MUSCLE GROWTH IN MEAT ANIMALS

William R. Dayton and Marcia R. Hathaway, Department of Animal Science, University of Minnesota, St. Paul, MN 55108

INTRODUCTION

The ultimate goal of meat-animal agriculture is rapid and efficient production of relatively fat free muscle tissue. Because biological mechanisms operating during embryonic and postnatal muscle development play a major role in determining final muscle mass as well as efficiency and rate of muscle deposition, it is important for us to learn more about these mechanisms and the growth factors involved in their regulation at various stages of development. Utilisation of recombinant DNA technologies to produce relatively large quantities of biologically important and scarce growth factors such as somatotropin, insulin-like growth factor I and fibroblast growth factor has led to significant advanced in understanding the role these peptides may play in muscle growth *in vitro* and *in vivo*. In some instances (somatotropin and beta adrenergic agonists), treatment of animals with these compounds has lead to dramatic increases in muscle mass and decreases in fat deposition. However, despite these initial successes, researchers know relatively little about the mechanism or regulation of muscle growth in meat-producing animals. At the cellular level, muscle growth can be enhanced by altering proliferation or differentiation of myogenic cells (e.g., altering the number, type or DNA content of each fibre) or by increasing protein accretion in individual fibres. The aim of this review is to briefly describe what is known about these processes and their regulation in meat animals.

MYOGENIC CELL PROLIFERATION AND DIFFERENTIATION

The embryonic development of muscle tissue originates in muscle precursor cells, of mesodermal origin, that differentiate and ultimately fuse to form multinucleated myotubes. Myotubes synthesise muscle-specific contractile proteins, assemble them into myofibrils (Devlin and Emerson 1978), and further differentiate to form muscle fibres present in postnatal muscle tissue. As differentiation proceeds, slightly different isoforms of many of the individual contractile proteins are synthesized. Myosin, for example, undergoes several isoform transitions which appear to be related to developmental stage and fibre type (Robbins et al. 1986). Similarly, actin and troponin-T have been shown to undergo isoform transitions during muscle differentiation. No surprisingly, this diversity of contractile protein isoforms appears to have its origins at the gene level where myosin and actin genes have been shown to exist as multigene families. Expression of specific genes may be regulated by muscle-specific *trans*-acting factors that bind to specific regions of the gene. For example, Walsh and Schimmel (1987) have shown that myogenic and non-myogenic cells contain different nuclear factors that compete for the skeletal muscle α-actin promoter. It is possible that binding of these factors may differentially regulate expression of the α-actin gene. Additionally, Markham et al. (1987) have reported interaction of a protein factor within a thyroid hormone-sensitive region of the rat α-myosin heavy chain gene. Again, these workers have suggested that this factor may play a role in the regulation of the α-myosin heavy chain gene. A greater understanding of the control of gene expression is essential in order to regulate gene expression and insertion in transgenic animals. Until we understand more about their regulation successful insertion of specific genes in farm animals appears unlikely.

During *in vivo* embryonic development of both mammalian and avian muscle (reviewed by Kelly and Rubinstein 1986), myoblasts initially fuse to form a set of "primary fibres". The origin of primary and secondary fibres may be partially explained by the discovery that several different types of myogenic cells are actively proliferating and differentiating during different stages of embryonic development of avian muscle (Miller et al. 1985; Miller and Stockdale 1986a; 1986b; Stockdale et al. 1986; Narusawa et al. 1987). Cultured myogenic cells isolated at various stages of embryonic development demonstrate dramatic differences in morphology and medium requirements (Whit et al. 1975). "Early muscle colony-forming (MCF) cells" predominate in the early stages of embryonic development while "late MCF cells" predominate in the later stages of development of embryonic muscle. Despite this temporal relationship, late MCF cells do not appear to descend from early MCF cells (Rutz and Hauschka 1982; Seed and Hauschka 1984). Subsequent studies have shown that early MCF cells can be subdivided into three subtypes and that late MCF cells can be subdivided into two subtypes based on formation of distinct myotubes expressing a particular myosin heavy chain isoform (Stockdale and Miller 1987). Because the appearance of myoblasts in the early and late MCF lineages corresponds to the time of formation of primary and secondary myotubes, early MCF cells may form the primary fibres and late MCF cells may form the secondary fibres (Miller and Stockdale 1986b; Stockdale and Miller 1987). Factors that differentially affect the proliferation or differentiation of cells in these myogenic lineages could have profound effects on fibre type distribution and fibre number in postnatal muscle. Consequently, we need to learn more about the properties of these embryonic myogenic lineages in meat producing animals.

In meat animals the formation of muscle fibres is essentially complete prior to birth, thus, no increase in fibre number occurs during postnatal growth. Additionally, it is well established that the nuclei present in fibres are not able to divide (Stockdale and Holtzer 1961). Paradoxically, the amount of DNA present in muscle fibres has been reported to increase as much as 8-fold during postnatal muscle growth (Winick and Nobel 1966). This DNA accretion is closely associated with, if not casually related to, the rate and extent of muscle growth in chickens (Moss 1968), pigs (Powell and Aberle 1975; Harbison et al. 1976; Swatland 1977) and cattle (Trenkle et al. 1978). Mononucleated, myogenic "satellite" cells located between the plasmalemma membrane and basal lamina of each muscle fibre are thought to provide this additional DNA (Mauro 1961; reviewed by Camion et al. 1984) by proliferating and

fusing with existing muscle fibres during postnatal muscle growth (Moss and Leblond 1970; 1971). It is possible that satellite cells may be yet another myogenic cell lineage expressed during embryonic differentiation of muscle tissue. Because current information indicates that satellite cells play an important role in muscle growth, we need to know more about their origin and regulation of their proliferation, differentiation and fusion.

MUSCLE PROTEIN ACCRETION

Muscle proteins are in a continual state of flux, with both protein synthesis and degradation occurring simultaneously. As might be expected for such fundamental cellular processes, both protein synthesis and degradation appear to be highly, and in many cases coordinately, regulated by a large number of physiological factors including insulin, glucocorticoids, insulin-like growth factors, thyroid hormone, anabolic steroids, age, nutritional state, exercise and fibre type. Although accurate measures of the absolute rates of protein synthesis and degradation are difficult to obtain, available data suggests that both protein synthesis and degradation rates are high in young, rapidly-growing animals and that both decrease significantly as animals become older (Millward et al. 1975). In fact, it has been estimated that in rapidly growing muscles as much as 1030% of the total protein is degraded and must be replaced each day (Millward et al. 1975; Laurent et al. 1978; Maruyama et al. 1978; MacDonald and Swick 1981). Replacement of these degraded proteins represents a tremendous utilisation of energy that might otherwise be utilized to synthesize proteins that would increase muscle mass. Thus, if this basal degradation rate could be reduced without detrimentally affecting the physiology of the animal, the rate and efficiency of muscle growth should be increased. Unfortunately, at the present time, our ability to regulate protein synthesis and degradation is limited by our understanding of the mechanisms of these processes. This is especially true for protein degradation, where a universally accepted mechanism has yet to be elucidated.

Traditionally, protein degradation was thought to occur in membrane-bound organelles known as lysosomes. Protein to be degraded were transported into the lysosome where they were degraded by lysosomal proteases (cathepsins). However, it has proven difficult to establish that myofibrillar proteins, which make up over 50% of the total muscle protein, are degraded solely by lysosomal action. Although cathepsins B, D, H, and L (Schwartz and Bird 1977; Bird and Carter 1980; Matsukura et al. 1981) are able to degrade myofibrillar proteins *in vitro*, there is no evidence that myofibrils or myofibrillar structures can be taken into lysosomes. Additionally, recent studies have shown that treatment of muscle cells with lysosomal enzyme inhibitors did not reduce injury- or calcium-induced muscle protein degradation (Furuno and Goldberg 1986). Consequently, it has been hypothesized that the rate-limiting steps in myofibrillar degradation involve neutral cytosolic proteases that disassemble the myofibril and make solubilised and partially degraded myofibrillar proteins available for lysosomal degradation (Dayton et al. 1975). Currently, the calcium-activated proteinase system (Dayton et al. 1976a; 1976b; 1981; 1985) and the ATP-ubiquitin dependent proteinase (Hershko and Ciechanover 1982) appear to be the most likely candidates for this disassembly role.

The calcium-activated proteinase is a heterodimer with a molecular weight of 110 kd. The proteinase selectively removes z-disks and hydrolyses troponin T, troponin I, tropomyosin, desmin, C-protein, filamin, and possible titan. Two forms of the enzyme have been isolated; one requiring millimolar levels of calcium (Dayton et al. 1976a; 1976b) and one which requires micromolar levels of calcium for activation (Dayton et al. 1981).

Additionally, a specific inhibitor has been isolated (Lepley et al. 1985). Thus, it appears that the activity of the proteinase is very highly regulated. The enzyme has been localized in muscle cells and, possibly more significantly, in the z-disk region of the myofibril (Dayton and Schollmeyer 1982) where, if activated, it could begin disassembly of the myofibril by breaking down the integrity of the z-disk. It has been suggested that this protease may initiate myofibril degradation by selective hydrolyzing structural elements of the myofibril and, possible in combination with other neutral proteases, causing its eventual disassembly. Consequently, understanding the complex regulation of the calcium-activated proteinase system may be crucial to our eventual understanding of the mechanism of muscle protein degradation.

Another neutral protease system which could be involved in muscle protein turnover is the ATP-dependent system originally identified and characterised in reticulocytes (Ciechanover 1978; Hershko 1979). Proteins are "marked" for degradation by the binding of a small protein (ubiquitin) to the α-amino group of lysine or to the N-terminal amino group (Hershko et al. 1984). Ubiquitin-conjugated proteins are then degraded by an ATP-dependent protease with release of undergraded ubiquitin. Although this is probably the best characterised protease system, it has been difficult to demonstrate its presence in muscle cells and its specificity for myofibrillar proteins has not been characterised.

FACTORS AFFECTING MUSCLE GROWTH

As can be seen from the preceding discussion, muscle growth is an extremely complex process that can be affected by events that impact on proliferation, fusion, fibre type, development of myogenic lineages, protein synthesis or protein degradation. Although neither the mechanisms nor regulation of these processes is completely understood, several factors capable of influencing one or more of them have been identified. The remainder of this review will be devoted to a discussion of these factors and their *in vivo* effects on muscle growth.

Insulin-Like Growth Factors (Somatomedins):

Insulin-like growth factors (IGF's) or somatomedins are a class of polypetides originally isolated from sera and, at concentrations of 10^{-9} to 10^{-10} M, these polypetides are mitogenic for a variety of cultured cells. Although IGF's share considerable sequence homology with insulin and

possess insulin-like properties *in vitro*, they do not cross-react with insulin antibodies (Zapf et al. 1978). Two classes of insulin-like growth factors have been characterised: Insulin-like growth factor I (Rinderknecht and Humbel 1978a) or somatomedin C (SmC) (Svoboda et al. 1980); and Insulin-like growth factor II (Rinderknecht and Humbel 1978b). Additionally, another family of peptides collectively designated multiplication stimulating activity (MSA) has been isolated from media conditioned by a Buffalo rat liver cell line (BRL 3A) (Moses et al. 1980). Multiplication stimulating activity III (MSA III) appears to be the rat form of IGF II, since the primary structure of MSA III shows 93% identity with that of human insulin-like growth factor II (Marquardt et al. 1981). Circulating levels of IGF I/SmC and to a lesser extent IGF II appear to be regulated by somatotropin.

In cultured muscle cells IGF I/SmC stimulates amino acid uptake (Hill et al. 1986; Ewton et al. 1987), proliferation (Ballard et al. 1986; Hill et al. 1986; Ewton et al. 1987), differentiation (Ewton and Florini 1981; Schmid et al. 1983; Ewton et al. 1987) and protein synthesis (Ballard et al. 1986; Harper et al. 1987) while decreasing protein degradation (Ewton et al. 1987; Harper et al. 1987). IGF I has also been shown to stimulate RNA synthesis in isolated rate soleus muscle (Monier and LeMarchand-Brustel 1984) and to stimulate weight gain in hypophysectomised rats (Schoelne et al. 1982). IGF II/MSA behaves very similarly to IGF I in that it stimulates proliferation (Ewton and Florini 1980; Ewton and Florini 1981; Florini and Ewton 1981; Florini et al. 1984; Beguinot et al. 1985; Ballard et al. 1986; Hill et al. 1986; Ewton et al. 1987), differentiation (Ewton and Florini 1981; Florini and Ewton 1981; Florini et al. 1984; Ewton et al. 1987), amino acid transport (Merrill et al. 1977; Janeczko and Etlinger 1984; Hill et al. 1986; Ewton et al. 1987) and protein synthesis (Janeczko and Etlinger 1984; Ballard et al. 1986) while decreasing protein degradation (Janeczko and Etlinger 1984; Ballard et al. 1986; Ewton et al. 1987) in cultured myogenic cells. Additionally, ovine somatomedin and rat MSA/IGF II have also been reported to stimulate proliferation of cultured satellite cells (Dodson et al. 1985). Cell surface receptors that preferentially bind IGF I (type I IGF receptor) or IGF II (type II IGF receptor) have been identified on muscle cells (Yu and Czech 1984; Beguinot et al. 1985; Ballard et al. 1986; Ewton et al. 1987). The type I IGF receptor, a 350-kd heterotetramer, preferentially binds IGF I, but has substantial affinity for insulin and IGF II (Czech 1986). It possesses intrinsic tyrosine kinase activity which may play an important, but as yet undefined, role in its functionality. The type II IGF receptor is a 220-kd monomer which preferentially binds IGF II and, unlike the type I IGF receptor, has little or no affinity for insulin (Czech 1986). The preponderance of evidence suggests that the type II IGF receptors are not involved in the ability of IGF II to affect proliferation, differentiation and protein turnover. Rather, it has been hypothesised that effects of IGF II on these processes result from cross-reaction of IGF II with the type I IGF receptor (Yu and Czech 1984; Ballard et al. 1986; Ewton et al. 1987). The role of IGF II is important during fetal development and that a "switch over" to IGF I/SMc occurs postnatally (Adams et al. 1983).

Until relatively recently, the liver has been considered to be the source of circulating IGF's that influence growth and differentiation of other tissues such as muscle and bone. However, numerous fetal and adult tissues have recently been reported to contain and/or synthesise IGF (D'Ercole et al. 1984; 1986; Hill et al. 1985a; 1985b; Clemmons and Shaw 1986; Han et al. 1987a; 1987b; Jennische et al. 1987; Jennische and Hansson 1987). In the most convincing of these reports, in situ hybridisation histochemistry was utilised to localise IGF I and IGF II messenger RNA's in connective tissues or cells of mesenchymal origin in 14 organs and tissues (Han et al. 1987a). Thus, many tissues, including muscle, may produce IGF's that function locally via autocrine and/or paracrine mechanisms. However, the relative importance of autocrine, paracrine and telecrine mechanisms of IGF action is unknown.

An important question concerning the potential autocrine/paracrine effects if IGF's on muscle differentiation and growth is whether myoblasts and myotubes are able to produce IGF's. Although immunocytochemical studies have shown that immunoreactive IGF's can be localised in muscle fibres (Han et al. 1987b; Jennische et al. 1987; Jennische and Hansson 1987) and cultured embryonic muscle cells have been reported to synthesise and secrete approximately equal amounts of IGF I/SmC and IGF II/MSA (Hill et al. 1985a; 1985b), in situ hybridisation techniques have shown that IGF I and IGF II mRNA's are not detectable in myogenic cells. However, both IGF I and IGF II have been localised in connective tissue and fibroblasts in skeletal muscle tissue from human fetuses (16-20 wk of gestation) (Han et al. 1987a). These observations have led to speculation that fibroblasts may synthesise and secrete IGF's which act on other cells via paracrine mechanisms (Han et al. 1987a; 1987b). In summary, although IGF's are synthesised locally in muscle tissue, it is not clear whether myogenic cells are able to synthesise these growth factors.

Insulin:

Several lines of evidence suggest that insulin has an anabolic effect on muscle tissue. For example, wasting of skeletal muscle is a well-documented feature of diabetes mellitus and this wasting is reversed by administration of insulin to affected animals (Pain and Garlic 1974; Flaim et al. 1980). Additionally, ribosomes isolated from muscle of diabetic rats are less active *in vitro* protein synthesis systems than ribosomes from nondiabetic controls. Insulin also increases rate of protein synthesis and decreases rate of protein degradation in isolated muscles (Manchester and Young 1970; Fulks et al. 1975; Frayan and Maycock 1979) and in the perfused rat hemicorpus (Jefferson et al. 1977).

In cultured satellite cells and embryonic muscle cells, as well as fibroblasts and fibroblastic cell lines, supraphysiological concentrations of insulin 1 g/ml or higher, are required to elicit a maximal response. In muscle cell cultures these high concentrations stimulate both proliferation and differentiation of myogenic cells

(Merrill et al. 1977; Ball and Sanwall 1980; Ewton and Florini 1981). Additionally, insulin at high concentrations (10^{-6}M) is a component of synthetic media used to support growth and differentiation of myogenic cells in culture (Florini and Roberts 1979; Dollenmeier et al. 1981). It has been proposed that the stimulation of growth of fibroblasts by insulin is mediated by its weak binding to receptors for insulin-like growth factors. It is thought that insulin, at high concentrations, binds to the type I IGF receptor and in so doing affects cell growth in a manner similar to that observed for much lower concentrations of IGF I (King et al. 1980). Even though physiological levels of insulin do not appear to influence proliferation or differentiation of cultured myogenic cells, insulin may facilitate muscle cell proliferation and/or differentiation by maintaining cells in a metabolic state that allows them to respond to other hormones and growth factors which directly affect these processes.

Transforming Growth Factor-β significantly affects proliferation and differentiation of many cell types (reviewed by Massague 1987) including myogenic cells. It has been shown to inhibit differentiation of L6 myoblasts (Florini et al. 1986; Massague 1986) and proliferation and differentiation of cultured rat satellite cells and primary embryonic muscle cells (Allen and Boxhorn 1987). Florini et al. (1986) have shown that the biological activities, physical properties, and antigenic properties of TGF-β are very similar to those of a "differentiation inhibitor" (DI) (Evinger-Hodges et al. 1982; Florini et al. 1984) which they initially isolated from the culture media of Buffaclo Rat liver cells. Based on these observations, Florini et al., (1986) have concluded that the principle differentiation inhibitor in their partially-purified DI preparation is TGF-β or a very closely related molecule.

Many different types of cells synthesise TGF-β in culture. Consequently, it is currently believed that TGF-β acts as an autocrine or paracrine regulator of proliferation, differentiation and function in numerous cell types. In developing muscle, TGF-β might prevent differentiation of proliferating myoblasts to fusion-competent, nonproliferating myoblasts during the initial stages of embryonic development. This could be instrumental in maintaining the extensive myogenic cell proliferation presumably required to provide the basis for later formation of muscle tissue (Florini et al. 1986). In this regard, it is significant that there have been numerous reports of TGF-β-like activities in very early development. Alternatively TGF-β might regulate proliferation and/or differentiation of satellite cells in postnatal muscle (Allen and Boixhorn 1987). Although more study is needed to establish the role of TGF-β in muscle growth, this molecule appears to offer promise as a factor regulating this process.

Fibroblast Growth Factor (FGF):

Two closely-related forms of FGF (acidic and basic), possessing similar biological activities in a wide range of cell types, have been isolated and purified to homogeneity (Gimenez-Gallego et al. 1985; Esch et al. 1985; Gospodarowicz et al. 1987). Partially-purified FGF stimulates proliferation of cultured satellite cells and embryonic myogenic cells while inhibiting their differentiation (Gospodarowicz et al. 1976; Linkhard et al. 1981; Allen et al. 1984; Lathrop et al. 1985a; 1985b; Spizz et al. 1986; Wice et al. 1987). Both highly-purified acidic and basic FGF stimulate proliferation of MM14 myoblasts with half-maximal effects on proliferation occurring at 30 and at 1 pM, respectively (Olwin and Hauschka 1986). MM14 myoblasts appear to possess a single FGF receptor, with a molecular weight of 165,000 daltons (Olwin and Hauschka 1986), that binds both acidic and basic FGF.

Basic FGF has been isolated from a number of tissues and/or cultured cells including pituitary, brain, placenta, corpus luteum, macrophages and cartilage (Gospondarowicz et al. 1987). Additionally, an FGF-like myogenic factor has recently been isolated from chicken skeletal muscle tissue (Kardami et al. 1985a; 1985b). Considering the effective of FGF's on myogenic cells and the presence of G\FGF receptors on myoblasts, these observations suggest that FGF may function as a paracrine regulator of myogenesis. Thus, it is essential to learn more about the potential role of FGF in differentiation and growth of muscle.

Somatotropin:

The effect of somatotropin deficiency on muscle growth has been well established for many years. Additionally, long-term administration of somatotropin to pituitary-intact animals has been reported to increase muscling, decrease fat content and improve feed efficiency in swine (Machlin 1972; Chung et al. 1985). Despite these observations, it is not clear that somatotropin has a direct effect on muscle growth and differentiation. Skepticism about the direct effect of somatotropin on muscle growth stems primarily from repeated reports that somatotropin, even at concentrations obtainable in somatotropin, even at concentrations obtainable in somatotropin-injected animals, has no detectable effect on proliferation, differentiation, or rate of protein turnover in cultured satellite cells, myotrubes or embryonic myoblasts (Florini et al. 1977; Merrill et al. 1977; Ewton and Florini 1980; Allen et al. 1983; Harper et al. 1987; Kotts et al. 1987). However, because somatotropin sensitivity does not appear to develop until after birth, cultured muscle cells, which are analogous to embryonic muscle cells, may not be an appropriate model for studying the effects of somatotropin in postnatal muscle tissue. Attempts to study the effect of somatotropin on postnatal muscle by utilising *in vitro* muscle incubations have often shown positive effects of somatotropin on muscle. However, recent observations that many types of cells, including fibroblasts, can secrete somatomedin raise the possibility that responses seen in these isolated muscles are the result of locally produced somatomedins. Another problem with many of these studies is that very high concentrations of stomatotropin (10^{-7}M) were used. This raised the possibility that impurities in the somatotropin preparation or biologically active proteolytic fragments of somatotropin (Liberti and Miller 1978; Armstrong et al. 1983) are responsible for the observed effects on muscle. Even so, based on current data, it is not possible

to unequivocally state that somatrotropin has no direct effect on muscle growth.

Although the direct effect of somatotropin on muscle growth remains ambiguous, there appears to be little doubt that a significant portion of the action of somatotropin on muscle growth is mediated through the insulin-like growth factors. As already discussed, IGF's have significant increase in circulating IGF I which coincides with an increased ability of their sera to stimulate proliferation (Kotts et al. 1987), increase protein synthesis rate, and decrease protein degradation rate in cultured myogenic cells (Dayton unpublished data). Consequently, it is likely that the stimulation of muscle growth in somatotropin-injected pigs is, a least partially, the result of somatotropin-induced increases in IGF I levels.

Gluucocorticoids:

Glucocorticoids are generally thought to be catabolic for muscle tissue and have been shown to stimulate protein degradation in muscle *in vivo* and *in vitro*. It is thus surprising that these hormones are an essential component of several synthetic media which support proliferation and differentiation of myogenic cells (Florini and Roberts 1979; Dollenmeier et al. 1981). In cultured muscle cells, glucocorticoids stimulate proliferation, differentiation and glucose transport (reviewed by Florini 1985). Additionally, the synthetic glucocorticoid dexamethasone has been reported to enhance the mitogenic effects of ovine somatomedin and rat IGF II/MSA on cultured satellite cells (Dobson et al. 1985). Although the mechanism of glucocorticoid action on cultured myogenic cells in unclear, it has been suggested that they may be necessary to maintain carbohydrate metabolism (Florini 1987).

Thyroid Hormone:

It is well documented that experimental hyperthyroidism decreases muscle mass (Satoyoshi et al. 1963). Several studies have suggested that this decrease is primarily the result of increased protein degradation (Carter et al. 1982; Angeras and Hasselgren 1985). Additionally, a growing body of evidence indicates that thyroid hormone (tyriiodothyronine, T_3) may play a role in differentiation and development of muscle tissue. This evidence is based on the ability of thyroid hormone to alter the myosin isoform composition of muscle fibres. During the differentiation of skeletal and cardiac muscle, the normal transition of embryonic to adult myosin heavy chain isozymes is accelerated by hyperthyroidism and inhibited by hypothyroidism (Gustoafson et al. 1986; Izumo et al 1986). Additionally, in postnatal animals hyper- or hypothyroid conditions result in alterations in myosin heavy chain isozymes present in specific muscles (Gustafson et al. 1986; Izumo et al. 1986). It should be noted that, because thyroid hormone has not been shown to affect muscle cells in culture, it is possible that its effects on protein turnover and myosin isozyme expression are not the result of the direct action of thyroid hormone on muscle cells.

Anabolic Steroids:

Testosterone, estrogen and their derivatives have been shown to stimulate muscle growth in meat animals (Bohorov et al. 1987). It is well established that intact males grow more rapidly and efficiently than castrates and testosterone and analogs have received wide use as protein anabolic drugs. Estrogens and their derivatives have also been repeatedly shown to increase growth rate in castrated males (Bohorov et al. 1987). Combined implants containing estrogenic and androgenic compounds have been shown to be more effective in stimulating growth than either type of compound administered singly (Bohorov et al. 1987). At present, the mode of action of these anabolic steroids in stimulating growth rate is unclear. However, current evidence suggests that it is not a direct interaction of steroids with muscle cells (Florini 1987).

β-Adrenergic Agonists:

β-agonists are structurally related to the catecholoamines epinephrine and norepinephrine. When added to feed at concentrations of .1 to 10 ppm (depending on species) these compounds have been shown to dramatically improve carcass characteristics and growth performance (Baker et al. 1984; Dalrymple et al. 1984; Ricks et al. 1984; Muir et al. 1985; Beermann et al. 1986). Unfortunately, little is known about how β-agonists stimulate muscle growth in meat animals. Attempts to demonstrate a direct action of β-agonists on protein turnover, proliferation or differentiation of cultured myogenic cells have been unsuccessful (Forsberg and Merrill 1986; Young et al. 1987). Despite these finds, the limited number of *in vivo* studies that have been done suggest that b-agonist treatment decreases rate of muscle protein degradation (Reed et al. 1986; Bohorov et al. 1987) while having no detectable effect on protein synthesis rate. Moreover, the b-agonist clenbuterol has been reported to reduce muscle wasting in dystrophic mice (Rothwell and Stock 1985) and denervated rate hind limb muscles (Zeman et al. 1987). Viewed together, the data obtained *in vivo* and *in vitro* suggest that the effects of β-agonists on muscle may be indirect. This hypothesis is supported by recent reports that plasma insulin and insulin-like growth factor levels are reduced by 55 and 34%, respectively, in cimaterol-treated lambs (Beermann et al. 1987). In this same study, plasma somatotropin concentrations were also significantly increased in cimaterol fed lambs. It also appears that β-agonists may have differential effects on type I (slow contracting, oxidative) and type II (fast contracting, oxidative/glycolytic) muscle fibres. Several recent reports indicate that b-agonist treatment increases the diameter of type II fibres to a much greater extent than type I fibres (Maltin et al. 1986; Beermann et al. 1987; Kim et al. 1987). Although all of this information is useful, it is primarily observational in nature. More funadmental studies at the molecular level will be necessary to elucidate the mechanisms by which β-agonists stimulate muscle growth.

CONCLUSION

Although we have made significant progress in elucidating the mechanisms and regulation of muscle growth, many important questions remain to be answered. Many of these involve the fundamental biological processes regulating muscle growth in meat-producing animals. To answer these questions,

scientists who work with meat animals must make even greater use of current techniques in biochemistry and molecular biology.

REFERENCES

Adams, S.O., Nissley, S.P., Handwerger, S. and Rechler, M.M. (1983). *Nature* 302:150.

Angeras, U. and Hasselgren, P. (1985). *Acta Endocrinologica* **109**:90.

Allen, R.E. and Bohorn, L.K. (1987). *Journal of Cellular Physiology* **133**:567.

Allen R.E., Dobson, M.V. and Luiten, L.S. (1984). *Experimental Cell Research* **152**:154.

Allen, R., Masak, K.C., McAllister, P.K. and Merkel, R.A. (1983). *Journal of Animal Science* **56**:833.

Armstrong, J.M. Borstein, J., Bromley, J.O., Macaulay, S.L. and Ng, F.M. (1983). *Acta Endcrinologica* **102**:492.

Baker, P.K. Dalrymple, R.H., Ingle, D.H., Ricks, C.A. (1984). *Journal of Animal Science* **59**:1256.

Ball, E.H. and Sanwall, B.D. (1980). *Journal of Cell Physiology* **102**:27.

Ballard, F.J., Read., L.C. Francis, G.L., Bagley, C.J. and Wallace, J.C. (1986). *Biochemical Journal* **233**:223.

Beermann, D.H., Hogue, D.E. Fishell, V.K., Dalrymple, R.H. and Ricks, C.A. (1986). *Journal of Animal Science* **62**:370.

Beermann, D.H., Butler, W.R. Hogue, D.E., Fishell, W.K., Dalrymple, R.H., Ricks, C.A. and Scanes, C.G. (1987). *Journal of Animal Science* **65**:1514.

Beguinot, F., Kahn, C.R., Moses, A.C. and Smith, R.J. (1985). *Journal of Biological Chemistry* **260**:15892.

Bird, J.W.C. and Carter, J.H. (1980). In "Degradative Processes in Heart and Skeletal Muscle" (K. Wildenthal, ed.), pp. 51, Elsevier/North Holland Biomedical Press, New York.

Bohorov, O., Buttery, P.J., Correia, J.H.R.D. and Soar, J.B. (1987). *British Journal of Nutrition* **57**:99.

Campion, D.E., Purchas, R.W., Merket, R.A. and Romsos, D.R. (1984). Proceedings of the Society for Experimental Biology and Medicine **176**:143.

Carter, W.J.,, Benjamine, W.S.W. and Faas, F.H. (1982). *Biochemical Journal* **204**:69.

Chung, C.S., Etherton, T.D. and Wiggins, J.P. (1985). *Journal of Animal Science* **60**:118.

Ciechanover, A., Hod, Y. and Hershko, A. (1978). *Biochemical and Biophysical Research Communications* **81**:1100.

Clemmons, D.R. and Shaw, D.S. (1986). *Journal of Biological Chemistry* **261**:10293.

Czech, M.P. (1986). *Journal of Animal Science* **63**(Suppl. 2):27.

Dalrymple, R.H., Baker, P.K, Gingher, P.E., Ingle, D.L., Pensack, J.M. and Ricks, C.A. (1984). *Poultry Science* **63**:2376.

Dayton, W.R., Goll, D.E., Stromer, M.H., Reville, W.J., Zeece, M.G. and Robson, R.M. (1975). *In* Cold Spring Harbor Series on Cell Proliferation. II. Proteases and Biological Control, pp. 551. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Dayton, W.R., Goll, D.E., Zeece, M.G., Robson, R.M. and Reville, W.J. (1976a). *Biochemistry* **15**:2159.

Dayton, W.R. Schollmeyer, J.V., Lepley, R. and Cortes, L. (1981). *Biochemica et Biophysica Acta* **659**:48.

Dayton, W.R. and Schollmeyer, J.V. (1982). *Experimental Cell Research* **136**:423.

D'Ercole, A.J., Hill, D.J., Strain, A.J. and Underwood, L.E. (1986). *Pedatric Research* **20**:253.

D'Ercole, A.J., Stiles, A.D. and Underwood, L.E. (1984). Proceedings of the National Academy of Science USA **81**:935.

Devlin, R.B. and Emerson, C.P., Jr. (1978). *Developmental Biology* **69**:202.

Dodson, M.V., Allen, R.E. and Hossner, K.L. (1985). *Endocrinology* **117**:2357.

Dollenmeier, P., Turner, D.C. and Eppenberger, H.M. (1981). *Experimental Cell Research* **135**:47.

Esch, F., Baird, A., Ling, N., Veno, N., Hill, F., Denoroy, L., Klepper, R., Gospodarowicz, D., Bohlen, P. and Guillemin, R. (1985). Proceedings of the National Academy of Science USA **82**:6507.

Evinger-Hodges, J.J., Ewton, D.Z., Seifert, S.C. and Florini, J.R. (1982). *Journal of Cell Biology* **93**:395.

Ewton, D.Z., Falen, S.L. and Florini, J.R. (1987). *Endocrinology* **120**:115.

Ewton, D.Z. and Florini, J.R. (1980). *Endocrinology* **106**:577.

Ewton, D.Z. and Florini, J.R. (1981). *Developmental Biology* **86**:31.

Flaim, K.E., Copenhaver, M.E. and Jefferson, L.S. (1980). American Journal of Physiology **239**:E88.

Florini, J.R. (1985). *Journal of Animal Science* **61**(Suppl. 2):21.

Florini, J.R. (1987). *Muscle and Nerve* **5**:577.

Florini, J.R. and Ewton, D.Z. (1981). *In Vitro* **17**:763.

Florini, J.R. Ewton, D.X., Evinger-Hodges, M.J., Fallen, S.L., Lau, R.L., Rgan, J.F. and Vertel, B.M. (1984). *In Vitro* **20**:942.

Florini, J.R. Nicholson, M.L. and Dulak, N.C. (1977). *Endocrinology* **101**:32.

Florini, J.R. and Roberts, S.B. (1979). *In Vitro* **15**:983.

Florini, J.R., Roberts, A.B. Ewton, D.Z., Falen, S.L., Flanders, K.C. and Sporn, M.B. (1986). *Journal of Biological Chemistry* **261**:16509.

Forsberg, N.E. and Merrill, G. (1986). *Journal of Animal Science* **63**(Suppl. 1):222.

Frayan, K.N. and Maycock. P.F. (1979). *Biochemical Journal* **184**:323.

Fulks, R.M., Li, J.B. and Goldberg, A.L. (1975). *Journal of Biological Chemistry* **250**:290.

Furuno, K. and Goldberg, A.L. (1986). *Biochemical Journal* **237**:859.

Gimenez-Gallego, G., Rodkey, J., Bennet, C., Rios-Candelore, M., DiSulvo, J. and Thomas K. (1985). *Science* **230**:1385.

Gospodarowicz, D., Ferrara, N., Schweigerer, L. and Newfeld, G. (1987). *Endocrine Reviews* **8**:95.

Gospodarowicz, D., Weseman, J., Moran, J.S. and Lindstrom, J. (1976). *Journal of Cell Biology* **70**:395.

Gustafson, TA., Markham, B.E. and Morkin, E. (1986). *Circulation Research* **59**:194.

Han, V.K.M., D'Ercole, A.J. and Lund P.K. (1987a). *Science* **236**:193.

Han, V.K.M., Hill, D.J. Strain, A.J., Towle, A.C., Lauder, J.M., Underwood, L.E. and D'Ercole, A.J. (1987b). *Pediatric Research* **22**:245.

Harbison, S.A., Goll, D.E., Parrish, F.C., Wang, V. and Kline, E.A. (1976). *Growth* **40**:253.

Harper, J.M.M., Soar, J.B. and Buttery, P.J. (1987). *Endocrinology* **112**:87.

Hershko, A., Ciechanover, A. (1982). *Annual Review of Biochemistry* **51**:335.

Hershko, A., Ciechanover, A., and Rose, I.A. (1979) Proceedings of the National Academy of Science USA **76**:3107.

Hershko, A., Heller, H., Eytan, E., Kaklij, G. and Rose, I.A. (1984). Proceedings of the National Academy of Science USA **81**:7021.

Hill, D.J., Crace, C.J. and Milner, R.D.G. (1985a). *Journal of Cell Physiology* **125**:337.

Hill, D.J., Crace, C.J., Nissley, S.P., Morrell, D., Holder, A.T. and Milner R.D.G. (1985b). *Endocrinology* **117**:1061.

Hill D.J., Crace, C.J., Strain, A.J. and Milner, R.D.G. (1986). *Journal Clinical Endocrinology and Metabolism* **62**:753.

Izumo, S., Nadal-Ginard, B. and Mahdavi, V. (1986). *Science* **231**:597.

Janeczko, R.A. and Etlinger, J.D. (1984). *Journal of Biological Chemistry* **259**:6292.

Jefferson, L.S., Li, J.B. and Rannels, S.R. (1977). Proceedings of the National Academy of Science USA **69**:816.

Jennische, E. and Hansson, H.-A. (1987). *Acta Physiologica Scandinavica* **130**:327.

Jennische, E., Skottner, A., and Hansson, H.-A. (1987). *Acta Physiologica Scandinavica* **129**:9.

Kardami, E., Spector, D. and Strohman, R.C. (1985a). Proceedings of the National Academy of Science USA **82**:8044.

Kardami, E., Spector, D. and Storhman, R.C. (1985b). *Developmental Biology* **112**:353.

Kelly, A.M. and Rubenstein, N.A. (1986). *In* "Molecular Biology of Muscle Development" (C. Emmerson, D. Rischman, B. Nadal-Ginard and M.A.Q. Siddiqui, Eds.), Vol., 29, pp. 77-84. A.R. Liss, New York.

Kim, Y.S., Lee, Y.B. and Dalrymple, R.H. (1987). *Journal of Animal Science* **65**:1392.

King, G.L., Kahn, C.R., Rechler, M.M. and Nissley, S.P. (1980). *Journal of Clinical Investigation* **66**:130.

Kotts, C.E., White, M.E., Allen, C.E. and Dayton, W.R. (1987). *Journal Animal Science* **64**:623.

Lathrop, B., Olson, E. and Glaser, L. (1985a). *Journal of Cell Biology* **100**:1540.

Lathrop, B., Thomas, K. and Glaser, L. (1985b). *Journal of Cell Biology* **101**:2194.

Laurent, G.J., Sparrow, M.P. and Millward, D.J. (1978). *Biochemical Journal* **176**:407.

Lepley, R.A., Pampush, M. and Dayton, W.R. (1985). *Biochemica et Biophysica Acta* **828**:95.

Liberti, J.P. and Miller, M.S. (1978). *Endocrinology* **103**:29.

Linkhart, T.A., Clegg, C.H. and Hauschka, S.D. (1981). *Developmental Biology* **86**:19.

MacDonald, G.M.L. and Swick, R.W. (1981). *Biochemical Journal* **194**:811.

Machlin, L.J. (1972). *Journal of Animal Science* **35**:794.

Maltin, C.A., Delday, M.I. and Reeds, P.J. (1986). *Bioscience Reports* **6**:293.

Manchester, K.L. and Young, F.E. (1970). *Biochemistry Journal* **77**:386.

Markham, B.E., Bahl, J.J., Gustafson, T.A. and Morkin, E. (1987). *Journal of Biological Chemistry* **262**:12856.

Marquardt, H., Todaro, G.J., Henderson, L.E. and Oroszlan, S. (1981). *Journal of Biological Chemistry* **256**:6859.

Maruyama, K., Sunde, M.L. and Swick, R.W. (1978). *Biochemical Journal* **176**:573.

Massague, J. (1987). *Cell* **49:437.**

Matsukura, Y., Okitani, A., Nishimuro, T. and Katt, H. (1981). *Biochemica et Biophysica Acta* **662**:41.

Mauro, A. (1961). *Journal of Biophysical Biochemistry Cytology* **9**:493.

Merrill, G.F., Florini, J.R. and Dulak, N.C. (1977). *Journal of Cell Physiology* **93**:173.

Miller, J.B., Crow, M.T. and Stockdale, F.E. (1985). *Journal of Cell Biology* **101**:1643.

Miller J.B. and Stockdale, F.E. (1986a). Proceedings of the National Academy of Science USA **83**:3860.

Miller, J.B. and Stockdale, F.E. (1986b). *Journal of Cell Biology* **103**:2197.

Millward, D.J., Garlick, P.J., Stewart, R.J.C., Nanyelugo, D.O. and Waterlow, J.C. (1975). *Biochemical Journal* **150**:235.

Monier, S. and LeMarchand-Brustel, Y. (1984). *Molecular and Cellular Endocrinology* **37**:109.

Moses, A.C., Nissley, S.P., Short, P.A., Rechler, M.M. and Podskalny, M.M. (1980). *American Journal of Anatomy* **122**:555.

Moss, F.P. and Leblond, C.P. (1970). *Journal of Cell Biology* **44**:459.

Moss, F.P. and Leblond, C.P. (1971). *Anatomical Record* **170**:421.

Muir, L.A. (1985). *Journal of Animal Science* **61**(Suppl. 2):154.

Narusawa, M., Fitzsimons, R.B., Izumo, S., Nadal-Ginard, B., Rubinstein, N.A. and Kelly, A.M. (1987). *Journal of Cell Biology* **104**:447.

Olwin, B.B. and Hauschka, S.D. (1986). *Biochemistry* **25**:3487.

Pain, V.M. and Garlick, P.J. (1974). *Journal of Biological Chemistry* **249**:4510.

Powell, S.E. and Aberle, E.D. (1975). *Journal of Animal Science* **40**:476.

Reeds, P.J., Hay, S.M. Doorwood, P.M. and Palmer, R.M. (1986). *British Journal of Nutrition* **56**:249.

Ricks, C.A., Dalrymple, R.H., Baker, P.K. and Ingle, D.L. (1984). *Journal of Animal Science* **59**:1247.

Rinderknecht, E. and Humbel, R.E. (1978a). *Journal of Biological Chemistry* **253**:2769.

Rinderknecht, E. and Humbel, R.E. (1978b). *FEBS Letters* **89**:283.

Robbins, J., Horan, T., Garlick, J. and Kropp, K. (1986) *Journal of Biological Chemistry* **261**:6606.

Rothwell, N.J. and Stock, M.J. (1985). *Bioscience Reports* **5**:755.

Rutz, R. and Hauschka, S.D. (1982). *Developmental Biology* **91**:103.

Satoyoshi, E., Murokami, K., Kowa, H., Kinoshita, M., Noguchi, K., Hoshina, S., Nishiyama Y. and Kinihiko, I. (1963). *Neurology* **13**:645.

Schwartz, W.N. and Bird, J.W.C. (1977). *Biochemical Journal* **167**:811.

Schmid, Ch., Steiner, Th. and Froesch, E.R. (1983). *FEBS Letter* **161**:117-121.

Schoenle, E., Zapf, J., Humbel, R.E. and Froesch, E.R. (1982). *Nature* **296**:252

Seed, J. and Jauschka, S.D. (1984). *Developmental Biology* **106**:389.

Spizz, G., Roman, D., Strauss, A. and Olson, E.N. (1986). *Journal of Biological Chemistry* **261**:9483.

Stockdale, F.E. and Holtzer (1961).

Stockdale, F.E. and Miller, J.B. (1987). *Developmental Biology* **123**:1.

Stockdale, F.E., Miller, J.B., Schafer, D.A. and Crow, M.T. (1986). *In* "Molecular Biology of Muscle Development" (C. Emerson, D. Fischman, B. Nadal-Ginard and M.A.Q. Siddiqui, Eds.), Vol. 29, pp.213-223. A.R. Liss, New York.

Svoboda, M.E., Van Wyk, J.J., Klapper, D.G., Fellows, R.R., Grissom, F.E. and Schlueter, R.J. (1980). *Biochemistry* **19**:790.

Swatland, H.J. (1977). *Journal of Animal Science* **44**:759.

Trenkle, A., DeWitt, D.L. and Topel, D.G. (1978). *Journal of Animal Science* **46**:1597.

Walsh, K. and Schimmel, P. (1987). *Journal of Biological Chemistry* **262**:9429.

White, N.K., Bonner, P.H., Nelson, D.R. and Hauschka, S.D. (1975). *Developmental Biology* **44**:346.

Wice, B., Milbrandt, J. and Glaser, L. (1987). *Journal of Biological Chemistry* **262**:1810.

Winick, M. and Noble, A. (1966). *Journal of Nutrition* **89**:300.

Young, R.B., McGee, C.E., Moriarity, D.M. Richter, H.E., Campbell, M.R., Maupin, J.A. and Hudson, J.R. (1987). *Federation Proceedings* **46**:1020.

Yu, K.-T. and Czech, M.P. (1984). *Journal of Biological Chemistry* **259**:3090.

Zapf, J., Rinderknecht, E., Humbel, R.E. and Froesch, E.R. (1978). *Metab. Clin. Exp.* **27**:1803.

Zeman, R.J., Ludemann, R. and Etlinger, J.D. (1987). *American Journal of Physiology* **252**:E152.