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Annual Report NASA Grant No. NAG 2-212: "Alterations in Skeletal Muscle with Disuse Atrophy".

Principal Investigator: Robert H. Fitts.

I. Objectives in FY '84.

In FY '84 our research centered on the development of new micro-techniques that will in the future allow us to evaluate the effect of disuse atrophy on single fiber or needle biopsy samples. Basically, we concentrated on three techniques: 1) microgel electrophoresis identification of single fibers, 2) skinned fiber preparation, and 3) microbiochemical techniques for assaying important enzymes and substrates in single fibers. In the following paragraphs I will review our progress in each of these areas.

II. Accomplishments.

A. Skinned Fiber Studies.

1. Identification of fiber type. The use of single muscle fibers for functional studies eliminates confusion that may result from employing mixed fiber populations. However, in order for single fiber analysis to be correctly interpreted, unequivocal characterization of fiber-type is mandatory. The classical histochemical technique for identification of fiber-type, depends upon relative staining intensities, a problem that is exacerbated with single fiber analysis. Furthermore, there is evidence that the histochemical ATPase reaction does not always reflect the biochemical and physiological characteristic of the fiber.

We have found that solubilization of single muscle fibers, or parts of a fiber, followed by electrophoresis (one-dimensional SDS-PAGE) in the presence of detergent, results in a protein banding pattern, after silver staining, that is characteristic of fiber-type. The method described by Guillian et al. (Anal. Biochem. 129, 277-287, 1983) is used without modification. Vertical SDS-discontinuous PAGE is carried out using a Hoefer Model SE600 gel cell on slab gels 0.75 mm thick. The acrylamide concentration in the stacking gel is 9% and in the separating gel 15%. Samples are loaded in a volume of 2-10 µl, the wells overlaid with electrode buffer (Tris Glycine, pH 8.5). The bromophenol blue dye front takes about 6 hours to reach the bottom of the gel at a current of 19 ma/slab. A representative SDS-polyacrylamide gel of single fibers prepared from rat striated muscles of known fiber-type distribution is illustrated in Figure 1. The figure shows a slow type I fiber from the

soleus, a fast type IIa fiber from the red region of the lateral gastrocnemius, and a fast type IIb fiber from the superficial region of the vastus lateralis (SVL). Type I fibers can be identified by their characteristic myosin light chains, LC_{1s} and LC_{2s}, while type IIa and IIb fibers can be separated by the appearance of a 30,000 M Wt. band in the IIa but not the IIb fiber. Since this band appears in gels from type I as well as type IIa fibers, it is most likely a mitochondrial protein or the soluble protein carbonic anhydrase.

2. Physiological properties. In order to carry out experiments evaluating the effects of disuse atrophy (produced by hindlimb immobilization or weightlessness) on type I, IIa, and IIb fibers, it was first necessary to perfect the skinned fiber technique. The skinned fibers are prepared as described by Julian et al. (J. Physiol. 311, 201-218, 1981). We are presently collecting preliminary data on type I fibers isolated from the soleus muscle. Our pCa-force (Fig. 2) and length-tension (Fig. 3) data agree well with the published results of Stephenson and Williams (J. Physiol. 317, 281-302, 1981) on soleus skinned fibers.

The solid line in Fig. 2 (x——x) represents the published data of Stephenson and Williams, while the dots (·) represent our data. The optimal pCA is between 5.6 and 5.2.

Sarcomere length will be determined both by photomicroscopy and He-Ne laser technique, and length-tension curves established for all three fiber types. Preliminary data on type I fibers from the soleus are illustrated in Fig. 3. The dots (·) represent our data, the crosses (x) the data of Stephenson and Williams, and the solid line is a theoretical length-tension relation constructed using the known filament lengths.

The maximal shortening velocity (V_{max}) of all three fiber types will be measured using both the force step and the slack test techniques. Preliminary data for type I fibers using the slack test technique are shown in Fig. 4. At 22°C V_{max} averaged 5.77 fiber length·sec^{-1}, while at 15°C the V_{max} was 1.61 fiber lengths. Our results compare well with the published data of Moss et al. (J. Biol. Chem. 257, 8588-8591, 1982) on rabbit muscle. The dots (.) and crosses (x) represent our data on the type I fiber at 22° and 15°C respectively, while the circles (o) represent data on a rabbit psoas muscle fiber at 15°C.

We are presently developing 2 techniques for measuring the ATPase of a single skinned fiber. The first is a fluorescence technique in which ADP production is linked through enzyme reactions to the oxidation of NADH to NAD, while the second procedure detects ADP production using HPLC. When fully developed we will be able to directly determine if disuse-mediated changes in V_{max} are due to an altered myofibrillar ATPase.

B. Microbiochemical Techniques.

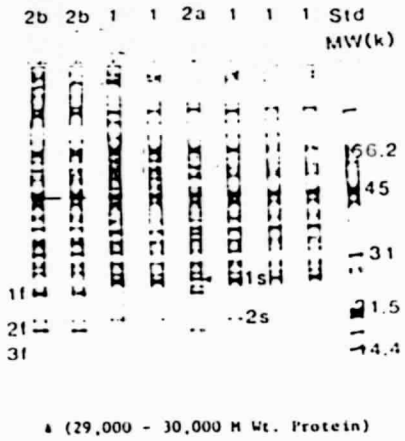
We have previously shown that disuse atrophy causes an elevated anaerobic metabolism during contractile activity (J. Appl. Physiol. 54, 1242-1248, 1983), thus one goal is to establish a cellular mechanism for this observed metabolic shift. This year, we have developed a new microbiochemistry laboratory which will allow us to evaluate the effect of disuse on the important

metabolic pathways in single fibers. To date we have perfected assays for the glycolytic enzymes lactic dehydrogenase and phosphofructokinase, and enzymes controlling glucose entry into the glycolytic pathway, hexokinase and phosphorylase. In the coming year we will determine if the disuse-mediated change in glycolysis is a direct result of alterations in any of these key enzymes. Importantly, these studies will be carried out on single fibers, such that, fiber type-specific changes can be elucidated.

III. Publications.

1) Fitts, R.H. and Brimmer, C. Effect of Long Term Disuse on the Contractile Properties of Skeletal Muscle. J. Appl. Physiol. In review.

2) Troup, J.P. and Fitts, R.H. Membrane Properties of Skeletal Muscle Fiber Types Following Hindlimb Immobilization. J. Appl. Physiol. In review.



A (29,000 - 30,000 M Wt. Protein)