THE EFFECT OF THYROTROPIN RELEASING HORMONE AND 3METHYL THYROTROPIN RELEASING HORMONE ON GROWTH AND HORMONAL RELEASE IN THE GROWING CALF

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This is to certify that the

thesis entitled

The Effect of Thyrotropin Releasing Hormone and 3Methyl Thyrotropin Releasing Hormone On Growth And Hormonal Release In The Growing Calf

presented by

Rodney Kenneth McGuffey

has been accepted towards fulfillment of the requirements for

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Date 5/15/75

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ABSTRACT

THE EFFECT OF THYROTROPIN RELEASING HORMONE AND 3METHYL THYROTROPIN RELEASING HORMONE ON GROWTH AND HORMONAL RELEASE IN THE GROWING CALF

By

Rodney Kenneth McGuffey

An experiment was designed: (1) to determine the effects of thyrotropin releasing hormone (TRH) and 3 methyl thyrotropin releasing hormone (3MET) on growth in calves; (2) to determine serum concentrations of growth hormone (GH), thyroxine, prolactin, and insulin during chronic injection of TRH or 3MET; and (3) to determine the effects of age and time after feeding on serum hormone concentrations.

Thirty-nine Holstein calves, 30 males and 9 females, received daily intramuscular injections of either 500 ug of TRH, 500 ug of 3MET or 0.85% saline beginning at 45 days of age and continuing to 135 days of age. Weight gain and feed intake were measured at 15-day intervals. Jugular blood was sampled at 4 ages (45, 60, 105, and 135 days) at frequent intervals after injection for the determination of serum concentrations of GH, thyroxine, prolactin, and insulin. Nine calves, 3 from each treatment,

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were slaughtered and tissue weights and gastrocnemius muscle and liver nucleic acids determined.

Calves receiving TRH gained 7.8 kg more weight ($P<0.05$) and consumed 28.8 kg feed ($P<0.05$) than control calves while differences between 3MET and control calves were not significant. Feed to gain ratio was not significant between treatments. Weight gain and feed intake of calves receiving TRH were greater ($P<0.01$) than controls from day 76 to 90 and continued to be nonsignificantly greater than controls through day 135. Calves receiving 3MET gained less weight ($P<0.01$) and consumed less feed ($P<0.01$) than control calves from day 45 to 60 but other period differences were not significant.

Serum concentration of insulin but not GH, thyroxine, and prolactin in control calves was increased by feeding ($P<0.01$). In control calves, serum concentration of insulin, thyroxine, and prolactin increased ($P<0.01$) while GH decreased ($P<0.01$) with age. The increase in serum prolactin concentration was related to environmental factors moreso than age of calf.

Overall, serum GH concentration in calves given TRH was greater than that of calves given 3MET or saline at 5, 10, 15, and 30 minutes after injection. Calves given 3MET had greater ($P<0.05$) serum GH concentration than calves given saline at 10 and 15 minutes post-injection. From 45 to 480 minutes post-injection serum GH concentration was not different between treatments.

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The difference between maximum GH (ng/ml) after injection and at injection at days 45, 60, 105, and 135 was 47.5, 35.8, 38.0, and 35.1 for TRH and 20.9, 16.8, 4.3, and 62.4 for 3MET, respectively. The difference for calves given TRH was greater ($P<0.05$) at days 45, 60, and 105 than that of 3MET.

Overall serum thyroxine concentration was increased 3.60, 4.64, and 0.81 ng/ml-hr for calves receiving TRH, 3MET, and saline injections, respectively. The increase was greater ($P<0.01$) for TRH and 3MET than that for saline. At day 45 serum thyroxine concentration was increased 6.25 and 8.65 ng/ml-hr for TRH and 3MET. Relative to day 45, the increase in serum thyroxine concentration on days 60, 105, and 135 was decreased 50 ($P<0.08$), 55 ($P<0.05$), and 60% ($P<0.05$) for TRH and 50 ($P<0.05$), 77 ($P<0.01$) and 72% ($P<0.05$) for 3MET, respectively.

Overall serum prolactin concentration in calves given TRH was greater ($P<0.01$) than that of saline at 15, 30, 45, and 60 minutes post-injection and greater ($P<0.05$) than that of 3MET at 60 minutes. Calves given 3MET had greater ($P<0.05$) serum prolactin concentration than saline-injected calves at 15, 30, and 45 minutes. Prolactin release was not depressed by repeated injections of TRH and 3MET.

Overall serum insulin (ng/ml) was 1.51, 1.19, and 1.14 for calves given TRH, 3MET, and saline, respectively, and the difference between TRH and the other two treatments

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was significant ($P<0.05$). At days 45 and 60 serum insulin concentrations were relatively stable and similar between treatments. At days 105 and 135 variable responses in serum insulin concentration to treatments especially TRH were noted.

Tissue weights were greater on an absolute basis from calves receiving TRH but these differences disappeared when expressed on a percent of body weight with the exception of the thyroid gland. Thyroid weight was greater ($P<0.05$) in calves receiving TRH than that from calves receiving 3MET or saline. Concentration of RNA and DNA in liver and gastrocnemius muscle were not different between treatments but muscle RNA/DNA ratio was 50% greater in calves receiving TRH than that of calves receiving saline. Total liver and muscle nucleic acids were greater for calves receiving TRH.

Overall plasma non-esterified fatty acids were lower ($P<0.05$) in calves receiving TRH with the major difference at day 135. Plasma glucose and nitrogen balance were unaffected by treatment.

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I. INTRODUCTION

Growth is a phenomenon of all species. Like many other scientific phenomena, description of growth cannot be embodied in simple terms and present a complete definition. Yet, the definition of growth is more simple than a complete description of factors causing growth (or lack of).

Long ago animal producers recognized that animals fed a "superior" ration grew faster than animals fed one of lesser quality. Other factors such as genetic improvement, special breeds, and environment have been shown to affect growth. More recently growth has been shown to be strongly influenced by hormones both synthetic and natural.

Today, the human population has created competition for both land and feedstuffs for animal production. An increase in animal production on less land and from lower quality rations has become a necessity to meet the pressure of this expanding population.

To meet population demands for increased production the previous factors affecting growth, i.e. nutrition, superior genetic strains or capabilities, environment, and specific growth stimulants will have to be integrated in

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some manner. For this to occur animal scientists must first formulate basic, simple questions about animal growth. Only after these questions are satisfactorily answered can the above factors be integrated to produce superior and faster growing animals.

This study was designed to investigate a new source of increasing growth in young calves and determine hormonal parameters resulting from this treatment and explore those that may alter growth.

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II. REVIEW OF LITERATURE

Description of Growth

Brody (1945) analyzed growth curves of populations of yeasts, flies, individual pumpkins, and rats and found growth curves from each species similar and that they coincided in his mathematical expression of growth. He divided the growth curve into two phases: (1) a self-accelerating phase and (2) a self-inhibiting phase. The self-accelerating phase is characterized by reproducing units to reproduce at a constant percentage rate when allowed to do so. In the absence of inhibiting factors the percentage growth rate remains constant. Finally, there comes a time when inhibiting factors override the accelerating factors until growth processes cease.

This idealized description of growth was mathematically described by Laird (1966a, 1966b). Growth before and after birth was described by an equation having an exponential and linear component. The exponential component described hyperplasia or an increase in cell numbers while the linear component described hypertrophy or an increase in cell size.

Enesco and Leblond (1962) studied growth of many tissues in rats from birth to 160 days of age. From their data they divided tissue growth into three phases. First, tissues grew by hyperplasia with minute changes in cell size; secondly, growth was characterized by both hypertrophy and hyperplasia and finally, a period that continued until mature body weight was attained where growth was characterized by hypertrophy with hyperplasia playing only a small part in the increase in size.

Changes in Chemical Constituents During Growth

Chemical composition of an animal changes throughout the growth process. At birth, water constitutes about 82% of fat-free body weight, protein about 14%, and minerals about 4% (Forbes, 1968). During growth water decreases, protein increases, and they plateau at 70-75% and 21-24%, respectively. Mineral contribution to body weight remains relatively constant on a percentage basis during growth.

Palsson and Verges (1952) reported the order of development is from inside to outside, i.e. central nervous system, bone tendon, muscle, intermuscular fat, and finally subcutaneous fat. At birth the central nervous system is essentially complete. Changes in percentage of bone, fat, and muscle then constitute body components undergoing postnatal changes.

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Berg and Butterfield (1968) described the development of bone, muscle, and fat in cattle from birth to maturity. At birth, bone represented a greater proportion of the carcass than at any other time. Fat, conversely, represented a greater proportion of the carcass at maturity than at any other time. Muscle represented a greater proportion of the carcass intermediate in development. Factors such as nutrition, breed, and sex affected the rate of growth of tissues but not order of development.

Moulton (1923) introduced the concept of chemical maturity and defined it as the age which the concentration of water, protein, and ash become constant in the fat free cell. Armsby and Moulton (1925) reported the age mammals reach chemical maturity as follows: rat, 50 days; guinea pig, 50 days; swine, 150 to 300 days; cattle, 150 to 300 days; and humans, 500 to 1000 days or at approximately 3.9 to 4.6% of the total expected life span of each species.

Reid et al. (1955) summarized published data on body composition of the bovine as influenced by sex, age, and breed. A total of 256 cattle representing 139 beef (133 males) and 117 dairy (2 males) animals ranging in age from 1 to 4860 days were used to establish relationships of body fat, water, protein, and ash. Fat and water were the body constituents exhibiting the most variation. Expression of water, protein, and ash on a fat-free empty body weight basis tended to make these components relatively constant.

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Body constituents were divided into two groups: (1) variable, which included fat and water; and (2) constant, which included protein and ash. Correlation between fat content (%) and water content (%) for 256 cattle of various ages was -0.987. Expression of protein and ash content on the fat-free dry basis rather than fat-free basis reduced variation of these components by a factor of 3 and 2, respectively. There were no differences between breed or sex of cattle in amount of protein and ash content when expressed on the fat-free dry basis. However, age of animal was found to be highly significantly correlated with protein and ash content (-0.42 and +0.42, respectively) when expressed on the fat-free dry basis. Thus, expression of protein and ash on the fat-free dry basis gave the most accurate prediction of these components. Reid et al. (1955) presented a series of equations so that with an accurate estimate of body water content and age of the animal, body composition and body energy from cattle fed under different nutritional regimes can be estimated with a relatively high degree of precision.

Nutritional Requirements of Growth

Quantitatively and probably qualitatively, nutrient requirements for growth are different than those for maintenance. Relative to maintenance, a larger proportion of protein, energy, minerals, and vitamins is required for a young growing animal than a mature animal on a body

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weight basis. A decrease in growth occurs in a young growing animal when any single or combination of these nutrients is sub-optimal.

Osborne and Mendel (1915) demonstrated that under-nutrition in the growing period delayed growth. Upon realimentation growth resumed at a more rapid rate than normally observed. McCay et al. (1935) restricted caloric intake in rats at weaning to allow approximately 10 grams in gain every 2 to 3 months. At 766 or 911 days of age these rats were fed ad libitum and an increase in body weight was noted. Final body weights were however less in these animals than those fed at a normal rate from weaning and these restricted animals lived longer.

Meyer and Clawson (1964) reported 5 month-old sheep and 1 to 2 month-old rats were able to maintain body weight, body fat, and body protein percentage when fed 52% of the amount consumed by ad libitum fed animals. When refed an amount of feed to give the same total intake as consumed by ad libitum controls, restricted rats and sheep did not attain the same body weight as controls, and final body energy in restricted rats but not sheep was less than controls. When fed to the same body weight as controls, restricted animals had a higher body fat thus greater final body energy than controls. The time required to reach control weight was proportional to severity of restriction.

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McCance and Widdowson (1962) correlated growth of suckling rats with the protein content of maternal milk. Rat pups in litters of 16 grew less than did rat pups in litters of 4 (Sinha et al., 1973). Pups from dams consuming 50% of ad libitum intake during gestation and lactation grew slower than did pups from ad libitum fed dams (Stephan et al., 1971).

Weanling rats fed 6, 12, or 18% crude protein diets grew less in 14 days than did similar rats fed 24% crude protein (Howarth, 1972). Weight of gastrocnemious muscle and its DNA, RNA, and protein content decreased with decreasing dietary protein level. Relative to accumulation of muscle constituents on the 24% crude protein diet, decreasing dietary protein resulted in a greater decrease in accumulation of DNA than protein. RNA accumulation decreased intermediate to DNA and protein with the exception of the 6% diet where there appeared to be a loss of muscle RNA. Feeding a protein free diet for 14 days resulted in a marked loss of muscle RNA but insignificant changes in DNA and protein content from that initially present.

Howarth and Baldwin (1971) fed weanling rats ad libitum and restricted some to 50 to 60% of ad libitum intake. As a result gastrocnemius muscle weight and RNA, DNA, and protein content, were less in the restricted group. This dietary restriction resulted in lowered ^{32}P-orthophosphate incorporation into RNA and DNA and

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reduced protein synthetic capacity of muscle microsomes. Refeeding the restricted group resulted in delayed recovery of protein synthetic capacity with no compensatory acceleration due to dietary restriction, yet rates of RNA and DNA synthesis during refeeding were greater than normal.

Trenkle (1974) observed a reduced growth rate in rats when fed either a calorie or protein deficient diet. Limiting protein or energy resulted in reduced muscle growth, reduced DNA, RNA, and protein content. Limiting either nutrient singly or together did not change protein/DNA and muscle weight/DNA ratios. During dietary restriction muscle continued to grow but the rate of growth was retarded. Dietary restriction limited accumulation of muscle constituents but had no effects on rate of accumulation relative to muscle growth.

Muscle is of primary interest in meat production. Dietary restrictions such as reduced protein or energy intake influences muscle growth. During tissue growth by hyperplasia, dietary restriction limits total DNA accumulation therefore limiting future growth capacity of muscle although concentrations of muscle constituents may not decrease. To attain maximum growth during hypertrophy adequate nutrition is imperative early in life.

For reasons such as time, money, and animal size the amount and detail of investigation into large animal growth is limited. There is considerable literature where growth rate under various nutritional regimes has

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been quantitated. Since many basic anabolic and catabolic mechanisms are common to all animal species, patterns of cellular growth would be expected to be similar for farm and laboratory animals. This section will review the influence of nutrition on ruminant growth and make assumptions concerning nutritional alterations of ruminant growth based on data from laboratory animals.

Jones and Hogue (1960) in a 2 x 3 factorial experiment compared energy and protein fed at 90 and 120% of Morrison standards with and without oral stilbestrol on growth in lambs. Lambs fed the high protein ration (regardless of energy) had higher average daily gain (0.34 lb) than those fed the low protein ration (0.28 lb) and ate more feed than lambs fed the low protein level. No significant effect on weight gain occurred with different energy levels. Lambs fed high protein-high energy gained faster than other combinations with low energy-high protein the most efficient combination for growth. Lambs fed high energy-low protein gained slower and were the least efficient of all treatment groups with a much lower expected consumption of estimated net energy (78.6%). Stilbestrol increased average daily gain and feed efficiency in all protein and energy combinations. These authors concluded that low protein intake relative to energy influences food intake through the adverse ratio of the two nutrients. Baile and Forbes (1974) concluded from a review of the literature that growing cattle can compensate for diet

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Willey et al. (1952) fed fattening steers low and high energy rations (58.5 and 64.5 therms respectively) with low and high levels of fat (3.0% and 7.5%) in the diet with a 12.5% crude protein content in all diets. Steers did not differ in average daily gain but animals fed high levels of fat required about 45.4 kg less feed per 45.4 kg gain during the fattening period.

Struedemann et al. (1968) raised beef calves from birth to slaughter on five nutritional levels as follows:

	Treatment Group				
	Very Restricted	Restricted	Normal	High	Very High
Weaning age, days	170	240	240	240	240
Milk production of dam	Average	Low	Average	Average	High
Supplemental feed	---	---	---	---	Creep

Between 170 and 240 days of age calves in the very restricted group were raised on limited hay and pasture with calves receiving creep feeding between 90 and 240 days of age. Calves in restricted, normal, high, and very high gained .04, .16, .25, and .23 kg per day more than very restricted calves, respectively.

When placed in the feedlot there was no significant difference in average daily gain for calves from the different nutritional regimes. The time required to reach

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a final weight of 430 kg was less for calves in the higher nutritional levels (high or very high) but, the amount of feed per unit of gain was less for the lower nutritional levels (normal, restricted, very restricted). As the level of nutrition increased during early life, carcasses from these animals tended to contain a greater percentage of lean and bone and a lower percentage of fat. Waldman et al. (1971) fed steers ad libitum or 70% of ad libitum intake. Steers fed ad libitum had higher average daily gain but less carcass protein at comparable weights than steers fed 70% ad libitum.

Gardner et al. (1960) compared the efficiency of growth of suckling single or twin lambs to 90 days of age when ewes were fed 95 or 115% National Research Council requirements for digestible energy. Lambs whose dams consumed the higher level of energy were significantly heavier at 90 days of age than lambs whose dams consumed the lower level of energy. The heavier lambs had a 12% increase in body energy gain and a 10% increase in protein gain. Body composition in lambs was not affected by energy intake of the dam.

Johns (1974) fed weanling lambs for 60 days either a 7% or 15% crude protein (C.P.) ration having equal digestible energy density. Lambs fed the 15% C.P. ration gained more, consumed more, and had a lower feed/gain ratio than did lambs fed the 7% C.P. ration. Lambs fed the

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15% C.P. ration had heavier liver and gastrocnemius muscle. Total liver RNA, DNA, and protein were increased with the higher protein diet while liver RNA/DNA ratio was unaffected by protein level. Similar results were obtained for muscle except that muscle RNA/DNA ratio was increased by the high protein diet. Total essential amino acids (TEAA) per gram of wet tissue were increased in muscle but not liver in the 15% C.P. ration. Total non-essential amino acids (TNEAA) were increased in muscle but not in liver for the 7% C.P. ration. Plasma TEAA increased and TNEAA decreased with feeding the 15% C.P. diet. These results indicate that muscle which is of economic interest for meat production is more adversely affected by low protein diets than is liver.

Endocrine Requirements of Growth

Cushing (1912) observed a marked loss in body weight following removal of the anterior pituitary from growing animals. Evans and Long (1922) restored growth of hypophysectomized rats to normal with the injection of anterior pituitary extracts. Since that early research, growth hormone (GH) has been isolated (Li and Evans, 1944) and identified as the single most important anterior pituitary hormone in improving growth in hypophysectomized animals. The following discussion will review the effects of hormones on altering growth in animals.

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Growth Hormone

Growth hormone affects growth in two ways: (a) acts on bone to cause bone growth especially long bones and (b) increases the retention of ingested nitrogen (Nalbandov, 1963). The possible cellular mechanism of GH is the subject of a latter part of this review.

Baird et al. (1952) using 2 lines of swine bred to gain at different rates found an increased pituitary GH activity of the line that gained faster. At any age or body weight the GH content of the pituitary was highest in the faster gaining line. Armstrong and Hansel (1956) reported a positive correlation between GH/gram of pituitary tissue or total pituitary GH/100 lb body weight and rate of growth for the 16 week period previous to slaughter in dairy heifers. Purchas et al. (1970) slaughtered bulls at monthly intervals from birth to one year of age and found neither plasma GH concentration, total GH in circulatory fluid, pituitary GH concentration, total pituitary GH nor pituitary GH per unit body weight to be closely correlated with observed rate of growth. Measures of serum GH in cattle (Grigsby, 1973) and lambs (Johns, 1974) were negatively correlated with growth fed feedlot type rations.

The concentration of serum GH is greater in young growing animals than older growing animals (Armstrong and Hansel, 1956; Purchas et al., 1970; and Trenkle, 1971b). Nalbandov (1963) and Curl et al. (1968) have suggested

there is less GH per unit of body weight in heavier animals and this results in growth statis. In agreement with this hypothesis, Trenkle (1971b) observed a 5-fold decrease in GH secretion rate in cattle from 3 to 17 months of age.

Machlin (1972) injected 1.1 mg/kg body weight purified porcine GH into pigs and noted death in 8 of 12 pigs due to liver damage, kidney degeneration, and stomach hemorrhage. When this GH preparation was injected into rats at 10 times the dosage given pigs no lethality was observed. A dose of 0.13 mg GH/kg body weight to pigs produced no fatal symptoms. This dose increased average daily gain and percentage lean cuts and decreased feed/gain ratio and dressing percentage. The hypothalamic peptide somatostatin, which inhibits release of GH from the pituitary, decreased body weight gains in male rats during 8 days of injection (Brazeau et al., 1974).

Thyroid Hormones

Brody (1945) thyroidectomized a calf at 50 days of age. At maturity this animal's mature body weight was half normal and energy metabolism per unit body area was 40% of normal.

Scow (1959) thyroidectomized (T) and hypophysectomized (H) rats at 21 and 26 days of age, respectively. At 56 days of age either GH, thyroxine, or both were given daily for 36 days. Rats without pituitaries and thyroids gained 8.0 grams but when given 0.5 mg GH or 2.5 mg

thyroxine alone or in combination each day the gain was 68, 27, and 81 grams, respectively. For the 70 day period following removal of these glands gains for HT, H and T rats were 32, 35, and 67 grams respectively. Quantitation of muscle myosin and collagen showed different sites of action for thyroxine and GH in HT rats. Myosin to collagen ratio was 12 in HT rats given 2.5 mg thyroxine alone and this ratio decreased to 9.3 when 0.5 mg GH was also provided. Scow (1959) suggested thyroxine to be myotropic and GH to be collagenotropic. Trenkle (1974) reported that GH and thyroxine given in combination completely restored muscle DNA synthesis in hypophysectomized rats to levels comparable to that of normal rats of the same age.

Pituitary thyroid-stimulating hormone (TSH) concentration like GH is highest in young cattle and decreases with age (Armstrong and Hansel, 1956). Heifers fed a high plane of nutrition tended to have higher pituitary TSH content than those fed a low plane of nutrition. Armstrong and Hansel (1956) found a positive correlation between pituitary TSH content and rate of growth during the 16 week period previous to slaughter in heifers.

Hatch et al. (1972a, 1972b) classified steers and lambs by thyroid status, i.e. hypo-, eu- or hyperthyroid from estimates of thyroid activity of the animals. Steers classified hyperthyroid gained significantly more weight during the 28 day period following classification than either hypo- or euthyroid steers while lambs showed no

difference in weight gain following thyroid status classification. During a 147 day feedlot period steers classified hyperthyroid gained 5.6 kg and 3.7 kg more than hypo- or euthyroid classed steers, respectively.

Growth Promotants

The suggestion that hormones have a profound influence of growth led researchers to begin looking for agents that would stimulate growth by alteration of endogenous hormone production. Although not included in this review, the reader is reminded of growth stimulation by antibiotic additions to feed especially in monogastric animals.

In 1954, two reports appeared describing increased weight gain following addition of the synthetic estrogen diethylstilbestrol (DES) to feed of ruminants (Burroughs et al., 1954; Clegg and Cole, 1954). Surprisingly, DES had little or no positive effects on milk production in ruminants (Wrenn and Sykes, 1957) or increasing weight gain in monogastric animals. Clegg and Cole (1954) postulated that the action of stilbestrol in promoting growth may be mediated by pituitary hormones. These workers noted larger pituitaries from animals implanted with stilbestrol. With the introduction of the radioimmunoassay to endocrinology, scientists were better able to determine the role DES played in altering hormone levels.

Davis et al. (1970a,b,c) in a series of papers noted a high degree of similarity of action between purified ovine GH and DES. With fasting sheep, the authors noted an initial decline in plasma non-esterified fatty acids (NEFA) and glucose concentrations followed by an elevation at one hour following injection of 15 mg GH. Eight hours following GH injection, plasma NEFA and glucose concentrations reached maximum values while plasma urea nitrogen and free amino acid nitrogen concentrations were decreased from the level at injection. There was no change in plasma insulin concentration as the result of GH injection (Davis et al., 1970a).

Davis et al. (1970b) proceeded to do a longer trial in which 10 mg of GH was injected daily for 9 days. A 9 and 7 day control period preceded and followed GH treatment, respectively. Plasma NEFA were elevated by daily injections of GH. Plasma insulin and glucose concentrations showed an initial increase after GH treatment began but both were depressed midway through the treatment period. A high positive correlation (0.95) was found between insulin and glucose concentrations. Again plasma urea nitrogen concentration decreased and nitrogen retention increased more during GH treatment.

The third paper (Davis et al., 1970c) reported on the metabolic effects of DES. Two 3 week periods during which 4 mg DES per day was administered were spaced

between 3 one-week control periods giving a sequence as follows: control, DES, control, DES, and control. Glucose and insulin concentrations in plasma tended to increase weekly over each 3 week DES treatment period and decline during control periods. Weekly means of plasma urea nitrogen concentration and nitrogen retention were inversely related ($r = -0.95$). Plasma urea nitrogen concentration decreased and nitrogen retention increased with DES treatment.

Trenkle (1970b) fed finishing rations to fattening steers and observed no effect of ration on plasma GH concentration and only a slight increase in plasma insulin concentration. Addition of DES (10 mg/day) to the ration increased plasma GH and insulin concentrations with no effect on protein-bound iodine. DES feeding to lambs for 2 weeks resulted in a 32% increase in serum GH (Hutcheson and Preston, 1971). Steers fed DES gained significantly more weight and had 15.7% larger pituitaries than control animals.

Davis and Garrigus (1971) fed DES either continually (7 weeks) or intermittently (3 weeks fed, 1 week without, 3 weeks fed) to lambs. Nitrogen retention was significantly increased in both DES treatment groups with intermittent DES being significantly greater than continual DES. During the week without DES, nitrogen balance remained equal to continual DES and when DES feeding was resumed a further stimulation in nitrogen retention occurred. As

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previously reported (Davis et al., 1970c), plasma urea nitrogen concentration was decreased in sheep fed DES for 3 weeks. Between 3 and 7 weeks of continuous DES treatment, plasma urea nitrogen concentration increased gradually to a similar concentration of controls. Hutcheson and Preston (1971) also found DES to initially depress plasma urea nitrogen followed by a return to control values after 6 weeks of DES feeding.

In 1973, legal action banned the use of DES for ruminants because of its possible carcinogenic activity. Later that year an injunction by the courts allowed use of DES in ruminants until the matter could be settled legally. As a result the search for other growth promoting agents (natural and synthetic) has intensified with the identification of some active compounds. To date the mechanism of each has not been elucidated but certain agents show sufficient promise to warrant further research. A few will be discussed briefly.

Zearalonol or Zeranol (same) is similar in structure to DES and is the lactone of resorcylic acid. In nature it is produced by a mold which grows on corn and it has known estrogenic activity. Sharp and Dyer (1971) observed a 12% increase in feedlot gain of steers and lambs implanted with zearalonol with a decrease in feed/gain ratio and no intake differences. Borger et al. (1973) noted a 7.8% increase in gain of steers implanted with 36 mg Zeranol at 1 and 84 days of a 169 day feedlot trial. Implanted steers

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had significantly higher serum GH concentration than controls with no difference in insulin.

Goodrich and Meiske (1973) implanted Holstein steers with a variety of growth promotants. Steers implanted with DES, 200 mg progesterone plus 20 mg estradiol benzoate, or Zeranol gained 17.0, 15.0, and 5.9% faster than controls. An implant of 120 mg of testosterone plus 24 mg DES did not improve gain. Feed per 100 lb of gain was less with those treatments showing a positive response in weight gain.

Raun et al. (1974) added monensin a compound produced by Streptomyces cinnamonensis to feedlot rations and observed a 14 to 15% improvement in feed/gain ratio. The exact mechanism producing the positive response is not completely understood but a 45% increase in ruminal propionic acid production has been observed (Richardson et al., 1974). The composition of carcass gain was not affected by monensin treatment (Potter et al., 1974).

The previous section has shown that growth rate and body composition can be altered by many factors such as nutrition, breed, sex, hormones, and compounds termed growth promotants. There is general consensus that an elevation of serum hormones especially insulin and GH produce an increase in rate of gain and more often than not improved feed utilization. To more completely understand mechanisms regulating growth, improved methods for

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changing growth rate and patterns and evaluating changes associated with improved growth are needed.

The Hypothalamus and Its Hormones

Located in the diencephalon at the base of the brain, the hypothalamus forms the floor and lateral walls of the third ventricle and is bordered anteriorly by the optic chiasma and posteriorly by the mammillary bodies. The hypothalamus is connected to the pituitary by a stalk containing the portal vessels. Located dorsal to the stalk is an expanded area known as the median eminence. The hypothalamus consists of a series of nuclei with nerve fibers running toward the pituitary stalk and ending at the portal vessels.

Green and Harris (1947) made a major discovery in neuroendocrinology when they described the portal circulation of the hypothalamus and pituitary. This discovery provided the final connection for the anatomical basis of the "neurohumoral" concept for pituitary secretion. Simply stated this hypothesis suggested that neurohumors were synthesized in hypothalamic nuclei and stored in nerve fibers of the median eminence. At this point the neurohumors were released into the portal circulation and carried to the anterior pituitary causing release or inhibition of release of pituitary hormones. This theory was later given impetus with the discovery of a corticotrophin-releasing factor (Saffran and Schally, 1955).

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To date nine other hypothalamic hormones have been described that control release or inhibition of release of pituitary hormones (Table 1).

Thyrotropin Releasing Hormone

Thyrotropin-releasing hormone (TRH) was demonstrated by Schrieber et al. (1961) in Czechoslovakia. These workers noted an increase in pituitary acid phosphatase *in vitro* when rat pituitaries were treated with acid extracts of bovine hypothalamic tissue. When media from the incubation mixture was injected into hypophysectomized rats an increase in the uptake of radio-iodine by the thyroid was observed (Schrieber et al., 1962).

Not until five years later did Schally et al. (1966) report TRH to be made of equimolar amounts of 3 amino acids: glutamic acid, histidine, and proline. Interestingly enough, Schrieber et al. (1962) had reported TRH to be composed of 9 amino acids.

Boler et al. (1969) and Burgus et al. (1970) reported the structure of TRH isolated from porcine and ovine hypothalamic tissue, respectively. The structure is shown in Figure 1 and undoubtedly the cyclized glutamic acid and the prolineamide group contributed to the long delayed structural determination. Today, TRH is made in a relatively large and synthetic TRH has all the functions of natural TRH. Vale et al. (1971) reported an analog of TRH having a methyl group in the 3-N position on the imidazole

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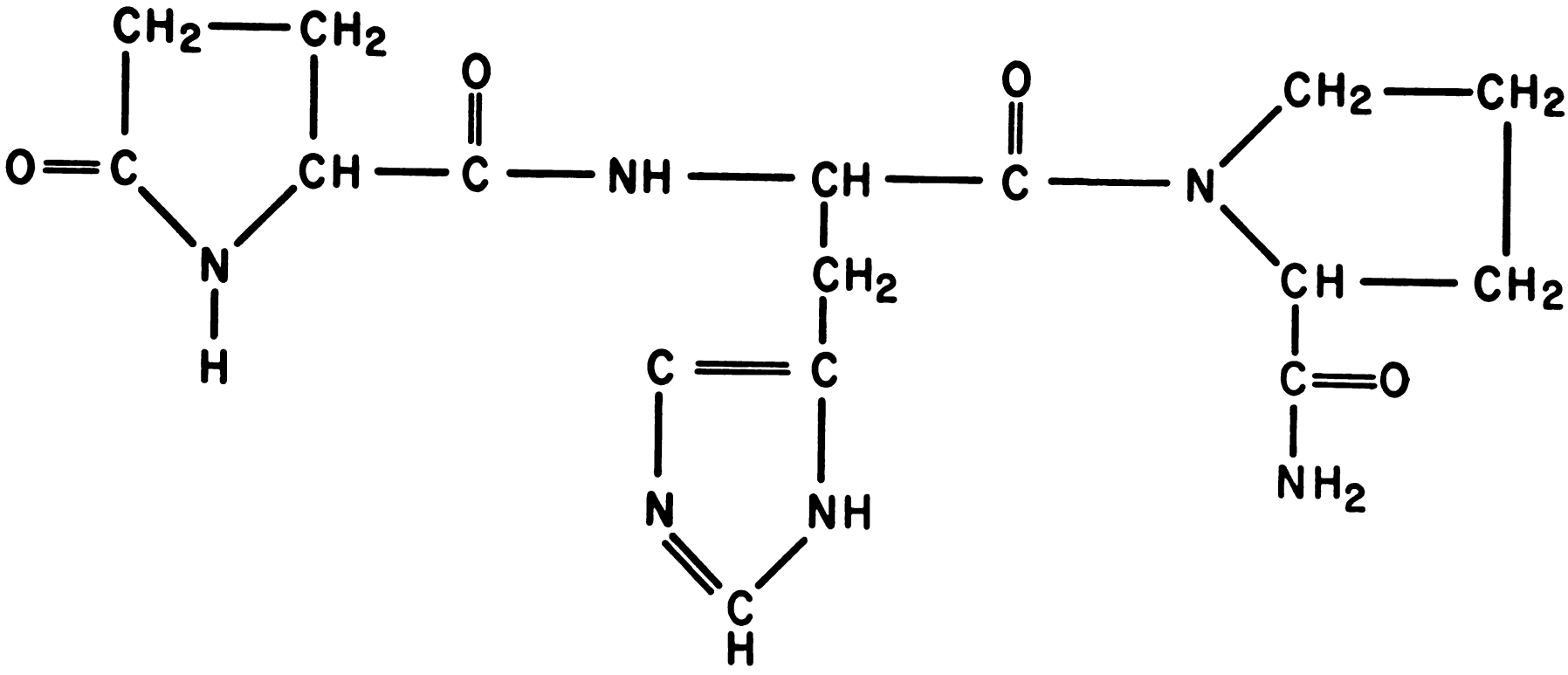
TABLE 1.--Hypothalamic hormones controlling release of pituitary hormones.

Hypothalamic Hormone
Corticotropin (ACTH)-releasing hormone
Thyrotropin (TSH)-releasing hormone
Luteinizing hormone (LH)-releasing hormone
Follicle stimulating hormone (FSH)-releasing hormone
Growth hormone (GH)-releasing hormone
Growth hormone (GH)-releasing-inhibiting hormone
Prolactin release-inhibiting hormone
Prolactin-releasing hormone
Melanocyte-stimulating hormone (MSH) release-inhibiting hormone
Melanocyte-stimulating hormone (MSH) releasing hormone

Figure 1. Thyrotropin releasing hormone.

TRH



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ring of histidine to be 8-fold more potent than TRH in releasing TSH from mice pituitaries *in vivo*.

Mitnick and Reichlin (1972) reported *in vivo* synthesis of TRH to require an ATP dependent soluble enzyme system. The authors named the enzyme "TRH synthetase." The incorporation of amino acids into the tripeptide molecule was not inhibited by the protein synthetic inhibitors cycloheximide and puromycin.

Labrie et al. (1972) and Wilber and Shaw (1973) have reported TRH to be bound to membranes of pituitary cells. Wilber (1971) observed a TRH stimulated incorporation of ^{14}C-glucosamine into TSH *in vitro* when the incubation medium was assayed for TSH but not when pituitary tissue was assayed. Protein synthetic inhibitors caused a decrease in the appearance of label into TSH in tissue and medium, but total TSH secreted into medium was unaffected by inhibitors. When thyroxine was added to the incubation medium TRH stimulated synthesis of TSH but release was inhibited. Bowers et al. (1968) demonstrated that triiodothyronine inhibited TRH-induced TSH release and the effect was abolished by incubation with protein synthetic inhibitors. This result suggests that a protein is involved in triiodothyronine inhibition.

The thyroid status (hypo-, eu-, or hyperthyroid) of an animal affects the response of the pituitary to TRH-induced TSH release. Wilber and Shaw (1973) reported increased TRH binding to pituitary membranes in the

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hypothyroid state and decreased binding in hyperthyroid state relative to the euthyroid state. Vale et al. (1971) and Wilber (1971) reported the amount of TSH released from TRH stimulation to be less when either triiodothyronine or thyroxine was incubated with pituitary explants. Smith (1974) observed a decreased TRH-induced prolactin release from bovine pituitary cell cultures incubated with triiodothyronine or thyroxine.

Martin and Reichlin (1970), Adams et al. (1973), and Debeljuk et al. (1973) measured an increase in circulating TSH after administration of TRH. Martin and Reichlin (1970) were also able to measure an increase in plasma TSH by electrical stimulation of the medial basal hypothalamus. In each case the rise in TSH was rapid and peak levels reached within 30 minutes after TRH administration.

The response of thyroxine to TRH has been reported in mice (Chopra and Solomon, 1973) and cattle (Convey et al., 1973a and Vanjonack et al., 1974). Twice daily injections of 1 ug of TRH for 7 days increased serum thyroxine concentrations at 2 hours after TRH only on day 1 with no change in thyroid size or ^{131}I uptake by the thyroid noted in mice (Chopra and Solomon, 1973). Convey et al. (1973a) reported maximal thyroxine response 5 hours after TRH injection and that magnitude of response was unrelated to dose given. Vanjonack et al. (1974) noted a biphasic response of thyroxine to TRH. An increase of

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107% in plasma thyroxine was observed 30 minutes after TRH administration followed by a decline to only 10.8% increase above controls at 2 hours after administration. At 4 and 6 hours after TRH administration, plasma thyroxine concentration was 34 and 41% greater than controls.

Thyroid Hormones and Growth

Peake et al. (1973) made rats hypothyroid by including propylthrouracil (PTU) in the drinking water. Within 12 days protein bound iodine (PBI) had decreased from 4.0 to less than 1.6 ug/100 ml. During this same time period pituitary GH content decreased to 75% of control. After 40 days of PTU treatment pituitary GH content was less than 10% of control. When PTU therapy ceased pituitary GH concentration returned to pretreatment levels. Injection of thyroxine thrice weekly into PTU treated rats prevented the decline in GH. Rats rendered hypothyroid by PTU grew slower than normal controls.

Armstrong and Hansel (1956) slaughtered heifers at 1, 16, 32, 48, 64, and 80 weeks of age and observed greater pituitary TSH activity in younger animals than in older animals. Mixner et al. (1966) using cattle noted a decline with age in PBI, thyroxine turnover, and thyroid secretion rate (TSR) when expressed per 100 kg body weight. The total TSR increased with age which coincided with an increase in body weight. For each 1% increase in body weight, there was a 0.60% increase in TSR.

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Administration of exogenous thyroxine increases oxygen consumption (Mukherjee and Mitchell, 1951; and Yousef and Johnson, 1966) and milk production (Thomas et al., 1954; Moore, 1958; and Yousef and Johnson, 1966). Thomas (unpublished observations) noted a 0.33 kg per day reduction in gain in 6 to 10 months old calves when fed 1 g thyroprotein per day.

The exact mechanism of increased milk production resultant from thyroprotein feeding in unknown. Animals become hyperthyroid and may exhibit a 2-3 fold increase in serum thyroxine concentration (Shaw et al., 1975 in press). No change in the concentration of serum prolactin, GH, or total corticoids were noted during 35 days of thyroprotein feeding. There was an increase in excretion of endogenous nitrogen and creatinine nitrogen during thyroprotein feeding. Maracek and Feldman (1973) have observed an increase in glucose utilization and decreased insulin half-life in hyperthyroid rabbits. Blaxter et al. (1949) and later Tucker and Reece (1961) suggested thyroid secretion to be the limiting factor in milk production but no concrete evidence presently exists for this idea. Stimulation of milk production by supplemental thyroxine administration ceases after 3-8 months of continued thyroxine administration (Thomas et al., 1974).

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Control of Growth Hormone Release

The release of growth hormone can be illicited by mild electrical stimulation of the ventromedial nucleus of the hypothalamus (Kokka et al., 1972; Martin, 1972; and Martin, 1974). A GH-releasing hormone has been proposed (Wilber and Porter, 1970).

Brazeau et al. (1973) isolated a peptide from ovine hypothalami that inhibited GH release, named somatostatin. Daily injections of the synthetic peptide reduced body weight gain in 180 gm rats over an 8 day period (Brazeau, et al., 1974).

Conflicting evidence appears in the literature concerning TRH-induced GH release. TRH has been shown to consistently increase serum GH concentration in lactating cows (Convey et al., 1973b) ovariectomized heifers (Smith et al., 1974; Smith, 1974) and 4 to 5 month old heifers (Vines et al., 1974). Smith (1974) reported estradiol implants to sensitize overiectomized heifers to TRH induced GH release. Davis and Borger (1972) failed to observe an increase in serum GH concentration after TRH injections into lambs.

Administration of TRH increased serum GH concentrations in acromegalics (Saito et al., 1971; and Schalch et al., 1972) and normal humans (Fleischer et al., 1970; Bowers et al., 1971; and Torjesen et al., 1973). However,

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Anderson et al. (1971) and Saito et al. (1971) failed to observe an increase in GH after TRH in normal humans.

Yousef et al. (1969) and Trenkle (1971b) independently estimated the plasma half-life of GH in cattle to be 22-24 minutes. Mitra et al. (1972) found elevated environmental temperature prolonged GH half-life in cattle. Their measured half-life was 26 minutes at 18° and 39 minutes at 35° but GH secretion rate was decreased by 43% at the higher temperature. Their data suggest a slight decrease in circulating GH concentration at higher environmental temperatures.

Purchas et al. (1971) reported heifers fed 0.9 kg grain per day between 4 and 10 months of age had higher serum GH concentration than heifers fed 4.5 kg grain per day. Trenkle (1970a) found plasma GH concentration higher in sheep fed hay than in sheep fed 30 or 70% of their diet as grain. Trenkle (1970b) reported plasma GH concentration remained unchanged in cattle fed finishing rations for 80 or 142 days. These results indicate a negative relationship between circulating GH and dietary energy content.

Sinha et al. (1973) adjusted litter size to either 4 or 16 rat pups at birth. At 21 days of age pups from the smaller litter were larger, had 50% larger pituitaries, and 3 times higher pituitary GH concentration than pups from the larger litter. Pups from the smaller litter had an increased rate of incorporation of ^{3}H-leucine into GH. When pups from the larger litter were weaned and

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allowed 2 weeks of free access to food, then the differences in body weight, pituitary size, and pituitary GH concentration disappeared. Maternal restriction of feed intake (to 50% of control) resulted in decreased pituitary GH content in newborn rat pups (Stephan et al., 1971).

Machlin (1972) restricted feed intake in 8 pigs weighing 90 kg and injected 4 pigs with porcine GH purified to have no lipolytic activity. Pigs receiving GH for 21 days gained 10 kg more weight than restricted controls receiving saline with both groups consuming equal amounts of dry matter. The author suggested caloric restriction inhibited endogenous GH production. Plasma GH concentration is elevated in kwashiorkor but not in marasmus (Raghuramulu and Jayo Rao, 1974).

These results with pigs plus the results from rat experiments previously mentioned are at odds with results from cattle (Trenkle, 1970a; and Purchas et al., 1971) and sheep (Trenkle, 1971a) with respect to caloric intake and serum GH level. This difference between monogastric and ruminant species may be responsible for the favorable growth response seen in ruminants to diethylstilbestrol.

Metabolic Actions of Growth Hormone

Houssay and Biasotti (1931) observed a lessening of the diabetic symptoms in depancreatized dogs when these dogs were hypophysectomized. They postulated a substance

was released from the pituitary which antagonized insulin action. Since that time numerous workers have shown GH to be both glycogenolytic and lipolytic. Davis et al. (1970a) observed an increase in plasma glucose after daily injections of GH. Radloff and Schultz (1966) administered GH to goats and observed an increase in blood glucose. Rathgeb et al. (1970) noted a hyperglycemic state in chronic GH-treated dogs. Head et al. (1970) and Manns and Boda (1965) have administered GH to calves and sheep and failed to observe an increase in plasma glucose concentration. The reason for the difference in response is not apparent.

Goodman (1965) administered GH to hypophysectomized rats and observed a two-fold increase in glucose uptake, CO_2 production from glucose and conversion of glucose carbon into fatty acids by adipose tissue 30 minutes after GH administration. At 60 minutes after GH administration glucose conversion to fatty acids was not different from controls while uptake and transport were still increased. However, glucose uptake, CO_2 production from glucose, and glucose conversion to fatty acids were all decreased 3.5 hours after GH administration.

Goodman (1968) classified the early and late actions of GH on adipose tissue as being insulin-like and anti-insulin-like, respectively. The early effects include increased glucose transport, CO_2 production, and glucose conversion to glycogen and occur within one hour after GH

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administration. The late effects include reduced glucose utilization, decreased glucose conversion to fatty acids, and fatty acid release from adipose tissue and these changes occur more than two hours after GH administration.

Many early workers observed a growth increase in hypophysectomized animals when given an injection of pituitary extract. One of the major changes that occurred when the extract was given was an improved nitrogen balance. This led to the hypothesis that a material in the extract improved nitrogen or protein utilization.

In hypophysectomized animals there is a decrease in cellular protein and RNA content and amino acid transport. Kostyo (1964) and Rillema and Kostyo (1971) have observed increased amino acid accumulation in diaphragm muscle from hypophysectomized rats when treated with GH. However, this effect on amino acid transport is not necessary for the stimulation of protein synthesis by GH.

Beach and Kostyo (1968) found an increase in total muscle DNA in hypophysectomized rats given GH injections. There was no differences in concentration of DNA per mg of wet muscle between hypophysectomized controls and hypophysectomized rats given GH. Cheek and Hill (1970) suggested GH administration to hypophysectomized rats was necessary for normal muscle nuclear proliferation. Jasper and Brasel (1973) found GH treatment of hypophysectomized animals increased liver DNA polymerase activity.

Kostyo (1966) failed to observe an increase in the incorporation of uridine into RNA from diaphragms of GH treated hypophysectomized rats. Jefferson and Korner (1967) found an increased incorporation of orotic acid into liver nucleic acids during perfusion with GH. Cheek and Hill (1970) found an increase in the amount of RNA in muscle after GH treatment of hypophysectomized rats. Kostyo and Rillema (1971) suggested the simulatory effect of GH on protein synthesis was related to the ability of ribosomes to promote peptide bond formation. This suggestion is in agreement with the earlier finding of Florini and Breuer (1966) in which a single injection of GH into hypophysectomized rats increased ribosomal activity before a stimulation in RNA polymerase activity was observed.

Regardless of the mechanisms of GH (and certainly more than one is involved) the stimulation of amino acid incorporation into protein by GH has been confirmed by many studies (Kostyo, 1964; Jefferson and Korner, 1967; Kostyo, 1968; Reeds et al., 1971; Rillema and Kostyo, 1971; and Jaspar and Brasel, 1973). Kostyo (1968) suggested the early action of GH on protein synthesis involved the synthesis of a few specific proteins which caused the later stimulation of protein synthesis observed after GH treatment in hypophysectomized animals.

Control of Insulin Release

The release of insulin by pancreatic beta cells is influenced by neural inputs, plasma metabolites, and hormones. This section will discuss these factors as they related to insulin release.

The islets of Langerhans are innervated by sympathetic and parasympathetic nerve fibers (Honjin, 1956; and Esterhuizen et al., 1968). Insulin secretion is increased by cholinergic agents (Malaisse et al., 1967) and blocked by atropine (Porte et al., 1973) a drug which blocks cholinergic receptors. Adrenergic agents have mixed effects due to differential receptor types. Drugs that stimulate alpha adrenergic receptors inhibit insulin release while insulin release is increased by beta-adrenergic drugs (Porte et al., 1966; Burr et al., 1971; and Frohman et al., 1967).

The destruction of the ventromedial nucleus (VMN) of the hypothalamus leads to hyperphagia and obesity in rats (Cox et al., 1968; Goldman et al., 1970; and York and Bray, 1972) and ruminants (Baile and Mayer, 1969). This condition is associated with hyperinsulinemia (Frohman and Bernardis, 1968; Frohman et al., 1969). Several lines of evidence indicate that the hyperinsulinemia is a cause of the hyperphagia and obesity. Animals made obese and hyperphagic by insulin treatment fail to gain further weight with VMN lesion (Hoebel and Teitelbaum, 1966). Pancreatic beta-cell destruction prevents VMN lesioned animals from

becoming obese unless excess insulin is administered (York and Bray, 1972). Finally, after obesity developed in VMN lesioned animals a subdiaphragmatic vagotomy reversed the obesity and hyperphagia and produced normal sized rats once again (Powley and Opsahl, 1974).

That the central nervous system (CNS) is involved in insulin secretion was further substantiated recently (Curry and Joy, 1974). These workers were able to measure an increase in insulin secretion by electrical stimulation of the VMN during glucose infusion into the pancreas. When VMN stimulation ceased, insulin secretion returned to a rate similar to that in animals receiving only glucose.

Manns and Boda (1965) injected either prolactin or GH into male castrate sheep and failed to observe an increase in plasma insulin levels. Similarly, Davis et al. (1970a) failed to observe an increase in plasma insulin concentration with the injection of ovine growth hormone. It would appear that acute injections of either GH or prolactin fail to elevate plasma insulin in ruminants. This lack of response to exogenous hormones does not however rule out the possibility that prolonged endogenous changes in circulating hormones have a direct effect on insulin release or synthesis.

Curry and Bennett (1973) reported the diphasic pattern of insulin release following glucose perfusion of the pancreas was reduced 70% and 35%, for the first and second phase, respectively by hypophysectomy.

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Administration of ACTH and hydrocortisone for 7 days before perfusion restored both phases to normal while GH administration restored only the second phase.

Rathgeb et al. (1970) administered GH to dogs and observed no change in plasma insulin concentration. Continued daily GH administration produced an increase in plasma insulin concentration with dogs exhibiting many diabetic symptoms. Young (1963) in similar experiments reported increased insulin requirement of dogs made diabetic by GH treatment.

Koerker et al. (1974) have reported insulin secretion to be decreased during intravenous infusion of somatostatin to baboons. These and other workers (Curry et al., 1974; Devane et al., 1974) have proposed a direct inhibition by somatostatin on insulin release from the pancreas.

The best known effector of insulin release is undoubtedly glucose. Ingestion of a high carbohydrate meal gives rise to insulin postprandial (Manns and Boda, 1967; and Trenkle, 1970a). Intravenous infusion of glucose or perfusion of the pancreas with glucose results in a rise of circulating insulin (Trenkle, 1970a, Stern et al., 1971; and Feldman and Jackson, 1974).

The volatile fatty acids propionate and butyrate stimulate insulin release while acetate has little stimulatory effect in ruminants (Manns and Boda, 1967; Horino et al., 1968; and Trenkle, 1970a). Horino et al.

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(1968) found VFAs to have no stimulatory effect on insulin release in non-ruminant species. Trenkle (1970a) concluded VFAs especially propionate and butyrate when infused intravenously stimulate insulin release and that diets affect the concentration of these fatty acids and in this manner diet affects insulin release by altering the pattern of VFAs produced in the rumen. Bassett et al. (1971) fed sheep diets differing both quantitatively and qualitatively. Plasma insulin concentration was most highly correlated with digestible organic matter intake ($r = 0.74$) and intestinal non-ammonia nitrogen digested ($r = 0.74$). Correlation between individual or total VFA production to plasma insulin concentration was less (0.40-0.51). Acetate accounts for 95% of peripheral plasma VFA concentration with propionate and butyrate removed by the liver (Bergmann and Wolff, 1971). Thus, the significance of propionate and butyrate as stimulators of insulin release under normal physiological conditions remains speculative.

Intravenous infusion of amino acids to ruminants increase serum insulin (Stern et al., 1971; and Davis, 1972), or with other species arginine is the most effective amino acid in causing release while leucine and phenylalanine also stimulate release (Davis, 1972). Tyrosine, valine, isoleucine, and phenylalanine were most highly correlated to plasma insulin concentration. Stern et al. (1971) reported a higher serum insulin for mature goats than weanling or suckling goats. Young et al. (1970)

failed to observe a statistical increase in insulin in calves from birth to 105 days of age but reported highly variable plasma insulin concentrations for the calves.

Metabolic Actions of Insulin

Even more so than with GH, insulin exerts a powerful influence on the intermediary metabolism of carbohydrates, fats, protein, and amino acids. The action of insulin on carbohydrate metabolism is probably foremost when one discusses insulin action. In diabetic animals insulin increases metabolism of carbohydrates, lipids, protein, and amino acids.

Volumes have been written concerning insulin action on all aspects of metabolism. For a more detailed analysis of the specific metabolic action of insulin reviews covering that aspect will be cited for the reader.

The administration of exogenous insulin has been shown to lower blood glucose in a variety of species. Resultant to this decrease in blood glucose is an increase in glucose transport into a variety of tissues (muscle: Morgan et al., 1965; adipose: Rodbell et al., 1968; and liver: Fritz, 1972), glycogen synthesis from glucose (Whelan and Cameron, 1964; and Larner et al., 1968) and inhibition of gluconeogenesis (Jefferson et al., 1968; and Hanson et al., 1975).

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Protein Metabolism

Insulin lowers circulating plasma amino acids and has more of an effect on essential than nonessential amino acids (Luck et al., 1928; and Cahill et al., 1972). The transport of many amino acids has been shown to be stimulated by insulin (Kipnis and Noall, 1958; and Manchester and Young, 1958). Urea production is reduced in the presence of insulin (Exton et al., 1971) and insulin has been demonstrated to increase the rate of protein synthesis in muscle (Manchester, 1970; and Morgan et al., 1971) and also to decrease protein degradation in muscle (Jefferson et al., 1974).

The mechanism whereby protein synthesis is stimulated is not clear but a number of steps in the synthetic pathway are stimulated by insulin. RNA synthesis but not DNA synthesis is increased by insulin. In perfusion studies with muscle, Morgan et al. (1971) found insulin to increase the level of polysomes and decrease the level of monosomes suggesting insulin involvement in peptide chain initiation. Davey and Manchester (1969) observed an increased activation of the amino acyl t-RNA for leucine and tyrosine suggesting a role for insulin regulation of protein synthesis by peptide elongation. These data provide evidence that the action of insulin in controlling protein synthesis is not confined to one particular step but acts at each step along the protein synthetic pathway to make protein synthesis more efficient.

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III. MATERIALS AND METHODS

Design of Experiment

Animals

A total of 39 Holstein calves were used in this study. Nine intact male calves and nine female calves originated from the Michigan State University dairy herd. These calves were assigned to a randomized complete block design (Sokal and Rohlf, 1969) according to sex and genetic history. The remaining 21 intact male calves were purchased at one week of age from a nearby dairy herd. These calves were assigned to treatment at 45 days of age according to their body weight at that time.

Calves were housed individually in pens with wood shavings for bedding. All calves were fed whole milk 2X daily at 0800 and 1600 hours to 30 days of age with free access to alfalfa hay, calf starter, and water.

At 30 days of age the evening feeding was discontinued and the calves offered the ration shown in Table 2. This ration had a calculated crude protein content of 15.4%, 73% TDN, and 3250 Kcal/kg digestible energy on a dry matter basis. The ration was fed as a complete

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TABLE 2.--Composition of ration fed to calves from 30 to 135 days of age.

Ingredients	30 to 135
Corn	39
Oats	10
Soybean meal	15
Corn cobs	25
Molasses	5
Alfalfa meal	3
T.M. salt	1
Dicalcium phosphate	1
$CaCO_3$	0.4
K_2SO_4	0.4
MgO	0.2
Vit. ADE[a]	+++

[a]Vit. ADE to provide: A: 11,000 I.U./kg of ration
D: 1,100 I.U./kg of ration
E: 137 I.U./kg of ration

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mixed ration with corn cobs as the roughage source to give a more uniform ration throughout the experimental period. The ration was fed once daily at 1300 hours at a rate to give about 10% feed refusal. After 3 to 4 days the morning milk feeding was discontinued.

All calves were weighed at 30 days and at 15 day intervals thereafter to 135 days of age. Feed given was weighed daily and that not eaten was weighed and discarded every third day. Consumption was summed by 15 day periods giving weight gain and feed intake data for 7 periods. At 135 days of age, calves were removed from individual pens, placed in a common pen, and fed hay and any available grain. Thirty days later, calves were weighed for observation of residual effects of treatments.

Treatments

Beginning at 45 days of age and continuing to 135 days of age, calves received a daily intramuscular injection of either of the following solutions (5 ml):

(a) thyrotropin-releasing hormone (TRH) 500 ug

(b) 3-N-methyl histidyl-TRH (3MET) 500 ug

(c) Saline (0.9 g/100 ml)

Calves were injected within 30 minutes of their once daily feeding using a 5 ml syringe and a 20 gauge needle.

Sufficient TRH, 3 MET, and saline were prepared to give enough volume to inject for one week. TRH and

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3 MET were dissolved in isotonic saline to give a final concentration of 100 ug/ml and stored at 4°C.

Collection of Samples

Blood

To determine the pattern of hormone release as a result of injections, blood was obtained by indwelling jugular cannula implanted the evening before the following day's collection. The jugular area of the neck was shaved and washed with 70% ethanol. A 10 gauge needle was used to puncture the vein and a sterile 35 cm cannula (Vinyl IV Tubing, Clay Adams, Inc., New York) inserted into the vein leaving about 10 cm to the exterior. The exposed cannula was flushed with 3.5% sodium citrate, sealed, and affixed to the neck area with tag cement (Nasco, Fort Atkinson, Wisconsin) on 6.6 x 13.2 cm adhesive tape.

On the day of collection of sequential blood samples, the following regime was used: (1) approximately 30 minutes prior to injection about 5 ml of fluid (citrate and blood) were removed and discarded to insure proper cannula function. (2) The cannula was resealed with 3.5% sodium citrate until the following sample was obtained. (3) At time intervals indicated in Table 3 approximately 10 ml of blood were withdrawn and dispensed into 15 x 85 mm glass test tubes. (4) Step 2 was repeated.

Blood was stored overnight at 4°C and sera obtained by centrifugation at 2500 x g for 30 minutes. Plasma for

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TABLE 3.--Bleeding schedule and samples used for hormone analysis[1].

Hormone	Time relative to injection[2]											
	-10	0	5	10	15	30	45	60	90	120	240	480
Growth hormone	X	X	X	X	X	X	X	X	X	X	X	X
Prolactin	X	X			X	X	X	X				
Thyroxine	X									X	X	X
Insulin	X					X		X		X	X	X

[1]Calves were bled at 45, 60, 105, and 135 days of age and hormone concentration determined at the designated time (X) for all ages.

[2]Calves received an IM injection at 0. Remaining times are minutes relative to injection.

the determination of glucose and nonesterified fatty acids was obtained by mixing blood with potassium oxalate and sodium fluoride, chilling immediately, and centrifuging within one hour at 2000 x g for 30 minutes.

Male calves were bled at 45, 60, 105, and 135 days of age. Female calves were bled at 60 days of age only. Plasma was obtained at 60, 105, and 135 days of age. Serum and plasma were stored at -20°C in 7 dram vials until analyzed.

Nitrogen Balance

To determine the effects of treatments on nitrogen retention, 5 calves per treatment were placed in metabolism

cages at 90 days of age. After a 3 day adjustment period urine and feces were collected for 7 days. Urine was collected in bottles containing 25 ml of 50% H_2SO_4. Daily 10% aliquots of urine were pooled and stored at 4°C until analyzed. All feces voided were collected daily and stored at 4°C. At the end of the 7 day collection period feces were weighed and a sample obtained for the determination of dry matter, nitrogen, and ash. Calves were weighed at the end of the 7 day collection period and returned to their pens.

Each calf was offered an amount of feed that approximated 90% of their ad libitum intake during the previous week. After 3 days feed intake was adjusted and maintained at that level for the remaining 7 day collection. Feed refusal was weighed and a sample obtained for dry matter, nitrogen, and ash determination. All feed was weighed prior to the collection period and stored at 4°C. Samples were obtained for dry matter, nitrogen, and ash.

Tissues

A total of 9 calves (3/treatment) was designated for slaughter at the end of the trial. Because of a prior commitment, 3 calves (one/treatment) were slaughtered at approximately 105 days of age and the remaining animals were slaughtered at 135 days of age. Animals were transported by truck to the abattoir on the morning of slaughter.

Calves were stunned with a blow to the head, raised and exsanguinated. The abdomen was opened and the internal organs removed. The liver was removed immediately, weighed, samples taken from different lobes and frozen in liquid nitrogen. Other tissues removed included the pituitary, adrenals, testis, thyroid, pancreas, and gastrocnemius muscle. All tissues were weighed, frozen in liquid nitrogen, and stored at -20°C until analyzed.

Laboratory Analysis

Plasma Glucose

Glucose in plasma was assayed using glucose oxidase (Worthington). A plasma volume of 0.5 ml was deproteinized by addition of 1.0 ml of 1.8% $Ba(OH)_2$ (w/v) and 1.0 of 2.0% $ZnSO_4$ (w/v). After centrifugation at 2000 x g for 30 minutes duplicate 0.5 ml aliquots of the supernatant were incubated at 37°C for 45 minutes with 3.0 ml of glucose oxidase. At the end of the incubation period, 1 ml of 0.1 N HCl was added to stop the reaction. Optical density was obtained for all at 400 nm using a Gilford spectrophotometer fitted with a flow through cell. Water was used as a blank.

Nonesterified Fatty Acids (NEFA)

NEFA were determined using the method of Ho (1970) as modified by Bieber (1974). NEFA were extracted from

0.2 ml plasma with 1.0 ml of Dole extraction mixture (isopropanol:heptane:1NH_2SO_4, 40:10:1) (Dole, 1956). Samples were vortexed and placed in ice. After 10 minutes of standing, 0.2 ml of heptane and 0.2 ml H_2O were added, vortexed, and placed in ice to allow the separation of organic and aqueous phases. An aliquot of the heptane layer (0.2 ml) was removed and placed in a 1.5 ml polypropylene centrifuge tube with an attached cap (Brinkman Instruments, Inc., Westbury, N.Y.). Chloroform (0.8 ml) was added to each tube, the tube capped and placed on ice.

Nickel reagent was made by dissolving 0.4050 g of $NiCl_2 \cdot 6H_2$) in 100 ml of 1 M triethanolamine giving a final Ni concentration of 1 mg/ml of reagent. Radioactive ^{63}Ni (1.17mCi/100ugNi; Amersham Searle Corp., Arlington Heights, IL) was added to give approximately 10^6 cpm per 0.1 ml of Ni reagent.

Nickel reagent (0.1 ml) was added to each tube, vortexed vigorously for 45 seconds, and placed in an ice bath. The organic and aqueous phases were separated by centrifugation at 500 x g for 5 minutes. The aqueous phase was removed by aspiration and a 015 ml aliquot of the organic phase transferred to a scintillation vial and evaporated to dryness. Ten ml of scintillation fluid (Appendix Table 1) were added and ^{63}Ni counted in a Nuclear Chicago liquid scintillation counter.

Palmitic acid was used as standard for calculation of unknown NEFA concentration. The concentration of NEFA

in plasma was obtained from an equation derived by the regression of palmitic acid (Y) in concentrations from 0 to 100 nmoles on counts corrected for blank activity (x). Standards were carried through all the manipulations as for serum.

Hormone Determinations

Growth Hormone

Serum samples described in Table 3 were thawed overnight at 4°C. Dilution of the sample in phosphate-buffered saline, 1% bovine serum albumin (PBS-BSA), pH 7.4 was accomplished using an automatic pipette (Micromedic Systems Inc., Philadelphia, PA). Because of different concentrations of hormone in serum samples, a range of dilutions (10 ul-250 ul) was used with 500 ul final volume of the serum-PBS-BSA mixture. Composition of all reagents used in radioimmunoassays is shown in Appendix Table 2.

Growth hormone (GH) was quantitated using the double antibody radioimmunoassay of Purchas et al. (1970). After the appropriate dilution (10-250 ml) was obtained, 200 ul of guinea pig antibovine growth hormone serum (GPABGH) was added to each tube (except background and total count tubes). After a 24 hour incubation at 4°C, 100-200 ul (depending on specific activity) of ^{125}I-GH was added to all tubes. Following a second 24 hour incubation at 4°C, 200 ul of sheep antiguinea pig gamma globulin

(SAGPGG) was added to all tubes (except total counts). All tubes were incubated for an additional 72 hours.

At the end of the 72 hour incubation, 2 ml of 0.01 M PBS, pH 7.0 was added to each tube (except total counts) followed by centrifugation at 2500 x g for 30 minutes. The liquid was decanted, tubes inverted to drain for 30 minutes or longer, dried and the precipitate counted in a Nuclear-Chicago Model 4230 autogamma scintillation counter. A standard curve was constructed for each assay with NIH-GH-B 2 ranging from 0.1 to 5.0 ng/tube as a standard. Regression coefficients for the standard curve were calculated on the C.D.C. 3600 or 6500 and entered into an Olivetti calculator (Programma 101, Olivetti Underwood New York, NY) which corrected for dilution and each serum GH concentration was then calculated.

Prolactin

Prolactin was determined in serum samples described in Table 3 (Koprowski and Tucker, 1971). Sera (10 to 100 ul) was added to a tube, diluted to 500 ul with PBS-BSA, then 100 ul of guinea pig antibovine prolactin serum (1:30,000) added to all tubes (except background and total count tubes) followed by mixing and incubating 24 hours at 4°C. Following the initial incubation, 100 ul of ^{125}I-prolactin (20,000 cpm) was added to all tubes, mixed and incubated for an additional 24 hour period.

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Following this incubation, 100 ul of SAGPGG was added, mixed and incubated for 72 hours. The remainder of the procedure was similar to that for GH determination. A standard curve was constructed for each assay with NIH-P-1 as a standard.

Insulin

Insulin was obtained in serum samples described in Table 3 using the method described by Grigsby (1973). After dilution of serum samples (150-250 ul) to 500 ul, 200 ul with buffer of guinea pig antibovine insulin (GPABI, 1:105,000) was added to each tube (except background and total count tubes), mixed and incubated for 24 hours at 4°C. Following the initial incubation 100 ul of ^{125}I-insulin (approximately 16,000 cpm, Amersham Searle Co., Arlington Heights, IL) with a specific activity of 50 uCi/ug were added to all tubes, mixed, and incubated for 24 hours at 4°C. After this incubation 200 ul of SAGPGG were added to all tubes (except total counts), mixed and incubated for 96 hours at 4°C. Subsequent handling was similar to that for GH assay. A standard curve was constructed using highly purified bovine insulin (Eli Lilly and Co., Indianapolis, IN, lot 795372, 24.2 units per mg) at a concentration ranging from 0.04 to 5.0 ng of insulin per tube.

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Calculation of Standard Curve and Results

The autogamma counter was electronically connected to a teletype (Teletype Corp., Skokie, IL) which printed tube number and counting time. In addition these two parameters were simultaneously punched onto a paper tape which was used later to calculate results. Regression coefficients were generated by computer (C.D.C. 6500) by entering hormone concentration and corresponding counting time. Coefficients generated described a sigmoid curve with a negative slope and included cubic, quadratic, and linear terms. The proportion of within assay variation explained by the coefficients exceeded 0.98 in all assays.

Regression coefficients were entered into an Olivetti calculator (Programma 101, Olivetti Underwood, New York, NY) which corrected for dilution and calculated hormone concentration of unknown sera. The paper tape was used to enter tube number and counting time via a punch tape editor (Beckman Model 6912 Tape Editor, Beckman Instruments, Inc., Fullerton, CA).

Thyroxine

Thyroxine was quantitated using Tetra-Sorb kits donated by Abbott Laboratories, North Chicago, Illinois. A mixture of 1 ml of plasma and 2 ml of absolute ethanol were vortexed vigorously for 20 seconds, allowed to stand in ice for 10 minutes, and centrifuged at 2000 x g for 15 minutes at 0°C. A 0.3 ml aliquot of the ethanolic

supernatant was added to polypropylene tubes supplied in the kit and dried at 45-50°C with a continuous flow of air.

After the tubes were dried, 1 ml of thyroid binding globulin-thyroxine-^{125}I was added to each tube, mixed gently avoiding foaming, and incubated for 12 minutes at room temperature. After this incubation period, tubes were placed in a water bath maintained at 0 to 1°C with ice. After 5 minutes a sponge impregnated with a resin which binds displaced ^{125}I-thyroxine was added to each tube and the liquid absorbed into the sponge. The tubes and its contents were incubated for 60 minutes in the water bath. At the end of the 60 minute incubation period liquid was removed from the tubes and each tube washed three times with cold distilled water.

To determine thyroxine concentration random tubes were counted for 1 minute during the 60 minute incubation period to determine initial counts added to tubes. After washing, all tubes were counted for one minute. Remaining counts represented displaced ^{125}I-thyroxine from TBG-^{125}I-thyroxine.

The ratio of final counts to initial counts gave a percent uptake from the TBG-^{125}I-thyroxine. Percent uptake was converted into uncorrected thyroxine concentration from the graph of percent uptake vs. thyroxine concentration supplied with each kit.

Since ethanol does not extract thyroxine from serum completely efficiency of extraction was determined as

follows. To 0.2 ml of TBG-^{125}I-thyroxine, 1 ml of serum was added and incubated for 10 minutes at 45°C. Tubes were counted for one minute, 2 ml of absolute ethanol added, mixed for 30 seconds, and allowed to stand for 5 minutes. Following centrifugation, 1 ml of supernatant was added to polypropylene tubes and counted for one minute. Extraction efficiency was calculated as follows:

$$\frac{\text{final counts} \times 3.2}{\text{initial counts}} \times 100$$

Extraction efficiency was 79, 77 and 76% determined on 3 different days. An extraction efficiency of 77% was used. The product of the reciprocal of extraction efficiency and uncorrected thyroxine concentration yielded corrected serum thyroxine concentration.

Determination of Tissue Nucleic Acids

Liver and muscle were used for the determination of RNA and DNA by methods outlined by Munro and Fleck, (1966). Each tissue was homogenized in cold distilled water in the ratio of 20:1 and 10:1 for liver and muscle, respectively. Four samples representing different sections of liver and two samples from different areas of the gastrocnemious muscle were assayed in duplicate.

Two ml of homogenate were dispensed into glass centrifuge tubes and 5 ml of cold 2.5% (w/v) perchloric acid (PCA) added. Tubes were vortexed and placed in an

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ice bath for 10 minutes, vortexed again, and centrifuged at 35,000 x g for 15 minutes. The supernatant was discarded and 5 ml of cold 1% PCA added. The pellet was broken apart, vortexed, and centrifuged.

After the second centrifugation, the supernatant was discarded, 4 ml of 0.3 N potassium hydroxide added, pellet broken apart, and tubes incubated at 37°. Tubes were agitated frequently until all tissue was digested. Tubes were removed from a water bath and placed in ice; then 5 ml of 5% cold PCA added, vortexed, and centrifuged. The supernatant was added to 25 ml graduated tubes and the pellet washed twice with 5 ml of 5% cold PCA, vortexed, and centrifuged at 35,000 x g for 10 minutes each time. The washings were added to the tubes containing the original supernatant and volume brought to 20 ml with 5.0% PCA. This fraction represented RNA.

To the pellet remaining after washing, approximately 5.0 ml of 10% PCA was added. The extraction of DNA was accomplished by incubating the pellet at 70°C for 25 minutes shaking at the beginning, middle and end of the incubation period. After incubation, tubes were placed in ice. When cold, centrifugation at 35,000 x g for 15 minutes produced the DNA-containing supernatant. This was decanted into 10 ml calibrated tubes. The pellet was washed with 4.8 ml of cold 10% PCA and centrifuged. The washings were added to the original supernatant and volume brought to 10 ml by addition of 10% PCA.

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The concentration of RNA was determined by a colorimetric procedure utilizing orcinol. A two ml aliquot of the 20 ml RNA solution was added to test tubes and 2 ml of orcinol reagent (Appendix Table 3A) added. A reagent blank of 2.0 ml of 5% PCA and RNA standards of 12.5, 25.0, 37.5, and 50.0 mg per ml were used for deriving a standard curve. Marbles were placed on top of each tube and tubes incubated in a boiling water bath for 30 minutes, cooled in running cold water, allowed to reach room temperature, and optical density determined using a Gilford Spectrophotometer at 680 nM.

DNA concentrations were determined colorimetrically using diphenylamine and acetaldehyde. A 2 ml aliquot of the 10 ml DNA solution was added to test tubes and 2.0 ml of 4.0% diphenylamine in glacial acetic acid (Appendix Table 3B) and 0.1 ml of acetaldehyde solution (Appendix Table 3C) were added and mixed. A reagent blank of 10% PCA and DNA standards of 12.5, 25.0, 37.5, and 50.0 mg per ml were treated in the same manner. Marbles were placed on top of the tubes and samples incubated for 16 hours at 30° in a water bath. Tubes were removed, cooled to room temperature, and optical density determined at 595 nM in a Gilford Spectrophotometer. Duplicate determinations agreed within 10%.

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IV. RESULTS

Weight Gain, Feed Intake and Their Ratio

A 15 day pre-treatment period (31-45 days of age) allowed adequate time for calves to be weaned and adjusted to the experimental ration. Weaning was usually accomplished by 35 days of age and treatment began at 45 days of age. Weight gain, feed intake and feed to gain ratio are shown in Table 4 for each 15 day period. Although differences between groups were not significant ($P>0.10$) calves assigned to receive saline gained more and had a lower feed to gain ratio during the 15 day adjustment period than calves assigned to TRH or 3 MET treatment groups.

During the first 15 day treatment period (46-60 days of age) weight gain of calves injected with 3 MET was less ($P<0.01$) and feed to gain ratio was higher ($P<0.01$) than calves receiving saline. Total feed intake was not different ($P>0.10$) between groups. Differences in weight gain, feed intake, and feed to gain ratio between TRH and saline treated calves were not significant ($P>0.10$).

TABLE 4.--Weight gain (WG), feed intake (FI), and feed to gain ratio (F/G) by 15 day periods for calves receiving daily injections of thyrotropin releasing hormone (TRH), 3-methyl

TABLE 4.--Weight gain (WG), feed intake (FI), and feed to gain ratio (F/G) by 15 day periods for calves receiving daily injections of thyrotropin releasing hormone (TRH), 3-methyl thyrotropin releasing hormone (3MET) or saline (Sal).

Treatment	Parameter	31-45	46-60	61-75	76-90	91-105	106-120	121-135
		Days						
TRH	WG (kg)	8.5±1.3[1]	9.6±0.9[A]	12.0±2.0	16.7±0.7[A]	12.4±1.5	18.9±1.4	19.5±1.1
	FI (kg)	18.6±1.9	31.4±1.6	41.2±2.6	51.8±2.5	56.1±2.7	64.0±2.6	68.6±2.5
	F/G	2.24	3.32[A]	3.70	3.03	4.51	3.39	3.51
3-MET[2]	WG (kg)	7.6±1.4	6.2±0.6[B]	12.2±1.1	15.3±0.7[B]	12.6±1.9	15.1±1.5	16.9±2.0
	FI (kg)	16.7±2.2	26.9±2.5	36.7±2.3	46.6±2.0	50.1±2.2	58.2±3.2	61.5±2.5
	F/G	2.50	4.17[B]	2.95	3.04	4.11	3.84	3.70
Sal	WG (kg)	9.7±1.1	10.1±0.8[A]	12.0±0.6	14.0±0.8[B]	11.0±1.4	16.2±1.5	18.0±1.2
	FI (kg)	18.3±2.1	28.9±1.6	37.0±1.8	45.0±1.7	51.8±2.9	56.8±2.9	64.8±3.0
	F/G	1.83	2.86[A]	3.04	3.21	4.72	3.40	3.61

[ab]Items in same column with different superscripts significantly different from control, P<.05.

[AB]Items in same column with different superscripts significantly different from control, P<.01.

[1]Standard error of mean.

[2]Means for 3-MET based on 13 animals through 105 days and 9 animals for the remaining periods and overall.

Weight gain, feed intake, and feed to gain ratio for the second 15 day period (61-75 days of age) were not different between treatment groups. During this period two calves receiving TRH were sick with respiratory infections and required veterinary attention. On the average these two calves gained no weight during the period while other calves in the TRH group averaged 14.2 kg gain and 42.7 kg feed intake. Deletion of these two calves from the period totals resulted in an average gain which was greater than that of calves receiving saline. This trend toward greater gain in calves given TRH relative to controls continued without exception throughout successive periods.

During the third 15 day period calves injected with TRH gained more weight ($P<0.01$) than controls and differences in gain for calves given 3 MET and saline approached significance ($P<0.10$). However, differences in feed intake and feed to gain ratio between treatments were not significant. During the remaining periods calves given TRH gained more weight, consumed more feed and had a lower feed to gain ratio than calves given saline but in no instance were treatment differences within a period significant.

During the last 3 periods (91-135 days of age) period weight gains, feed intake and feed to gain ratios for calves given 3 MET or saline were virtually identical. From 91 to 105 days of age 5 calves from each treatment

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were used for a nitrogen balance trial. Calves used in this balance study gained less weight than calves kept in individual pens for each treatment and average period gain was less than the preceding or subsequent period. Gain was similar between treatment groups during each of the remaining two periods.

Overall, performance for the 90 day experimental period is shown in Table 5. Calves receiving TRH gained 7.8 kg more weight ($P<0.05$) than saline-injected calves, consumed 28.8 kg more feed ($P<0.05$), but had a higher feed to gain ratio ($P>0.10$). On a daily basis TRH-injected calves gained 87 grams (0.10 lb.) per day more than did saline-injected calves during the 90 day experimental period. Differences in growth parameters between calves given saline and those given 3 MET were not significant.

Analysis of weight gain during the 90 day experimental period adjusted by 15 day pretreatment gain resulted in negligible adjustments (less than 1.0 kg) of actual treatment means. The level of significance was similar ($f = .05$) for treatment effects on weight gain using either 2-way ANOVA or covariance analysis.

Serum Hormones

Double split-plot analysis of variance (Table 6) was calculated 3 ways as a result of failure to receive 3 MET late in the trial. In the first analysis (Analysis I) the hormonal response of 6 animals from each of 3 treatments

TABLE 5.--Total weight gain (WG), feed intake (FI), and feed to gain ratio (F/G) in calves receiving daily injections of thyrotropin releasing hormone (TRH), 3-Methyl thyrotropin releasing hormone (3MET), or saline (Sal) from day 45 to day 135.

Treatment	n	WG	FI	F/G
		kg		
TRH	13	$89.1^{a} \pm 3.6^{1}$	$313.1^{a} \pm 12.6$	3.51 ± 0.29
3-MET	9	$79.3^{b} \pm 5.2$	$276.8^{b} \pm 14.9$	3.49 ± 0.35
Sal	13	$81.3^{b} \pm 3.6$	$284.3^{b} \pm 11.4$	3.41 ± 0.40

^{ab}Means not sharing like superscripts significantly different $P<.05$.

1 Standard error of mean.

on days 45, 60, and 105 were used. In the second analysis (Analysis II), 6 animals given TRH or saline at days 45, 60, 105, and 135 were used. Finally, a third analysis (Analysis III) was based on 3 animals from each treatment on all days. Generally the results of each analysis relating significance of main effects (treatment, day and time) and the interaction of main effects were similar for each analysis. Exceptions to this generality were related to a decrease in degrees of freedom for those effects tested. The following discussion will concern itself with Analysis I and II because of the greater number of animals involved.

TABLE 6.--Double split-plot analysis of variance table for testing significance of main effects and their interactions.

Item	Source	Adjusted df	Items Used For Adjusted SS
1	Treatment (T)	T-1	1
2	Animals/ Treatment (A/T)	A(T-1)	2-1
3	Day (D)	D-1	3
4	T X D	(T-1) (D-1)	4-3-1
5	A/T X D	A(T-1) (D-1)	5-2-4+1
6	Time (M)	M-1	6
7	T X M	(T-1) (M-1)	7-1-6
8	A/T X M	A(T-1) (M-1)	8-2-7+1
9	D X M	(D-1) (M-1)	9-3-6
10	T X D X M	(T-1) (D-1) (M-1)	10-2-4-9+1+3+6
11	A/T X D X M	A(T-1) (D-1) (M-1)	Total-Σ Adj SS
12	Total	ATDM-1	Total

Growth Hormone (GH)

The effect of age and time after feeding on serum GH concentration was determined by split-plot analysis of variance using 6 calves given saline and sampled on each day tested. There appeared to be an increase in serum GH concentration occurring between 60 and 90 minutes after saline injection which persisted through 240 minutes but the increase was not significant at any time. Injection and feeding occurred within 30 minutes of each other. Serum GH at four ages is shown in Table 7. Means are the average of 12 samples per calf for 6 calves each day. At 45 days of age serum GH averaged 17.5 ng/ml during the 8 hour period following injection. Serum GH decreased to an average of 13.2 ng/ml at 60 days of age and this decrease approached significance ($P<0.10$). The decrease in serum GH occurring at 105 and 135 days of age from 45 days of age was significant ($P<0.01$).

Serum GH concentration of calves given TRH, 3 MET, or saline injections are shown in Figure 2. Serum GH concentration after TRH was greater ($P<0.01$) than 3 MET or Sal at 5 minutes after injection and persisted through 30 minutes. Injections of 3 MET resulted in a greater ($P<0.05$) serum GH concentration than did saline injection at 10 and 15 minutes post-injection. Maximal GH concentrations were attained between 10 and 15 minutes after TRH and 3 MET injection. But at 30 and 45 minutes after 3 MET and TRH respectively differences in GH concentrations

Figure 2. Average serum growth hormone concentration in calves after intramuscular injections of thyrotropin-releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

OVERALL

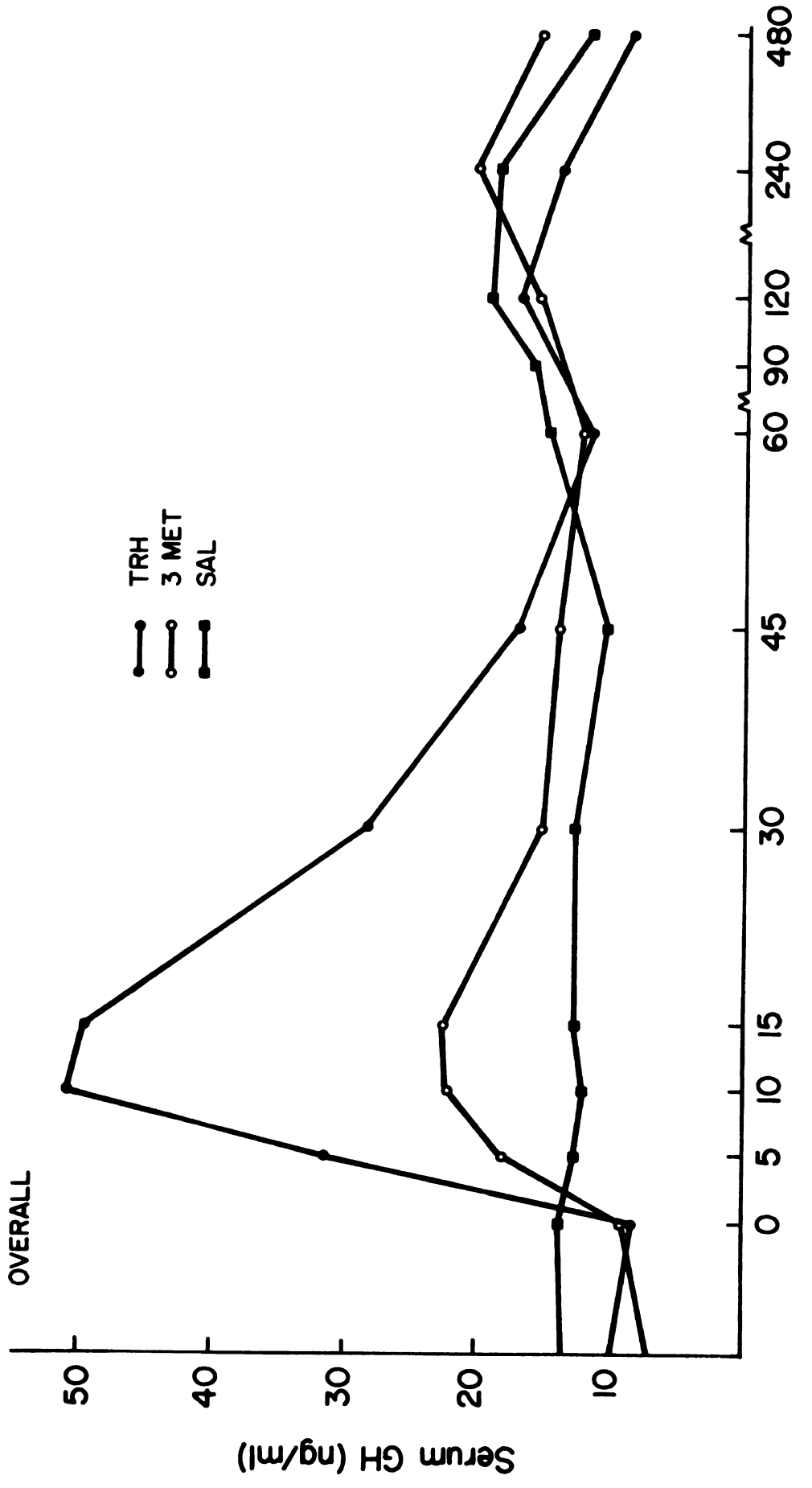

OVERALL
TRH
3 MET
SAL
Serum GH (ng/ml)
10
20
30
40
50
0
5
10
15
30
45
60
90
120
240
480
Min. after Injection

TABLE 7.--Serum growth hormones (GH), Prolactin (prl) and insulin (Ins) (ng/ml) at day 45, 60, 105, and 135 in calves receiving saline.

Hormone	n	Days				MS_E
		45	60	105	135	
GH	72	17.5^{a}	13.2^{ab}	11.6^{b}	11.3^{b}	113.4
Prl	36	6.6^{b}	7.5^{b}	27.4^{a}	----	284.1
Ins	36	1.04^{B}	1.03^{B}	1.32^{B}	2.13^{A}	0.69

$^{ab}P<0.05$; $^{AB}P<0.01$. Means within a row not sharing same superscript significantly different.

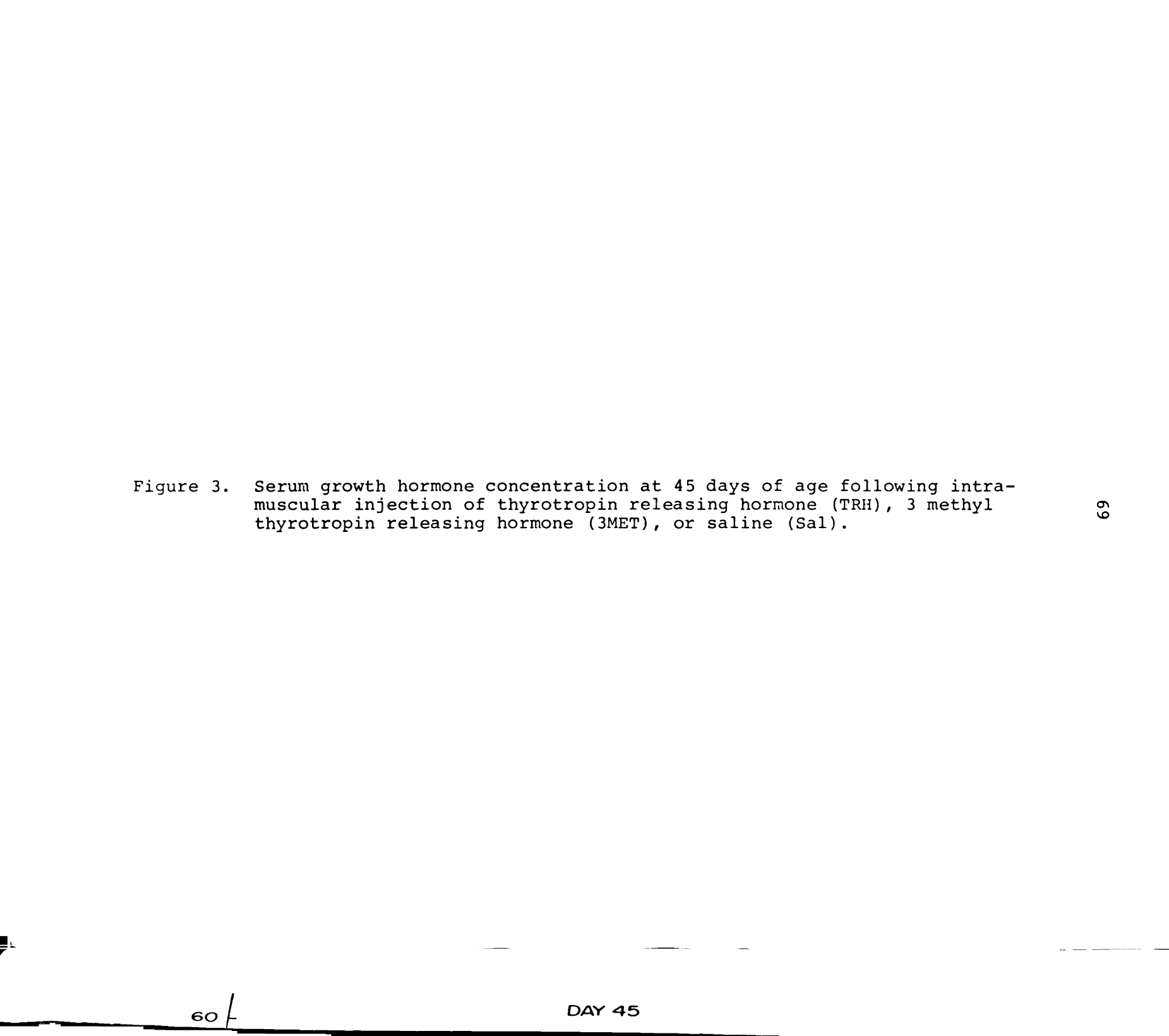
MS_E means square error.

n = number of samples used for determination of hormone concentration at each day.

were not significantly different than those for saline injected calves and this relationship existed to 480 minutes post-injection.

Serum GH after TRH, 3 MET, and saline for each sampling date is shown in Figures 3, 4, 5, and 6. These data illustrate the effectiveness of TRH in causing release of pituitary GH throughout the 90 day experimental period. Detectable increases in serum GH concentration from pre-injection levels occurred on each sampling date after TRH and 3 MET injections. On all days observed, serum GH concentration after TRH was greater ($P<0.05$) than pre-injection levels at 10 and 15 minutes post-injection but serum GH concentration after 3 MET was

Figure 3. Serum growth hormone concentration at 45 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).



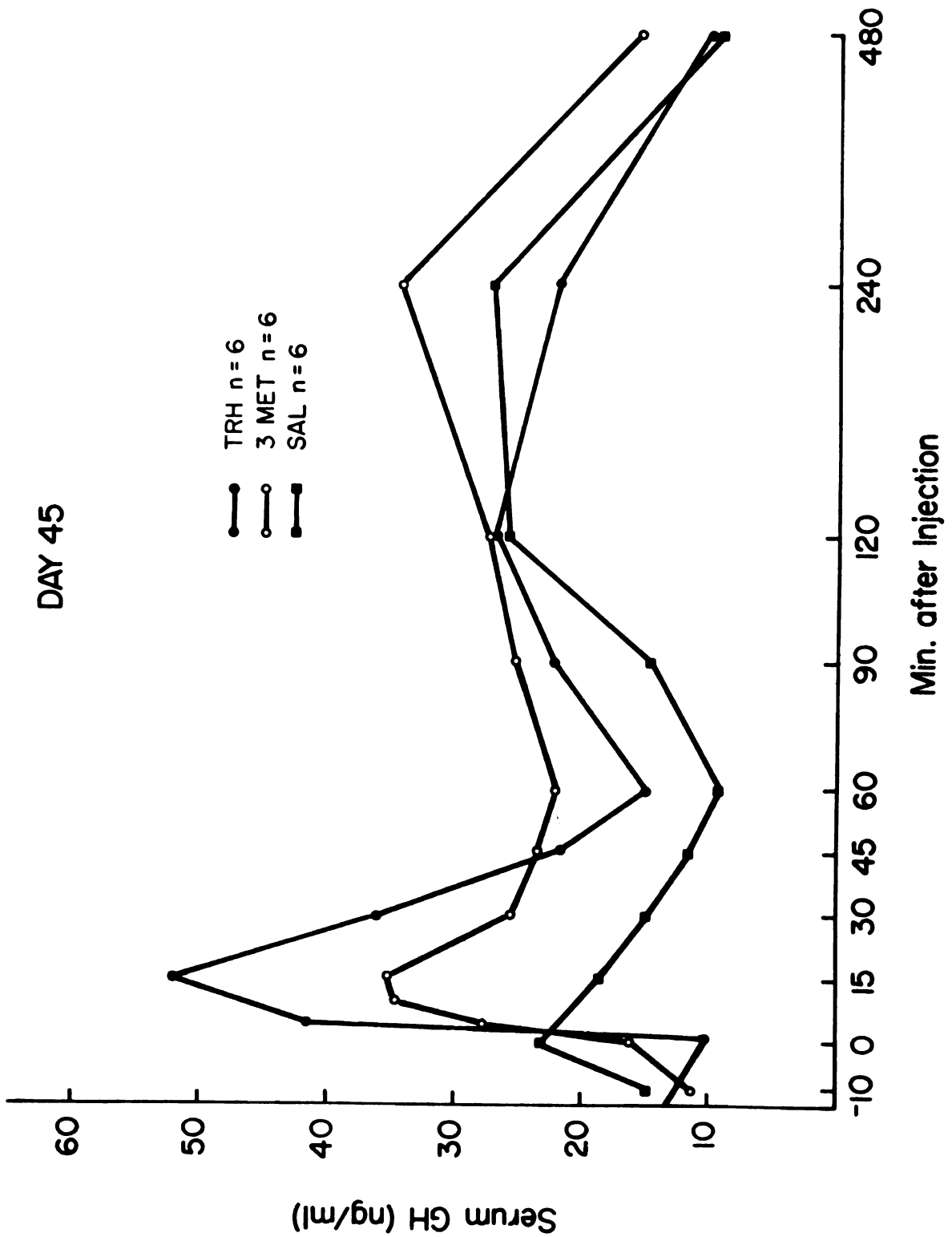

DAY 45
TRH n=6
3 MET n=6
SAL n=6
Serum GH (ng/ml)
60
50
40
30
20
10
-10 0 15 30 45 60 90 120 240 480
Min. after Injection

Figure 4. Serum growth hormone concentration at 60 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

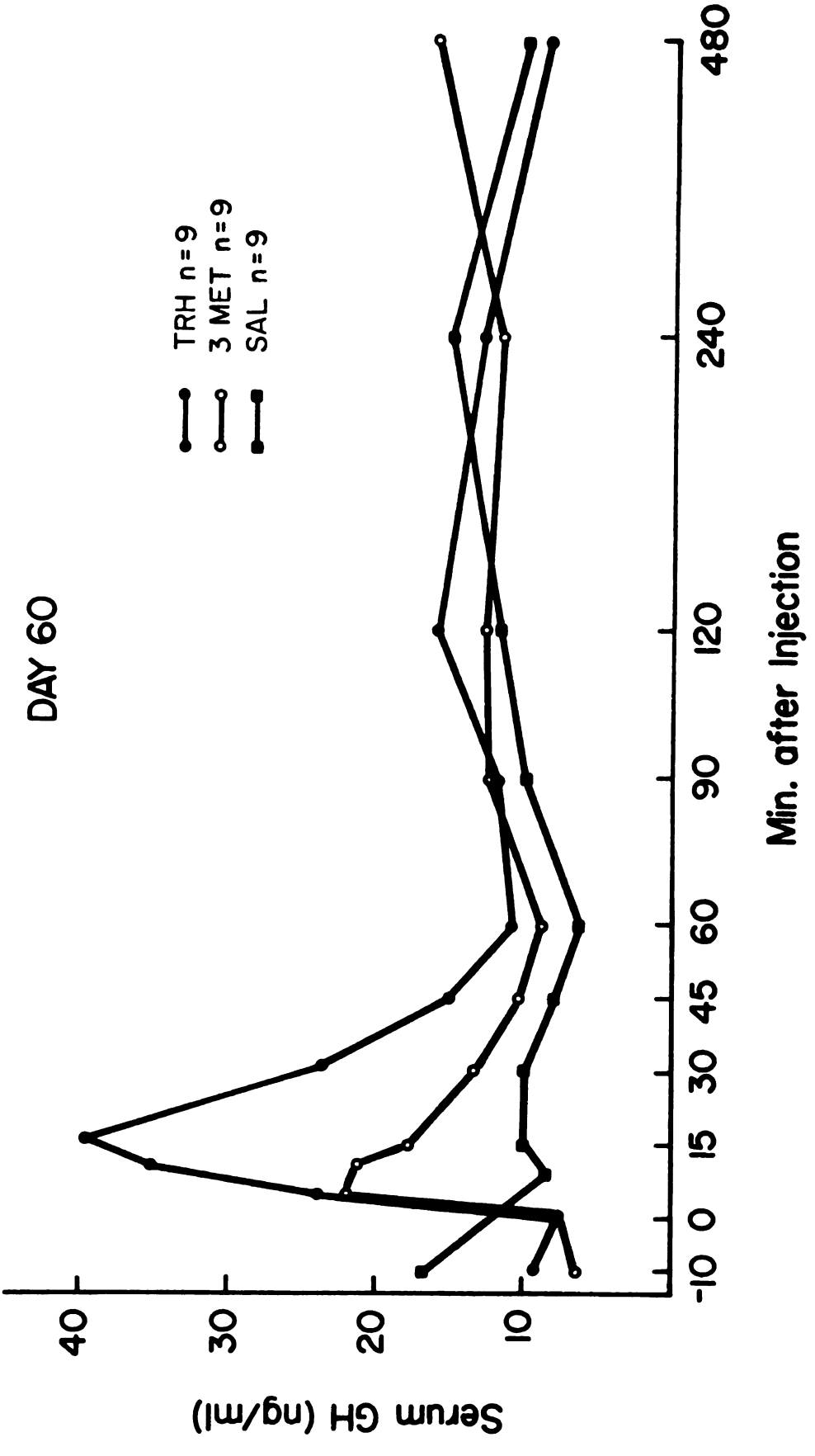

DAY 60
TRH n=9
3 MET n=9
SAL n=9
Serum GH (ng/ml)
40
30
20
10
-10 0 15 30 45 60 90 120 240 480
Min. after Injection

Figure 5. Serum growth hormone concentration at 105 days of age following intramuscular injection of thyrotropin-releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

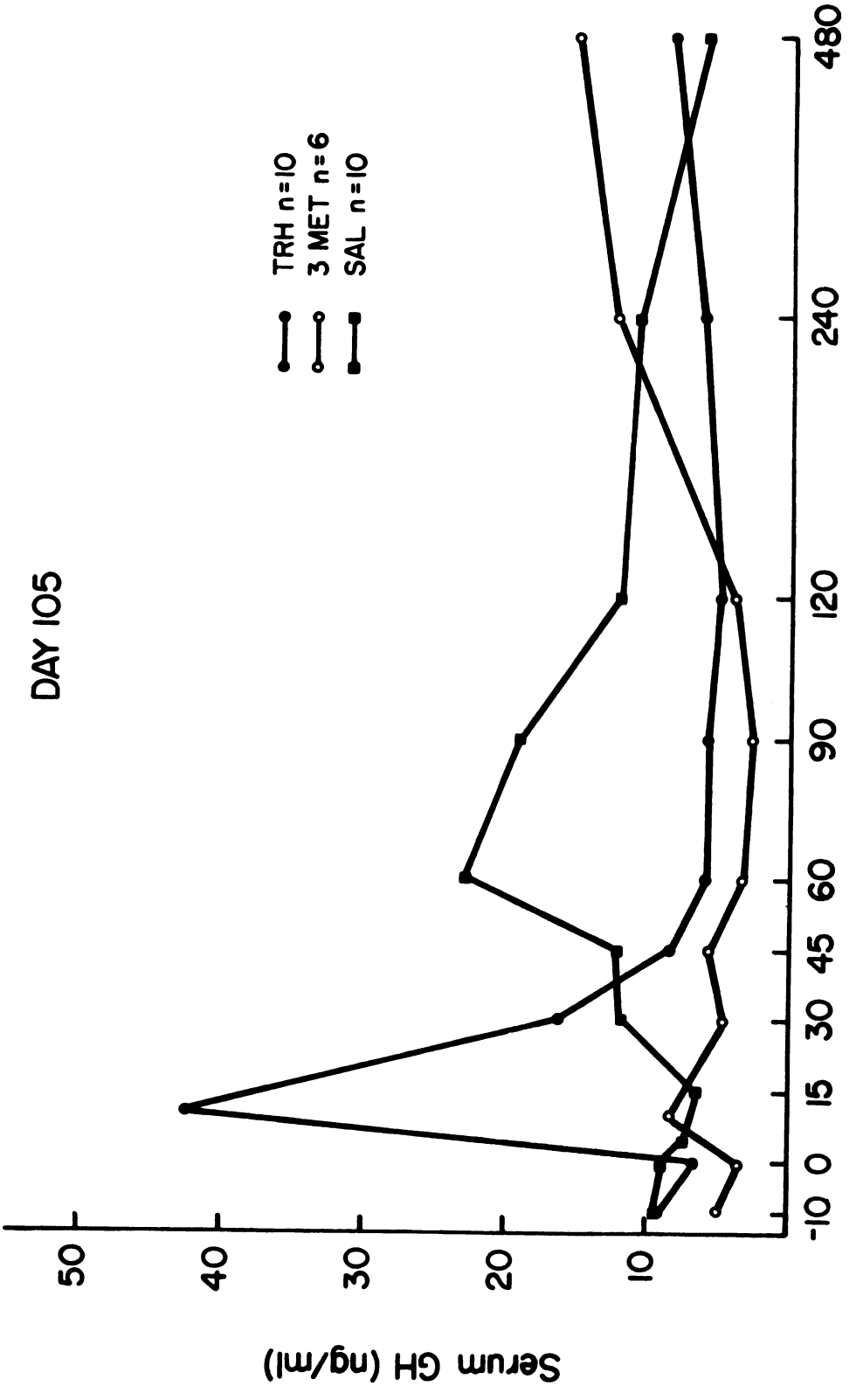
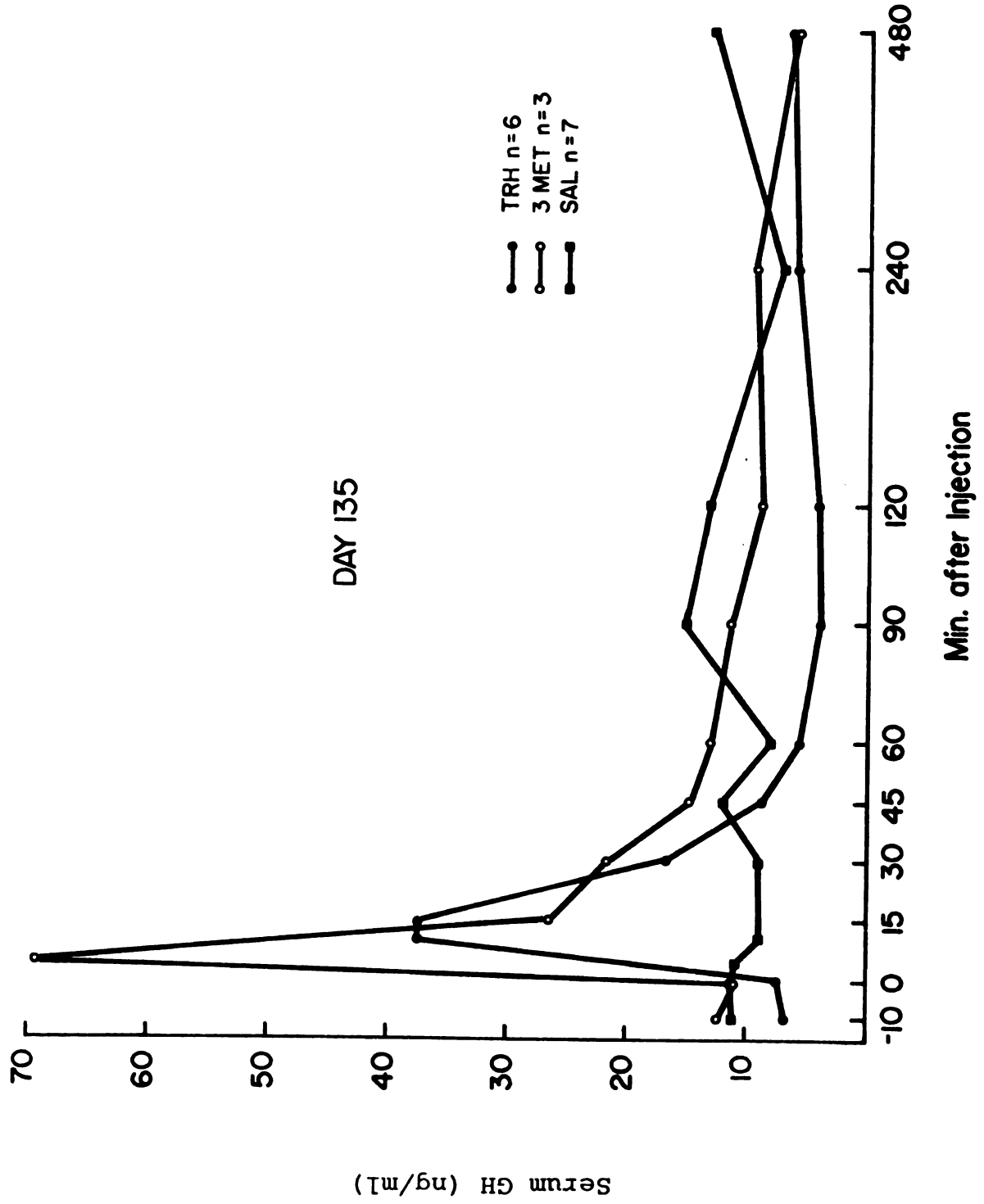

DAY 105
TRH n=10
3 MET n=6
SAL n=10
Serum GH (ng/ml)
50
40
30
20
10
-10 0 15 30 45 60 90 120 240 480
Min. after Injection

Figure 6. Serum growth hormone concentration at 135 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).


Serum GH (ng/ml)
70
60
50
40
30
20
10
-10 0 15 30 45 60 90 120 240 480
Min. after Injection
DAY 135
TRH n=6
3 MET n=3
SAL n=7

greater ($P<0.05$) than pre-injection levels at 5 minutes post-injection for only days 60 and 135.

Serum GH concentration of calves given TRH was greater ($P<0.05$) than for those given saline at 15 minutes post-injection at day 45 (Figure 3). A difference in serum GH concentration of at least 20 ng/ml between TRH and saline occurred at 5, 10, 15, and 30 minutes post-injections, but only differences at 15 minutes were significant. The greatest difference in serum GH concentration after giving 3 MET or saline was 16.9 ng/ml at 15 minutes post-injection but the difference was not significant.

At day 60 (Figure 4), serum GH concentration in calves after TRH was greater ($P<0.05$) than those given saline at 5, 10, 15, 30, and 45 minutes post-injection. Calves injected with 3 MET had lower ($P<0.05$) serum GH concentration than calves injected with saline at 10 minutes pre-injection. At no times post-injection was serum GH concentration in calves given 3 MET significantly greater than comparable values for calves given saline.

Serum GH concentration is shown in Figure 5 for calves at day 105. At 5, 10, and 15 minutes post-injection serum GH concentration of TRH-injected calves exceeded that for saline calves ($P<0.05$). However, at 60, 90, and 120 minutes post-injection serum GH concentration in saline treated calves exceeded that of TRH and 3 MET-injected calves. Serum GH concentration after 3 MET injection

increased only 4.9 ng/ml at 10 minutes post-injection from the level at injection.

At day 135 (Figure 6), serum GH concentration was greater ($P<0.05$) in calves given TRH than those given saline at 10 and 15 minutes post-injection. Serum GH concentration in calves given 3 MET was greater ($P<0.05$) than those given saline at 5 and 10 minutes post-injection.

The difference in magnitude and consistency of increases in serum GH concentration resulting from TRH and 3 MET injections is illustrated further by either the ratio or difference in maximum GH concentration occurring by 30 minutes post-injection when compared to GH concentration at injection (Table 8A). At 45, 60, 105, and 135 days the ratio was 6.3, 6.1, 6.9, and 5.7 respectively for TRH and 2.1, 3.7, 2.6, and 7.3 for 3 MET. At 45, 60, 105, and 135 days of age the difference for TRH was 47.5, 35.8, 38.0, and 35.1 ng/ml but only 20.9, 16.8, 4.3, and 62.4 ng/ml for 3 MET, respectively. The ratio and difference were not significant for TRH treatment between days. The difference was greater ($P<0.05$) at day 135 than at day 105 while the ratio at day 135 was greater ($P<0.05$) than day 45 and 105 in calves receiving 3 MET. Ratios and differences for TRH were greater ($P<0.05$) than corresponding values for calves given 3 MET at day 45, 60, and 105 but not at day 135.

The magnitude of increase in GH concentration during the 30 minute period post-injection of TRH and 3 MET

TABLE 8A.--Ratios to and differences between serum growth hormone at maximum concentration after injection of thyrotropin releasing hormone (TRH) or 3 methyl thyrotropin releasing hormone (3MET) concentration at injection for calves at day 45, 60, 105, and 135

		Days			
		45	60	105	135
Ratio	TRH	6.3^{a}	6.1^{a}	6.9^{a}	5.7
	3MET	2.1^{Bb}	3.7^{ABb}	2.6^{Bb}	7.3^{A}
Difference (ng/ml)	TRH	47.5^{a}	35.8^{a}	38.0^{a}	35.1
	3MET	20.9^{ABb}	16.8^{ABb}	4.3^{Ab}	62.4^{A}

$^{ab}P < .05$. Means for ratio or difference within a day not sharing same superscript significantly different among treatments.

$^{AB}P < .05$. Means within a row significantly different between days.

for each calf are categorized in Table 8B. At day 45, serum GH in each calf after TRH injections increased by at least 15 ng/ml with 3 of 6 exhibiting an increase serum GH greater than 50 ng/ml. In contrast, serum GH after 3 MET injections increased more than 15 ng/ml in only 2 of 6 calves. At day 60 TRH caused an increase in serum GH which exceeded 15 ng/ml in 7 of 9 calves while only 4 of 9 calves injected with 3 MET had a comparable increase in serum GH.

At day 105, a decrease in number of calves responding with an increase in serum GH concentration to TRH or 3 MET was observed. Thus serum GH increased more than 10 ng/ml in only 5 of 10 calves given TRH while 1 of 6 calves given 3 MET responded with an increase of 10 ng/ml or greater. Of the 5 calves exhibiting an increase greater in serum GH than 10 ng/ml, 4 responded with an increase of 40 ng/ml or greater. At day 135 all 6 calves given TRH responded with an increase in serum GH of 15 ng/ml or greater while the 3 calves given 3 MET responded with an increase of 30 ng/ml or greater.

Serum GH at -10 and 0 minutes were averaged to estimate basal concentrations for each treatment at each day and overall (Table 9). Calves injected with TRH had lower nonsignificant GH concentrations than that of saline at each day sampled and the overall mean for TRH was less ($P<0.05$) than saline. Calves injected with 3 MET had lower ($P<0.05$) basal GH concentration than saline at 105 days of

TABLE 8B.--Number of calves at each of 4 ages categorized as to magnitude of increase in serum growth hormone (ng/ml) after injection of thyrotropin releasing hormone (TRH) or 3 methyl thyrotropin releasing hormone (3MET).

Increased (ng/ml) above Baseline	Days							
	45		60		105		135	
	TRH	3MET	TRH	3MET	TRH	3MET	TRH	3MET
<5.0	-	2	2	1	3	4	-	-
5.0-10.0	-	-	-	1	2	1	-	-
10.1-15.0	-	2	-	3	-	1	-	-
15.1-20.0	1	-	1	1	-	-	2	-
20.1-30.0	-	1	2	2	1	-	1	-
30.1-40.0	2	-	-	1	-	-	1	1
40.1-50.0	-	-	1	-	2	-	1	1
>50.0	3	1	3	-	2	-	1	1
n	6	6	9	9	10	6	6	3

TABLE 9.--Baseline serum growth hormone (GH) (ng/ml) (average of -10 and 0 min) at day 45, 60, 105, and 135 for calves receiving daily injection of thyrotropin releasing hormone (TRH), 3-methyl thyrotropin releasing hormone (3MET), or Saline (Sal).

	Days				
	45	60	105	135	Average
	----------GH (ng/ml)----------				
TRH	11.5	8.5	8.0^{AB}	7.1	8.6^{B}
3MET	13.9^{a}	7.1^{ab}	4.3^{Ab}	11.6^{ab}	8.7^{AB}
Sal	18.9^{a}	14.2^{ab}	9.2^{Bb}	11.3^{b}	12.9A
Average	14.8^{a}	9.9^{ab}	7.6^{b}	9.8^{a}	

$^{ab}P<.05$. Means within a row not sharing same superscript significantly different.

$^{AB}P<.05$. Means within a column not sharing same superscript significantly different.

age. Overall, the magnitude of difference in serum GH concentration between 3 MET and saline treatments was similar to the magnitude of difference in serum GH concentration between TRH and saline and approached significance ($P<0.10$).

Thyroxine

Linear regression equations combining data from all test days and their graphic presentation for changes in serum thyroxine concentration with time after treatment are shown in Figure 7. Daily injections of TRH or 3 MET but not saline resulted in significant regressions ($P<0.0005$) indicating serum thyroxine concentration increased after injection of TRH or 3 MET. The y-intercepts for TRH and 3 MET regressions were lower than that of saline with the difference between 3 MET and saline significant ($P<.05$). The slope of each regression equation was used as an estimate of potency of treatments. Slopes ranked in order of magnitude (largest to smallest) were 3 MET, TRH, and saline with slopes for calves given 3 MET and TRH each greater ($P<.01$) than saline injected calves but not different from each other ($P>.10$).

Table 10 lists linear regression equations for each treatment at each day. Lines described by these equations and actual treatment means are plotted in Figures 8, 9, 10, and 11. At day 45, injection of 3 MET and TRH resulted in significant regressions for serum

Figure 7. Plots of linear regression equations for serum thyroxine concentration combined for all days following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET) or saline (Sal).

Overall		R^2	Sign of Regress	n
TRH	$\hat{Y} = 77.3 + 3.60X$	.22	<.0005	31
3 MET	$\hat{Y} = 69.3 + 4.64X$	.27	<.0005	24
SAL	$\hat{Y} = 81.6 + 0.81X$	.01	0.181	32

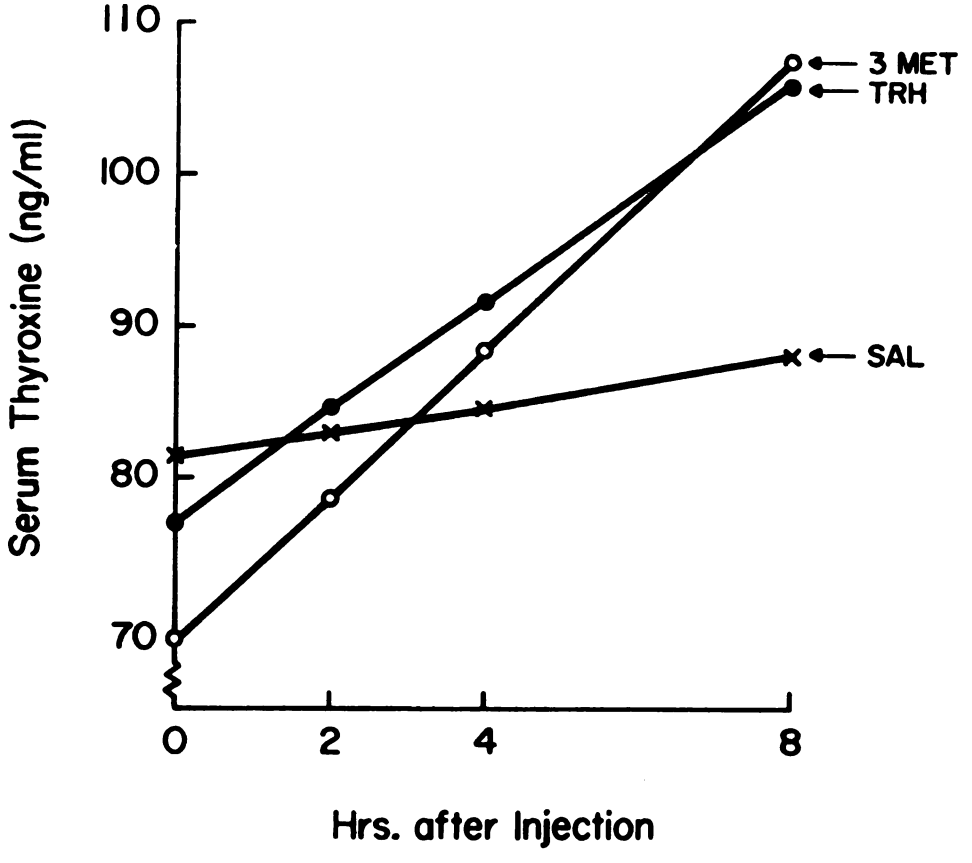


TABLE 10.--Linear regression equations of serum thyroxine concentration (Y) with time (X) (hrs) after injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or Saline (Sal) into calves at day 45, 60, 105, and 135.

Day 45		R^2	Sign. of Regress.
TRH	$\hat{Y} = 72.1 + 6.25\ (X)$	.37	.002
3-MET	$\hat{Y} = 47.7 + 8.65\ (X)$	.66	<.0005
Sal	$\hat{Y} = 73.9 + 1.29\ (X)$	.03	.423
Day 60			
TRH	$\hat{Y} = 81.5 + 3.15\ (X)$	.21	.004
3-MET	$\hat{Y} = 84.1 + 4.41\ (X)$	.26	.002
Sal	$\hat{Y} = 75.5 + 0.71\ (X)$	.02	.410
Day 105			
TRH	$\hat{Y} = 64.7 + 2.81\ (X)$	.33	<.0005
3-MET	$\hat{Y} = 61.3 + 1.96\ (X)$	.14	.069
Sal	$\hat{Y} = 69.8 + 0.64\ (X)$	.03	.290
Day 135			
TRH	$\hat{Y} = 97.6 + 2.50\ (X)$	.25	.014
3-MET	$\hat{Y} = 86.0 + 2.47\ (X)$	.31	.097
Sal	$\hat{Y} = 112.4 + 0.81\ (X)$	.06	.212

Figure 8. Plots of linear regression equations for serum thyroxine concentration in calves 45 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

T, M, and S symbols on graph represent actual thyroxine means for the three respective treatments.

DAY 45		R^2	Sign of Regress	n
TRH	$\hat{Y} = 72.1 + 6.25\ X$	.37	.002	6
3 MET	$\hat{Y} = 47.7 + 8.65\ X$	.66	<.0005	6
SAL	$\hat{Y} = 73.9 + 1.29\ X$	.03	.423	6

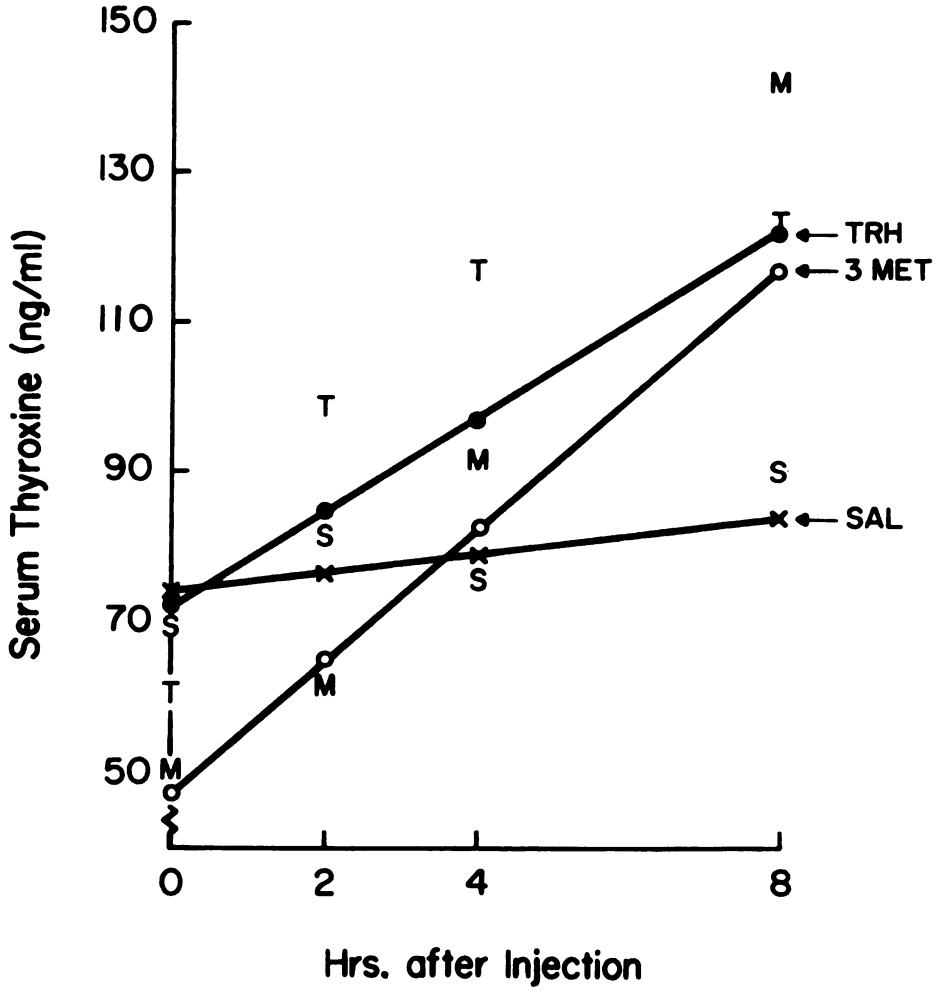


Figure 9. Plots of linear regression equations for serum thyroxine concentration in calves 60 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

T, M, and S symbols on graph represent actual thyroxine means for the three respective treatments.

DAY 60		R^2	Sign of Regress	n
TRH	$\hat{Y} = 81.5 + 3.15\ X$	.21	.004	9
3 MET	$\hat{Y} = 84.1 + 4.41\ X$	.26	.002	9
SAL	$\hat{Y} = 75.5 + 0.71\ X$	.02	.410	9

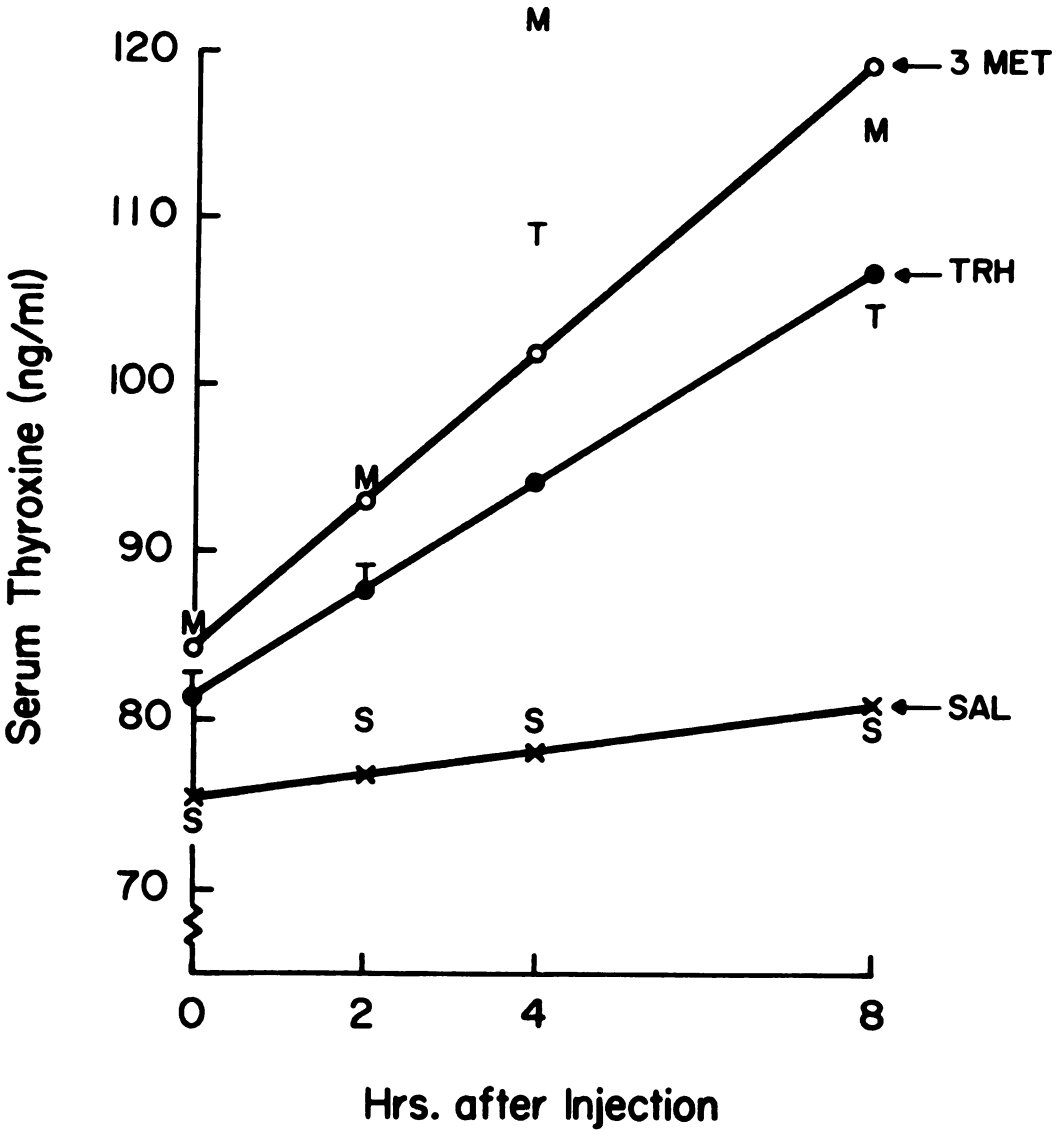


Figure 10. Plots of linear regression equations for serum thyroxine concentration in calves 105 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

T, M, and S symbols represent actual thyroxine means for the three respective treatments.

DAY 105		R	Sign of Regress	n
TRH	$\hat{Y} = 64.7 + 2.81\ X$	.33	<.0005	10
3 MET	$\hat{Y} = 61.3 + 1.96\ X$	.14	.069	6
SAL	$\hat{Y} = 69.8 + 0.64\ X$	.03	.290	10

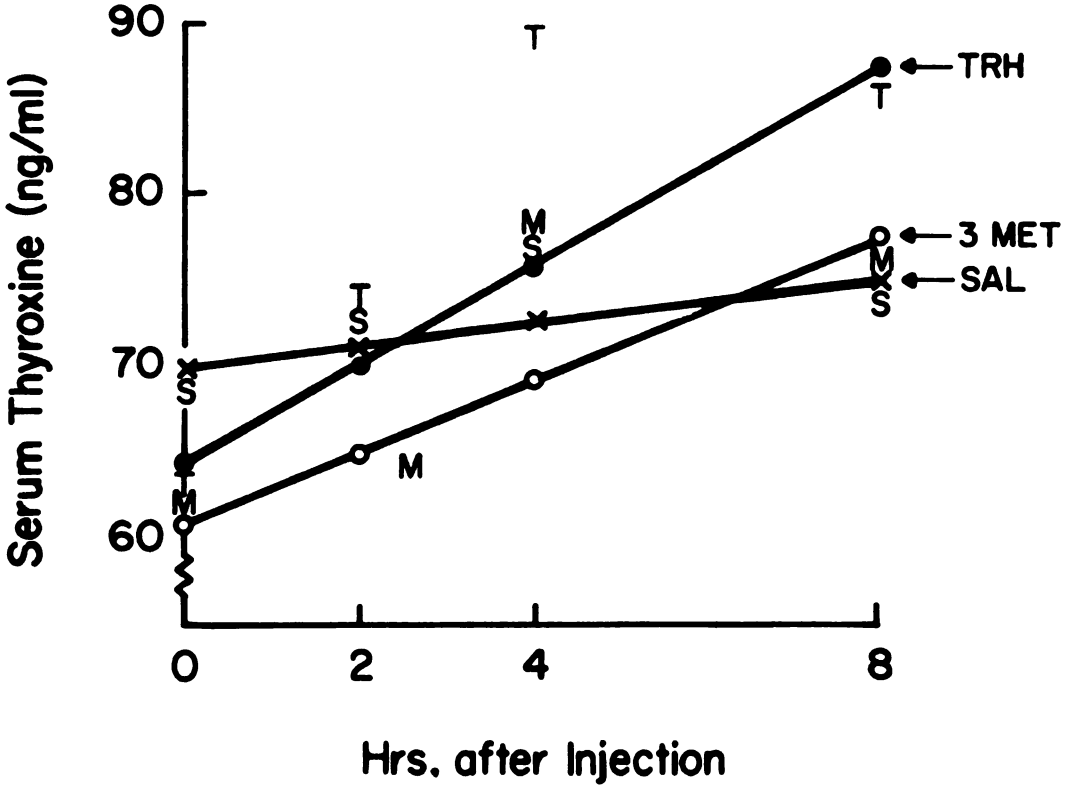
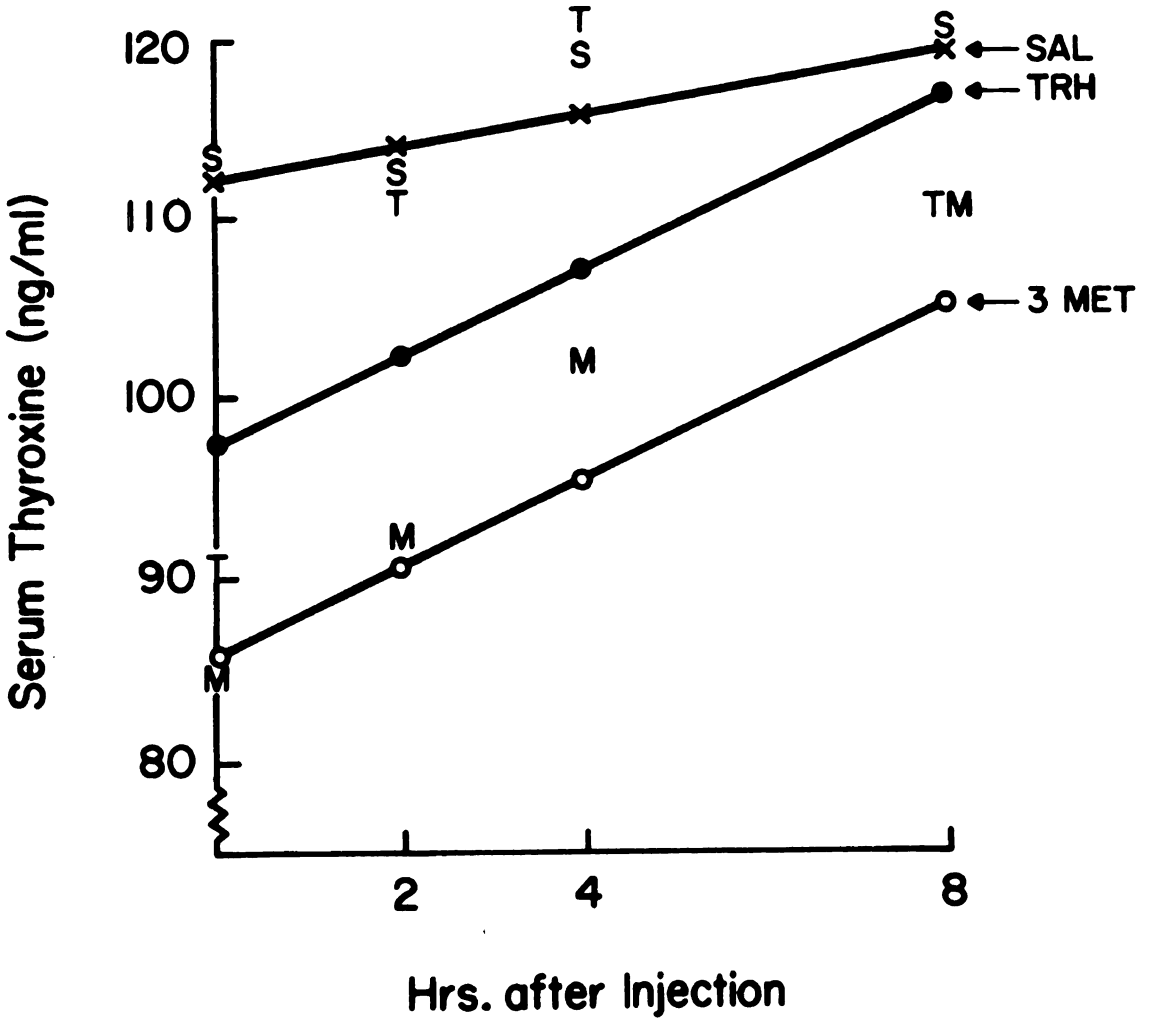


Figure 11. Plots of linear regression equations for serum thyroxine concentration in calves 135 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

T, M, and S symbols represent actual thyroxine means for the three respective treatments.

DAY 135		R^2	Sign of Regress	n
TRH	$\hat{Y} = 97.6 + 2.50\ X$	.25	.014	6
3 MET	$\hat{Y} = 86.0 + 2.47\ X$	.31	.097	3
SAL	$\hat{Y} = 112.0 + 0.81\ X$	.06	.212	7



thyroxine. The y-intercept for 3 MET was significantly less than either saline or TRH initially. This is purely random since calves had received no prior injections and had been assigned to treatments by weight at 45 days of age or by genetic background at birth. Using slopes of regression equations as an estimate of potency, 3 MET treatment was the most potent followed by TRH and saline. Slopes for 3 MET and TRH injections were greater ($P<0.05$) than saline but not different from each other. Examinations of the actual means shown in Figure 8 indicate that the increase in serum thyroxine induced by TRH was faster than that induced by 3 MET but the effect of 3 MET persisted longer. In fact, serum thyroxine concentration appears to be increasing even at 8 hours after 3 MET injection.

At day 60 (Figure 9), injection of TRH and 3 MET resulted in significant regressions. Comparisons of y-intercepts between treatments at day 60 were not significant. The y-intercepts were greater for TRH and 3 MET at day 60 than at day 45 but when compared to day 45, only 3 MET was greater ($P<.01$). The slope of the regression equation describing serum thyroxine response to injection of 3 MET was greater ($P<.05$) than that for saline but not different from that for TRH. Differences in the slope for TRH and control regression equations approached significance ($P<.08$). The patterns of actual means for 3 MET and TRH were similar at each time but always greater for 3 MET.

Injection of TRH at day 105 produced a highly significant regression ($P<0.01$) while that for 3 MET injection only approached significance ($P<0.10$) (Figure 10). Again, regression for saline was not significant. The y-intercepts for regression equations for 3 MET and TRH but not saline were lower ($P<0.05$) than comparable values at 60 days of age.

At day 135 injection of TRH resulted in a significant regression ($P<0.01$) (Figure 11). Due to small numbers involved, the regression for 3 MET treatment only approached significance ($P<0.10$) while that for saline was not significant ($P<0.10$). Serum thyroxine concentration at time zero (y-intercepts) for saline was greater ($P<0.01$) than that of TRH and 3 MET treatments and the difference between y-intercepts for TRH and 3 MET approached significance ($P<0.10$). Differences in slopes between the two treatments were not significant ($P>0.10$).

Serum thyroxine concentration for calves given saline at day 135 was greater ($P<0.01$) than comparable values for all previous days with similar y-intercepts at 45, 60, and 105 days. Differences in y-intercepts on different days followed a similar pattern for both TRH and 3 MET treatments. Intercepts at day 60 were greater than at day 45, but decreased at day 105 and increased at day 135.

Saline treated calves had similar slopes of regression equations for each test day which indicates a

relatively constant thyroxine output from day 45 to 135. Injection of 3 MET or TRH on day 45 produced a marked stimulation of thyroxine output (indicated by slopes of equations) but this response diminished with repeated injections. Difference in magnitude of slopes between day 45 and 60 was significant for 3 MET ($P<0.05$) and approached significance for TRH ($P = 0.10$). By day 105 differences in slopes from day 45 were significant for TRH and 3 MET treatments and remained significantly different from day 45 at day 135. Slopes at days 60, 105, and 135 were not different between days for TRH and 3 MET treatments. The decrease in thyroxine response to TRH and 3 MET occurred early in the repeated injection scheme followed by smaller depressions at later dates.

Prolactin

Results of split-plot analysis of variance were similar for analysis I and analysis II. Therefore only analysis I which includes all treatments will be discussed.

Average serum prolactin concentration increased with age (Table 7). This was determined by using all prolactin values from 6 calves injected with saline at days 45, 60, and 105. Calves at day 105 had greater ($P<0.01$) serum prolactin concentration than those at 45 or 60 days of age. Time relative to saline injection and day X time interaction did not affect ($P>0.10$) serum prolactin concentration.

Overall changes of serum prolactin concentration with time after injection of TRH, 3 MET, or saline are shown in Figure 12. Injection of TRH resulted in an increase ($P<0.01$) in serum prolactin concentration above controls at 15, 30, 45, and 60 minutes post-injection. Prolactin (ng/ml) in calves injected with TRH declined to 41.3 at 30 minutes and remained within 5 ng/ml of this at 45 and 60 minutes post-injections. Injection of 3 MET resulted in an increase ($P<0.05$) in serum prolactin concentration relative to saline at 15, 30, and 45 minutes post-injection while at 60 minutes post-injection the difference in serum prolactin concentration between calves given 3 MET and saline approached significance ($P<0.07$). At 60 minutes post-injection serum prolactin concentration in TRH-injected calves was greater ($P<0.05$) than that of calves injected with 3 MET. The differences in serum prolactin concentration between calves receiving TRH and 3 MET at earlier times were not significant.

The response of serum prolactin to each treatment at each time on the four test days is shown in Figures 13, 14, 15, and 16. At day 45 (Figure 13) calves receiving TRH had significantly greater serum prolactin at 15 minutes ($P<0.01$) and 30 minutes ($P<0.05$) than at the time of injection. Compared to calves given saline, serum prolactin concentration in TRH-treated calves was greater ($P<0.05$) at each post-injection time. Calves receiving 3 MET had greater ($P<0.05$) serum prolactin concentration

Figure 12. Average serum prolactin concentrations in calves following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

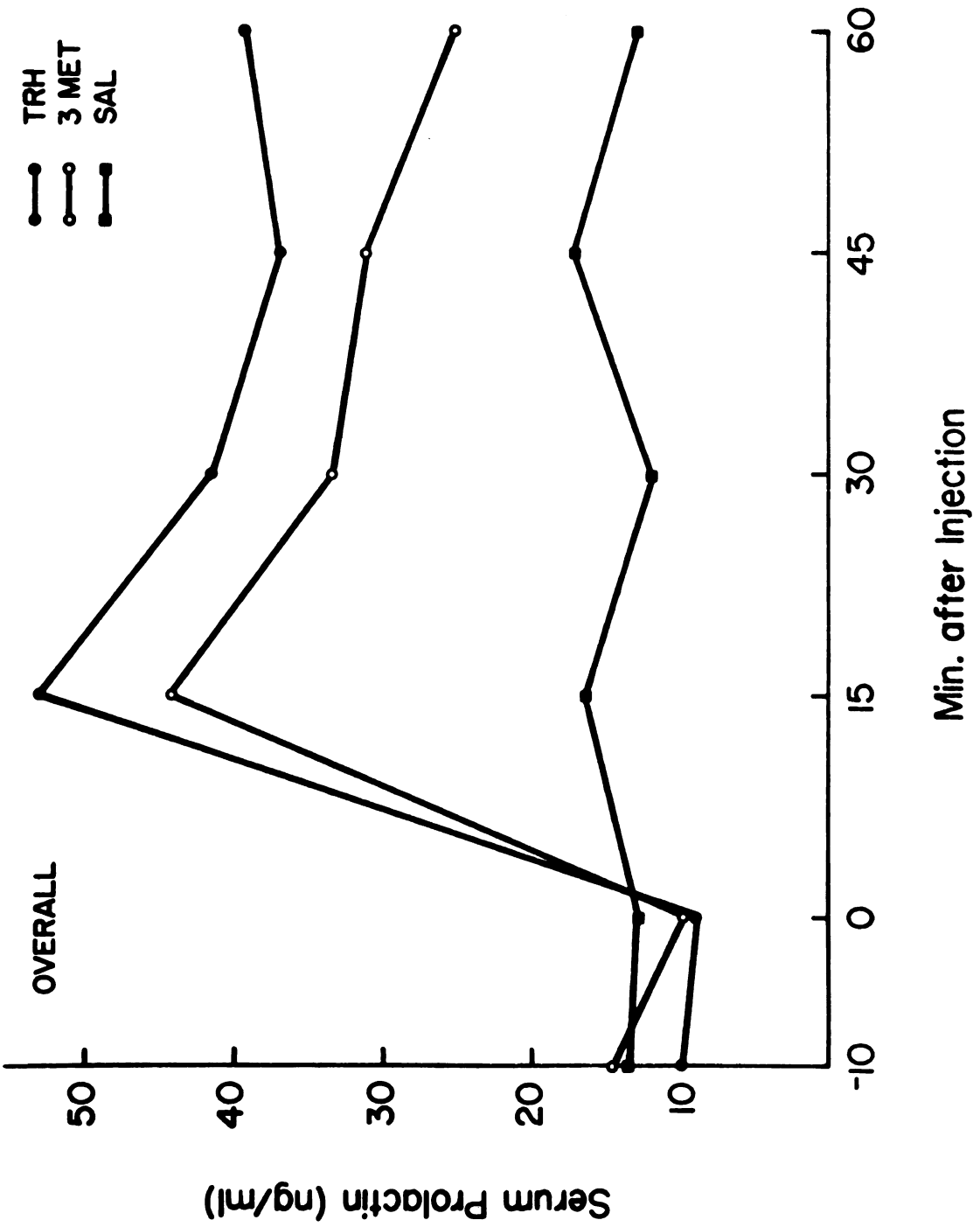

TRH
3 MET
SAL
OVERALL
Serum Prolactin (ng/ml)
50
40
30
20
10
-10
0
15
30
45
60
Min. after Injection

Figure 13. Serum prolactin concentration in calves 45 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

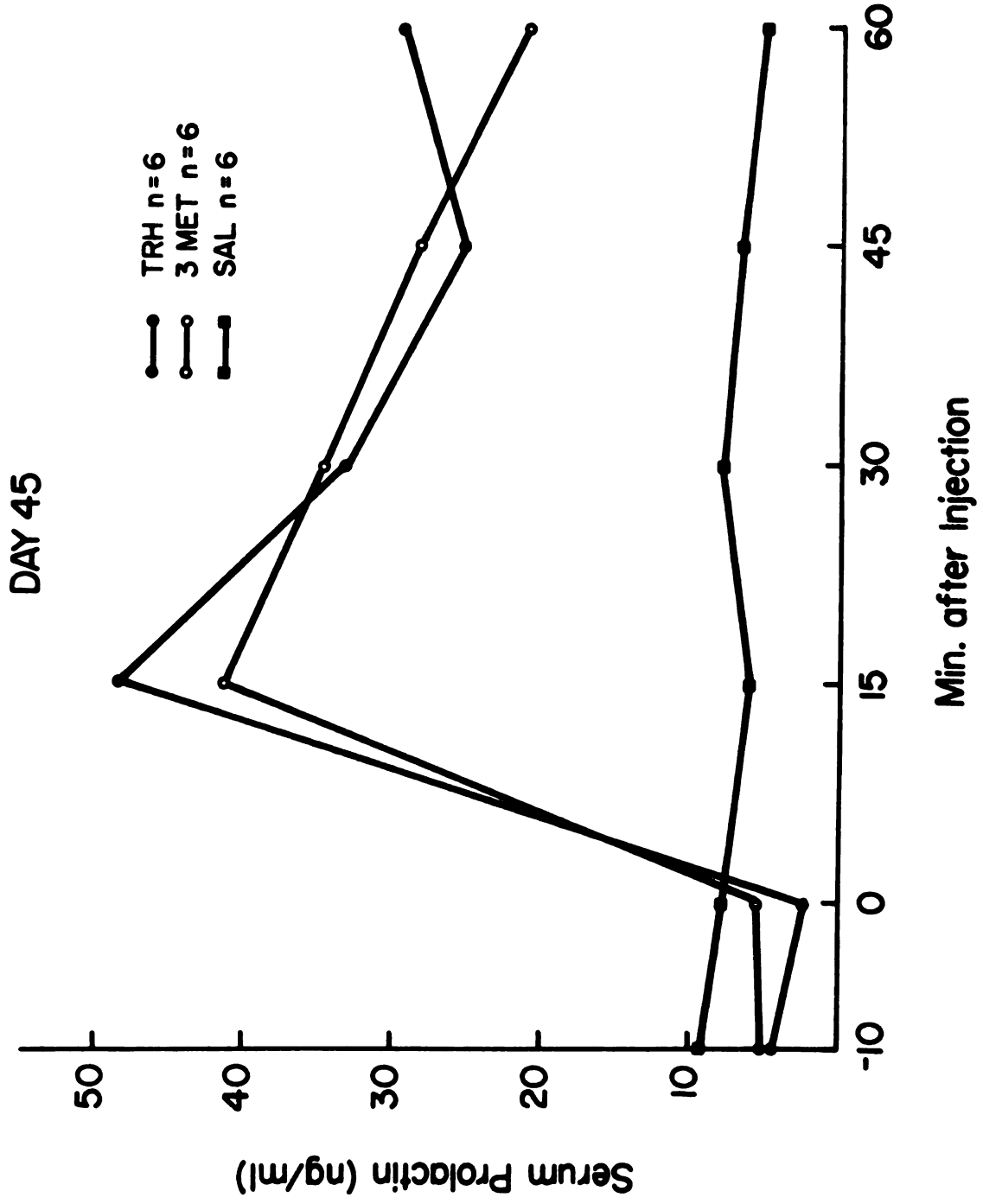

DAY 45
TRH n=6
3 MET n=6
SAL n=6
Serum Prolactin (ng/ml)
50
40
30
20
10
-10
0
15
30
45
60
Min. after Injection

Figure 14. Serum prolactin concentration in calves 60 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

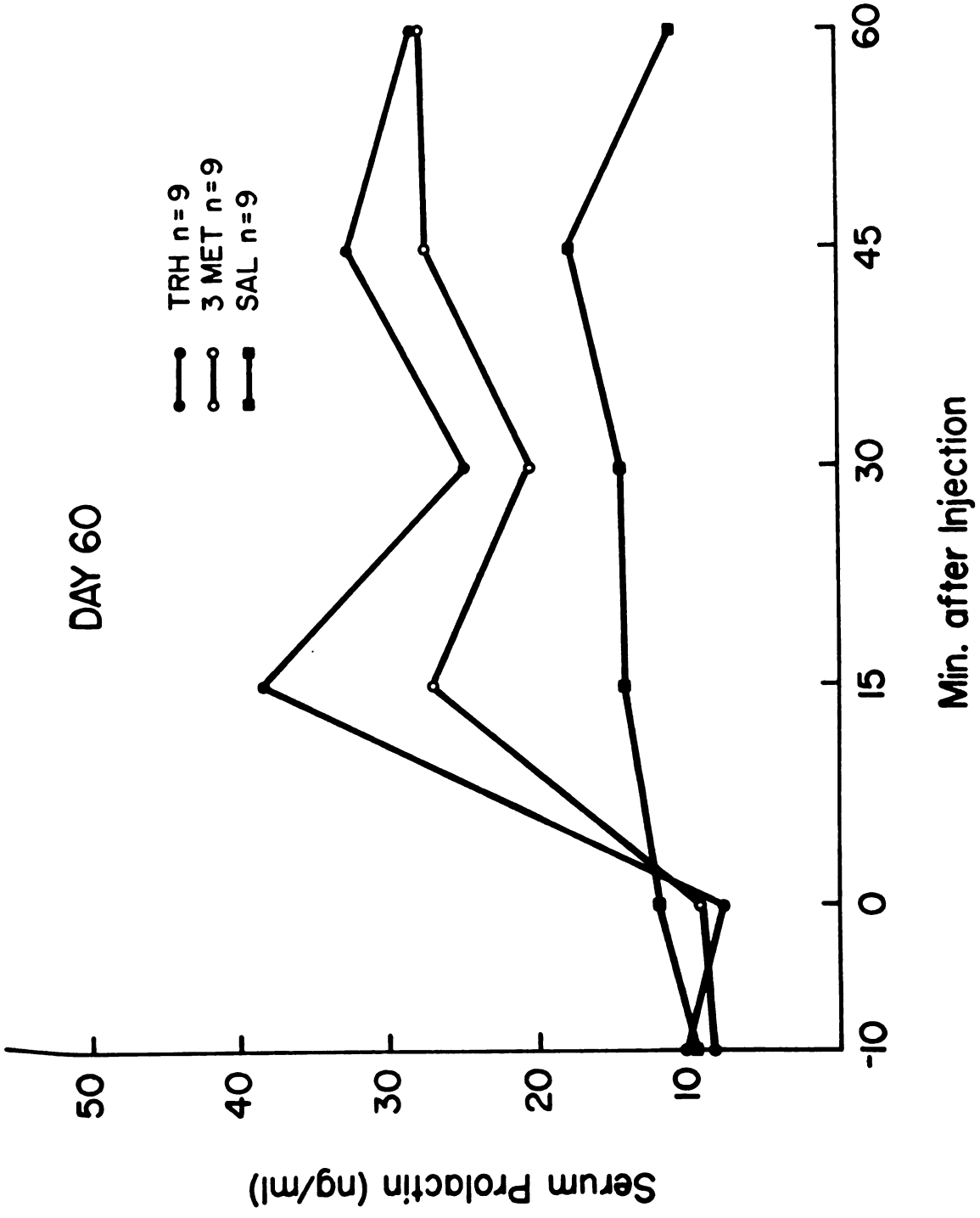

DAY 60
TRH n=9
3 MET n=9
SAL n=9
Serum Prolactin (ng/ml)
50
40
30
20
10
-10
0
15
30
45
60
Min. after Injection

Figure 15. Serum prolactin concentration in calves 105 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

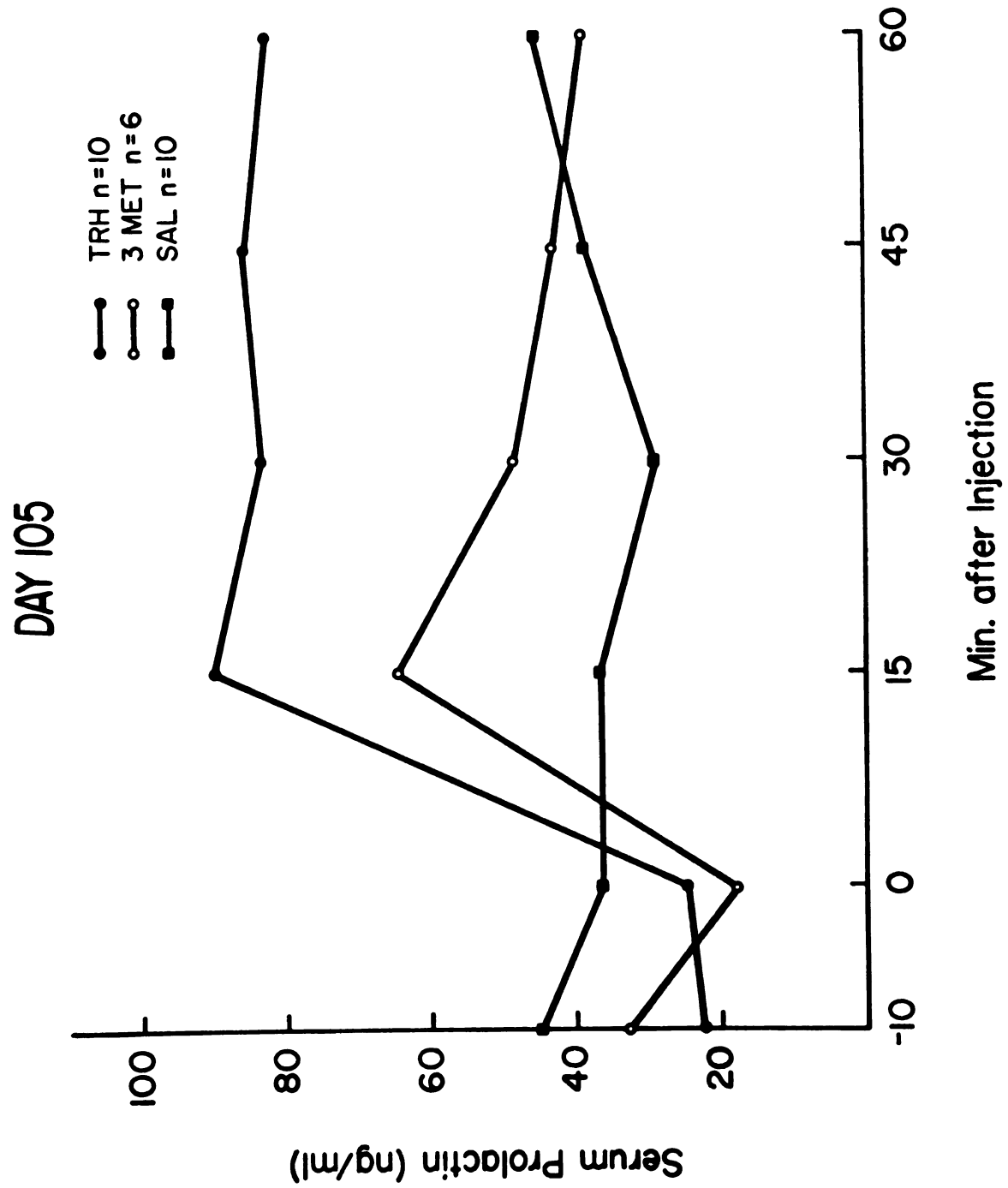
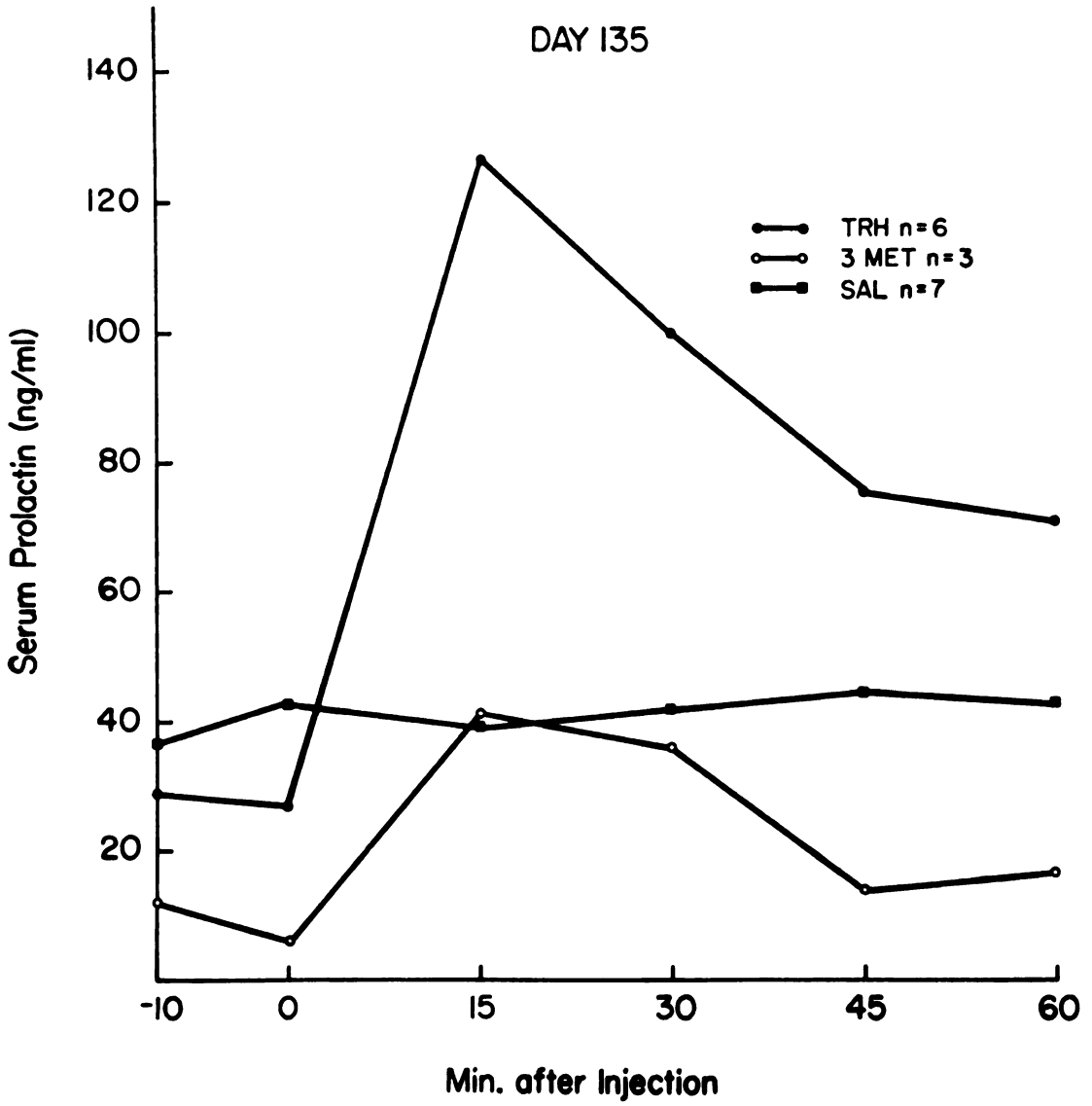

DAY 105
TRH n=10
3 MET n=6
SAL n=10
Serum Prolactin (ng/ml)
100
80
60
40
20
-10
0
15
30
45
60
Min. after Injection

Figure 16. Serum prolactin concentration in calves 135 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or Saline (Sal).


DAY 135
140
120
100
80
60
40
20
Serum Prolactin (ng/ml)
TRH n=6
3 MET n=3
SAL n=7
-10
0
15
30
45
60
Min. after Injection

than controls at 30, 45, and 60 minutes post-injection. Although serum prolactin concentration was increased at each time after 3 MET injection, the concentration at 15 minutes after injection was the only value which differed ($P<0.05$) from the level at injection.

At day 60 TRH injection resulted in an increase ($P<0.05$) in serum prolactin concentration at 15 and 45 but not at 30 and 60 minutes post-injection when compared to the level at injection (Figure 14). Compared to saline injected calves, serum prolactin concentration in TRH-treated calves was greater ($P<0.05$) at 15 minutes post-injection only. In contrast, there were no significant differences in serum prolactin concentration between 3 MET and control calves at any post-injection time and although serum prolactin concentration was increased over the pre-injection level, post-injection differences were not significant.

At day 105, calves receiving TRH had greater ($P<0.05$) serum prolactin concentration at all times after injection than did control calves. Serum prolactin concentration was increased ($P<0.05$) relative to pre-injection level at all times post-injection in calves receiving TRH. The increase in serum prolactin concentration in calves injected with 3 MET was not significant relative to that of control calves at all times post-injection and serum prolactin concentrations after 3 MET injection were not greater than that at injection.

At day 135, serum prolactin concentration at 15 minutes after TRH injection was greater ($P<0.05$) than that of control calves. At no other times post-injection were there any significant differences between TRH and saline treatments although calves receiving TRH averaged 57.1, 31.0, and 28.0 ng/ml more prolactin in serum collected at 30, 45, and 60 minutes post-injection respectively than did controls (Figure 16). Serum prolactin concentration increased after 3 MET injection but this increase was not significant at any time post-injection. Four of 7 calves receiving saline at day 135 were sampled in mid-July while all calves receiving 3 MET were sampled in late March and early April. Therefore comparison of serum prolactin between the two treatments is not justifiable due to environmental effects on serum prolactin concentration (Tucker, unpublished observations).

Insulin

The effects of age and time after injection and feeding were determined for insulin in the same manner as for growth hormone. Means were calculated from 6 samples from each of 6 calves receiving saline on the 4 sampling dates. The effect of age of average serum insulin is shown in Table 7. Serum insulin at day 45 and 60 were 1.04 and 1.03 ng/ml and increased to 1.32 ng/ml at day 105. However, at day 135 average serum insulin increased ($P<0.01$) to 2.13 ng/ml.

Average serum insulin concentration during the 8 hour period following injection of saline and feeding (concurrent) is shown in Figure 17. Serum insulin decreased during the 60 minute period following feeding from 1.57 to 1.09 ng/ml and this decrease approached significance ($P<0.10$). Insulin increased only slightly during the next hour to 1.18 ng/ml and by 4 hours after feeding returned to 1.42 ng/ml. At 8 hours after feeding serum insulin increased to 2.27 ng/ml which was higher ($P<0.01$) than at all previous times.

Serum insulin concentrations for calves injected with TRH, 3 MET, or saline are shown in Table 11. Analysis of the data to 105 days of age resulted in a mean serum insulin concentration of 1.51, 1.19, and 1.14 ng/ml for TRH, 3 MET, and saline respectively with calves injected with TRH having greater ($P<0.05$) serum insulin value than the other two treatments. Adding day 135 to insulin values to TRH and saline treatments increased these means 20.5 and 19.3%, respectively. Serum insulin was also greater ($P<0.01$) in calves receiving TRH in Analysis II.

No significant interactions were obtained in any analysis of these data. However, the insulin response to injections with time was different for each treatment. The concentration of insulin at 45 and 60 days of age is shown in Figure 18. At day 45 and 60 serum insulin concentrations were relatively stable following injections with TRH and 3 MET and no significant differences with time

Figure 17. Average serum insulin concentration in calves (n = 24) for 8 hr. period following intramuscular injection of saline.

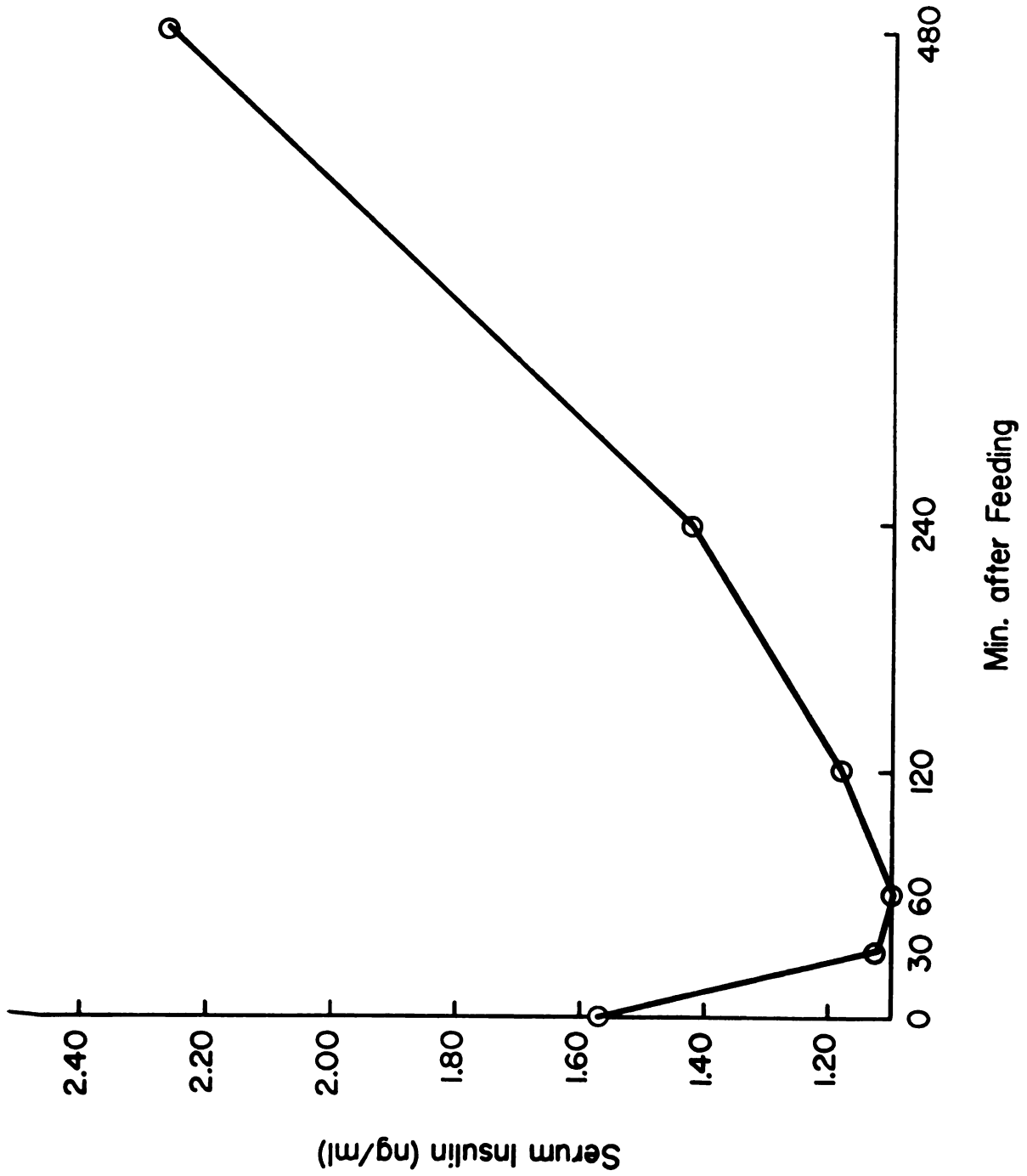

Serum Insulin (ng/ml)
2.40
2.20
2.00
1.80
1.60
1.40
1.20
0
30
60
120
240
480
Min. after Feeding

TABLE 11.--Average serum insulin concentration in calves after injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), and saline (Sal).

Analysis[1]	Treatment		
	TRH	3-MET	Sal
	----------ng/ml[2]----------		
I	$1.51^{a} \pm 0.12$	$1.19^{b} \pm 0.11$	$1.14^{b} \pm 0.06$
II	$1.82^{A} \pm 0.12$	-----	$1.36^{B} \pm 0.08$

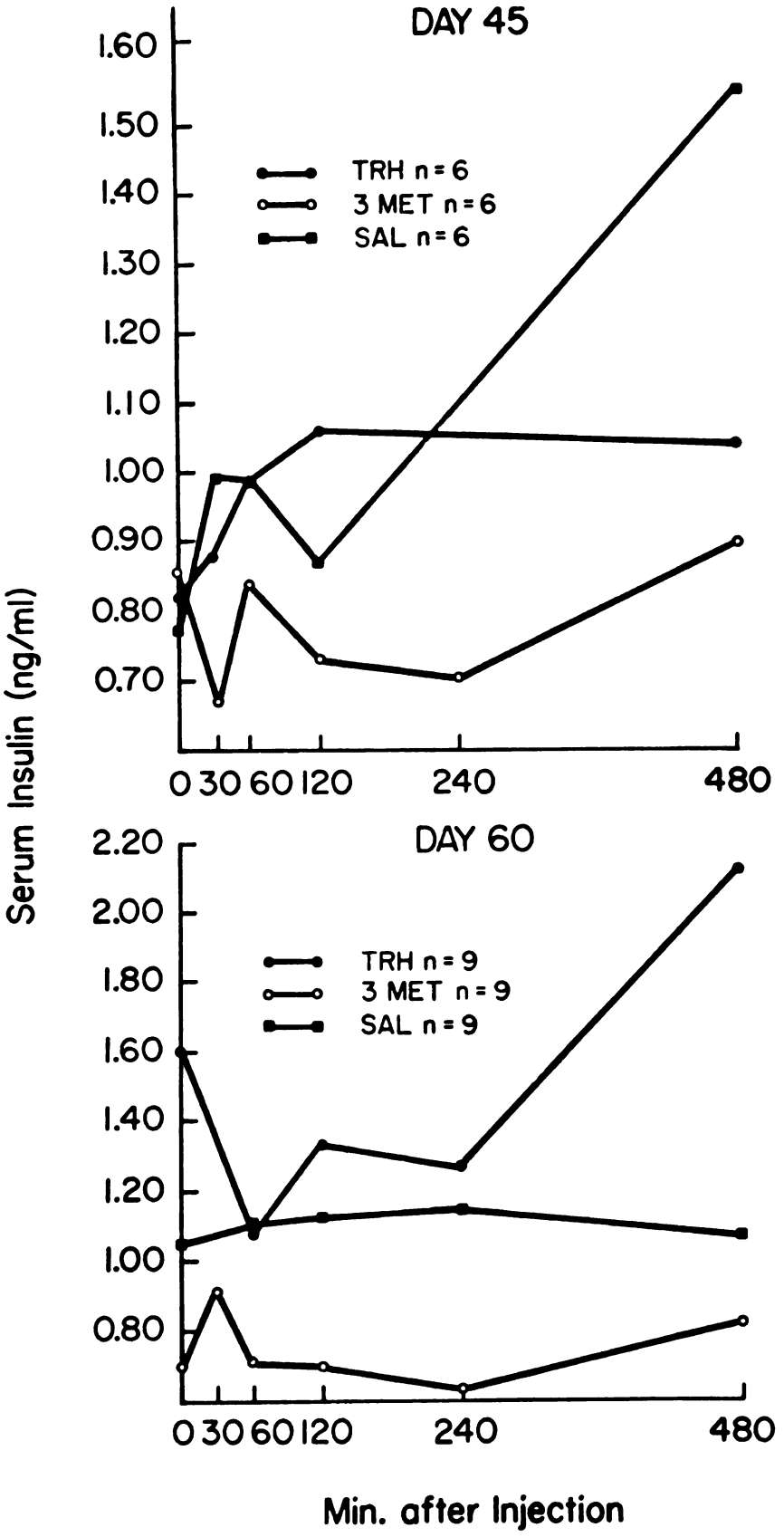
$^{ab}P<.05$. Means not sharing same superscript within a row significantly different.

$^{AB}P<.01$. Means not sharing same superscript within a row significantly different.

[1]Analysis I. 6 Animals per treatment at day 45, 60, and 105.
Analysis II. 6 Animals per treatment at day 45, 60, 105, and 135.

[2]1 ng equals 24.4 uU/ml crystalline bovine insulin.

Figure 18. Serum insulin concentration in calves 45 (A) and 60 (B) days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or Saline (Sal).


DAY 45
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
TRH n = 6
3 MET n = 6
SAL n = 6
0 30 60 120 240 480
DAY 60
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
TRH n = 9
3 MET n = 9
SAL n = 9
0 30 60 120 240 480
Serum Insulin (ng/ml)
Min. after Injection

or treatment were found, but at 105 and 135 days of age responses to treatments were different than those noted at days 45 and 60 and these are shown in Figures 19 and 20, respectively. On day 105 at 60 minutes post-injection, calves injected with 3 MET had higher ($P<0.05$) serum insulin concentrations than saline-injected calves and the difference in serum insulin concentration between TRH and saline approached significance ($P<0.10$). Serum insulin concentration was markedly higher with either TRH or 3 MET injections than with saline during the 4 hour interval following injection. Serum insulin concentration in calves receiving saline was higher ($P<0.05$) at 480 minutes post-injection than at all previously measured times for calves receiving saline.

At day 135 calves injected with TRH had greater ($P<0.05$) serum insulin concentration at 60 minutes post-injection than calves given 3 MET or saline. Although there was a greater difference in serum insulin concentration between calves injected with TRH and those given 3 MET or saline at 120 minutes than at 60 minutes statistical evaluation showed these differences non-significant.

Remaining Parameters

Glucose

Average plasma glucose is shown in Table 12 for each of 4 intervals according to treatments and are averaged for days 60, 105, and 135. No significant

Figure 19. Serum insulin concentration in calves 105 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

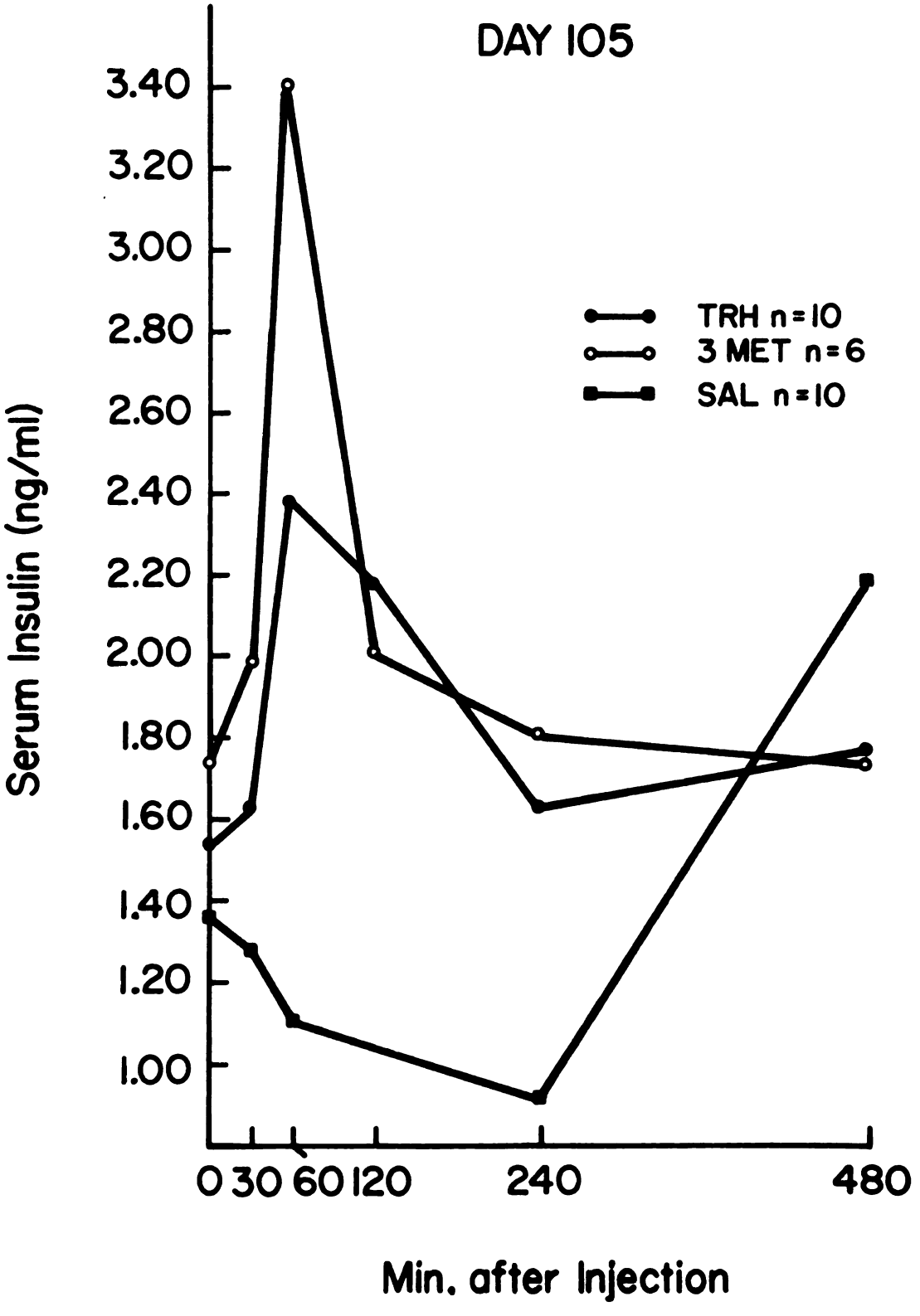

DAY 105
3.40
3.20
3.00
2.80
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
Serum Insulin (ng/ml)
TRH n=10
3 MET n=6
SAL n=10
0 30 60 120 240 480
Min. after Injection

Figure 20. Serum insulin concentration in calves 135 days of age following intramuscular injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

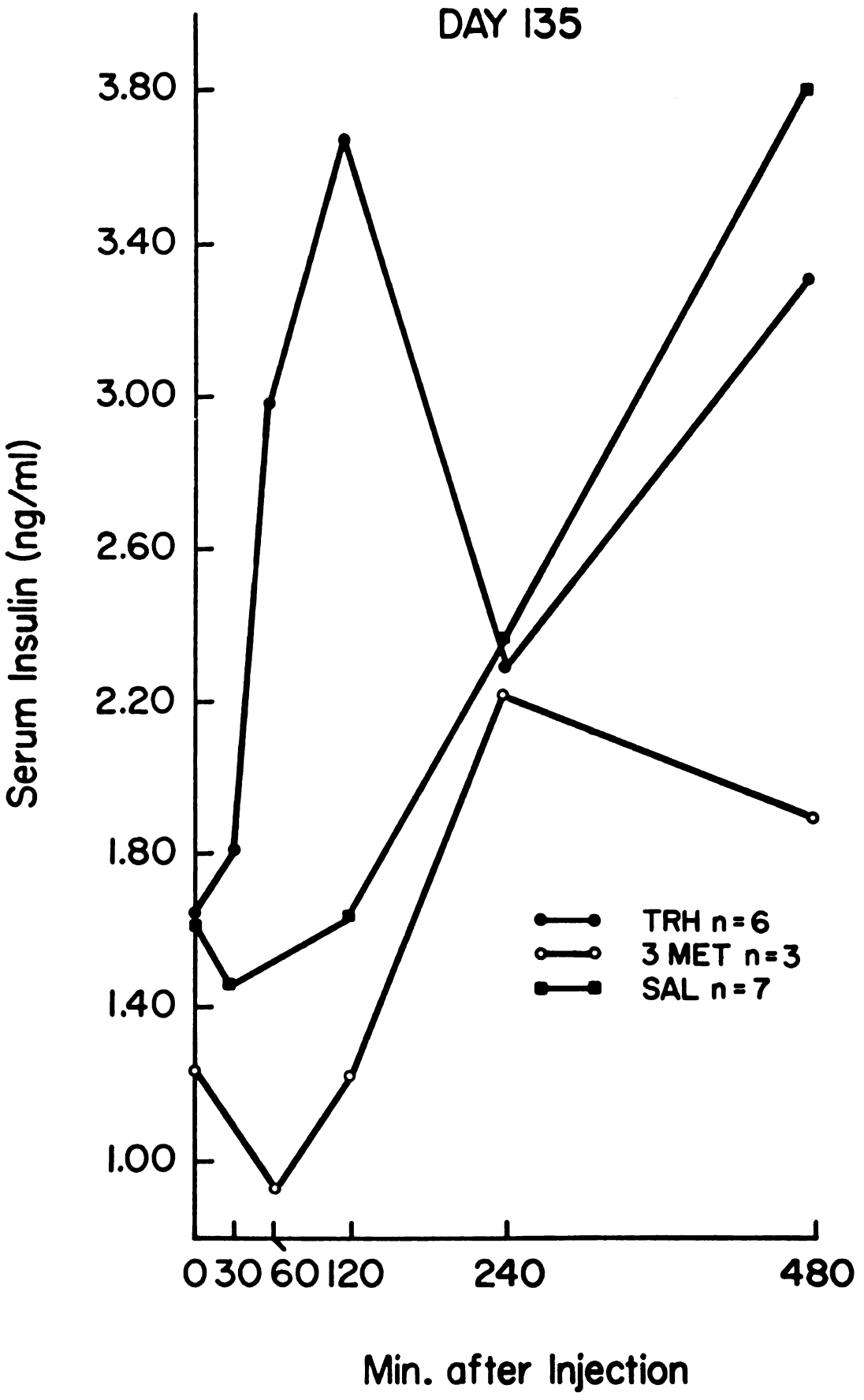

DAY 135
3.80
3.40
3.00
2.60
2.20
1.80
1.40
1.00
Serum Insulin (ng/ml)
TRH n=6
3 MET n=3
SAL n=7
0 30 60 120 240 480
Min. after Injection

TABLE 12.--Average plasma glucose concentration at 0, 2, 4, and 8 hr after injection of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

Treatment	Time (Hrs)			
	0	2	4	8
TRH	52.2[1]	54.5	58.0	60.6
3-MET	50.4	57.1	55.7	58.8
Sal	52.3	58.5	55.8	57.4

[1]mg/100 ml

differences in plasma glucose due to time, treatment, or day were noted. Plasma glucose concentration was higher at all times after injection than at injection. This increase with time was probably related to calves consuming a large portion of their daily feed soon after injection more than any other factor.

Non-esterified Fatty Acids (NEFA)

In analysis I, main effects and their interactions were not significant. Analysis II resulted in significant differences for treatment and day. Calves injected with TRH when compared to saline-injected calves had lower ($P<0.05$) plasma NEFA overall (Table 13). This lower plasma NEFA in TRH-injected calves was due mainly to values obtained on day 135. Plasma NEFA increased with age as

TABLE 13.--Non-esterified fatty acids (NEFA) in calves: Age and treatment effects.

	Age in Days			
	60	105	135	MS_E
	----------µeq per liter----------			
NEFA	168.9^{b}	182.0^{ab}	191.1^{a}	219.6
	n = 48			

	Treatment[1]		
	TRH	Sal	MS_E
	----------µeq per liter----------		
NEFA	170.6^{b}	190.7^{a}	226.9
	n = 72		

$^{ab}P<.05$. Means not sharing same superscript within a row significantly different.

[1]Analysis II data: 3 methyl thyrotropin releasing hormone not included.

MS_E mean square error.

calves at day 60 had lower ($P<0.05$) plasma NEFA than comparable values at days 105 and 135 (Table 13).

Nitrogen Balance

During the 3 day adjustment period feed intake decreased as much as 30% in some calves. The range in dry matter intake for the collection period was 2.43 to 3.71 kg per day with TRH, 3 MET, and Sal averaging 3.40, 2.86, and 2.92 kg intake per day, respectively. No significant differences in any aspect of N-balance were found (Table 14).

TABLE 14.--Nitrogen balance in calves receiving daily injections of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

	Treatment		
	TRH	3 MET	Sal
N-intake[1]	81.0	66.1	67.5
N-balance[1]	30.4	20.4	25.6
$\frac{\text{Retained}^N}{\text{Absorbed}^N}$ X 100	54.2	45.7	53.4

[1]grams per day.

Tissue Weights

Due to a commitment to provide carcasses for a teaching class on a predetermined date a complete block of calves was slaughtered at 110 days of age. The remaining

blocks designated for slaughter were sacrificed at 135 days of age. Table 15 gives the weights of several endocrine glands, liver, and gastrocnemius muscle expressed as total weight and as percent of body weight.

With the exception of the adrenal glands all organs in calves injected with TRH were heavier than other treatments but these differences were minimized when expressed as a percent of body weight. The exception was the thyroid gland. There was a marked enlargement of the thyroid from calves receiving TRH and differences due to treatments were significant when expressed as total weight ($P<0.05$) or as a percent of body weight ($P = 0.05$) (Table 15).

Tissue Nucleic Acids

Muscle and liver nucleic acids are shown in Table 16. Statistical analysis resulted in no significant differences but trends will be discussed. The concentration of DNA in liver and muscle was greatest for calves receiving Sal and least for TRH-injected calves. The concentration of RNA in liver was greatest for calves receiving TRH and least in muscle for calves receiving 3 MET. Calves receiving TRH had the highest RNA to DNA ratio in both tissues while calves receiving saline had the lowest ratio.

When liver and muscle weights are used to give total nucleic acid content differences became more marked especially the RNA fraction. Total RNA in calves receiving

TABLE 15.--Organ weights of calves receiving daily injections of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

Body Part	Treatments					
	TRH		3-MET		Sal	
	TW g	% BW[1]	TW g	% BW[1]	TW g	% BW[1]
Liver	2986	1.94	2652	1.81	2472	1.86
Muscle[2]	616	0.40	542	0.37	532	0.40
Thyroid ($X10^{-2}$)[3]	29.7[a]	1.93[a]	13.2[b]	0.90[b]	13.8[b]	1.04[b]
Pancreas ($X10^{-1}$)[3]	133.9	0.87	131.8	0.90	98.3	0.74
Pituitary ($X10^{-4}$)[3]	1.26	8.2	1.11	7.6	1.10	8.3
Adrenal ($X10^{-3}$)[3]	8.00	5.2	7.76	5.3	7.97	6.0

[1]Weight as a percent of total body weight.

[2]Gastrocnemius muscle.

[3]Refers to % body weight only.

[ab]$P<0.05$ means from respective columns not sharing same superscript significantly different.

TABLE 16.--Muscle and liver nucleic acids in calves receiving daily injections of thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), or saline (Sal).

	-------mg/g wet tissue----------		
Liver	TRH	3-MET	Sal
DNA	4.97	5.13	5.15
RNA	9.33	9.01	8.89
RNA/DNA	1.94	1.76	1.74
Muscle	TRH	3-MET	Sal
DNA	0.64	0.69	0.87
RNA	1.88	1.53	1.74
RNA/DNA	3.20	2.50	2.11
	Total Nucleic Acids		
Liver (g)	TRH	3-MET	Sal
DNA	14.9	13.5	12.5
RNA	27.5	23.6	21.5
Muscle (mg)	TRH	3-MET	Sal
DNA	392.4	382.6	444.0
RNA	1141.1	816.7	893.0

TRH was 28% greater for both liver and muscle, respectively than saline-injected calves and 17% and 40% greater than 3 MET-injected calves. The differences in total DNA were less than 20% between treatments for both liver and muscle with no discernible trends apparent.

V. DISCUSSION

One objective of this study was to determine effects of daily injections of thyrotropin-releasing hormone and 3 methyl thyrotropin-releasing hormone on growth rate, blood metabolites and serum hormone concentrations. These treatments were known to temporarily increase circulating levels of hormones known to be related to growth. A further objective was to monitor hormone patterns as influenced by age and time after feeding of young growing calves fed a ration to provide a normal growth rate. Additionally, the response of hormones to successive daily injections of TRH or 3 MET was characterized since this response was considered important in ascertaining any future medical usage of either releasing hormone.

In early stages of postnatal growth serum concentrations of GH is high and decreases with age and is negatively correlated with energy density of the ration fed to ruminants (Armstrong and Hansel, 1956; Purchas et al., 1971; and Trenkle, 1971b). Contrasted to the decrease in serum GH concentration with age, serum insulin concentration increased with age and energy concentration of rations

fed to ruminants (Trenkle, 1970b; Stern et al., 1971; and Johns, 1974). Like GH, pituitary thyroid-stimulating hormone (TSH) concentrations was higher in young calves and decreased with age but showed a tendency to be positively correlated with level of nutrition (Armstrong and Hansel, 1956). Mixner et al. (1966) using the bovine noted a decrease in thyroxine-secretion rate per 100 kg body weight with increasing age. Feeding a single ration to all calves in this study eliminated ration as a variable. In the present study, serum hormone concentrations changed with age in that GH decreased while insulin and thyroxine increased with age. Wetteman and Tucker (1974) observed higher concentrations of serum prolactin in calves at elevated environmental temperatures and this phenomena occurred in the present study. Calves at day 135 and sampled in mid-July averaged more than 40 ng/ml greater serum prolactin at injection than the similar aged calves sampled in late March and early April.

In adult sheep, a biphasic pattern in serum insulin concentration occurs with eating. Within 5 min after feeding 800 g of lucerne hay and oats, serum insulin concentration increased but returned to the prefeeding level within 30 min (Bassett, 1974a). At 2-4 hr after feeding serum insulin concentration increased and the magnitude of increase was related to the amount of feed consumed. Plasma GH concentration decreased during the post-feeding period and the decrease was not related to amount of feed

consumption (Bassett, 1974b). In heifers, plasma prolactin concentration increases after feeding and decreases with fasting (McAtee and Trenkle, 1971). In the present experiment, serum insulin concentration was increased at 8 hr after feeding ($P<0.05$) but other hormones (thyroxine, prolactin, and GH) did not exhibit any changes related to feeding.

The serum concentration of GH, prolactin, and thyroxine increases in the bovine after a single injection of TRH (Convey et al., 1973b; Vines et al., 1973, 1974; Smith et al., 1974; and Vanjonack et al., 1974). No published reports exist concerning hormonal response in the bovine to 3 MET injection.

In general, the injection of 500 ug of TRH or 3 MET resulted in an increase in the concentration of serum GH, prolactin, and thyroxine. However, hormonal responses were variable from day to day especially after injection of 3 MET. For example, serum GH concentration was greater ($P<0.05$) each test date after TRH injection that that after saline injection. In contrast, injection of 3 MET resulted in an increase ($P<0.05$) above control calves only on day 60 and 135 although detectable increases occurred on day 45 and 105. In addition to differences in persistency of increase there was a difference in magnitude of response calculated by comparing baseline values to maximum increase in serum GH concentration after TRH and 3 MET injections (Table 8). At 45, 60, 105, and 135 days the respective

increases of serum GH (ng/ml) for TRH and 3 MET were 47.5 vs 20.9; 35.8 vis 16.8; 38.0 vis 4.3; and 35.1 vs 62.4. Maximum increase after TRH was greater ($P<0.05$) than that after 3 MET at days 45, 60, and 105 but not at day 135.

The serum concentration of prolactin after TRH or 3 MET injections relative to time zero was increased on all days. Due to seasonal differences of sampling mentioned previously, a comparison of serum prolactin concentrations within treatments is more valid than comparison between treatments.

Slopes of regression equations relating serum thyroxine concentration to hours after injection in control calves were positive but not different from zero indicating a relatively constant thyroxine output by the thyroid during the 8 hour period following saline injection. At day 45, TRH and 3 MET injections resulted in significant regressions of thyroxine concentration with time after injection indicating stimulation of thyroxine secretion by TRH and 3 MET. The low serum thyroxine concentration at day 45 in calves receiving 3 MET is purely random since calves received no injections prior to this day. The increase in serum thyroxine from pre-injection to 8 hours after injection at day 45 was 62.1 and 93.1 ng/ml for TRH and 3 MET, respectively. The greater response of thyroxine to 3 MET than to TRH can be explained by biological potency (Vale et al., 1971) and serum thyroxine concentration at injection (Wilber, 1971; and Vale et al., 1971).

Vale et al. (1971) found 3 MET to be 8 fold more potent than TRH for increasing serum TSH concentration in mice. Vale et al. (1971) and Wilber (1971) reported a greater stimulation of TSH release by TRH at low thyroxine concentrations than at high thyroxine concentrations. Similar results with TRH-stimulated prolactin release have been observed (Smith, 1974). Since calves at day 45 injected with 3 MET had a lower serum thyroxine concentration at injection than did calves receiving TRH, a greater response from 3 MET injections due to lower serum thyroxine concentration would be expected.

By days 60, 105, and 135 thyroxine response to daily injections of TRH and 3 MET had decreased ($P<0.05$) and the depression of thyroxine response was greatest for 3 MET injections. Daily injections of gonadotropin releasing hormone (GnRH) result in a decrease in the release of luteinizing hormone (LH) (Mongkonpunya, 1974; and Rippel et al., 1974). Mongkonpunya (1974) injected GnRH into 2-6 month old bulls at 12 hour intervals for 28 days. At 30 minutes after the second GnRH injection, serum LH concentration was only 40% of that attained at 30 minutes after the initial injection. Similarly, Rippel et al. (1974) injected ewes with GnRH and observed a 70 ng/ml increase in serum LH at 90 minutes post-injection. Twenty-four hours later a second GnRH injection resulted in a 10 ng/ml increase in serum LH from a baseline

value comparable to that on the first day. The increase in serum LH concentration was less with continued GnRH injections. Repeated daily injections of TRH and 3 MET resulted in a continued increase in serum concentrations of GH (TRH only) and prolactin but thyroxine response to repeated TRH and 3 MET injections was depressed from initial response. Injections of 3 MET in the initial days of the 90 day experimental period resulted in a greater increase in serum thyroxine concentration than that observed with TRH injections but, during later days of the experiment, TRH injections were more effective than 3 MET injections in elevating serum thyroxine concentration.

Daily injections of TRH but not 3 MET resulted in increased ($P<0.01$) serum insulin concentration above that for control calves especially at days 105 and 135. This difference occurred during the 4 hour period immediately following injection of TRH (Figures 19 and 20). The responses of GH and insulin to TRH and 3 MET injections differed on days 105 and 135. On both days, injection of TRH resulted in an increase in serum concentration of both GH and insulin. However, on day 105, 3 MET injection produced an increase in serum insulin concentration only while on day 135, 3 MET injection produced a rise in serum GH concentration, only. The hypothalamic peptide, somatostatin inhibits pituitary GH secretion (Brazeau et al., 1974). Moreover, somatostatin has a direct effect of the pancreas to inhibit both insulin and glucagon secretion

(Curry et al., 1974; and Koerker et al., 1974). The effects of TRH on insulin secretion has not been reported previously and TRH might be similar to but opposite in effects to those of somatostatin, i.e. TRH stimulates insulin secretion. However, two factors indicate this not to be true. Firstly, Koerker et al. (1974) reported serum insulin concentration returned to the pre-infusion level within 5 minutes after somatostatin infusion ceased. In this study, serum insulin increased in some cases for as long as 2 hours after injection. Secondly, serum insulin concentration was decreased at 60 days of age immediately following TRH injection.

This experiment was started December 4, 1973, with the first injections beginning December 19. The last injection was given on July 15, 1974, for calves started April 15. 1974. Consequently, there was a 4 month difference between starting the first and last calves on the daily injection schedule.

A factor which has a profound influence on calf performance is health. Those calves which started the trial early were more prone to infectious disease especially respiratory infection than those starting at a later date. The 2 most extreme cases have been discussed previously. Some calves required veterinary attention during the trial but no attempt was made to select these calves according to treatment. Originally, 45 calves were started on this experiment but 2 blocks were dropped

because one calf had chronic pneumonia with a respiratory capacity of less than 50% of normal. When this calf was removed from the barn a noticeable reduction in respiratory problems in other calves occurred. In the other block which was removed one calf developed a bloody urine which persisted for 5-6 days and at the same time a second calf in that block developed severe pneumonia (temp. 106° for 4-6 days).

Evans and Long (1922) injected bovine anterior pituitary extracts into hypophysectomized rats and made the following statement, "Such animals are invariably much heavier than their littermate sisters." Some 20 years later GH was isolated from bovine pituitaries (Li and Evans, 1944). The growth promoting activity of the injected pituitary extract may also have resulted from another hormone, TSH. It has been suggested that GH and thyroxine act synergistically to promote increased body size in several species of animals (Brody, 1945; Armstrong and Hansel, 1956; and Scow, 1959). The increased rate of gain and better feed efficiency resulting from diethyl-stilbestrol treatment has been attributed to increased serum GH (Davis et al., 1971; and Trenkle, 1970b) and insulin (Trenkle, 1970b, 1970c) concentration.

Calves in this study injected with TRH had a greater weight gain and feed intake with no difference in feed to gain ratio when compared to calves of other treatments. The increased gain and feed intake in

TRH-treated calves began to appear between 76 and 90 days of age and persisted to 135 days of age. Serum GH concentration was increased after TRH at all days of age but not until day 105 of age was there a significant increase in serum insulin concentration. At all days except day 135 the increase in serum GH concentration was greater for TRH than that of 3 MET.

Nalbandov (1963) postulated a dilution in GH availability per unit of tissue as a possible mechanism of growth stasis as animals aged. Trenkle (1971b) observed higher circulating GH levels in 3 month-old bulls than in 7 month-old bulls. Total GH secretion decreased from 47 ug per kg body weight per day at 3 months of age to 10 ug per kg body weight per day at 7 months of age and the correlation between plasma GH concentration and secretion rate was +0.97. Simpson et al. (1950) began injecting GH into old rats that had reached a growth plateau. Initially, injection of 0.4 mg GH per day resulted in resumption of growth which plateaued 23 days later. When GH was increased to 0.6 mg/day, growth resumed to a new plateau. Subsequently, doses of 1.0, 1.5, and 2.0 mg GH per day were required to increase weight gain in the old rats.

At the age the calves started the experiment sufficient GH would be available to promote similar gains in all treatments. With an increase in age serum GH concentration would decrease. Daily TRH-injections allowed a time-period of the day for tissue exposure to

elevated GH levels not noted in saline and to a lesser degree from 3 MET-injections. Concomitant to the elevated GH, an elevated serum insulin occurred in TRH injected calves only. Reeds et al. (1971) have suggested the full action of GH on protein synthesis may occur only if insulin is present.

Machlin (1972) reported increased weight gains in pigs with daily injections of purified porcine GH. Clegg and Cole (1954) noted larger pituitaries in sheep fed diethylstilbestrol (DES) and suggested the increase gain resulting from DES was a function of GH. Trenkle (1970b) reported plasma GH and insulin concentrations in steers fed 10 mg DES per day was greater than that in steers receiving no DES. Hutcheson and Preston (1971) observed a 32% increase in serum GH concentration in sheep fed DES for 2 weeks. Davis et al. (1970 a,b,c) noted similar metabolic actions of DES and purified ovine GH in sheep.

During the initial 15 day treatment period (day 45-60) calves receiving 3 MET injections had a decreased weight gain and increased feed to gain ratio but no difference in feed intake when compared to control calves. The increase in serum thyroxine concentration was greater in calves receiving 3 MET than those receiving TRH during this period. Thomas (unpublished observations) found thyroprotein feeding to calves reduced average daily gain 0.33 kg and increased TDN required per kg of gain by 60%. Weight gain, feed intake, and feed to gain ratio for calves

receiving 3 MET or saline injections for remaining periods were not different. Two factors changed that could have negated any negative effects of excessive thyroxine levels on growth rates. Firstly, thyroxine response to TRH and 3 MET injections diminished with age and secondly, thyroxine levels in control calves increased with age and at 135 days of age were significantly greater than pre-injection levels for calves receiving TRH or 3 MET.

Muscle RNA concentration and content were higher and the RNA/DNA ratio increased in calves receiving TRH. Johns (1974) reported a positive correlation between muscle nucleic acid content and averaged daily gain. The trends in this study are in agreement with this finding. The increased serum insulin concentration observed in calves receiving TRH may explain the increased RNA content and RNA/DNA ratio of muscle. The elevation of serum GH may have produced increased DNA directed RNA synthesis resulting in a greater efficiency in RNA synthesis hence the higher RNA/DNA ratio.

Clearly, the elevation of serum hormone concentrations especially insulin and GH result in an increased growth rate. Results from this study illustrate the importance of sampling blood for an extended time period during the day rather than single daily samples to relate hormonal patterns to growth. Besides many samples per day, more than one sampling day is necessary to assure

repeatability of observed changes to properly explain differences in growth rate.

VI. CONCLUSIONS

Growth and hormonal responses to thyrotropin releasing hormone (TRH), 3 methyl thyrotropin releasing hormone (3MET), and saline were investigated in calves.

Daily injections of 500 ug of TRH into calves beginning at 45 days of age and continuing for 90 days resulted in increased weight gain and feed intake. The difference in weight gain and feed intake occurred during the last 60 days of the experimental period.

Daily injections of TRH resulted in a greater serum growth hormone (GH) concentration during the 30 minute period following injection than either 3MET or saline injections. The increase in serum growth hormone concentration after 3MET but not TRH was decreased with repeated injections. Injection of TRH or 3MET resulted in a significant increase in serum thyroxine concentration. Relative to day 45, the increase in serum thyroxine concentration with repeated injections of TRH or 3MET was decreased with repeated injections. Overall, serum prolactin concentration was greater during the 60 minute period following injection of TRH than that of saline with the response

after 3MET greater for 45 minutes. Daily injections of TRH or 3MET did not depress prolactin response. Serum insulin concentration was greater in calves receiving TRH than that of calves receiving 3MET or saline.

The concentration of serum GH decreased while insulin and thyroxine increased with age. Serum prolactin concentrations increased as a result of increasing ambient temperature and day length. Feeding resulted in an increase in serum insulin concentration.

These data suggest the increased weight gain observed in calves receiving TRH is the result of: (1) stimulation of feed intake by TRH; (2) temporary elevation of serum GH concentration; (3) lower response of thyroxine to TRH than 3MET; (4) and greater serum insulin concentration in calves receiving TRH. Limited data suggest increased muscle RNA/DNA ratio as a possible site of hormonal action.

VII. APPENDIX

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Table A-1.--Composition of scintillation fluid.

Xylenes	770 ml
p-Dioxane	770 ml
Absolute ethanol	460 ml
Napthalene	160 g
2,5-Diphenyloxazole	10 g
1, 4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene	0.1 g

TABLE A-2.--Composition of reagents for radioimmunoassays.

A. 0.05 M PBS-1% BSA pH 7.4

NaCl	9.0 g
Dissolve with 1 liter of Buffer A_1	

B. Buffer A_1

$NaH_2PO_4 \cdot 2H_2O$	6.2 g
Merthiolate	0.25g
BSA (Fraction V, Sterils, 35% solution serological, NBC, Cleveland, Ohio)	14.6 ml
Add 950 ml distilled water	
pH to 7.4 with 5 N NaOH	
Dilute to 1 liter	

C. Guinea Pig Anti-bovine Insulin and Guinea Pig Anti-bovine Growth Hormone

Antisera diluted 1:400 with 0.05 m PBS-EDTA, pH 7.0. On day of use, dilute 1:400 antisera to required concentration using 1:400 NGPS as diluent

D. 0.05 M PBS-EDTA pH 7.0

Disodium EDTA	18.612 g
Add about 950 ml PBS	
Adjust pH to 7.0 with 5 N NaOH	
Dilute to 1 liter	

E. 0.01 M phosphate buffered saline, pH 7.0 (PBS)

NaCl	143 g
Monobasic phosphate	120 ml
Dibasic phosphate	240 ml
Merthiolate	1.75 g
Dissolve in distilled water and transfer to large container	
Dilute to 17.5 liters with distilled water	
Adjust pH to 7.0 with NaOH if necessary	

F. Monobasic phosphate (0.5m)

$NaH_2PO_4 \cdot H_2$)	69.05 g
Dissolve in distilled water and dilute to 1 liter	

TABLE A-2.--Continued.

G. Dibasic phosphate (0.5m)

Na_2HPO_4---------- 70.98 g
Dissolve in distilled water, heat to dissolve and dilute to 1 liter

H. 1:400 Normal Guinea Pig Serum (NGPS)

Obtain blood from guinea pigs not used for antibody production
Clot the blood, recover serum and store
Add 2.5 ml of serum to 1 liter volumetric flask and dilute to 1 liter with 0.05 m PBS-EDTA and store

I. Sheep Anti-Guinea Pig Gamma Globulin Antibody (SAGPGG)

Dissolve 50 mg guinea pig gamma globulin (Pentex, Kankakee, Illinois, Fraction II) in 5 ml of .85% sterile saline
Emulsify in 5 ml Freund's complete adjuvant by continuous flux through an 18 gauge needle. (Emulsified when a droplet retains a bead form when dropped on a water surface)
Antigen is injected subcutaneously in 6-8 sites on side of animal
Injections repeated every two weeks with Freund's incomplete adjuvant substituted for the complete
Antisera is collected about six weeks after first injection (collect about 600 ml blood from a 70 Kg sheep)

J. Guinea Pig Anti-Bovine Growth Hormone Antibody (GPABGH)

Two mg of bovine GH is dissolved in 0.5 ml saline and emulsified with Freund's complete adjuvant as described above
Injections were started as above with subsequent injections of 0.5 mg emulsified in Freund's incomplete adjuvant made every two weeks for up to seven injections
Blood is collected by heart puncture and serum recovered

TABLE A-3A.--Orcinol Reagent.

Make a stock solution of 0.1% $FeCl_2 \cdot 6H_2$) in concentrated HCl. Before each use, prepare a 1.0% orcinol solution using the stock solutions.

TABLE A-3B.--Diphenylamine Reagent.

Prepare a 4.0% solution of diphenylamine in glacial acetic acid. Store at 4°C.

TABLE A-3C.--Acetaldehyde Solution.

Add 0.4 ml of acetaldehyde to a 250 ml volumetric flask. Dilute to 250 ml distilled water and store at 4°C.

VIII. BIBLIOGRAPHY

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