ENDOCRINOLOGY OF THE MYOGENIC CELL[1]

by
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Many tissues and organs are influenced by circulating hormones. Some hormones regulate short term metabolic activities in cells while other hormones exert a longer ranged influence on differentiation and growth. The long range hormone actions, in particular, the role of hormones in regulating muscle growth, will be focused on in this discussion. Other presentations in this series have dealt with the integrated regulation of muscle and adipose tissue growth at the whole animal and tissue level. In contrast, the purpose of this review is to take a very narrow, in-depth view of hormones and growth factors that impinge directly on myogenic cells, the response elicited, the possible mechanism of action, and ultimately, the impact these events may have on muscle growth. Because our goal is the efficient production of muscle protein, understanding the factors that regulate muscle mass is essential.

For the purpose of this discussion we are defining myogenic cells as cells in the pathway of differentiation between mesenchyme cells of the embryonic mesoderm and terminally differentiated myoblasts (Figure 1). This last point deserves some elaboration. Stating that a myoblast is a biochemically differentiated muscle cell reflects the fact that mononucleated myoblasts possess the same biosynthetic capabilities as multinucleated myotubes or muscle fibers. Changes occurring as myoblasts transformed into muscle fibers are the result of increased accumulation and utilization of muscle specific molecules and not activation of new genes and synthesis of new, different molecules (reviewed by Young and Allen, 1979). For example, myoblasts are post-mitotic cells, capable of synthesizing and assembling myofibrillar proteins and capable of synthesizing molecules required for cell fusion. Myotubes and muscle fibers possess the identical capabilities.

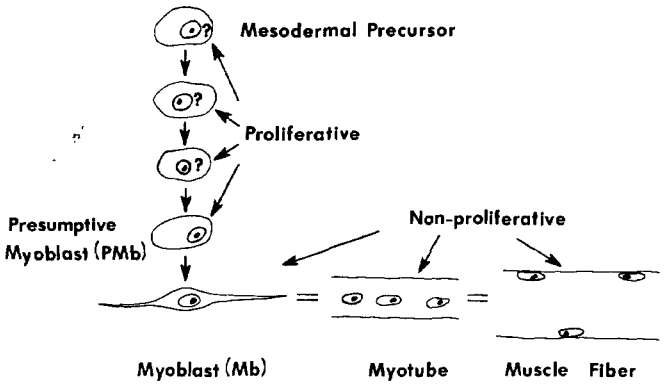

FIGURE 1

Myogenic cells differentiate from precursor cells in the embryonic mesoderm. Several states of differentiation have been postulated to exist between mesodermal cells and terminally differentiated myoblasts. These cells have the ability to proliferate, thus, increasing the size of the myogenic cell population. Presumptive myoblasts are the last proliferative cells in the myogenic lineage, and they differentiate directly into non-proliferating myoblasts, which have identical biosynthetic capabilities as myotubes and muscle fibers.

Little Known About Cells Which Precede Myoblasts

Cells that precede myoblasts in the myogenic lineage are actually in a different compartment or state of differentiation. Unfortunately, very little is known about these cells. Presumptive myoblasts are cells in the compartment immediately antecedent to myoblasts. They are capable of DNA synthesis and cell division and are ultimately capable of dividing to form one or two terminally differentiated myoblasts. They do not synthesize any muscle specific proteins and, at this time, no one has documented any specific proteins that are synthesized exclusively by presumptive myoblasts. At least one or two presumptive myoblast precursor cell types or states of differentiation have been postulated, although even less is known about these cells than about presumptive myoblasts. The crucial point regarding these myogenic cells is that they are cells with proliferative potential, and, therefore, any hormone or growth factor that regulates muscle cell number in the embryo must be acting on these myogenic cells.

Muscle cell proliferation and differentiation are

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generally considered to be a prenatal activities. However, most of the DNA that ultimately accumulates in a muscle is synthesized after birth (Cheek et al., 1965; Enesco and Puddy, 1964; Harbison et al., 1976; Johns and Bergen, 1976; Moss, 1968; Moss et al., 1964; Winick and Noble, 1966). Consequently, cells with some of the same capabilities as presumptive myoblasts must be present and active in post-natal muscle. In fact, this is precisely the role of muscle satellite cells. Satellite cells are small mononucleated cells residing between the plasmalemma and the basement membrane of muscle fibers. They have the ability to synthesize DNA, divide, and make molecules necessary for fusion into adjacent muscle fibers. These are clearly properties of embryonic presumptive myoblasts, and satellite cells actually demonstrate this myogenic potential in vitro (Figure 2). Satellite cells start as mononucleated cells, proliferate, differentiate, fuse to form myotubes and synthesize myofibrillar proteins. At this point, no biochemical evidence distinguishes presumptive myoblasts from satellite cells. It is conceivable that they are identical cell types. Again, the crucial point is that any hormone or growth factor that regulates DNA synthesis in muscle must be acting on satellite cells or presumptive myoblasts.

Assuming myogenic cell proliferation is an important aspect of muscle growth, the relationship of proliferative activity to muscle growth and development becomes an essential element in our understanding of the total growth process. The proliferative activity of cells in developing embryonic muscle has been extensively documented (Marchok and Herrmann, 1967). Using light microscope autoradiographic techniques, the percentage of actively proliferating muscle cells was monitored in chick leg muscle from 7 days of embryonic life through 8 days post-hatching. In a 7 day embryo, 69% of the cells were proliferating; this percentage dropped to 29% by 11 days, 5.4% by day 18 of embryonic life and 1% in 5 and 8 day hatched chicks. Although the percentage of the total muscle nuclei population participating in cell division is drastically reduced during the latter stages of embryonic life, this population of proliferating myogenic cells (satellite cells) remains active during post-natal life as the data of Moss (1968) indicates (Figure 3). The information in Figure 3 was adapted from Moss (1968) and illustrates the increase in muscle nuclei number in the gastronemius muscle of male white leghorn chickens. Nuclei numbers were originally calculated from total muscle DNA content. As indicated, a tremendous increase in muscle nuclei occurs during the first 18 weeks post-hatching. In addition, Figure 3 also demonstrates the intimate relationship between DNA accretion and post-natal muscle growth. Consequently, myogenic cell proliferation may be considered as an integral part of prenatal growth and the rapid phase of post-natal muscle growth.

Trophic Factor Activity

If circulating hormones or growth factors are involved in regulating the activity of myogenic cells in growing muscle, the level of such factors could conceivably be elevated during the period of time when cell proliferation is most active. Kohama and Ozawa (1978) monitored the "muscle trophic factor" activity in chicken serum during development from 11 days of embryonic life through 200 days post-natally. Trophic factor activity, as defined in this work, is the ability of serum to stimulate the proliferation of chick embryo muscle cells in culture. During embryonic life, a peak of activity occurred from days 11 to 13, and the activity diminished to a minimum value on day 16. So far, these results are consistent with the high proportion of proliferating cells in 11-day chick embryos and the subsequent decline in this cell population during latter embryonic life (Marchok and Herrmann, 1967). Beyond 16 days, however, the level of trophic factor activity increases to a maximum while the size of the proliferating myogenic cell population continues to decline (Kohama and Ozawa, 1978). About 18 days post-hatching, the level of activity increases back to concentrations similar to that found in 11-day embryo serum, and in 30-day males the activity increases again and then maintains a relatively high level through 200 days of age; the increasing level of trophic factor activity at 30 days coincides with the increased rate of DNA accretion occurring in chicken gastrocnemius muscle from 20-40 days (Moss, 1968). Noteworthy in this regard is the observation that the activity in female serum does not increase during this time period and actually decreases slightly, although DNA accretion is still occurring. While trophic factor activity in chicken serum is not perfectly correlated with changes in the proliferating myogenic cell population in embryonic muscle or the pattern of DNA accretion in post-hatching growing muscle, a relationship seems to be present that warrants further investigation.

Even though a relationship between serum trophic factor activity and muscle growth was suggested, serum is such a complex mixture that the role of specific hormones or factors and their regulation remains a mystery. Consequently, we shall approach this problem from a different angle and seek to determine which hormones or growth factors have the biological potential for regulating myogenic cell growth, based on their actions in muscle as well as other tis-

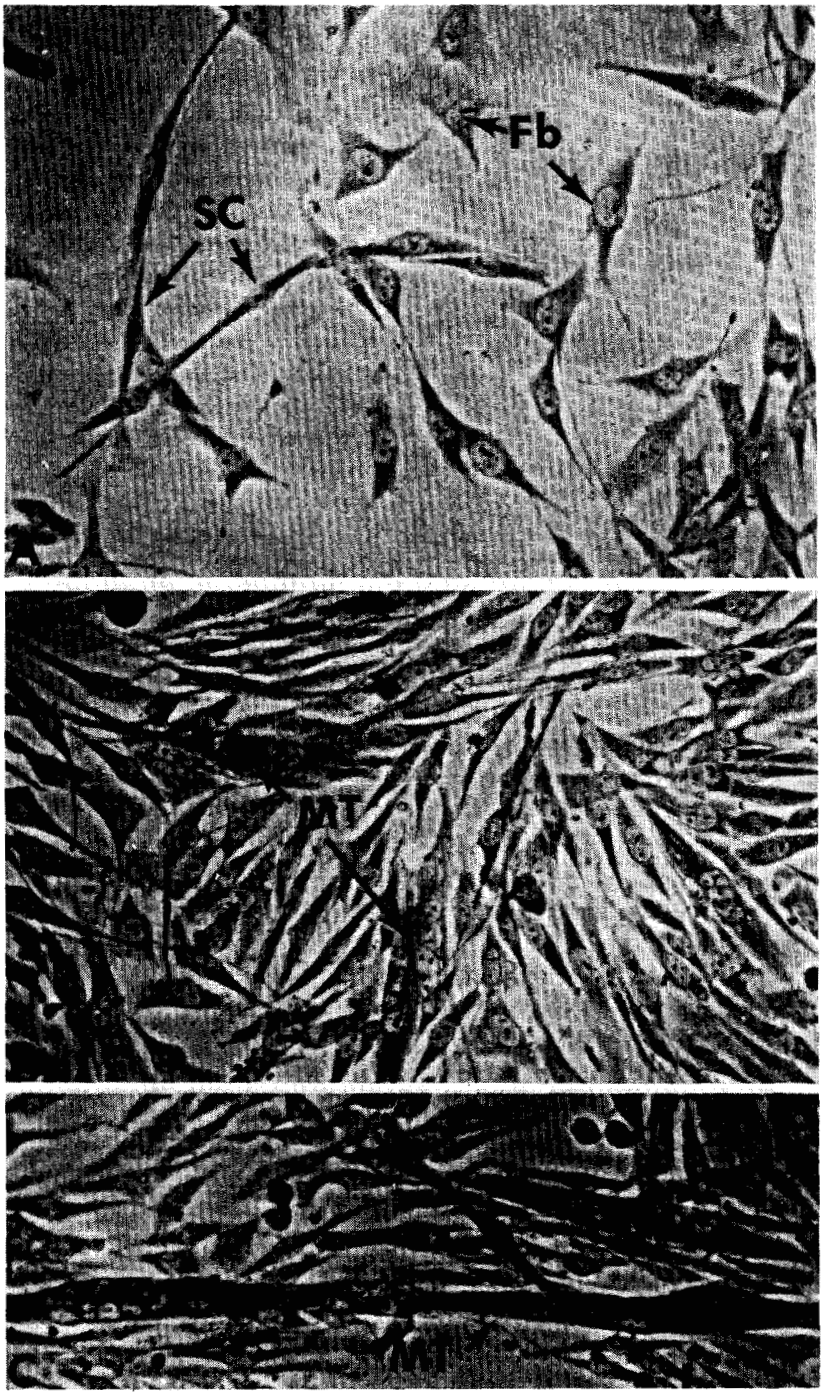

FIGURE 2

In vitro differentiation of rat muscle satellite cells. (A) Mononucleated satellite cells (SC) and fibroblasts (Fb) from young cultures. (B) Initial aggregation and fusion of satellite cells into myotubes. (C) Multinucleated myotube from 4-day satellite cell culture.

sues. The potential effector molecules will be arbitrarily grouped as steroid and protein molecules. The possible effect of these molecules and their mode of action will be discussed.

Steroid Hormones Having Potential to Stimulate Myogenic Cell Proliferation

In considering steroid hormones that could have the potential to stimulate myogenic cell proliferation and

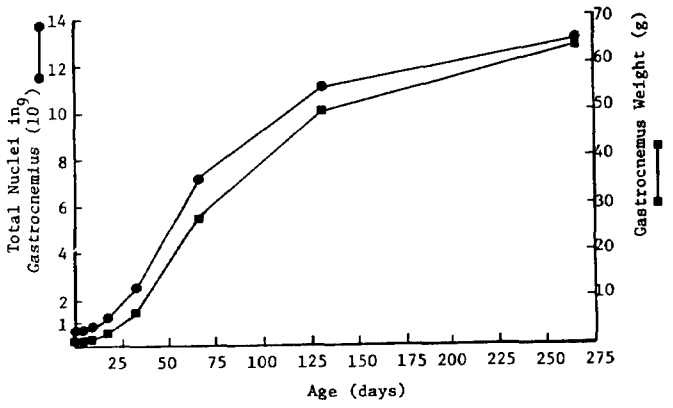

FIGURE 3

Gastrocnemuis muscle nuclei number (●) and weight (■) from growing white leghorn male chickens (adapted from data reported by Moss, 1978).

differentiation, testosterone would appear to be the most likely candidate. The first experiments to demonstrate a direct effect of testosterone on myogenic cells were conducted by Powers and Florini (1975). In these experiments cultured cells from a rat muscle cell line were exposed to 10^{-8} M testosterone for 48 hr, with a 1 hr pulse of ^{3}H-thymidine during the final hour. Afterwards, cells were prepared for autoradiographic analysis, and the percentage of ^{3}H-labeled cells was determined. This percentage is referred to as a "labeling index." Cells treated with testosterone consistently had a 25% greater labeling index than untreated cultures, thus indicating that testosterone was able to stimulate myogenic cell proliferation. Other investigators, however, have not been able to detect any mitogenic action on the part of testosterone (Gospodarowicz et al., 1976; Ozawa and Kohama, 1978).

Even though the action of testosterone on muscle is not completely clear, testosterone has been shown to stimulate cell proliferation in target organs other than muscle. Cell proliferation in the seminal vesicle and coagulating gland of castrated mice was stimulated by testosterone (Alison et al., 1976; McHanwell et al., 1976), and recently, Mainwaring (1976) found increased synthesis of certain prostate proteins when castrated rats were given testosterone injections. Furthermore, proteins in this fraction possessed DNA binding and unwinding activity, which led the authors to suggest that this protein may be part of the mechanism responsible for testosterone stimulation of DNA synthesis and proliferation.

Recently, glucocorticoids have been used to stimulate rat myogenic cell proliferation and differentiation (Guerriero and Florini, 1978). Dexamethasone (10^{-7}M) caused a 30% increase in colony formation when cells were plated at clonal densities. Myogenic cell differentiation, as measured by creatine phosphokinase activity, was also stimulated by naturally occurring glucocorticoids, cortisol and corticosterone (10^{-7}M), as well as synthetic glucocorticoids, such as dexamethansone, prednisolone, triamcinolone, and betamethasone. Other closely related steroids, however, had no demonstrable effect on differentiation. Included in this group were cortisone, dehydro-corticosterone, testosterone, progesterone, deoxycorticosterone, 17-β estradiol, 5-α dihydrotestosterone, androstenedione and aldosterone (Powers and Florini, 1975; Guerriero and Florini, 1978). The apparent mitogenic effect of glucocorticoids is somewhat surprising in view of the fact that glucocorticoids are often considered as catabolic hormones in so far as muscle metabolism is concerned.

The possible mechanism of glucocorticoid action is unknown; in other cell types, however, glucocorticoids have been shown to have a permissive or potentiating effect and not a direct mitogenic effect. For example, glucocorticoids alone did not stimulate DNA synthesis in cultures of 3T3 cells; whereas, glucocorticoids in combination with fibroblast growth factor (FGF) enhanced FGF stimulation of DNA synthesis to the same level as serum (Gospodarowicz, 1974). The extent to which glucocorticoids served in a potentiating role in myogenic cell proliferation and differentiation (Guerriero and Florini, 1978) cannot be resolved at this time.

Protein Hormones Having Potential To Stimulate Myogenic Cell Proliferation

Turning from steroid to protein hormones, several hormones are encountered that are commonly associated with muscle growth and body growth in general. The two most prominant hormones, growth hormone and insulin, have no direct stimulatory action on myogenic cells at concentrations resembling physiological levels (Gospodarowicz et al., 1976; Florini et al., 1977). Insulin can be stimulatory but only at concentration two to three orders of magnitude higher than physiological ranges (de la Haba et al., 1966). The ability of other polypeptide hormones to activate myogenic cell proliferation and differentiation has also been investigated (Gospodarowicz et al., 1975). Follicle-stimulating hormone (FSH) had the least activity, while luteinising hormone (LH) and thyroid stimulating hormone (TSH) possessed measurable activity. The authors, however, attributed this activity to mitogenic contaminants in the LH and TSH preparations. As a result of these investigations, most of the common, well characterized, protein hormones have been assumed to have no direct action on myo-

genic cells. This situation is a bit confusing especially when considering the experiments of Beach and Kostyo (1968). Cheek and Hill (1970), and Trenkle (1976) which demonstrated stimulation of DNA accretion and muscle growth in hypophysectomized rats treated with growth hormone.

In recent years the mechanism of growth hormone action has been postulated to involve an intermediate hormone synthesized by the liver and stimulated by growth hormone. This intermediate hormone interacts directly with the ultimate target cells to elicit a growth response. This hormone(s) is called somatomedin (SM). Somatomedin activity was originally described by Salmon and Daughaday (1957) as a substance in normal serum that promotes sulfate incorporation into proteoglycans of cartilage *in vitro*. This activity originally known as "sulfation factor activity," was present in normal rat serum but not in the serum of hypophysectomized rats. However, after growth hormone administration, sulfation factor activity appeared in the serum of hypophysectomized rats. During the last decade somatomedin activity has been purified into three primary proteins, somatomedin A, B, and C (SM-A, SM-B and SM-C). All three are single polypeptide chains with molecular weights between 5,000 and 10,000 daltons, and all three have insulin-like properties. The three have been distinguished on the basis of their isoelectric points and the bioassay used to purify them. SM-A is a neutral polypeptide with the ability to promote SO_4 incorporation into chick embryo pelvic leaflets; SM-B an acidic protein, stimulates thymidine incorporation into glial cells, and SM-C is a basic protein that promotes thymidine and SO_4 incorporation into costal cartilage of hypophysectomized rats (the reader is referred to reviews by Gospodarowicz and Moran, 1976; Van Wyk et al., 1974 for more extensive treatment of this topic).

Although SM's are regulated, in part, by growth hormone and have biological activities that can be related to skeletal growth, the role of SM in muscle growth has not been documented. Indirect evidence, however, links SM to muscle growth. This evidence is based on the observation that a SM-like protein, multiplication stimulating activity (MSA) has the ability to stimulate rat myogenic cell proliferation in culture (Florini et al., 1977). MSA is a 10,000 dalton polypeptide produced by rat liver cells in culture; it possesses insulin-like activity, sulfation factor activity (Temin et al., 1974) and has been postulated to be closely related, if not identical, to SM. Incidentally, this hypothesis aids in rationalizing the stimulatory effect of high insulin concentration; because insulin and SM are closely related molecules, insulin may be acting as a SM analogue when it promotes muscle precursor cell replication.

We do not have direct evidence for SM action on myogenic cells, much less a description of the biochemical mechanism of action. A preview of this growth regulating event may be possible, however, by studying the action of SM and closely related proteins on other non-muscle types. SM and MSA are members of a group of growth factors (Table 1), many of which are insulin-like. Other protein factors in this group include insulin and acid-ethanol soluble non-suppressible insulin-like activity (NSILA-s). Of these insulin-like growth factors, only MSA and insulin have been demonstrated to have an effect on myogenic cell growth. Two non-insulin-like growth factors have been included in Table 1, epidermal growth factor (EGF) and fibroblast growth factor (FGF); FGF is a potent mitogen for myogenic cells but EGF has no significant effect (Gospodarowicz and Mescher, 1977). The significance of these various growth factors to this discussion, however, stems from the fact that they share some degree of sequence homology or common biological activity. Furthermore, knowledge of the mechanism of action of individual factors is beginning to accumulate, and by pooling new information about various related growth factors, we can postulate or at least speculate on the mode of action of similar protein hormones or factors that stimulate myogenic cells.

Regarding the possible mechanism of polypeptide hormone action, the first step involves recognition and binding to specific receptors on myogenic cells. Of the previously mentioned growth factors, only insulin receptors have been studied on myogenic cells (Sandra and Przybylski, 1978). High and low affinity binding sites with binding constants of 10^{10} and 2×10^9 M^{-1}, respectively, were discovered on cultured chick embryo myogenic cells. Growth hormone, thyrotropin, insulin A chain and insulin B chain could not compete with insulin for binding to these receptors. Approximately 500 sites/nucleus were present in 24 and 49 hr myoblasts and 72 hr fibroblasts; 72 and 144 hr myotubes bound 1000 and 3000 molecules of insulin per nucleus, respectively. Even though receptors were present and able to bind insulin at physiological concentrations, physiological levels of insulin did not stimulate 2-deoxyglucose and α-aminobutyric acid uptake, or uridine incorporation. Myogenic cells, therefore, do not respond to physiological concentrations of insulin even though specific receptors are present on their plasma membranes.

The presence on myogenic cells of specific receptors for the hormones and factors most likely involved

in myogenic cell growth has not been reported. However, receptors for these proteins (NSILA-s, MSA, SM-A and SM-C) have been found on other cell types (Table 2). These receptors have been shown to be specific growth receptors and not insulin receptors based on the inability of insulin in effectively compete for binding to these sites and/or the correlation between binding to these sites and the stimulation of certain cell growth responses. For example, MSA binds specifically and reversibly to chick embryo fibroblasts with a binding constant of 4.2×10^8 M^{-1} and a capacity of 58,000 molecules/cell. NSILA-s was a potent inhibitor of binding, whereas, insulin and proinsulin were only 60 and 25% as effective, respectively. The fact that these polypeptides compete for binding to growth receptors and stimulate fibroblast DNA synthesis at comparable levels tends to support the contention that proliferation is mediated through these specific growth peptide receptors (Rechler et al., 1977). Growth receptors such as these might be expected to be present on presumptive myoblasts and satellite cells.

As we attempt to bridge the gap between receptor binding and stimulation of DNA synthesis, we depart from traditional mechanisms of protein hormone action in which the protein hormone binds to a receptor on the cell membrane and generates a second chemical message, such as a cyclic nucleotide. The second message subsequently sets off a series of biochemical events ultimately resulting in a specific physiological response. Recently, Goldfine et al. (1978) have presented evidence that insulin may be internalized after initial binding. Using electron microscope autoradiography, they documented the time dependent entry of ^{125}I-labeled insulin into cultured lymphocytes; within 30 min 40% of the ^{125}I-insulin was inside the cell, mostly associated with the endoplasmic reticulum and nucleus. In an extension of this research, they demonstrated specific ^{125}I-insulin binding to intact, purified rat liver nuclei (Vigneri et al., 1978), and after fractionation, showed that 70% was associated with the nuclear membrane. Furthermore, ^{125}I-insulin also bound to purified nuclear membrane high and low affinity sites with dissociation constants of 6.1 and 65.6 nM. These constants differed significantly from high and low affinity insulin binding sites on the plasma membrane; therefore, insulin binding to nuclear membrane preparations could not be attributed to plasma membrane contamination. Neither growth hormone, prolactin, thyrotropin nor glucagon competitively inhibited insulin binding to nuclear membranes.

Epidermal growth factor has also been shown to move into the cell after binding (Gorden et al., 1978). Electron microscope autoradiography was used to demonstrate initial binding to the plasma membrane of human fibroblasts followed by a time-temperature dependent interalization of the ^{125}I-EGF. At steady state about one-third of the labeled EGF was bound to the plasma membrane and two-thirds internalized. Most of the internalized EGF was associated with lysosomal structures and not mitochondria or rough endoplasmic reticulum. Translocation to the nucleus was not reported as with insulin.

This same process was studied biochemically (Das and Fox, 1978) by covalently attaching ^{125}I-EGF to its receptor on mouse 3T3 cells using a photoaffinity labeling technique. The fate of the EGF-receptor complex could be followed by autoradiography of SDS-gels. Initially a labeled 190,000 dalton complex was present on SDS gels. With time the complex was internalized and sequentially degraded to labeled fragments of 62,000, 42,000 and 37,000 daltons. These products were fractionated exclusively with lysosomes, consistent with the EMAR results of Gorden et al. (1978). Furthermore, receptor internalization and degradation and DNA synthesis were stimulated half-maximally at the same EGF concentration, 0.1 nM.

At this point, we cannot complete the circuit from protein hormone binding to initiation of DNA synthesis and cell division. And again, none of the previously mentioned mechanistic investigations have been conducted with myogenic cells or with any of the somatomedins. These studies, however, serve to elicit the hypothesis that the mechanism of SM action on myogenic cells may be similar to the mode of action of other protein growth factors on their target cells. Possible mechanisms of action are presented schematically (Figure 4), as a specific hormone binds to a growth peptide receptor on a myogenic cell, several alternative actions are available. 1) Upon binding to the receptor a second message could be generated that would subsequently initiate DNA synthesis and cell division through some unknown mechanism. 2) The hormone-receptor complex or the hormone itself, could be internalized and translocated to the nucleus where the hormone would bind to specific receptors on the nuclear envelope. This complex could then initiate DNA synthesis directly or via a second message such as a cyclic nucleotide or polyamine. 3) The hormone-receptor complex could be internalized into lysosomes and gradually degraded to active fragments that could subsequently stimulate DNA synthesis directly or via a second message system. It is particularly difficult to complete the picture because the molecular mechanisms responsible for initiating and carrying out cell proliferation are unknown.

TABLE 1
PROTEIN GROWTH FACTORS

	Molecular Weight	Source	Stimulated by Growth Hormone	Sulfation factor Activity	Stimulates DNA Synthesis	Effect on Myogenic Cells	Reference
Somatomedin A	7000	Serum	yes	yes	yes	?	Hall et al., 1975
Somatomedin B	5000	Serum	yes	no	yes	?	Sievertsson et al., 1975
Somatomedin C	7000	Serum	yes	yes	yes	?	Van Wyk et al., 1975
Multiplication Stimulating Activity (MSA)	10,000	Liver Cell Cultures	yes?	yes	yes	DNA synthesis	Temin et al., 1974; Florini et al., 1977
NSILA-S Insulin-like growth factor I (IGF-I)	7,649	Human plasma	yes	yes	yes	?	Zapf et al., 1978
Insulin-like growth factor II (IGF-II)	7,471	Human plasma	yes?	yes	yes	?	
Insulin	6000	Pancreas	no	yes?	yes	DNA Synthesis	Van Wyk et al., 1975 de la Haba et al., 1966
Fibroblast growth factor (FGF)	13,400	Brain and pituitary	no	no	yes	DNA Synthesis	Gospodarowicz and Moran, 1976
Epidermal growth factor (EGF)	6,045	Mouse submaxillary gland	no?	no	yes	No Effect	Gospodarowicz and Moran, 1976

TABLE 2

SPECIFIC RECEPTORS FOR PROTEIN GROWTH FACTORS
(Insulin does not effectively compete for binding)

Growth factor	Cell Type	Binding	Number of Molecules Bound	Biological Response	Reference
Insulin	Cultured Muscle	$Ka=10^{10}$ & $2 \times 10^{9}\ M^{-1}$			Sandra and Przybylski, 1979
	PMB		500/cell	None?	
	MT		3000/cell		
MSA	Chick embryo fibroblasts	$Ka = 4.2 \times 10^{8} M^{-1}$	58,000/cell	DNA synthesis and MSA binding were half maximum at similar concentrations	Rechler et al., 1976 Rechler et al., 1977b
	Human fibroblasts	$Ka = 2 \times 10^{8} M^{-1}$	24,000/cell	DNA synthesis	Rechler et al., 1977a
SM-A	Placental membrane	$Ka = 2.7 \times 10^{7} M^{-1}$	19 nmoles/mg of protein	?	Hall et al., 1975
SM-B	?	?		?	
SM-C	Circulating mononuclear cells	Half-maximal binding 4 nM		?	Thorsson and Hintz, Van Wyk et al., 1975
	Placental membranes	Half-maximal binding 1.7 nM		?	
IGF I	Chick embryo fibroblast	Kd=0.8-1.1 nM		DNA synthesis half-maximal stimulation at 0.4 mM	Zaph et al., 1978
	Chick embryo chondrocytes	Kd=0.6 nM		SO_4 incorporation significant stimulation a 2.5 ng/ml	
IGF II	Chick embryo fibroblast	Kd=1.1 nM		DNA synthesis half-maximal stimulation at 0.4 nM	
	Chick embryo chondrocytes	Kd=0.6-1.2 nM		SO_4 incorporation significant stimulation between 2.5 and 8.3 ng/ml	
EGF	Human fibroblasts	Half-maximal binding 0.4 nM	40,000/cell	DNA synthesis half-maximal stimulation at 10^{-10} M	Hollenberg and Cuatrecass, 1975

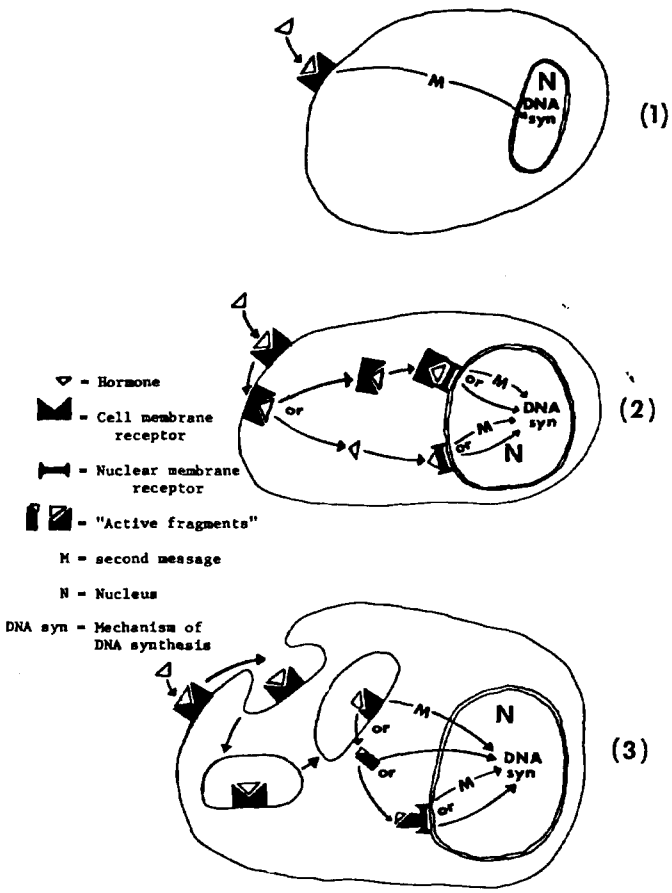

FIGURE 4

Possible mechanisms of polypeptide hormone action on proliferating myogenic cells.
(1) A polypeptide hormone (▽) binds directly to cell membrane growth receptors (◥) to stimulate synthesis of a second message (M). Through an unknown series of reactions the second message initiates DNA synthesis (DNA syn) and cell proliferation.
(2) the polypeptide hormone binds to a cell membrane receptor and is translocated with, or without, the receptor to the nuclear membrane. At the nuclear membrane binding occurs which generates a second message or acts directly to stimulate DNA synthesis.
(3) the polypeptide hormone binds to a cell membrane receptor and is internalized into lysosomes where it is proteolytically processed to generate active fragments of the hormone-receptor. The active fragments synthesize a second message or act directly, with or without binding to a nuclear receptor (⊢◥), to stimulate DNA synthesis and cell proliferation.

Conclusions

It should be obvious by now that our understanding of the endocrinology of myogenic cells is fragmented, at best. Much of what we know or suspect concerning hormone action on muscle cells, we have learned only by analogy–analogous hormones acting on analogous cells types. Of the hormones and cells discussed, some of the best candidates for muscle mass regulation are the growth hormone-controlled insulin-like growth factors (SM, NSILA-s, MSA) that may be acting on presumptive myoblasts and satellite cells. This area of investigation is clearly in its infancy.

As scientists interested in muscle growth, how should we approach the subject of endocrine control of myogenic cells, and what do we hope to gain from an understanding of this area? The problem is too complex to take a "shotgun" approach. We must be willing to make a long-term commitment without immediate payoffs. Our first goal should be to learn the biology of the cells involved and their role in muscle growth. Second, we must focus our energies on elucidating the specific effector molecules acting on important myogenic cells and their biochemical mechanism of action. Finally, we can set our sights on formulating strategies for directly manipulating muscle growth at the cellular level. For example, will we someday be able to use current recombinant DNA technology to mass produce myogenic cell-specific protein mitogens to stimulate muscle DNA accretion and consequently, muscle growth in livestock? Can we further apply our fundamental knowledge of muscle growth toward improved selection of breeding livestock? These are but a few of the challenges confronting "livestock biologists."

LITERATURE CITED

Alison, M. R., M. A. Wright, W. Taylor and S. McHanwell. 1976. Distribution and proliferative activity of testosterone and 5-dihydrotestosterone in accessory reproductive tissues of the castrated mouse. J. Endocrinol. 71:94P.

Beach, K. and J. Kostyo. 1968. Effect of growth hormone on the DNA content of muscles of young hypophysectomized rats. Endocrinology 82:882.

Cheek, D. B. and D. E. Hill. 1970. Muscle and liver cell growth: Role of hormones and nutritional factors. Fed. Proc. 29:1503.

Cheek, D. B., G. Powell and R. E. Scott. 1965. Growth of muscle cells (size and number) and liver DNA in rats and Snell Smith mice with insufficient pituitary, thyroid, or testicular function. Bull. Johns Hopkins Hospital 117:306.

Das, M. and C. F. Fox. Molecular mechanism of mitogen action: Processing of receptor induced by epidermal growth factor. Proc. Natl. Acad. Sci. USA 75:2644.

de la Haba, G., G. W. Cooper and V. Elting. 1966. Hormonal requirements for myogenesis of striated muscle *in vitro*: Insulin and somatotropin. Proc. Natl. Acad. Sci. USA 56:1719.

Enesco, M. and D. Puddy. 1964. Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Amer. J. Anat. 114:235.

Florini, J. R., M. L. Nicholson and N. C. Dulak. 1977. Effects of pepetide anabolic hormones of growth of myoblasts in culture. Endocrinology 101:1.

Goldfine, I. D., G. J. Smith, K. Y. Wong and A. L. Jones. 1977. Cellular uptake and nuclear binding of insulin in human cultured lymphocytes—Evidence for potential intracellular sites of insulin action. Proc. Natl. Acad. Sci. USA 74:1368.

Gorden, P., J. L. Carpentier, S. Cohen and L. Oric. 1978. Epidermal growth factor: Morphological demonstration of binding, internalization, and lysosomal association in human fibroblasts. Proc. Natl. Acad. Sci. USA 75:5025.

Gospodarowicz, D. and A. L. Mescher. 1977. A comparison of the responses of cultured myoblasts and chon-

drocytes to fibroblast and epidermal growth factors. J. Cell. Physiol. 93:117.
Gospodarowicz, D. and J. S. Moran. 1976. Growth factors in mammalian cell culture. Ann. Rev. Biochem. 45: 531.
Gospodarowicz, D., J. Weseman and J. Moran. 1975. Presence in brain of a mitogenic agent promoting proliferation of myoblasts in low density culture. Nature 256:216.
Gospodarowicz, D., J. Weseman, J. S. Moran and J. Lindstrom. 1976. Effect of fibroblast growth factor on the division and fusion of bovine myoblasts. J. Cell Biol. 70:395.
Guerriero, V. and J. R. Florini. 1978. Stimulation by glucocorticoids of myoblast growth at low cell densities. Cell Biology International Reports 2:441.
Hall, K., K. Takano, L. Fryklund and H. Sievertsson. 1975. Somatomedins. Adv. Metab. Disord. 8:19.
Harbison, S. A., D. E. Goll, F. C. Parish, Jr., V. Wang and E. A. Kline. 1976. Muscle growth in two genetically different lines of swine. Growth 40:253.
Hollenberg, M. D. and P. Cuatrecasas. 1975. Insulin and epidermal growth factor—Human fibroblast receptors related to deoxyribonucleic acid synthesis and amino acid uptake. J. Biol. Chem. 250:3845.
Johns, J. T. and W. G. Bergen. 1976. Growth in sheep. Pre- and post-weaning hormone changes and muscle and liver development. J. Anim. Sci. 43:192.
Kohama, K. and E. Ozawa. 1978. Muscle trophic factor: II. Ontogenic development of activity of a muscle trophic factor in chicken serum. Muscle and Nerve 1:236.
Mainwaring, W. J. P., P. S. Rennie and J. Keen. 1976. The adrogenic regulation of a prostate protein with a high affinity for deoxyribonucleic acid. Evidence for a prostate deoxyribonucleic acid unwinding protein. Biochem. J. 156:253.
Marchok, A. C. and H. Herrmann. 1967. Studies of muscle development. I. Changes in cell proliferation. Develop. Biol. 15:129.
McHanwell, S., M. R. Alison and N. A. Wright. 1976. Testosterone-induced cell proliferation in the prostate complex of the castrated mouse after unilateral removal of the seminal vesicle and coagulating gland. J. Endocrinal. 71:95P.
Moss, F. P. 1968. The relationship between the dimensions of fibres and the number of nuclei during normal growth of skeletal muscle in the domestic fowl. Amer. J. Anat. 122:555.
Moss, F. P., R. A. Simmonds and H. W. McNary. 1964. The growth and composition of skeletal muscle in the chicken. 2. The relationship between muscle weight and the number of nuclei. Poul. Sci. 43:1086.
Ozawa, E. and K. Kohama. 1978. Muscle trophic factor: III. Effect of hormones and tissue extracts on muscle-trophic factor activity. Muscle and Nerve 1:314.
Powers, M. L. and J. R. Florini. 1975. A direct effect of testosterone on muscle cells in tissue culture. Endocrinology 97:1043.
Rechler, M. M., S. P. Nissley, J. M. Podskalny, A. C. Moss and L. Fryklund. 1977a. Identification of a receptor for somatomedin-like polypeptides in human fibroblasts. J. Clin. Endocrinol. Metab. 44:820.
Rechler, M. M., J. M. Podskalny and S. P. Nissley. 1976. Interaction of multiplication-stimulating activity with chick embryo fibroblasts demonstrates a growth receptor. Nature 259:134.
Rechler, M. M., J. M. Podskalny and S. P. Nissley. 1977b. Characterization of the binding of multiplication-stimulating activity to a receptor for growth polypeptides in chick embryo fibroblasts. J. Biol. Chem. 252:3898.
Salmon, W. D. and W. H. Daughaday. 1957. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vitro*. J. Lab. Clin. Med. 49:825.
Sandra, A. and R. J. Przylbylski. 1979. Ontogeny of insulin binding during chick skeletal myogenesis *in vitro*. Develop. Biol. 68:546.
Sievertsson, H., L. Fryklund, K. Uthne, K. Hall and B. Westermark. 1975. Isolation and chemistry of human somatomedins A and B. Adv. Metab. Disord. 8:47.
Temin, H. M., G. L. Smith and N. C. Dulak. 1974. Control of multiplication of normal and rous sarcoma virus-transformed chicken embryo fibroblasts by purified multiplication-stimulating activity with nonsuppressible insulin-like and sulfation factor activities. In B. Clarkson and R. Baserga (Ed.). Control of Proliferation in Animal Cells, Cold Spring Harbor Conferences on Cell Proliferation, Vol. 1. p. 19. Cold Spring Harbor, N.Y.
Thorsson, A. V. and R. L. Hintz. 1977. Specific ^{125}I-somatomedin receptor on circulating human mononuclear cells. Biochem. Biophys. Res. Comm. 74:1566.
Trenkle, A. 1976. Endocrine regulation of skeletal muscle growth. J. Anim. Sci. 43:308, abstract.
Van Wyk, J. J., L. E. Underwood, J. B. Baseman, R. L. Hintz, D. R. Clemmons and R. N. Marshall. 1975. Explorations of the insulin-like and growth-promoting properties of somatomedin by membrane receptor assays. Adv. Metab. Disord. 8:128.
Van Wyk, J. J., L. E. Underwood, R. L. Hintz, D. R. Clemmons, S. J. Vorina and R. P. Weaver. 1974. The somatomedins: A family of insulin-like hormones under growth hormone control. Recent Prog. in Horm. Res. 30:259.
Vigneri, R., J. D. Goldfine, K. Y. Wong, G. J. Smith and V. Pezzina. 1978. The nuclear envelope. The major site of insulin binding in rat liver nuclei. J. Biol. Chem. 253:2098.
Winick, M. and A. Noble. 1966. Cellular response in rats during malnutrition at various ages. J. Nutr. 89: 300.
Young, R. B. and R. E. Allen. 1979. Transitions in gene activity during development of muscle fibers. J. Anim. Sci. 48:837.
Zaph, J., E. Rinderknecht, R. E. Humbel and E. R. Froesch. 1978a. Nonsuppressible insulin-like activity (NSILA) from human serum: Recent accomplishments and their physiologic implications. Metabolism 27:1803.
Zaph, J., Schoenle and E. R. Froesch. 1978b. Insulin-like growth factors I and II: Some biological actions and receptor binding characteristics of two purified constituents of nonsuppressible insulin-like activity of human serum. Eur. J. Biochem. 87:285.