AN ASSESSMENT OF THE ROLE OF MUSCLE BIOLOGY RESEARCH IN THE FIELD OF MEAT SCIENCE

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INTRODUCTION

It is a pleasure and honor for me to prepare this manuscript for the 31st EMMRW to be convened in Varna, Bulgaria. The task presents a distinct and clear opportunity for me to view an area from a slightly removed perspective and allows the expression of thoughts which have been building up for some time regarding trends in Meat Science.

At issue is the question of how basic science is being utilized by the Meat Industry. To examine the problem requires, of necessity, review of what is happening at present in various areas of fundamental science. Relating the state of knowledge and level of scientific activity to more practical utilization of the information involves some opinions on my part. Therefore, I must say at the outset that this manuscript is somewhat different from the usual. Each scientific area of specialized endeavor consists of a massive wealth of details and each, in itself, would qualify for a major review. No attempt has been made to do that here, but rather entry to the literature in each area is provided with one or two citation. Major, recent reviews of a number of specialized areas are present in the current literature and no need exists to repeat them here. I realize that my approach may overlook important contribution and I'm sorry about that. Likewise, some of my opinions may be contrary to those of my colleagues, but then I don't believe I was asked to write this assessment in such a fashion as to only repeat what has been said or recorded previously, or to agree with everyone.

In order to set the stage, some historical perspective will be useful. I have made deliberate use of the term Muscle Biology in the title because even though it is in common use today, some thoughts about its origin may be useful as we assess what it means today.

Meat Science by any measure is a relatively new area of endeavor. There is no argument over the fact that progress in the applied aspects can be made by empirical efforts. Likewise, there is no argument that a segment of resources must be directed at very fundamental work in order to not only understand mechanisms and thereby be able to exert some control, but also to keep an adequate storehouse of information for the future. Stockpiling of knowledge is critical in order to deal with unforeseen problems which will arise and to have a basis for making make informed decisions about future progress.

The early history of Meat Science was established by several individuals who understood problems of the industry yet had the insight, the ability and the determination to conduct rather basic efforts. They were leaders. We look today at their accomplishments and use them as examples of how scientific findings have indeed helped the industry. Examples are work on rigor mortis (Bate-Smith & Bendall, 1949, Bendall, 1973), water-binding (Hamm, 1960), role of the live animal in postmortem change (Howard and Lawrie, 1956), the relaxing factor (Marsh 1952), the PSE condition (Briskey, 1964; Hart, et al., 1963; Henry et al., 1955; Ludvigsen, 1954) and contraction and tenderness (Locker 1960). These efforts, are all characterized by one key fact. The research scientist involved if not having actual close working association with fundamental scientists were very much aware of fundamental work in the area and of who was doing it. Such association is absolutely critical. The two symposia on Biochemistry and Physiology of Muscle as a Food (Briskey, et al., 1966; 1970) exemplified the respect of and the exchange between the fundamental scientists and the scientist using basic techniques to generate information which would, in the future, have some practical use.

In my opinion, there has been an unfortunate decline in fundamental research by Meat Scientists. I must hasten to add that there are still excellent examples of basic science being done within the realm of long-term application by the Meat Industry. However, the problem is that there are very few such examples, especially in view of the already identified serious problems the Meat Industry is currently facing.

I can offer two possible explanations of why this drift has occurred during the past 15 years. One is the apparent attraction to meat research scientists of doing product-development type work. These efforts are short-term and do produce results. The problem is how the efforts are actually used by the industry. Also, such work has little appeal to the fundamental scientist and it is, therefore, most difficult to engage in needed dialogue let alone cooperative ventures which seek to

explain mechanisms. The other major drain from fundamental Meat Science research has been the high intensity interest in food safety and diet-health issues. The prime example of this is the amount of work directed at nitrite as a meat curing agent during the decade of the 1970's. Even though good work was done, and no one would disagree that it was necessary, it in total drained talent and resources away from Muscle Biology type work. Much of the effort in this latter area can best be described as defensive. During both of the above two movements, less and less interest was shown in the role of the live animal as an important controlling factor in the properties of the meat from it. This is also unfortunate.

I must focus more specifically on the USA at this point in order to conclude this section. In our country, the problem of two little fundamental research within the realm of the Meat Industry is recognized to the point it has been identified at a public forum (Hiegel, 1984). Again, I must caution that there are examples of excellent progress being made. This occurs primarily at a focus point provided by a large multidisciplinary Government-Industry financed laboratory or with a smaller group located in a large center of existing scientific expertise.

The Meat Industry, in my opinion, is not a healthy one just now. The problems are so apparent that two major symposia have been organized during the past years to consider the future trends of the Industry (Cassens et al., 1984; Krol et al., 1985). It seems most appropriate, therefore, to consider at this time trends in basic science (that is Muscle Biology) since science forms a foundation for the future.

I list and describe in the following sections 17 areas of basic science research which I believe represent unique opportunity for the Muscle Biologist working for the improvement of Meat Science.

NEW PROTEINS

While information about the basic contractile and regulatory proteins remains rather true and tested, there has been great activity lately in the discovery and characterization of new proteins in muscle especially the so-called cytoskeletal proteins which undoubtedly play some role in the maintenance of the structural integrity of the cell (see Robson et al., 1984; Robson and Huiatt, 1983; Greaser et al. 1981 for reviews).

Titin (also known at connectin) is a high molecular weight (1,000,000 dalton) very insoluble protein which accounts for about 10% of the total of myofibrillar proteins. Localization studies have shown it to be a major component of the sarcomere but not exclusively a component of either the thick or thin filaments. It may comprise a set of very thin longitudinally running filaments which give some elasticity to the sarcomere. It has also been suggested that it helps maintain myosin filaments in longitudinal register in the A band, regulates insertion of thin filaments into the ends of the A band and helps in maintaining longitudinal structural integrity of the myofibrils. It has been suggested as being important in influencing tenderness -- a topic we will return to under the section headed "gap filaments".

Desmin is a rather insoluble protein with molecular weight of about 55,000 daltons. Localization studies have shown it comprises a set of 10 nm diameter filaments that encircle the Z-line and radiate out perpendicularly to the myofibrillar axis to ensnare and connect adjacent myofibrils. It is thought to play a structural role in connecting myofibrils and in maintaining overall integrity of the skeletal muscle cells.

Nebulin is also highly insoluble, of high molecular weight (500,000 daltons) and comprises about 5% of total myofibrillar protein. Localization studies suggest it is found in the I band at the N_2 line. Therefore, it may be a structural component of the N_2 line and function in the organization of the changing three-dimensional lattice of the thin filaments in the I-band.

Other proteins of the myofibrillar complex are: C-protein found in the thick filament, M-protein found in the M-line, and Vinculin and filamin which are found in the Z-line periphery.

TURNOVER

Muscle proteins are in a dynamic state of degradation and replacement which is an important consideration for those interested in the efficiency of muscle growth, in the assembly of structural components and in postmortem change in the myofibrils. Very recent publications have given information about the effects of nutritional state and stage of growth. Bates and Millward (1983) found that the ratio of sarcoplasmic to myofibrillar protein synthesis was similar for all muscles and when change in overall turnover rate occurs, the relative turnover of the two major protein fractions stays constant. There was a faster overall turnover rate for diaphragm and soleus muscles (composed mostly of slow-oxidative fibers) compared to several other muscles which were composed primarily of glycolytic fibers. The synthesis rate of the slower-turning-over myofibrillar protein fraction was shown to be more sensitive to nutritional state than was that of the sarcoplasmic fraction. Lewis et al. (1984) found that age related growth in various types of muscle was accompanied by a progressive decline in both fractional rates of protein synthesis and breakdown, the changes in synthesis being more pronounced. The fall in synthetic rate with development was similar for different muscles and the rate at 105 weeks of age was about 30-35% of the values at weaning in rats. At the 105 week age, the half-lives of the mixed muscle proteins were 5.1, 10.4, 12.1 and 18.3 days for smooth, cardiac, soleus and tibialis muscles respectively.

PROTEOLYSIS

Proteolysis in postmortem muscle has long been of interest because of its potential relationship to tenderness. Early work attempted to measure activity of various proteolytic enzymes and more recent efforts have been directed at extensive study of breakdown products with electrophoretic techniques. Goll et al. (1983) have explained in detail the properties of the various proteinases found in muscle. They concluded from current evidence that of the 13 known lysosomal peptide hydrolases, only 7, cathepsin A, B, C, D, H, L and lysosomal carboxypeptidase B, are located inside of the skeletal muscle cells. Only one of the reported neutral and alkaline proteases is located inside of skeletal muscle cells. That is the neutral Ca^{2+}-dependent proteinase known as CAF. They further concluded that it seemed probable, with the possible exception of cathepsin N which can degrade collagen, that any protease which contributes to postmortem tenderization must be located inside the muscle cell. Because very little degradation of myosin or actin occurs in postmortem muscle, most of the small amount of proteolytic degradation of myofibrillar proteins that occurs in postmortem storage must be due to CAF which is unique in being unable to degrade myosin and actin.

CONNECTIVE TISSUE

The role of connective tissue in meat has been considered often because of its obvious role in tenderness and in structural integrity. In the broad definition, connective tissue consists of the proteins collagen and elastin, of various connective tissue cells, and of the mucopolysaccharides. Interest has invariably been focused on the protein component (especially collagen) and from a meat related viewpoint, seems to rise and fall depending on periodic advances in fundamental research which are often medically related.

Recent advances have been described by Aberle and Mills (1983). Procollagen is the biosynthetic precursor of collagen molecules, and it undergoes substantial posttranslational modification intracellularly before it is secreted from the cell and eventually packed into fibrils. The collagen molecule is a triple helix consisting of a coiled coil of three polypeptide units or alpha chains. Each alpha chain twists in a left-handed helix with three residues per turn and the three alpha chains are wound into a right-handed super helix to form a molecule about 1.4 nm in diameter and 300 nm long. At least seven genetically different alpha chains have been identified in higher animals and they comprise five or more different molecules.

Interest has been shown in the demonstrations that melting temperature of intramuscular collagen decreases during postmortem time.

PROTEIN ASSEMBLY

From the viewpoints of both growth and turnover, the mechanism by which the components of the myofibril are assembled into the functioning unit has been of intense interest. Recent work has resulted in a model to explain myofibrillar assembly and maturation (Dlugosz et al., 1984). Stress fibers are composed of numerous aligned microfilaments with associated contractile proteins and are found subtending the sarcolemma of many cell types. Some have proposed that these stress fibers are primitive myofibrils. The authors cited above used immunofluorence microscopy to examine the relationship between stress fiber-like structures (SFLS) and nascent myofibrils in cultured chick cardiac myocytes. They found the following (1) more SFLS were present in immature than mature myocytes, (2) in immature myocytes, a single fluorescent fiber would stain as a SFLS distally and as a striated myofibril proximally or towards the center of the cell, (3) in the region of the myocyte not yet penetrated by elongating myofibrils, SFLS were abundant but they had disappeared in regions containing numerous mature myofibrils. They also observed that spontaneously contracting striated myofibrils with definitive Z-band regions were present long before anti-desmin localized in the I-Z region and long before recognizable structures periodically linked Z-bands to the sarcolemma. Their model shows a transient one-on-one relationship between individual SFLS and newly emerging individual nascent myofibrils.

In other recent work (Bandman et al., 1982), it was established that 3 myosin heavy chain isoforms appear sequentially in development of chick pectoralis major muscle. The embryonic isoform is detected early and throughout development of the embryo. The second isoform appears just after hatching and predominates by 10 days ex ovo. A third isoform which is indistinguishable from adult type predominates by 8 weeks after hatching. This sequence of myosin isoform changes does not appear during myogenesis in vitro where only embryonic heavy chain is expressed. The authors suggested this information would be of great value in probing further the neural and humoral determinants of muscle fiber maturation and growth. Bandman (1985) subsequently did study heavy chain isoform expression in normal and dystrophic chicken with a monoclonal antibody specific for neonatal myosin. Adult dystrophic muscle continued to contain neonatal myosin long after it disappeared from adult normal muscle. He showed with other techniques that immunoreactive myosin in adult dystrophic was identical to that found in neonatal normal. It was also established that all fibers in the dystrophic muscle failed to express neonatal myosin heavy chain. It was concluded that muscular dystrophy inhibits the myosin gene switching that normally occurs during muscle maturation.

GAP FILAMENTS

The so-called gap filaments are synonymous with the name of Locker, and he produced an extensive review and commentary on the subject in 1982. The gap-filaments are a third set of filaments and have

been observed for about 20 years, but as Locker (1982) points out, they had not, at that time, attracted a great deal of attention from either Meat Scientists or Muscle Biologist. He considers them a major factor in tenderness in which weakening by autolysis was a major consideration. Since he offered this challenge, a considerable amount of new and somewhat conflicting information has appeared. The research is obviously at a high state of activity and will undoubtedly yield some most useful results in the near future.

Recent studies have been published by King (1984). He found in cold-shortened muscle that the breakdown of connectin by proteolysis was as rapid as in control samples suggesting connectin exists in an exposed environment rather than as a core to thick filaments. Breakdown of connectin during heating at 60 or 80° for 40 min was more extensive than during aging for 3 weeks at 2°C. Hence the partial proteolysis of connectin during storage at 2° was unlikely to be responsible for tenderization induced by aging.

Locker and Wild (1984) have also published results using electrophoresis to study what they term the large structural proteins of the myofibril. They state that despite the known decay of gap filaments during aging and the identification of connectin as their substance, their current results indicate that connectin survives prolonged aging at 15°C. Cold shortening did not affect analysis. The only two changes on the same time scale as tenderization are the disappearance of nebulin and an increase in a protein lying between connectin and nebulin on the gels. They propose that nebulin may be a component of the G-filaments necessary for their stability. Cooking to various degrees apparently caused a random splitting of connectin.

GELATION

Gelation of the muscle proteins has been considered responsible for the so-called binding ability and also for the stabilization of fat and water in comminuted products. For the purpose of this assessment on basic science, I point out that some researchers have examined the more basic aspects of this phenomenon. Ishioroshi et al. (1979) measured the shear modulus of myosin at pH 6.0 in 0.6 M KCl which had undergone heat-induced gelation between 60° and 70°C. Replacing KCl with NaCl gave no difference. Increasing myosin concentration increased shear modulus. Ziegler and Acton (1984) found that with myosin, rapid aggregation and subsequent gelation began at about 55°C and were brought about by changes in the native structure of the myosin rod. The interactions between myosin rod or tail segments which stabilize the gel seemed to be noncovalent in nature.

STRUCTURE

Structural studies have always played a key role in basic Meat Science. The sliding filament theory, for example, was a major scientific accomplishment and allowed subsequent theory to arise regarding the role of contraction in tenderness of muscle.

Elegant scanning electron microscopic studies by Rowe (1981) have increased our appreciation for the manner in which connective tissue forms such an all-pervasive network and thereby influence tenderness. In this work, endomysium was a dense feltwork of collagen fibrils completely surrounding each individual fiber. He was able to categorize the perimysium into three components: (1) coarse crimped fibers laid down in a well-ordered criss-cross pattern, (2) a loose feltwork of non-crimped fibrils, and (3) fine non-crimped bundles of fibrils with no directional organization.

Offer and Trinick (1983) have used phase microscopy to develop an explanation for water holding in meat. Their hypothesis is that swelling or shrinking of the myofibrils caused by expansion or shrinking of the filament lattice is responsible for gains or losses of water in meat. Myofibrils were seen to swell to about twice their original volume in salt solutions. It was suggested that Cl^- ions bind to the filaments and increase the electrostatic repulsive force between them. A given salt concentration may also cause extraction of components causing structural constraint and thereby allow the filament lattices to expand. Their results help explain conditions of meat processing but also, for example, of water loss in rigor, PSE and cooking.

Heuser and Cooke (1983) have published a new technique which allows better visualization of certain structures by electron microscopy. It was developed because they considered it important to re-examine myosin-actin interactions utilizing a technique that does not require fixation, exposure to heavy metals or dehydration, and which provides three-dimensional surface views rather than projected images. Therefore, they quick froze the sample by contact with a block of copper cooled to about 4K. It was then freeze-fractured, deep-etched, rotary-replicated with platinum and viewed with transmission electron microscopy. Outstanding photos of the actin filament were produced. They also applied the technique to insect flight muscle and concluded that cross-bridge shape and orientation was strongly affected by different methods of sample preparation. This will make it difficult to visualize natural cross-bridge movements by electron microscopy.

Structure is determined by means other than microscopy. An important paper (Sundralingam, 1985) has used the X-ray technique on crystallized troponin C from chicken skeletal muscle. They found the protein to be about 70 Angstrom long with an unusual dumbbell shape. The carboxyl and amino domains are separated by a single long alpha helix of about 9 turns. Only the two high-affinity Ca^{2+}-Mg^{2+} sites of the COOH-domain are occupied by metal ions resulting in conformational differences between the

COOH- and NH_2-domains. The authors consider these differences as probably important in triggering of muscle contraction by troponin C.

SATELLITE CELL

Considerable interest has been shown in these cells because of their possible role not only in regeneration of muscle but also in growth. They were described by Mauro (1961). Their nuclei appear indistinguishable from the nuclei of the myofiber upon examination with light microscopy. Careful study with electron microscopy reveals, however, that they are not true nuclei within the myofiber but rather lie outside of the sarcolemma but within the basement membrane of the myofiber. Recent work (Allbrook, 1981) has shown that under normal adult conditions, satellite cell nuclei comprise less than 5% of total muscle nuclei. A review of available information favors the hypothesis that satellite cells are the stem cells necessary for regenerative myogenesis. Intactness of the basal lamina has been shown to be important for regeneration. Satellite cells cannot escape the basal lamina even though other invasive cells may move through it.

CELL CULTURE

I mention this subject as a technique that has potential wide application to Meat Science. It consists of growing individual cells in culture and allows not only measurement of growth and division but also the ability to expose the cell population to various substances at will. The technique has found very wide use and I site here only two instances pertinent to Meat Science.

Allen et al. (1979) have reviewed the factors associated with the cell proliferation aspects of muscle growth. As they state -- the proliferation of embryonic myogenic cells and their differentiation into multinucleated fibers, as well as the proliferation of myogenic cells in postnatal muscle (satellite cells) are major factors determining the ultimate mass of muscle that can be produced by an animal. Cell culture techniques allow careful study of these factors.

Cell culture has also been used as a technique to study the control of deposition of adipose tissue in meat animals (Novakofski, 1981). A technique has been developed which indicates the stromal vascular cells (from rat or pig) begin accumulating lipid after 3-5 days in culture and become monolocular cells in 5-7 days . The cells apparently accumulated lipid in a manner similar to adipocytes in vivo. The cultures remained viable and cell numbers increased for at least 21 days.

NERVE-MUSCLE-HORMONAL

I have given this concept of controlling properties of muscle (and thereby quality attributes) by regulating the nervous and hormonal systems (Cassens et al., 1983) a separate section in order to emphasize the importance of it. It is a classic example of how fundamental research can be used to improve meat for the consumer. This is a novel approach some time off from actual application, but of such potential significance as to deserve serious discussion. The regulation I speak of is via manipulation of the nervous and hormonal systems in the live animal. My proposal then is that fundamental properties of muscle, and therefore meat quality, can be regulated by manipulating the nervous and hormonal systems. There is, in the literature, a considerable amount of information indicating that the properties of the myosin molecule in the muscle of the live animal can be changed by influence of either the nervous or hormonal system. The critical issue is that muscle is plastic. It can in some manner adapt to its environment or to demands placed on it by changing its properties. This allows us as Meat Scientists a tremendous tool to work with -- if we can understand and control it.

The work most frequently cited which establishes that the properties of muscle are under neural control is that of cross-reinnervation (Buller et al., 1960). Two muscles in close proximity and having different properties (i.e., red and white) are selected and the nerves to them are surgically switched. After a period of time, it has been established by numerous techniques (physiological, histochemical, biochemical) that the properties of the muscle will have been transformed. The former white muscle is converted to red and the red to white. There is no doubt that in some manner the nerve dictates to the muscle what properties to express. The control is so strong that by changing innervation, the properties of the muscle can be substantially altered even in the mature animal.

Another series of observation has provided rather strong evidence that the thyroid hormones greatly influence the properties expressed in skeletal muscle (Ianuzzo et al. 1977). Apparently, increased level of thyroid hormone causes the muscle to become whiter. While it is still uncertain how the thyroid hormones cause the change the fact that they do so has been substantiated a number of times in the literature.

The potential exists for developing procedures to control directly the fundamental properties of muscle by manipulating the nervous and or hormonal systems in the live animal. Such control procedures would allow the technologist to regulate meat quality with the aim of improving acceptability of fresh meat and/or the potential of the muscle for utilization to processed products.

GROWTH

A strong renewed interest has occurred in growth and is associated with the great current interest in biotechnology. Study of growth has always been an important segment of Meat Science beginning with the early body and carcass composition studies. In this manuscript, reference is made elsewhere to topics such as protein assembly, satellite cell and cell culture. These topics also are concerned with growth. However, the new interest in growth focuses on hormonal control (by gene insertion or hormonal injection) and on repartitioning agents.

Bauman et al. (1982) propose that a higher order of endocrine regulation, over and above that provided by homeostatic mechanisms, directs the flow of nutrients to support the physiological or developmental process of highest prevailing priority. They apply the term homeorhesis to this regulatory phenomenon. It is distinct from the more familiar homeostasis. They conclude that despite the varied nature of supporting data, growth hormone, prolactin and the closely related placental somatomammotropin exhibit the properties and actions of homeorhetic hormones. They direct the flow of nutrients to the process of highest priority, partly by coordinating nutrient utilization by competing tissues.

Two recently published papers provide information about testing of a repartitioning agent in domestic livestock (Ricks et al., 1984; Baker et al., 1984). The data on lambs suggested that treatment with clenbuterol caused a repartitioning of nutrients resulting in improved feed conversion, reduced fat deposition and increased muscle deposition. In regard to feeding the compound to finishing steers, improvements in muscle accretion with concomitant reductions in fat deposition were achieved. The exact mode of action of clenbuterol is unknown at present. It is thought, however, that it causes a release of free fatty acids via beta-adrenergic stimulation of adipose tissue and the free fatty acids are then used as an alternative energy source to amino acids. Beta-adrenergic stimulation of muscle results in hypertrophy of skeletal muscle via a decrease in protein degradation and (or) an increase in muscle contraction. These processes may be amplified by increased serum growth hormone levels.

POSTMORTEM

Work on postmortem metabolism has been rather scarce during recent years, probably because of effort being directed at electrical stimulation and postmortem proteolysis (see other sections this manuscript). A major review of the area has been made by Greaser (1985).

Oualie (1984) studied modification of Mg-Ca-enhanced ATPase activity and sensitivity to ionic strength during conditioning of beef. As storage time increased so did ATPase activity at low ionic strength whereas it decreased at higher. It was concluded that the slope value which quantifies the sensitivity of ATPase activity to ionic strength could be an accurate indicator of degree of aging of the myofibrillar structure. It has been called the Biochemical Index of Myofibrillar Aging (BIMA).

Vogel et al. (1985) have used a non-invasive phosphorus-31 nuclear magnetic resonance technique to study postmortem energy metabolism. They found good agreement with enzymatic assays and suggested the new procedure provides a useful compliment to existing procedures.

SKINNED FIBERS

The use of preparations of skinned fibers has been available for some time, but there has been a great interest in their use recently because the techniques have become more commonplace and because the preparations offer certain unique advantages. Skinned fibers are prepared by actual physical removal of the sarcolemma or by disrupting the sarcolemma by chemical means. Stephenson (1981) has written a major review and pointed out that skinned fiber preparations with removed or disrupted sarcolemma bridge the gap between properties of isolated subsystems and the constrained operation in intact fibers. In general, use of the skinned fibers has provided evidence that the surface action potential propagates along transverse tubules but not the sarcoplasmic reticulum which appears to be a distinct intracellular component. The signal from the t-tubule to the sarcoplasmic reticulum is very likely to be initiated and controlled by small currents attributed to intramembrane charge movements that can grade calcium release. The nature of the junctional transmission itself is unknown.

Single cells of skeletal and cardiac muscle can be mechanically skinned and the effect of ions and substrate on the contractile machinery can be studied. For smooth muscle, however, the small cells and large amount of connective tissue surrounding them have prohibited mechanical skinning techniques (Gordon, 1978). In striated muscle, a 50% glycerol solution modifies permeability of the cell to CA^{+2} and short term exposure to EDTA increases the membrane permeability to ions. These procedures do not work well for smooth muscle. The above author exposed segments of rabbit taenia coli to the nonionic detergent Triton X-100. Following treatment, tension could be induced by increasing the calcium concentration in the micromolar range. In the presence of saturating calcium concentration, the preparation developed nearly 100% of control tetanus tension recorded from the intact muscle prior to the detergent treatment. The Triton X-100 treatment, therefore, seemed a suitable method for chemical skinning of smooth muscle fibers. Miller et al. (1979) have exchanged a series of letters regarding proper procedure for chemical skinning of cardiac cells. Knowledge about the process of contraction has been improved by the skinning technique which greatly enhances access to the intracellular organelles without the hindrance of an intervening cell membrane.

Eddinger et al. (1985a) have demonstrated that chemical (EGTA) skinning of rat skeletal muscle fibers has no detrimental effect on subsequent staining with the myosin ATPase procedure. This is useful because skinned fibers used for mechanical measurements can be typed subsequently thereby allowing correlational studies on the same fiber.

IMMUNOLOGY

Immonulogy is a strong and specific technique which has been uniquely useful to structural localization studies in Muscle Biology research. This is especially true for specific location of highly purified proteins. While some of the early studies were hindered by a certain lack of specificity, the recent technique of production of monoclonol antibodies has given very specific results. Several results discussed in previous sections of this manuscript (new proteins, proteolysis, and protein assembly, for example) have indeed been made possible by advances in immunology.

In order to illustrate the possibilities of the technique, work of Carpenter et al. (1985a, b) will be discussed here. Antibodies were developed against fast twitch myosin heavy chain and against slow tonic myosin heavy chain. This allowed subsequent, immunocytochemical studies. Chicken anterior latissimus dorsi (ALD) and pectoralis were both found to contain a population of type IRB myofibers as identified by ATPase. However, the IRB myofibers of pectoralis were demonstrated to be immunocytochemically distinct, in respect to their MHC isozyme, from IRB myofibers of ALD. Pectoralis IRB myofibers reacted with only anti-slow-MHC but IRB myofiber of ALD reacted with anti-slow-MHC and anti-fast-MHC. The IRB myofibers of ALD are believed to be transitional and contain a neonatal MHC isozyme antigenically similar to both fast MHC and slow MHC. Inconsistency and inaccuracy of myofiber typing by ATPase is suggested due to the identical ATPase based classification of IRB myofibers containing different MHC isozymes.

Histochemically slow myofibers of one-day-ex-ovo pectoralis were observed to react with only anti-slow-MHC or with both anti-fast-MHC and anti-slow-MHC while histochemically fast myofibers only showed reaction with anti-fast-MHC. Embryonic MHC appears to show only a reaction with anti-fast-MHC based on these results. In other studies, these authors found that feeding thyroxin (T_4) to chickens induced slow-tonic myofiber to fast-twitch myofiber conversion. It was also observed that newly synthesized myosin heavy chain was incorporated uniformly across the cross-sectional area of transitional myofibers and homogeneously into each sarcomere.

AGING

The effects of senescence on muscle are familiar and include decreased speed and endurance as well as atrophy The underlying mechanism of these changes is not completely understood but is at present, a challenging problem. In order to control senescence, the underlying basis of the associated changes must be understood. Because of obvious health and social benefits of being able to deal with it, senescence is under vigorous investigation. It may be an area of study which could offer a clue of importance to the meat industry. By way of example, two experiments are described in the following paragraphs.

It has been suggested rather widely in the literature that with advancing age, the percentage of type I fibers increases and total fiber number decreases. However, it has been shown this is not the case with the Fisher 344 rat (Eddinger et al., 1985b). In further work (Eddinger et al. (1985c), physiological measurements and fiber type histochemistry were conducted on living fiber bundles and skinned single fibers from extensor digitorum longus (EDL) and soleus (SOL) of control (a month) and aged (30 month) Fisher 344 rats. The results suggested three major points. First, in Fisher 344 rats, there were changes in V_{max} and P_o with aging in only the SOL muscle, which became faster and stronger. There was no discernable effect of aging on V_{max} or P_o in EDL fibers, however, velocities at intermediate relative loads were diminished. These changes are not due to changes in the neuromuscular junction or in the SR. The results suggest that changes in external factors such as motivation, central and peripheral nervous system, hormone levels, and articulation of joints, along with disease and simple disuse atrophy must be more closely scrutinized as the cause for decreased functional capacity with senescence. Secondly, excellent agreement was found when physiological (V_{max}) and histochemical (M-ATPase) properties and myosin light chain composition were measured in the same fibers. All fast fibers contained fast LC's and typed as fast (IIb) by M-ATPase while all slow fibers contained slow LC's and typed as slow (I) with M-ATPase. Thirdly, there were small but significant differences within each of these fiber types with aging that were detected by the mechanical measurements but were not related to detectable changes in the LC composition or fiber type. The latter finding suggests that factors other than M-ATPase activity and LC composition influence muscle P_o and V_{max}.

ELECTRICAL STIMULATION

I have chosen this topic as the final point not because of the great wealth of information which has been generated but because reference can be made to an excellent review of the topic (Marsh 1985) in which an important point is made pertinent to the future of Meat Science. He uses the work on electrical stimulation as an example to draw the conclusion of a critical need for a much greater understanding (only possible through accumulating background information) rather than an empirical approach depending primarily on manipulation and recycling of old knowledge.

SUMMARY AND CONCLUSION

It was suggested in the introductory statements that Meat Science researchers have drifted away from fundamental aspects. In the body of the text, several very active areas of Muscle Biology type research were described. The question then is how to encourage greater interest in and utilization of current Muscle Biology efforts for the betterment of the Meat Industry.

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