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Growth Factor Involvement in Tension-Induced  
Skeletal Muscle Growth

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### Description of Research

Long-term manned space travel will require a better understanding of skeletal muscle atrophy which results from the microgravity found in space. Astronaut strength and dexterity must be maintained for normal mission operations, for emergency situations, and for the rigors of reentry into the earth's atmosphere. A biochemical understanding of how gravity, by increasing the tension on muscle cells, helps to maintain muscle size and strength, should ultimately allow pharmacological intervention to prevent muscle atrophy in microgravity. Because of the limited availability of performing experiments in space, a number of earth-based model systems have been designed to study the relationship of muscle tension to muscle growth (1). These models include the casting of animal's limbs such that the limb muscles are held under increased (stretched) or decreased (shortened) tension; placing weights on limb muscles to increase their load; and suspending an animal's hindquarters above the ground in order to reduce skeletal muscle use.

Our laboratory has developed several tissue culture model systems for studying the relationship of tension to skeletal muscle growth (2,3). Tissue culture cells have been extensively used for studying basic biochemical growth processes in other cell types and allows easier manipulation of experimental conditions than whole animals. Cultured cells have been especially helpful in studying the role of growth factors in normal and pathological cell growth (4). We are utilizing one of our tissue culture model systems to determine the role played by exogenous and endogenous growth factors in tension-induced skeletal muscle growth. Briefly, this model involves isolating muscle cells from 12 day old chick embryos and growing them on a highly elastic substratum

material in a humidified CO<sub>2</sub> incubator at 37°C (3). The muscle cells, growing in a complex medium, initially proliferate, and then fuse to form multinucleated, spontaneously contractile myotubes in a manner very similar to growth and differentiation in vivo (5). These cells actively synthesize actin and myosin and incorporate them into cross-striated myofibrillar material. These in vitro differentiated, "embryonic" myofibers, can be subjected to different patterns of mechanical activity with our "Mechanical Cell Stimulator" which simulates in vivo muscle activity (Figure 1, Appendix). The activity patterns used to stimulate muscle growth are generated by an Apple IIe computer. In effect, we can subject muscle fibers in a defined in vitro environment to different types of "exercise" programs, and study their growth response on a molecular level. The results obtained using these avian muscle should be applicable to mammalian muscle, since most basic molecular mechanisms utilized by Nature are identical or very similar in different classes of vertebrates.

The specific goal of this project is to determine the role played by exogenous and endogenous growth factors in mechanical activity-induced growth of cultured skeletal muscle. We have shown previously that increased tension on these cells in a complex medium stimulates their growth (2). These factors include the medium growth factors found in serum and chicken embryo extract supplements that the cells are grown in, as well as endogenous growth factors produced by the muscle tissue itself (6,7). The importance of endogenously synthesized and secreted prostaglandins to mechanically-stimulated muscle growth is being studied.

By understanding the interactions between growth factors and mechanically-stimulated growth, the mechanisms by which gravity-induced tension are transduced into the biochemical alterations leading to muscle growth will be more clearly defined, and methods for prevention of muscle loss from disused muscles in microgravity can be developed.

#### Accomplishments

The first hypothesis being tested on our grant is whether extracellular serum factors present in the growth medium are essential for mechanical stimulation of skeletal muscle growth in vitro (Hypothesis I). The protocol being used is to allow the avian muscle cells to differentiate into spontaneously contractile "embryonic" myofibers in the Cell Growth Chamber of our Mechanical Cell Stimulator in complete culture medium (with 10% horse serum, 5% chicken embryo extract). Once developed, the cells are switched to medium with or without added growth factors. Repetitive mechanical stimulation of the cells is then initiated and at varying times, groups of cells are removed for determination of their growth rate by measuring protein:DNA ratios. Mechanically-stimulated growth of the muscle cells in medium containing added growth factors is compared to mechanically-stimulated growth in defined medium without any added growth factors. If the stimulated growth rates are identical in the two groups, then mechanically-stimulated muscle growth in our in vitro model system would be independent of exogenous growth factors and only dependent on internal cellular processes. Conversely, if by removing medium growth factors, mechanically-stimulated muscle growth is significantly reduced, then the interaction of as yet undefined exogenous

growth factors will be essential, and their role examined more closely.

When we began these studies we immediately found that there was a significant increase in the cultured "embryonic" myofiber's spontaneous contractile activity when they were placed into defined medium in the absence of serum or chick embryo extract. These contractions were so rigorous that within 12-24 hours in defined medium, the myofibers would contract off the substratum and degenerate. This obviously made the experiments outlined in the above paragraph difficult to run. We therefore spent a significant period of time attempting to find a method for attaching the contractile cells more strongly to the substratum. We found that by embedding the already formed myofibers in a collagen gel (collagel), combined with a perimeter nylon support ring, the myofibers could be maintained attached to the substratum and under active tension for 7-14 days in defined medium without serum or embryo extract (8,9). This new culturing technique thus allows us to continue with our mechanical stimulation experiments in examining the role played by exogenous growth factors in the tension-induced growth process. An additional benefit of using this collagel technique on the cultured myofibers is that they are able to differentiate into myofibers which are more "neonatal-like" rather than "embryonic" (9). These "neonatal" myofibers have subsarcolemmal nuclei, a more complete external lamina cell coat, and a more extensively organized myofibrils. This significant advance in muscle cell culturing should make the results of our growth-factor studies more directly applicable to in vivo studies using adult muscle cells.

Significance of Accomplishments:

New muscle tissue culture techniques have been developed to grow embryonic skeletal myofibers which are able to differentiate into more adult-like myofibers. Studies on mechanical stimulation of cultured muscle cell growth will now be more directly applicable to mechanically-induced growth in adult muscle, and lead to better models for understanding muscle tissue atrophy caused by disuse in the microgravity of space.

Publications

1. See Reference 8
2. See Reference 9

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