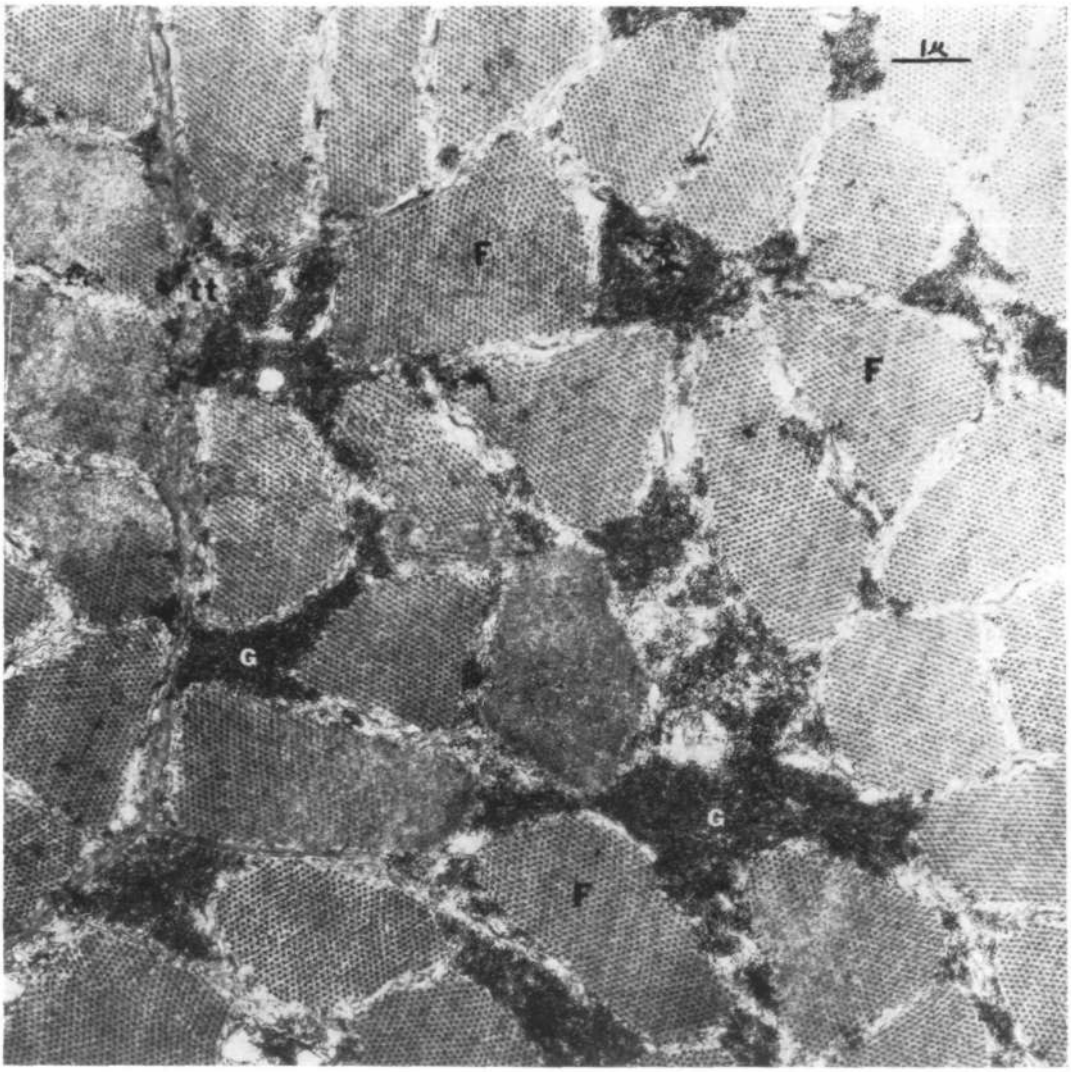

FIG. 5. Cross section through a crayfish phasic abdominal extensor muscle fiber showing dense glycogen deposits (G) around the myofibrils (F) (tt) transverse tubules.

tained when the dissection was done in ethanol. Since the superficials contained a large amount of glycogen, we separated the medial and lateral fibers in the tonic muscles as well as in the phasic muscles. Each of the lateral fibers contained more glycogen than its counterpart medial fibers. The lateral and medial deeps contained 6.3 μg/mg and 4.9 μg/mg, respectively, while the lateral and medial superficials contained 66.0 μg/mg and 24.0 μg/mg, respectively (Fig. 12).

DISCUSSION

We are confident that the granules seen in our preparations are glycogen deposits for several reasons: (1) They have a diameter of 350-400 Å; (2) they have an affinity for lead staining; (3) they conform to the β type glycogen seen in most striated muscles; and (4) they are removed with enzyme treatment.

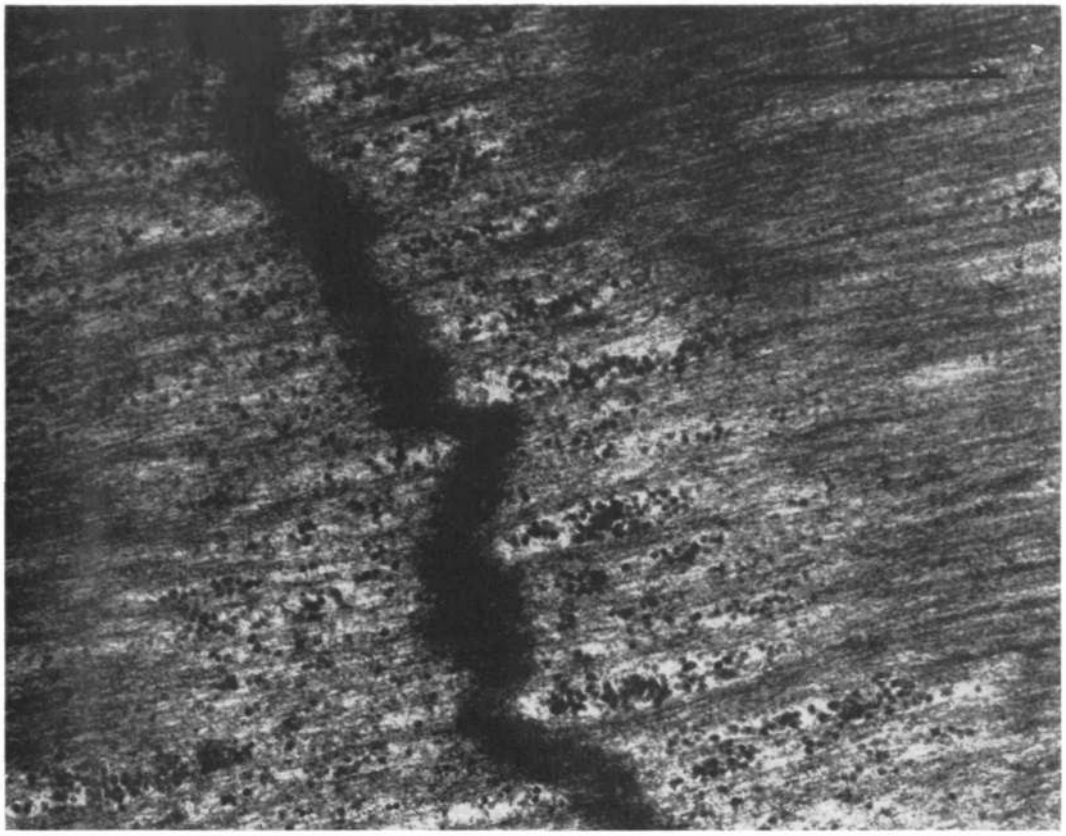

FIG. 6. Same as Figure 2 but higher magnification showing glycogen deposits in I band and between the contractile proteins.

Based on the homogeneity of the muscles and the clear identification of glycogen granules, these results indicate that there is a distinct difference in distribution and quantity of glycogen between the phasic and tonic abdominal extensor muscles.

Localization of glycogen in the I band region also occurs in the flight muscle of black flies, but only in newly emerged females for it is absent from the I band area in four-day old flies (Liu and Davies, 1971). Glycogen accumulation in the I band of the superficial fibers may indicate a functional difference between these and the deep fibers, but this possibility needs further investigation. Glycogen is probably the main energy supply in the extensor muscles, since staining for lipids using light microscopy proved negative and no lipid droplets were observed in electron micrographs. Furthermore, preliminary findings indicate that after prolonged stimulation, the glycogen content is depleted in the deep fibers (McLaughlin, unpublished).

It is interesting to speculate on the reason for the I band localization of glycogen in the superficials. These fibers can maintain tension for prolonged periods and perhaps energy is supplied directly by glycolytic reactions. Investigations of other systems have revealed that glycolytic enzymes are associated with the structural components of striated muscle. The enzymes involved in the synthesis and breakdown of glycogen in rabbit skeletal muscles are bound to the protein associated with the glycogen granules (Wanson and Drochmans, 1968, 1972). Phosphorylase *a* is also

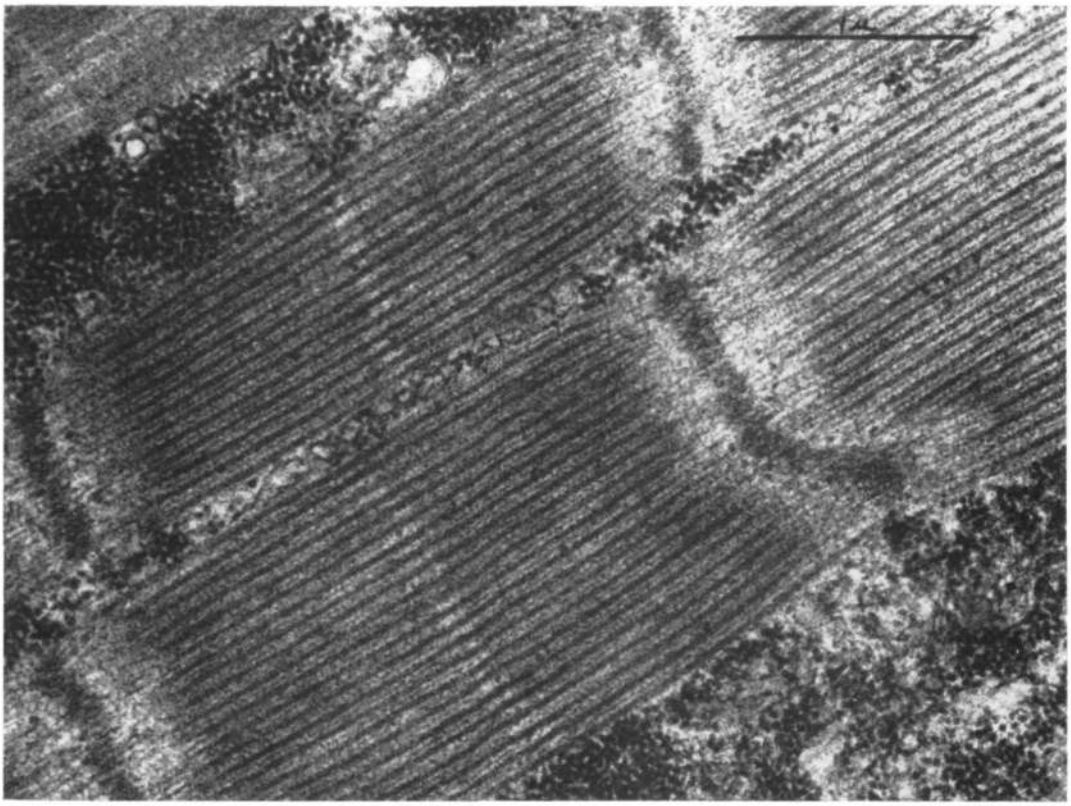

FIG. 7. Same as Figure 3 but higher magnification showing absence of glycogen in the I band but densely accumulated between the myofibrils.

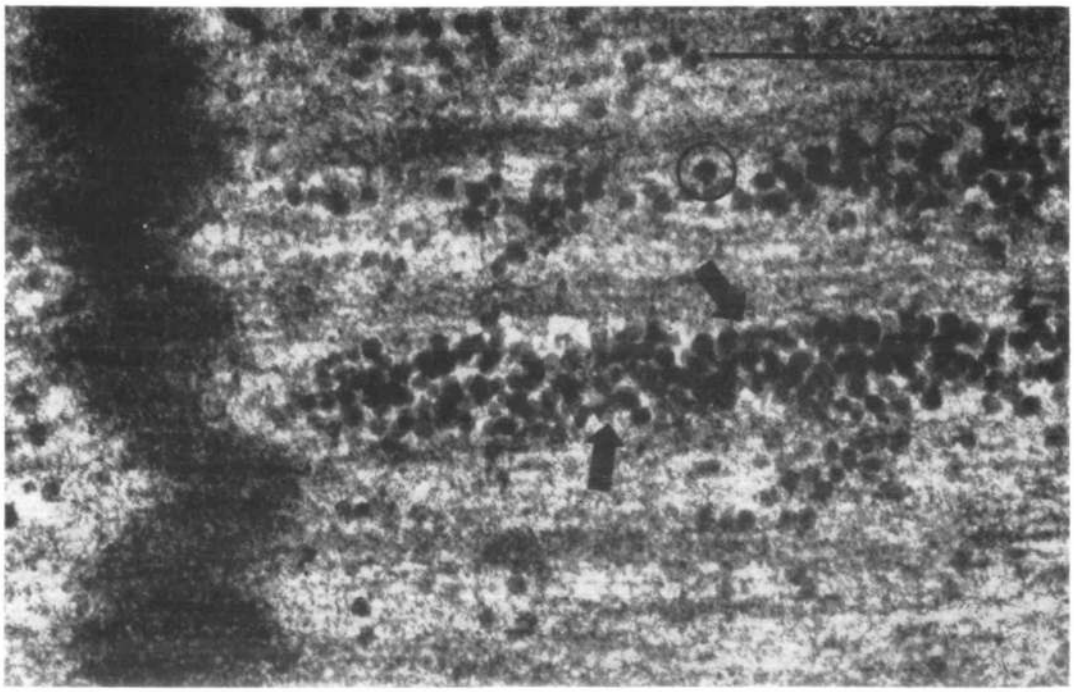

FIG. 8. Same as Figure 6 but higher magnification showing glycogen granules (O) (arrows) consisting of single particles composed of smaller subunits.

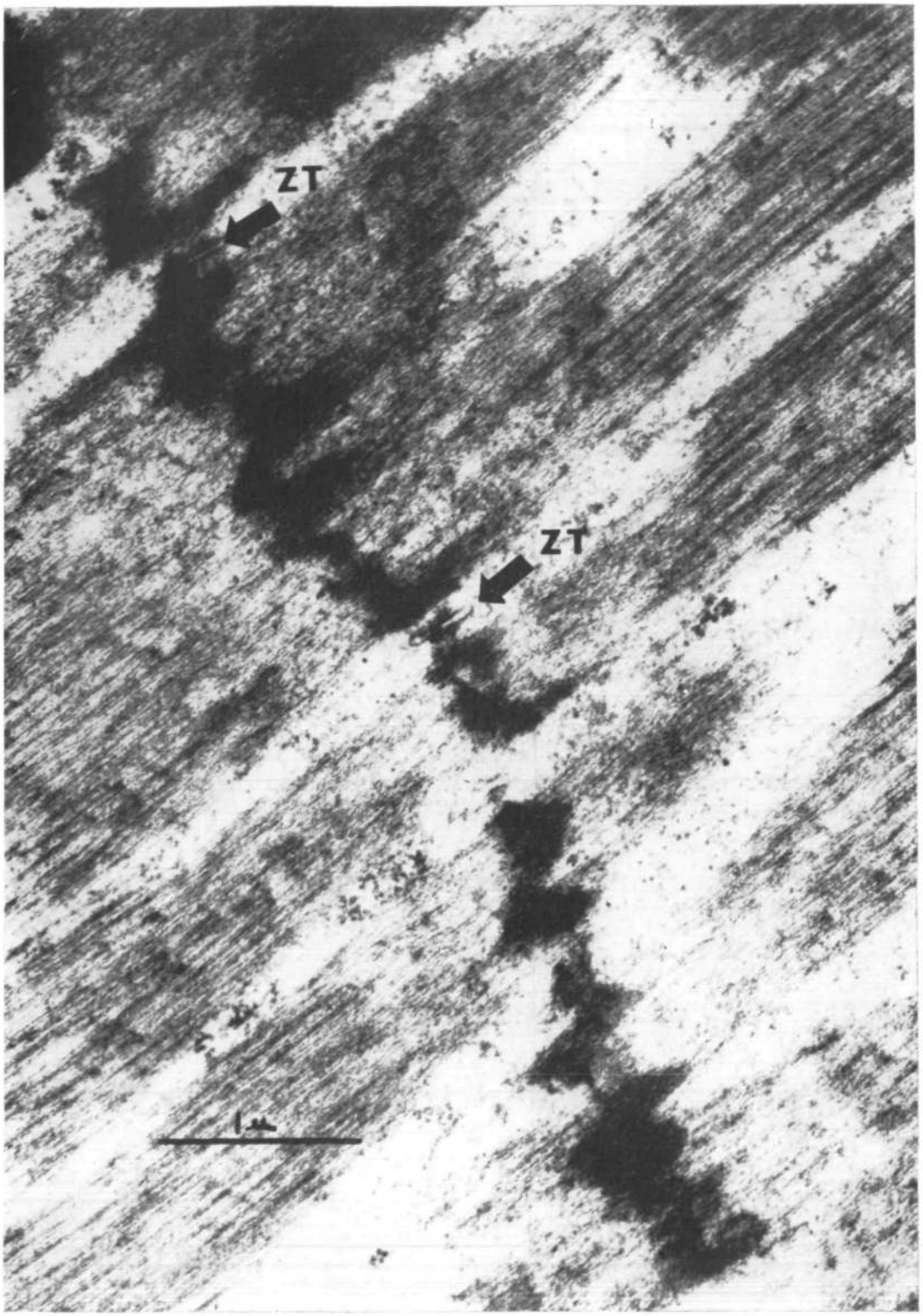

FIG. 9. Crayfish tonic extensor abdominal muscle treated with 1% diastase showing removal of glycogen deposits from I band (compare with Fig. 6). Z tubules (ZT) are evident between the Z lines of adjacent myofibrils.

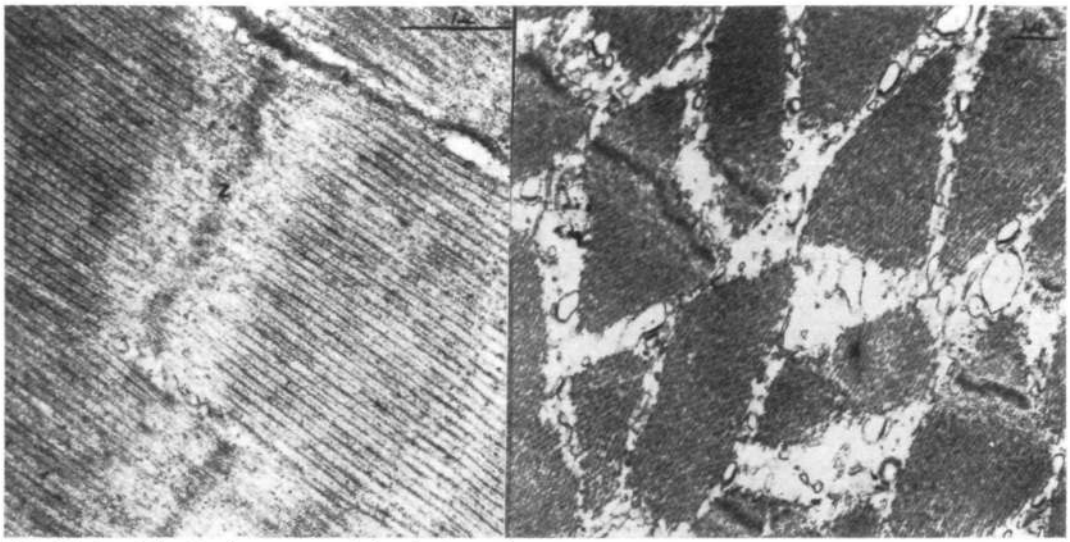

FIG. 10. Crayfish phasic extensor abdominal muscle treated with 1% diastase solution showing the removal of glycogen from the interfibrillar areas. *A*, longitudinal section; *B*, cross section (compare with Fig. 5).

(Chiddress et al., 1970). Arnold and Pette (1968) have found that the glycolytic enzymes, aldolase and phosphoglyceraldehyde dehydrogenase, are bound to the F-actin filaments in the rat and rabbit skeletal muscle fibers. If glycolytic enzymes are also bound to the actin filaments in the I band of the superficial muscle fibers and if the tension development in these fibers is dependent on glycolysis, one might expect to find the localization of glycogen deposits in close proximity to the contractile machinery.

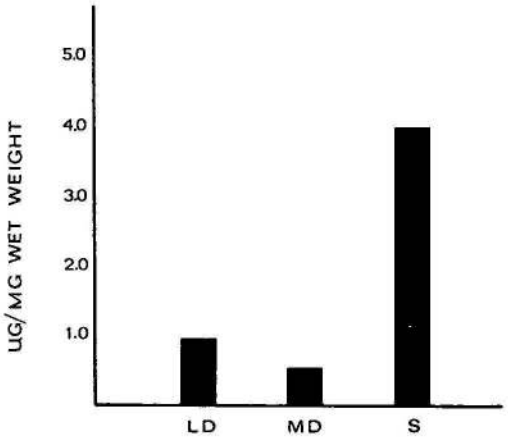

FIG. 11. Histogram of glycogen content in crayfish extensor muscles. Pooled superficial fibers (S) compared with lateral fibers (LD) and medial deep fibers (MD).

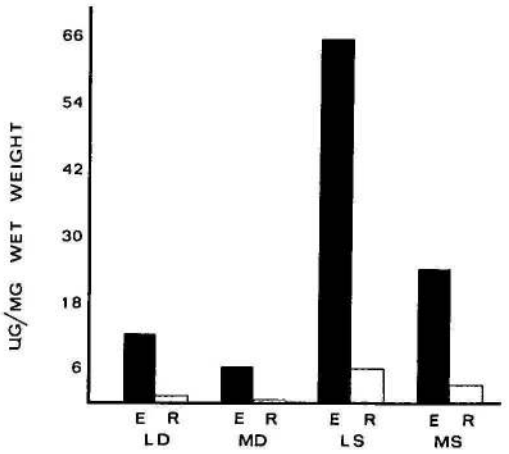

FIG. 12. Histogram of glycogen content in crayfish extensor muscles, comparing the amounts of glycogen in lateral deep fibers (LD), medial deep fibers (MD), lateral superficial fibers (LS), and medial superficial fibers (MS) dissected in ethanol (E) and crayfish ringer (R).

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