Muscle Regeneration and Compensatory Growth

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Regeneration and compensatory growth of skeletal muscle can be thought of as mechanisms for increasing the amount of skeletal muscle tissue. Many factors affecting these processes are similar to those which affect other aspects of muscle growth. Recent Reciprocal Meat Conference reviews in this area include Kent (1979), Martin (1978) and Allen (1978).

Muscle Regeneration

Regeneration of skeletal muscle has been documented from an anatomical perspective for a number of years. In recent times the mechanisms that underline skeletal muscle regeneration have been extensively investigated and the topic of a comprehensive edited review by Mauro (1979).

This brief review will focus on cellular proliferation aspects of skeletal muscle regeneration and will not attempt to completely cover the large volume of literature in this field. This article will deal only with muscle regeneration in mammalian systems which should not be equated with amphibian limb regeneration (Carlson, 1973, 1979).

Course of Events

The course of events involved in mammalian muscle regeneration are shown in Table 1. On day 0 the injury occurs and within 24-48 hours the damaged muscle in areas close to blood vessels are invaded by phagocytic cells. Fibers not adjacent to blood vessels are slowly degraded by necrotic processes. At approximately the same time, the appearance of large oval nuclei between the basement membrane and degenerating fiber are observed.

After 2-3 days, the maximum myoblastic cell population can be observed and myotubes begin to form beneath the basement membrane. It has been estimated by a number of investigators that myoblastic cells divide approximately every 20 hours and these events appear to be very similar to those observed during embryonic development of skeletal muscle. After formation of myotubes, the assembly of myofilaments can be observed. The formation of myotubes, their enlargement, and the formation of myofilaments in myotubes continues throughout the early phases of the regeneration process (Table 1).

Table 1. Early events occurring during skeletal muscle regeneration

1. Day 0:
   Injury.
2. Day 1-2:
   a) Infiltration of muscle cell by phagocytic cells.
   b) Appearance of cells with large oval nuclei.
3. Day 2-3:
   a) Maximum number of myoblastic cells.
   b) Start formation of myotubes.
4. Day 3-5:
   Formation of myofilaments in myotubes.

Regeneration events requiring more time include enlargement of muscle fibers and the formation, when necessary, of new basement membrane. After approximately three weeks, the formation of components of motor end plates has been observed (Zhenevskaya, 1962), and by 30-40 days differentiation into muscle fiber type has been reported (Snow, 1971) (Table 2).

Table 2. Late events occurring during skeletal muscle regeneration

1. Day ~14:
   Formation of new basement membrane.
2. Day ~21:
   Formation of motor end plate.
3. Day 30-40:
   Differentiation of muscle fiber type.

The influence of innervation of regeneration muscle tissue has been studied by Peterson and Crain (1972). These investigators reported that the presence of a functional nerve had a stimulatory effect on muscle regeneration. On the other hand, Mong (1977) provided evidence that early cytodifferentiation and morphogenesis do not require nerves. Experiments by several investigators (see review—Carlson, 1973) demonstrated that when the nerve and muscle cell were injured at the same time the regeneration process proceeded through the normal course of events. However, when the nerve was injured first, there was a quickened recruitment of myoblasts for regeneration. The physiological significance of these experiments is not clear, but it is interesting to speculate on the

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events which initiate the regeneration process, one of which could be loss of innervation.

Orientation and Shape

If the regenerated muscle is to regain its initial functional potential, orientation of the regenerating muscle fibers is an important parameter. The newly formed fibers will have a similar orientation to the original muscle when the old basal membrane is present during regeneration. However, if the basal membrane has been severely disrupted by procedures such as mincing (Carlson, 1972), the regenerated muscle fibers grow in a chaotic manner. By applying tension to the muscle, the regenerating tissue will tend to become more organized, whereas in the absence of tension the muscle fibers will grow in a more chaotic manner.

The final shape of the regenerated muscle tissue will vary depending on the amount of tissue regenerated, amount of scar tissue formed, tension on the muscle, degree of injury as well as numerous other parameters. Smaller injuries are often affected by the quantitative loss of regenerative fibers as well as the formation of scar tissue, and with massive injuries the final shape can be further molded by the pressures of surrounding tissues resulting in shapes distinctly different from the original muscle.

Functional Properties

The contracticle properties of regenerated muscle fibers have been investigated. Carlson and Gutmann (1972) showed that muscle fibers regenerated from minced muscle initially had slow contractile properties which changed to faster contractile properties after 30-40 days of regeneration. Snow (1971) found histological differences between newly regenerated skeletal muscle and 30-40 day regenerated tissue. These studies indicate that the regenerated muscle tissue can change fiber type, a process that resembles differentiation of embryonic muscle tissue.

Regenerated skeletal muscle has many of the functional properties of a "normal muscle" including the ability to maintain tension. The degree of tension exhibited by the regenerated tissue is usually reduced by 10-75% of the normal muscle and is dependent on the completeness of the regeneration process. There appears to be a relationship between histological properties and the functional properties of the regenerated muscle.

The origin of the myogenic cells responsible for the regenerative process has been investigated. The hypothesis that the myogenic cells were dormant myoblasts activated by the injury is widely held (Snow, 1977); however, several other hypotheses have been put forth. Two such alternate hypotheses include the possibility that these cells result from a reorganization of the cytoplasmic membrane around striated muscle nuclei or that these cells were initially circulating cells that migrate into muscle (Table 3).

In summary, the regenerating mammalian skeletal muscle requires a source of myoblastic cells, blood supply and motor nerve to maintain integrity. Also, the basement membrane supplies a satisfactory substratum for regeneration and tension helps functional organization. There are many common features between normal growth and development of skeletal muscle and the regeneration process and it is possible a large number of the regulatory parameters governing muscle cell proliferation are common to both processes.

Table 3. Origin of myogenic cells

1. Dormant myoblasts.
2. Circulating cells that migrate into muscle.
3. Pericytes of blood vessels that migrate into muscle.
4. Reorganization of the cytoplasmic membrane around the nuclei of striated muscle.

Compensatory Growth

The growth of skeletal muscle is a complex process in which both protein and DNA are accumulated. Goss (1976) has defined the potential for enlargement of an organ into four increments. The smallest minimal mass of an organ is called the basic size. For muscle, this is the size to which the muscle tissue will degenerate if not used (e.g. loss of muscle nerve). The next largest increment is the "normal size" which corresponds to the size of a normal muscle performing ordinary physiological activities. The next increment of enlargement is compensatory hypertrophy such as increasing muscle size by overwork. The final increment is the enlargement of a tissue during a pathological situation. In meat animals, our concern usually focuses on the "normal size" of the muscle.

Skeletal muscle cells are multinucleated and it has been well established that the nuclei within muscle cells do not divide under normal conditions. The cells that are thought to be responsible for increasing the number of muscle within a muscle cell are myogenic stem cells. These stem cells (located adjacent to the muscle fiber) divide giving rise to new stem cells and cells that will terminally differentiate and fuse with the existing muscle cell, thus increasing the number of nuclei within the muscle cell (Moore, 1979). The stem cell concept has also been used to explain the development of other multinucleated cells such as osteoclasts.

Mature mammalian skeletal muscle fibers normally have fiber diameters of 30-70 μm. Therefore, it is apparent that differences in muscle size are due in large part to differences in muscle cell number and length. Although the muscle cell diameter of comparable mature mammalian muscle cells are similar, there are large differences in the rate of growth of the muscle cell diameters. An example of this phenomenon is that it can take only 15-16 weeks to attain the maximum diameter of a rat skeletal muscle cell, while taking over two years for a comparable bovine muscle fiber and 12 or more years for those from man (Burleigh, 1976).

DNA Content and Growth Rate

The mg of protein per mg of DNA from different muscle types varies (Waterlow et al., 1978; Layman et al., 1981). Millward reported greater than a two-fold difference in the ratio of protein to DNA between predominantly red and white skeletal muscles from avian and mammalian species. It is of interest to note that genetic selections for muscle content in meat animals will often result in an increased number of white muscle fibers which contain more protein per unit DNA than is found in red muscle fibers.

The relationship between protein and DNA in strains of animals with different growth rates was examined by Millward (Waterlow et al., 1978). Determinations were made of protein to DNA ratios of muscle derived from rat strains with markedly different growth rates. Results indicated that although the rats had different growth rates, the protein to DNA ratios of comparable muscles were similar. Millward et al. (1975) have also reported that muscles from animals that had their growth rate altered by early malnourishment had the same ratio of protein to DNA as their well fed counterparts. These studies indicate that although the growth rates of animals may vary, the protein to DNA ratio may be a species specific number that exhibits minimal variation in "normal size" muscle.

Studies have shown that the DNA content of muscle in growing rats is sensitive to both energy restriction and protein restriction (Trenkle, 1974) when compared to controls. The status of the protein to DNA ratio in skeletal muscle during "catch up" growth following diet restriction is complicated due to the large number of variables which include the degree and type of restriction, duration of restriction and age of animal during restriction. An additional factor that has complicated studies is the interpretation of nutrition restriction experiments in growing animals. Skeletal muscle protein to DNA ratios increase with age until maturity; therefore, the questions arise as to what constitutes the appropriate control which have often included animals of the same chronological age (usually more physiologically mature) or the same weight (often younger animals). If an animal is allowed to "catch up" after a nutritional restriction, there is little evidence that it will ever have a greater protein to DNA ratio than the unrestricted control at maturity. The bulk of the nutrition studies indicate that by alteration of nutrition regiment, the protein to DNA ratios can be reduced, and few reports indicating that protein to DNA ratios greater than the normal control can be obtained in this manner.

Muscle Stretching

Possibly the most important physiological mechanism for increasing the DNA content in growing muscle is stretching of muscle over a prolonged period of time. Investigators have used different models to investigate the mechanisms of stretch induced muscle growth including use of weights (Laurent and Sparrow, 1977) and the use of spring clips (Barnett et al., 1980). Muscles that are stretched increase in weight, protein and DNA (e.g. Barnett et al. (1980) reported a 43% increase in total DNA content of chick skeletal muscle that was subjected to stretch induced muscle growth). Stretch induced muscle growth is a result of increases in both DNA and protein content, although there are some temporary deviations in the ratio of protein to DNA during the growth process. On a cellular level, the mechanisms of growth involved by the stretching process are not understood. However, there appears to be an increase in the number of satellite cells surrounding the stretched tissue as well as increases in rates of transcription and translation within the muscle cells.

The question arises, is there a common element to aid our understanding of muscle growth during regeneration and compensatory growth? The ratio of protein to DNA does vary with fiber type and the age of the animal; however, there is little evidence that these ratios can be altered in "normal size" mature animals. The fact that the ratio of protein to DNA can be temporarily altered during hypertrophy (stretching) has been shown but the ratios rapidly approach those in control tissues. Altering the protein to DNA ratio of muscle by nutritional means can reduce the ratio but there is little evidence that the ratio can be increased. If there is a common element, it may be that "real" muscle growth is the result of increased DNA content and that it is difficult to increase the protein to DNA ratio of muscle without altering its metabolic (fiber type) profile.

Discussion

Question: Would you expect the same sort of a regeneration path following injury to heart tissue?

Bechtel: No, because there are very few stem cells in cardiac tissue. Basically, right now there is no known satellite population for heart tissue, so I wouldn't expect much regeneration.

Question: In the last diagram, you showed that, with stretch induced growth, you get an increase in protein degradation in muscle. How does this fit in with what Bill Dayton has told us?

Bechtel: Well, I think the general idea is that both muscle protein degradation as well as protein synthesis go up together and that, in the balance, you have more synthesis than degradation. They do appear to go up together: it's kind of interesting. I should add that, in Bill's talk (that is, generally speaking) the amount of degradation that you have in the tissue at this point is correlated with the amount of lysosomes.

John Romans, University of Illinois: Sometimes in carcasses we see what appears to be an injury which was infiltrated with fat. Does that mean that too much muscle was removed, or how does that fit in?

Bechtel: If you have infiltration problems with fat in a carcass, yes, you have taken out a large enough mass of muscle that you can't get regeneration. What's happened is that, in order to get regeneration, you have to have the myogenic cells. Now, those are endogenous in the muscle, but, if you remove too big a piece of muscle, there aren't enough myogenic cells to give you enough proliferation to get back to where you started from.

C. E. Allen, University of Minnesota: Have you seen any work that would indicate that, after extended periods of malnourishment, the animal is not able to catch up in terms of this DNA and consequently ends up with an elevated protein to DNA ratio?

Bechtel: I can't remember seeing much work that, in the end, you see an elevated protein to DNA ratio. There are a fair number of studies that show, once you do physically impair the DNA content to a significant degree, that there is no final catch up. But I have to back off the latter part of the question.

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