GROWTH HORMONE AND INSULIN RESPONSE TO INTRAVENOUS ARGININE INJECTION IN THE LAMB

by

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(ABSTRACT)

Crossbred lambs were injected with L-arginine hydrochloride (arg) to determine the effects of single of multiple arg challenges on growth hormone (GH) secretion. Indwelling jugular catheters were inserted. At the beginning of each of 3 trials, lambs were injected with saline and blood samples were collected for 90 min to establish baseline GH. Blood sampling continued at 5-min intervals until 1 h after the final injection, then at 10-min intervals for one additional hour. In trial 1 arg (.5g/kg) was injected into 6 lambs while the other 6 received a second saline injection. Trial 2 consisted of 3 arg injections given at 1-h intervals. Trial 3 utilized 4 arg injections given at 15-min intervals. Trials 2 and 3 were replicated using a switchback design. Serum GH and insulin were measured by double antibody RIA. Mean GH for treated lambs in trial 1 was 3.89 ng/ml versus 1.74 ng/ml in controls ($P<.01$). GH peaked 30 min after injection (9.47 ng/ml), declined to 5-times baseline and remained near that level throughout the sampling

period. Serum insulin was not different between treatments. In trial 2 arg treated lambs had higher mean GH (2.57 ng/ml) than controls (.86 ng/ml; P<.01). Peaks of GH were observed 20 min after the first injection (7.59 ng/ml) and 1 h after the second injection (5.6 ng/ml). No increase in GH was observed after the third injection. Insulin tended to follow the same pattern, but was not significantly elevated in treated lambs. Differences in trial 3 mean GH between arg-treated lambs (5.25 ng/ml) and controls (1.16 ng/ml) were significant (P<.01). GH peaked (13.8 ng/ml) at 25 min after the first injection, surged again 100 min later (7.4 ng/ml), declined to levels 3-times baseline and remained elevated. Trial 3 insulin levels were significantly higher in treated lambs (.64 ng/ml) compared to control lambs (.15 ng/ml; P<.01). Control lambs showed no significant GH or insulin increases at any time. GH secretion patterns were significantly altered in lambs injected with arg. One arg injection caused GH to peak within 30 min. Further challenges resulted in smaller, delayed rise and persistence of elevated GH levels. Insulin levels tended to increase with arg stimulus, but were not significantly elevated except at extremely high doses of arg. The data reflects a consistent relationship between arg stimulus and serum GH.

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INTRODUCTION

The establishment of growth hormone and insulin as hormones critical to growth and development has lead to extensive research in an effort to define this relationship clearly. Use of exogenous anabolic steroid hormones to enhance natural growth of meat animals has been a common practice for many years. The potential of growth hormone is indicated by documentation of increased growth rate and nitrogen retention in lambs, pigs and cattle resulting from exogenous growth hormone administration. Perhaps even more promising are the amino acids such as arginine, leucine, and lysine, which stimulate endogenous secretion of growth hormone and insulin. The available data suggests that arginine is the most potent inducer of protein hormone release. Elucidation of the pattern of hormonal response to arginine may provide clues to the mechanism of this action, and help indicate the potential capacity of arginine for use in the meat animal industry.

Chapter II

REVIEW OF LITERATURE

GROWTH HORMONE AND INSULIN

Structure and Function

Meat animals are selected for maximum growth potential, but altering the endocrine status of these highly selected animals enhances growth performance still further. Clearly, selection has not yet tested the limits of growth performance, nor have the hormonal mechanisms which limit growth rate been defined. Growth hormone (GH) and insulin play critical roles in directing nutrient utilization for growth. Manipulation of GH and insulin secretion would provide a potential tool to increase meat animal production.

Ovine insulin is a protein hormone composed of two peptide chains of 21 and 30 amino acid residues respectively. Insulin is synthesized in the β -cell islets of Langerhans of the pancreas as pro-insulin with a connecting peptide of 30 amino acid residues between the A and B chains (Blunt, 1975). Major functions of insulin include the stimulation of glucose uptake in peripheral tissue and decreased glycogenolysis, gluconeogenesis, lipolysis, and blood glucose levels (Efendic et al., 1971). Insulin is the primary hormone modulating the energy homeostasis leading to uptake of nutrients.

Growth hormone, on the other hand, is a diabetogenic factor which induces hyperglycemia and hyperinsulinemia in fasted animals (Lewis et al., 1980). A direct trophic effect of GH on the islets of Langerhans promotes insulin and glucagon release (Sirek et al., 1979). Ovine GH contains 191 amino acid residues (MW = 22,000), and is synthesized by acidophilic somatroph cells of the adenohypophysis (Blunt, 1975). Secretory granules associated with the Golgi apparatus comprise storage for GH, although secretion without prior storage is also posseble (Greenwood, 1967). Regulation of GH secretion is directed by secretory neurons in the hypothalamus, which release growth hormone releasing factor (GRF) and somatostatin (Blunt, 1975). The suprapituitary control center for GH was demonstrated by Glick et al. (1965), when monkeys lesioned in the ventromedial nucleus of the hypothalamus did not respond to hypoglycemia with GH release. Human patients with sectioned pituitary stalks had measurable GH but no GH response to hypoglycemia, suggesting that a neural or vascular connection between the hypothalamus and pituitary must be intact for normal control of hGH release (Glick et al., 1965). Following feeding, when glucose levels are high, levels of GH decline. A neural reflex is thought to be involved since sham feeding with esophageal-fistulated wethers induced a decrease in serum concentrations of GH (Blunt, 1975).

Factors Affecting Hormone Secretion

Endogenous factors influencing GH effect include the metabolic clearance rate (MCR) of GH, tissue sensitivity to GH, and humoral factors such as GRF and somatostatin.

The basic pattern of secretion consists of episodic pulses which induce time dependent changes in serum GH concentration. Variation between individuals is large, but daily secretory patterns of individual animals are similar (Davis et al., 1979). Secretory patterns may be genetically predetermined, and dependent upon the animal's ability to respond to changes in internal and external stimuli (Davis et al., 1979).

Numerous external factors have been reported to influence endogenous GH levels. Advancing age has been associated with increased overall and baseline plasma GH in sheep (Morrison et al., 1981). However, other researchers noted decreasing GH levels with age in sheep (Falconer et al., 1979), cattle (Armstrong and Hansel, 1956; Trenkle and Irvin, 1970; Hafs et al., 1971; Joakimsen and Blom, 1976) and man (Glick et al., 1965). Ovine fetal plasma contained extremely high GH levels, attributed by Blunt (1975) to increased secretion rate, not decreased MCR. Nalbandov (1963) proposed that vigorous growth can only occur as long the circulating GH to body weight ratio is high enough to stimu-

late protein synthesis in both muscle and bone. Several studies have indicated that GH content per gram of anterior pituitary or per unit body weight is elevated in growing calves, compared to mature cattle (Armstrong and Hansel, 1956; Curl et al., 1968). Other researchers, however, have not been able to support this relationship (Purchas et al., 1970).

Sex differences in circulating GH levels show a trend toward higher GH in steers, compared to heifers (Trenkle and Burroughs, 1967; Trenkle and Irvin, 1970) and rams, compared to ewes or wethers (Blunt, 1975). In humans, females responded to venipuncture with a spontaneous increase in serum GH concentrations followed by a later, less marked release, while only 50% of men displayed a similar response (Spitz et al., 1972). Spitz and coworkers postulated the existence of two pools of hGH, one releasable and the other a storage pool, then speculating that nonresponding men had no readily releasable spontaneous pool of GH.

The effect of breed on GH has been reported to be minimal according to Eaton et al.(1968) and Trenkle and Topel (1978), but Ohlson et al. (1981) found significantly higher baseline and overall GH levels in Simmental bull calves compared to Herefords raised under similar situations. Targhee rams selected for rate and efficiency of gain were reported

to have higher overall GH than unselected Targhee rams (Dodson et al., 1983). This suggests that selection for growth rate and efficiency of gain results in greater ability to secrete GH by increasing the gene- controlled rate of secretion of GH by the acidophils of the adenohypophysis (Nalbandov, 1963).

Serum concentrations of GH vary with season in sheep, with increased spike amplitude occurring in December, compared to September (Davis et al., 1979). No circadian rhythm has been observed for GH or insulin (Trenkle, 1978).

Stress is another crucial factor influencing endogenous hormone levels. Several researchers report no stress-associated change in GH levels (Wagner et al., 1970; Davis et al., 1972). However, Eaton et al. (1968) reported that GH in cattle increased 10-fold immediately following catheterization, and declined to baseline within 30 min. Trenkle (1978) reported a similar stress response in sheep with GH increase, but no change in insulin level. Stress-related stimuli including sudden changes in environment, pain and hemorrhage have been observed to increase GH levels in the rhesus monkey. These responses may be midiated by a variety of humoral factors. For example, histamine, epinephrine and vasopressin augment ACTH in addition to stimulating GH release (Meyer and Knobil, 1967).

Type of diet may also influence GH. Sheep fed a high concentrate ration had significantly lower overall plasma GH, compared to sheep fed alfalfa hay (Trenkle, 1971). Feeding in general suppresses GH release, as somatostatin is released in response to gastrointestinal hormones (Trenkle, 1978). Conversely, fasting increases plasma GH concentrations in sheep (Driver and Forbes, 1981), rats, pigs (Atinmo et al., 1978), and man (Glick et al., 1965). Starved rats exhibited depressed GRF and GH content of pituitary extracts, suggesting an adverse effect upon the hypothalamic-pituitary pathway (Atinmo et al., 1978). Atinmo and coworkers studied the effects of protein and energy deprivation on plasma GH and insulin in pigs. Protein deprivation inhibited GH secretion. Pigs deprived of sufficient energy intake during gestation or after weaning displayed baseline GH levels two-fold those of control pigs. No response to GH stimulation was seen in insulin or glucose levels of deficient pigs (Atinmo et al., 1978). Increased GH during fasting is partially due to reduced MCR, according to Trenkle (1978). Decreased GH and insulin metabolism during fasting slows the rate of removal from the circulation by the tissues involved (Trenkle, 1978).

THE RELATIONSHIP OF GLUCOSE, GH, AND INSULIN

The relationship between GH and insulin is complex, and intimately related to plasma glucose levels. Plasma glucose was once thought to regulate GH release, since hypoglycemia in humans causes increased plasma GH, while feeding decreases GH. However, glucose levels increased during exogenous administration of GH in sheep (Manns and Boda, 1965; Wheatley et al., 1966; Wallace and Bassett, 1966; Muir et al., 1983) and pigs (Baile et al., 1983). The correlation between plasma glucose and plasma insulin is high ($r = .95$; Davis et al., 1970). Davis et al. (1970) showed that plasma insulin remained high even after plasma glucose fell. They suggested that GH stimulated insulin, since when GH treatment was withdrawn, insulin levels fell immediately. The effect of insulin on glucose uptake appears to be inhibited by GH, so that higher concentrations of glucose may be necessary to maintain normal rates of glucose utilization (Wallace and Bassett, 1966). Insulin can block the lipolytic effect of GH. However, during GH-induced hyperglycemia, the amount of insulin needed to decrease blood glucose to control values increases four-fold (Wagner et al., 1970). Although insulin is essential for growth, it is not highly correlated with growth rate, feed efficiency or muscle and adipose tissue mass.

Enhancement of protein synthesis by GH requires the presence of insulin, but chronically administered GH eventually decreases liver binding of insulin and leads to insulin-resistant diabetes (Etherton and Kensinger, 1984).

EFFECTS OF EXOGENOUS GH

Physiological functions attributed to GH include increasing fat catabolism while decreasing fat synthesis, and increasing protein synthesis (Machlin, 1972). Stimulation of growth and N retention by administration of exogenous GH has been well documented in cattle (Brumby, 1959; Moseley et al., 1982), sheep (Struempler and Burroughs, 1959; Wheatley et al., 1966; Wallace and Bassett, 1966; Trenkle and Burroughs, 1967; Davis and Garrigus, 1968; Davis et al., 1970; Trenkle, 1974), and swine (Henricson and Ullberg, 1960; Machlin, 1972). When GH was injected onto the cartilage growth plate of the proximal tibia of hypophysectomized rats, significantly increased growth resulted, indicating that GH directly stimulated the plate cells and did not act through insulin or the somatomedins (Isaksson et al., 1982). Nalbandov (1963) described the mechanism of action as increasing the cell membrane permeability to natural amino acids, and activation of microsomal enzymes for formation of proteins. When the availability of nutrients is limiting,

such as during pregnancy or lactation, increasing GH levels help mobilize energy from the adipose tissue, transferring these calories to lean tissue for metabolism (Trenkle, 1978). Both glucose and insulin have been highly correlated with N balance in sheep when exogenous GH is administered. The positive relationship between insulin and N retention suggests that high insulin levels may be crucial for the full N retaining effect of GH, as well as restoration and maintenance of glucose homeostasis (Wallace and Bassett, 1966).

The primary natural stimuli for endogenous GH secretion are declining levels of energy, blood glucose, free fatty acids, and volatile fatty acids (Blunt, 1975). Inherent factors prompting insulin release include increased blood glucose, increased blood concentration of metabolites easily converted to ATP and production of gut hormones such as gastrin, secretin and cholecystokinin (Blunt, 1975).

HORMONAL RESPONSE TO EXOGENOUS AMINO ACIDS

Administration of exogenous amino acids provokes increased serum concentrations of GH and insulin. In theory, increased GH and insulin from amino acid stimulus serve a physiological role in stimulating the incorporation of amino acids into protein after feeding, when plasma amino acids

are elevated (Davis, 1972). However, Trenkle (1978) contended that plasma amino acids do not affect GH, since GH in sheep is negatively correlated with free amino acid levels in plasma and the amount of protein ingested.

Amino acids have been shown to elevate insulin. In a study by Floyd et al. (1965) infusion of a mixture of 10 essential amino acids elevated insulin above the control. The efficacy of the mixture was not accounted for by the sum of the individual amino acids, since arginine infusion alone also elevated insulin above the control. Both ketogenic amino acids such as leucine, lysine, phenylalanine and glucogenic amino acids like arg and glycine produce significant insulin elevationin humans (Malaisse and Malaisse-Lagae, 1968). However, different degrees of potency are reported for each amino acid in each species. Tao et al. (1974) observed insulinogenic potency in humans for arg, lys, leu, phe, alanine, valine, methionine, and histidine in decreasing order of effectiveness, while rabbits responded most to lys, followed by his, leu, and arg. Davis (1972) infused ewes and lambs with multiple and individual amino acids. Ewes infused with arg, leu or phe displayed elevated insulin and GH. Female lambs responded to infusion of multiple amino acids with more rapid and greater increases of GH and insulin than did male lambs (Davis, 1972). Dibasic amino

acids arg and lys were shown to be the most effective stimulators of GH and insulin in man. Fajans et al. (1967) suggested that hormone release with amino acids is a physiological response not due to pharmacological doses, since maximum hormone levels occurred before 20% of the amino acid dose had been administered. While the infusion of arg, his, and lys increased blood glucose initially, the increase in glucose was deemed insufficient to account for the hormone release (Knopf et al., 1965). Webber et al. (1961) infused lys, arg, and his in dogs and measured plasma amino acids, urine flow and tubular reabsorption rates. They postulated a conversion of arg to ornithine via Krebs-Henseleit cycle due to the 20-fold increase in plasma orn when arg was infused. Administration of arg also provoked increased plasma urea levels and decreased plasma ammonia. Infusion of lys or orn doubled plasma arg. Handwerger et al. (1981) infused urea cycle components ornithine and citrulline to determine whether metabolic factors increased by arg also stimulated GH levels. A seven-fold increase in GH was noted for orn; five-fold for citrulline. The arg effect on plasma hormones may be partially attributable to conversion to other urea cycle components. Infusion of basic amino acids (lys, arg, orn) decreased the percentage of the filtered load reabsorbed by the tubules, and increased the excretion of other

basic amino acids. Reabsorption transport mechanisms differ for groups of amino acids. These basic amino acids probably share a transport mechanism, and so display mutual inhibition of reabsorption (Webber et al., 1961; Hagemeier et al., 1983).

Pairs of amino acids were infused to investigate possible mutual inhibition or synergism. Synergism was exerted by arg with leu or phe, but not by arg and lys, arg and his, or leu and his. This response was ascribed to the amino acid effect on the pancreatic β -cell. Hagemeier et al. (1983) fed excess arg and lys to swine in different ratios, and found no effect on daily gain, feed intake, feed efficiency or plasma lys. Heightened plasma arg and orn and depressed plasma thr and met were observed. Swine performance was affected only when excess arg was combined with a lys deficiency (Hagemeier et al., 1983).

SPECIFIC HORMONAL RESPONSE TO ARGININE

The most effective amino acid in elevation of GH and insulin in most species is arg. Infusion or injection of arg has shown consistent results, stimulating five to 10-fold release of GH and insulin in sheep (Hertelendy et al., 1970), cattle (Hertelendy et al., 1970; McAtee and Trenkle, 1971) and man (Merimee et al., 1965; Merimee et al., 1966;

Waddell et al., 1969; Fajans et al., 1972; Palmer et al., 1976; Spitz et al., 1972). Results in swine reveal an inconsistent pattern with about 70% of experimental animals totally unresponsive to arg stimulus (Hertelendy et al., 1970). Additional results of arg infusion include decreased plasma free fatty acids and increased plasma glucose. Fasting prior to infusion did not alter response to arg, although fasted heifers had a lower baseline than fed heifers before infusion (McAtee and Trenkle, 1971).

Several stages of hormonal response to arg infusion have been defined. The initial period (10 min) reflects the acute component of α -cell secretion, analogous to the early insulin response to glucose. The later phase is the β -cell response (Fajans et al., 1972; Palmer et al., 1976). Palmer et al. (1975) reported that arg stimulus during normoglycemia elicited α -cell insulin response, while the hyperglycemic state caused β -cell secretion. If high levels of arg are administered, this biphasic pattern is obscured. Lower doses permit the early response to be dissociated (Waddell et al., 1969).

A differential sex response has been noted in ewe lambs and humans. Females virtually always respond to arg with increased hormones, while males respond to a lesser degree or fail to respond (Davis, 1972). Fifty percent of normal

human males failed to respond to arg challenge (Spitz et al., 1972; Merimee et al., 1966). In the Merimee study, insulin response to arg was then tested following treatment with stilbestrol. All nonresponders released GH but not insulin in response to arg. The role of steroids in GH secretion has been described as possibly sensitizing the GH releasing apparatus to plasma amino acid levels. Responsiveness to arg in females has been suggested to be related to regular increased protein synthesis, such as during the menstrual cycle or pregnancy (Merimee et al., 1966).

Arginine stimulated insulin release has been studied intensively in the normal, obese, and diabetic human. Insulin-independent diabetics who display diminished response of the β -cells to high plasma glucose still respond well to arg stimulus (Palmer et al., 1976). Non-diabetic obese subjects show similar insulin response to arg stimulus as normal subjects do, but plasma GH levels double rather than increase 10-fold (Copinschi et al., 1967). The pattern of insulin release in obese subjects reveals a more predominant initial secretory phase and much reduced secondary phase (Fajans et al., 1972). Burday et al. (1968) compared normal, insulin-dependent and insulin- independent diabetics in either a normoglycemic or hyperglycemic state. Normal subjects responded to arg infusion with significant GH release

in the normoglycemic state, but showed no response when hyperglycemic. In contrast, Rabinowitz et al. (1966) found no change in response due to glucose levels. Insulin-dependent diabetics responded with GH increase in both states. Obese maturity-onset insulin-independent subjects demonstrated a blunted response to arg, with smaller GH response regardless of blood glucose.

MECHANISM OF ARGININE STIMULATION

Efendic et al. (1971) proposed a mechanism explaining the insulin response to arg infusion through a glucose mediated interaction. Glucose directly stimulates both secretion and synthesis of insulin. Several possibilities were proposed by Efendic. A coupling between secretion and synthesis was proposed to explain this action, but this seem unlikely, as ribose, xylitol and tolbutamide prompt release but not synthesis of insulin. Proposal of glucose as a nonspecific energy source for islet protein synthesis also seems unlikely, since substrates such as oxaloacetate, glutamate and pyruvate are oxidized by the islet cells but do not provoke insulin release. The most well supported theory is that glucose stimulates RNA synthesis, thus increasing the protein synthesis machinery for increased insulin (Kipnis and Permott, 1972). No data has yet been reported suggesting

that arg infusion stimulates insulin synthesis as well as secretion. Plasma glucose and insulin interactions have a well defined negative feedback system. Efendic et al. (1971) propose that if insulin increases were not mediated through increased glucose, then severe hypoglycemia would occur due to insulin secretion, since insulin does not lower the concentration of gut hormones or plasma amino acids. So most stimulators of insulin, including arg, depend on modulation of glucose-induced insulin release. To support this theory, Efendic et al. (1971) gave arg as a priming injection, followed by a 30-min infusion of glucose. The rapid insulin rise seen immediately after the arg injection was attributed to unequilibrated secretion of insulin. Glucose infusion resulted in insulin levels three-fold above those at the peak of the arg-induced surge. Therefore Efendic and coworkers concluded that glucose was ultimately responsible for the insulin levels. They stated that normal blood glucose levels were a prerequisite to arg stimulation.

When blood glucose was 55% below fasting level no insulin response to arg was seen (Efendic et al., 1971). Explanations included first, the degree of hypoglycemia, and second, possible inhibition by high levels of epinephrine noted during this condition. Further study revealed that while epinephrine inhibited glucose- stimulated insulin, it had no

effect upon arg stimulated insulin increases. This, then represents synergism between arg and glucose (Floyd et al., 1970). Efendic et al. (1970) concluded that arg stimulation requires normal glucose metabolism in the β -cell, and that arg elicits its response by potentiating the glucose effect on insulin levels. The synergism between arg and glucose was not due to the gastrointestinal factors which stimulated insulin, but to a response by the pancreatic β -cells to arg and glucose (Floyd et al., 1970). Other researchers experimented with the priming effect of glucose injection on arg infusion, and found enhanced insulin release (Efendic et al., 1971; Levin et al., 1971). Levin et al. (1971) suggested that elevation of glucose metabolites in the β -cell may increase the release of insulin by arg. This interpretation implied a different mode of action for glucose and arg.

A number of similarities between glucose and arg stimulation of insulin exist (Fajans et al., 1967). Exogenous administration of hGH augments glucose and arg-induced insulin release. Prior administration of sulfonylurea compounds such as chlorpropamide decreases arg or glucose effect, but greatly enhances leu effect. Diazoxide treatment decreases the effect of glucose and leu, but has no influence on arg effect. Diabetic individuals display a blunted insulin res-

ponse to infusion of arg, leu, or glucose. Glucagon release is stimulated by both arg and glucose. Dissimilarities (Fajans et al., 1967) include the epinephrine blockage of glucose effect, but not arg. Also, leu and arg apparently stimulate insulin directly, while glucose must first be metabolized (Fajans et al., 1967). Hypoglycemia normally triggers hGH release. Infusion of glucose inhibited hGH release in hypoglycemic patients. However, simultaneous infusion of glucose and arg elicits a normal increased hGH surge. Thus hormone release was maintained after arg infusion even when measures known to inhibit GH secretion were employed (Rabinowitz et al., 1966).

Sensitivity to arg stimulus in diabetics whose β -cells did not respond to high blood glucose provided strong support for non-glucose mediated mechanism (Palmer et al., 1976). Arg evoked a marked plasma insulin and glucagon response in obese diabetic mice, reflecting an indirect glucagon-mediated mechanism (Flatt and Bailey, 1982). Grasso et al. (1968) determined that premature infants are generally hypoglycemic, and show no insulin response to glucose. Infusion of multiple amino acids or arg alone in these infants provoked a 12-fold rise in plasma insulin with no change in plasma glucose (Grasso et al., 1971). An insulin reserve which is releasable by amino acids, not glucose, seems pro-

bable. Perhaps the function of insulin in premature infants centers on the uptake of amino acids for increased protein synthesis during this period of extremely rapid growth (Grasso et al., 1968).

Direct administration of arg to the pancreas provided further support to the separate mechanism theory. Thus, the preponderance of evidence seems to support the existence of different mechanisms for glucose and arg stimulation of insulin. Isolated rat pancreas was perfused with glucose with arg perfusion superimposed on the middle third of the glucose period. With no glucose or low levels, arg stimulated insulin release clearly. Increased levels of glucose served only to increase the height of the early secretion peak. So insulin secretion occurs without glucose in the rat pancreas, demonstrating a direct effect of arg. The early insulin secretion peak was not prominent with arg stimulus alone, but became so with added glucose. These studies support a difference in the stimulatory mechanism of glucose and arg. Action may be directed toward different cellular or compartmental systems, which are related but comparatively independent (Colwell et al., 1970; Levin et al., 1971). Intermediate factors of extrapancreatic origin may be ruled out as part of the mechanism.

Although arg and leu both have a stimulatory effect on insulin and GH, the mechanisms by which they accomplish this action are thought to be quite different. The discrepancies which led to this conclusion have been described by Fajans et al. (1967). When sulfonylurea compounds such as chlorpropamide are administered prior to leu, increased sensitivity of the β -cells to leu induces greatly elevated insulin levels. No alteration in secretion is observed with chlorpropamide prior to arg. Diazoxide and trichloromethiazide suppress all leu-induced insulin release, but actually increase arg-stimulated release.

Patients with pancreatic islet cell tumors show an insulin response to leu which greatly exceeds that displayed in healthy subjects. Both groups of subjects secrete similar levels of insulin in response to arg (Fajans et al., 1967). High leu levels in ingested protein were proposed as a stimulus for elevated insulin, but insulin levels peaked and fell before plasma leu levels had significantly increased (Floyd et al., 1966). Malaisse and Malaisse-Lagae (1968) utilized the isolated perfused rat pancreas and various glucose perfusion levels to compare leu and arg. All levels of glucose permitted normal arg stimulation of insulin. Insulin stimulation by leu is not accompanied by increased plasma glucose (Fajans et al., 1967). Efficacy of leu stimula-

tion was limited at low and very high glucose levels, unless leu concentration was greatly increased. The conclusion drawn from this study is that leu cannot alter the basal rate of secretion, but at glucose-threshold values glucose itself stimulates insulin.

Both leu and arg were shown to change hormone response directly, not through a metabolite. A non-metabolizable synthetic leu analogue, 2 aminobicyclo[2,2,1] heptane-2-carboxylic acid (BCH) was determined to be only slightly less effective than leu in inducing insulin secretion from the rat pancreas in vitro, and in increasing plasma insulin levels of dogs (Fajans et al., 1970; Fajans et al., 1971). McAtee and Trenkle (1971) suggested that a metabolite of arg may be important since insulin continued to rise after the arg infusion was completed. The metabolite, however, was not urea, as it did not stimulate insulin in sheep. Arginine is hydrolysed by arginase contained in the liver (Smith et al., 1983), skeletal muscle and erythrocytes (Pardridge et al., 1982) to ornithine, a component of the urea cycle. Ornithine is an effective stimulator of hormone release, but not as potent as arg. Another metabolite, guanidinoacetic acid is less effective than orn. A nonmetabolizable structural arg analogue 4-amino-1-guanylpiperidine- 4-carboxylic acid (GPA) stimu-

lates insulin in the rat and dog. Insulin stimulation by arg and GPA may be related to their transport across the β -cell membrane or activation of the membrane receptor (Fajans et al., 1972).

Specific mechanism control of arg stimulation is probably centered in the hypothalamic-pituitary axis. Infusion of orn into sheep fetuses did not alter the endocrine status of the fetus or ewe, although infusion of orn into the pregnant ewe increased the secretion of placental lactogen by 73% and GH by 255% (Grandis and Handwerger, 1983). Incomplete maturation of the hypothalamic-pituitary axis was proposed to account for this disparity. Evidence indicates that glucose metabolism is crucial to the arg mechanism. Mannoheptulose inhibits glucose utilization, and terminates insulin response to arg and GPA (Fajans et al., 1972). So the stimulatory effect of arg depends on the concommitant utilization of glucose by the β -cells.

Interestingly enough, arg not only effectively elevates plasma GH, but is critical to proper binding of GH to its specific receptors. Treatment of bGH with 1,2-cyclohexanedione selectively modified 13 arg residues in the GH primary structure, causing total loss of ability to compete with ^{125}I labelled GH for rat liver binding sites. This reaction was reversible with hydroxylamine treatment (Wolfenstein- Todel and Santome, 1983).

Control of the arg mechanism may be affected by several factors. Efendic et al. (1971), who proposed that arg acts as a modulator of the insulin-releasing signal evoked by glucose, asserted that arg acts as a sensitizer. The site of action would be where cAMP is perceived as the specific inducer of the insulin-releasing machinery. Growth hormone release in rats induced by hypoglycemia is blocked by a variety of drugs known to suppress ACTH release (Meyer and Knobil, 1967). Hertelendy et al. (1969) observed the effects of catacholamines on arg induced insulin and GH release. Addition of epinephrine to the arg infusion blocked GH and insulin release in sheep. The α adrenergic blocker phentolamine prevented the epinephrine inhibition of insulin, but not GH. The β adrenergic blocker propanolol failed to prevent either inhibition. Pancreatic β cells respond to α adrenergic stimuli, so β blockers would not be expected to influence insulin secretion. Phentolamine may not be able to cross the blood brain barrier, and so not be able to blockade the hypothalamus from the epinephrine effect. Lack of a pancreatic barrier explains the effectiveness of phentolamine in preventing the epinephrine inhibition of insulin (Hertelendy et al., 1969).

RENAL INFLUENCE ON ARGININE STIMULATION OF HORMONE RELEASE

Metabolism of amino acids and hormones by the kidney may partially explain the hormonal response to amino acids. Influences of elevated GH on renal function include increased glomerular filtration rate, renal plasma flow, tubular reabsorption of Na, P, K, increased responsiveness to aldosterone and anti-diuretic hormone (Westby et al., 1977; Nicoll, 1982). The kidneys are crucial in GH turnover. Most low molecular weight proteins are removed by glomerular filtration then proximal tubular absorption and degradation. About 70% of GH is removed by the kidney (Rabkin et al., 1981), catabolized within the renal cells, and the metabolites are released to circulation. Renal failure leads to increased plasma GH and increased half-life of circulating GH.

Amino acids are normally filtered through the glomerulus, then completely reabsorbed in the proximal tubule. Renal tubular cells also take up amino acids across antiluminal membranes (Perez et al., 1980). In addition to glutamate, citrulline and urea, arg tends to be net released in normal kidneys. Arg excretion dramatically increases in damaged kidneys (Tizianello et al., 1980). The kidney contributes to total synthesis and metabolism of amino acids by altering the rate of absorption and release (Kopple and Fukuda,

1980). Studies with the isolated perfused rat kidney showed that absorption of insulin and GH increased 10-fold when a mixture of amino acids was included in the perfusate. Proximal tubular absorption is by pinocytosis, initiated by binding of the protein to the brush border, then internalization and lysosomal digestion. Amino acids stimulating increased absorption could serve as an energy supply for protein transport. Absorption could be enhanced by amino acid synthesis of new membranes used in formation of pinocytotic vesicles. A small portion of increased reabsorption is consequent to the osmotic pressure gradient established by transfer of filtered amino acids out of the tubular lumen. Transport systems may be coupled. Amino acids did not alter the kidney's ability to remove GH and insulin from the circulation, since organ clearance of GH and insulin was similar with and without amino acids (Rabkin et al., 1982).

In summary, the role of amino acids in regulating normal insulin and GH secretion cannot be defined as yet since levels and rates of infusion of amino acids exceed those normally absorbed from the gastrointestinal tract (McAtee and Trenkle, 1971). Excess exogenous arg reverses the pattern of fuel utilization characteristic of fasting; that is, increases glucagon, glucose production by the liver, insulin, GH and glucose utilization and turnover (Cherrington et al.,

1974). Arginine may stimulate the release of GRF from the hypothalamus, or act directly on the pituitary (Hertelendy et al., 1970; Davis et al., 1972). Burday et al. (1968) predicted the existence of specific amino acid receptors in the hypothalamus, directly regulated by the median eminence. Clearly arg stimulation of insulin and GH does not represent a transient release, but continuous and consistent activation of an as yet undefined secretory mechanism.

Chapter III

OBJECTIVES

Stimulation of growth hormone and insulin by arginine has been studied in rodents, domestic animals and man. The primary concern of these studies has been the short term hormonal response associated with a single injection or infusion of arginine. The objective of this study was to thoroughly characterize growth hormone and insulin patterns in lambs following multiple injections of arginine at various time intervals.

Chapter IV

GROWTH HORMONE AND INSULIN RESPONSE TO INTRAVENOUS ARGININE INJECTION IN THE LAMB

SUMMARY

Crossbred lambs were injected with L-arginine hydrochloride (arg) to determine the effects of single or multiple arg challenges on growth hormone (GH) and insulin secretion. Indwelling jugular catheters were inserted. At the beginning of each of three trials, lambs were injected with saline and blood samples were collected for 90 min to establish baseline GH. Blood sampling continued at 5-min intervals until 1 h after the final injection, then at 10-min intervals for an additional hour. In trial 1 arg (.5g/kg) was injected into six lambs while the other six received a .9% NaCl injection. Trial 2 consisted of three arg injections given at 1-h intervals. Trial 3 utilized four arg injections given at 15-min intervals. Trials 2 and 3 were replicated using a switchback design. Switchbacks consisted of repeating each trial two days later with identical sampling procedure, but reversed treatment groups. Serum GH and insulin were measured by double antibody RIA. Mean GH for treated lambs in trial 1 was 3.89 ng/ml versus 1.74 ng/ml in controls ($P<.01$). GH peaked 30 min after injection

(9.47 ng/ml), declined to 5-times baseline and remained near that level throughout the sampling period. Serum insulin was not different between treatments. In trial 2 arg-treated lambs had higher mean GH (2.57 ng/ml) than controls (.86 ng/ml; $P<.01$). Peaks of GH were observed 20 min after the first injection (7.59 ng/ml) and 1 h after the second injection (5.6 ng/ml). No increase in GH was observed after the third injection. Insulin tended to follow the same pattern, but was not significantly elevated in treated lambs. In trial 3 mean GH levels between arg-treated lambs (5.25 ng/ml) and controls (1.16 ng/ml) were different ($P<.01$). Growth hormone peaked (13.8 ng/ml) at 25 min after the first injection, surged again 100 min later (7.4 ng/ml), declined to levels 3-times baseline and remained elevated. Trial 3 insulin levels were higher in treated lambs (.64 ng/ml) compared to control lambs (.15 ng/ml; $P<.01$). Control lambs showed no significant GH or insulin increases at any time. GH secretion patterns were altered in lambs injected with arg ($P<.05$). One arg injection caused GH to peak within 30 min. Further challenges resulted in smaller, delayed rise and persistence of elevated GH levels. Insulin levels tended to increase with arg stimulus, but were not significantly elevated except at extremely high doses of arg. The data reflect a consistent relationship between arg stimulus and serum GH.

INTRODUCTION

Various economic benefits such as increased growth rate and increased nitrogen retention have been attributed to the effects of exogenous administration of growth hormone (GH) in cattle (Brumby, 1959), sheep (Wheatley et al., 1966; Davis et al., 1970) and pigs (Machlin, 1972). Machlin (1972) also demonstrated a significant decrease in carcass adipose tissue with GH treatment. Amino acids such as arginine, leucine, and phenylalanine have been shown to stimulate endogenous GH and insulin in sheep and cattle in vivo (Hertelendy et al., 1969), and prompt insulin release from sheep pancreas in vitro (Hertelendy et al., 1968). Arg has proven to be particularly efficacious in elevating hormone levels, but has not been clearly linked to measurable increase in economic traits. Before performance trials should be attempted, additional information is required concerning the secretory mechanism involved and the precise pattern of the response itself. Existing data describes the hormonal response to short term infusions of arginine. The present study was designed to thoroughly characterize the pattern of GH and insulin response to a single injection of arg or to multiple injections of arg administered at various time intervals.

MATERIALS AND METHODS

Twelve crossbred lambs, 4 to 6 mo of age, were housed in slatted-floor pens, temperature controlled (25°C), with photoperiod maintained at constant light. Subjects were maintained on a ground corn, soybean meal, mixed hay based diet supplemented with vitamins and minerals (Table 1).

Lambs were blocked by weight and sex into two groups. In all trials 5-ml blood samples were taken with syringes (Becton Dickenson & Co., Rutherford NJ) via the jugular catheter at 15-min intervals for 30 min to establish baseline hormone levels. A 20-ml .9% saline injection was then given to all lambs and sampling continued for 60 min. In Trial 1 a single infusion of sterile, aqueous L-arginine hydrochloride (Sigma Chemical Company, St. Louis, MO) was administered through the catheter. After the initial saline infusion half the lambs (three wethers and three ewes) received the arg injection (.5g/kg bodyweight), while the remaining three wethers and three ewes received a second saline injection of a volume equivalent to that of the arg dose.

Trial 2 followed the same presaline and saline period design, but treatment consisted of three arg injections at 1-hr intervals. Again, half the lambs received control saline injections corresponding in volume to the arg injection. Two days later a switchback was employed.

TABLE 1 COMPOSITION OF LAMB DIET[a]

Ingredient	%
Corn, ground (IFN 4-02-931)	56.44
Mixed hay (alfalfa, orchard grass)	29.65
Soybean meal (IFN 5-04-604)	12.87
Molasses (IFN 4-04-696)	4.95
Trace mineral salt[b]	.99
Selenium/Vitamin premix[c]	.10

[a] as fed basis

[b] Mixture contained a minimum of .2% Fe, .2% Mn, .35% Zn, .3% Cu, .07% I_2 with a minimum of 96% and a maximum of 98.5% NaCl.

[c] Premix contained a minimum of 8,160 IU/g Vitamin A, 1,350 IU/g Vitamin D, 60 IU/g Vitamin E, .2 mg/g Se.

This entailed repeating the previously described trial with identical experimental design, but with treatment groups reversed. Blood samples were collected at 5-min intervals until an hour past the final injections, then at 10-min intervals for the final hour.

Trial 3 was similar, but treated lambs received four injections of arg at 15-min intervals. A switchback design was also applied in Trial 3, with repetition of sampling procedure 2 d later reversing treatment groups.

Feed was withheld for 18 hr prior to each trial. Lambs were accustomed to handling and being restrained. Each lamb was haltered and tied to the side of the pen, allowing sufficient space for all individuals to lie down.

One day prior to trials 1 and 2, all lambs were fitted with indwelling jugular cannulae for collection of blood samples and injections. Cannulae were maintained from the start of trial 2 through the termination of trial 3. Catheterization procedure consisted of shearing and disinfecting the neck (Betadine, Purdue Frederick Co., Norwalk CN), and inserting a sterilized 12-gauge needle into the jugular vein. Tygon microbore tubing (Fisher Scientific Co., Pittsburgh PA) was introduced through the needle and attached to an adaptor and stopper (Becton Dickenson & Co., Rutherford NJ). The tubing was sutured to the skin and .2% nitrofura-

zone (Clay- Parks Labs, Bronx NY) was applied liberally. The cannulae were kept in place with surgical tape (Elastikon, Johnson and Johnson, Inc., New Brunswick NJ) and adhesive tape (Parke-Davis & Co., Detroit MI). Between blood samplings, approximately 2 ml 4% sodium citrate (Fisher Scientific Co., Fair Lawn NJ) was flushed into the cannulae to prevent obstruction.

Blood samples were refrigerated, permitted to clot overnight and centrifuged for 20 min at 3000 x g (Beckmann model J2-21). Serum was harvested and frozen at -20 C until analysis.

LABORATORY ANALYSIS

Serum GH and insulin were determined by double antibody radioimmunoassay as described by Barnes et al. (1985). Ovine growth hormone standards were prepared using NIH-GH-I-3oGH. Procedure utilized to prepare and use antibodies was as detailed by Eisenman and Chew (1983). Anti-ovine GH (NIAMDD-AoGH-1) was the first antibody used. Samples were assayed for growth hormone in triplicate. A pooled ovine serum sample was used to calculate intra- and interassay coefficients of variation, which were found to be 5.9% and 1.2% respectively. Analysis for insulin was performed in duplicate with intra- and interassay variation calculated from pooled serum at 6.8% and 1.8%.

STATISTICAL ANALYSIS

Data were analyzed by least squares analysis of variance for a fixed effects model using the general linear models procedure of the Statistical Analysis Systems (SAS, 1979). Variation among lambs within treatment by sex groups was used as an error term for treatment, sex, and treatment by sex interaction effects. Residual error was used to test time and treatment by time interaction effects. Secretory patterns were analyzed by the method of Christian et al. (1978) for overall mean hormone concentration, baseline level and occurrence of secretory spikes. Total GH secretion was calculated by integration of the area below the secretion curve. Comparison was made within trials across treatments of total secretion for the periods prior to and subsequent to injection of arg.

RESULTS AND DISCUSSION

Trial 1

Growth Hormone.

The least squares mean GH secretion curve is plotted in figure 1. Large standard error of the mean is usually a problem in comparing average GH, since baseline GH levels may differ by 4-fold between two lambs of equal weight, age and sex.

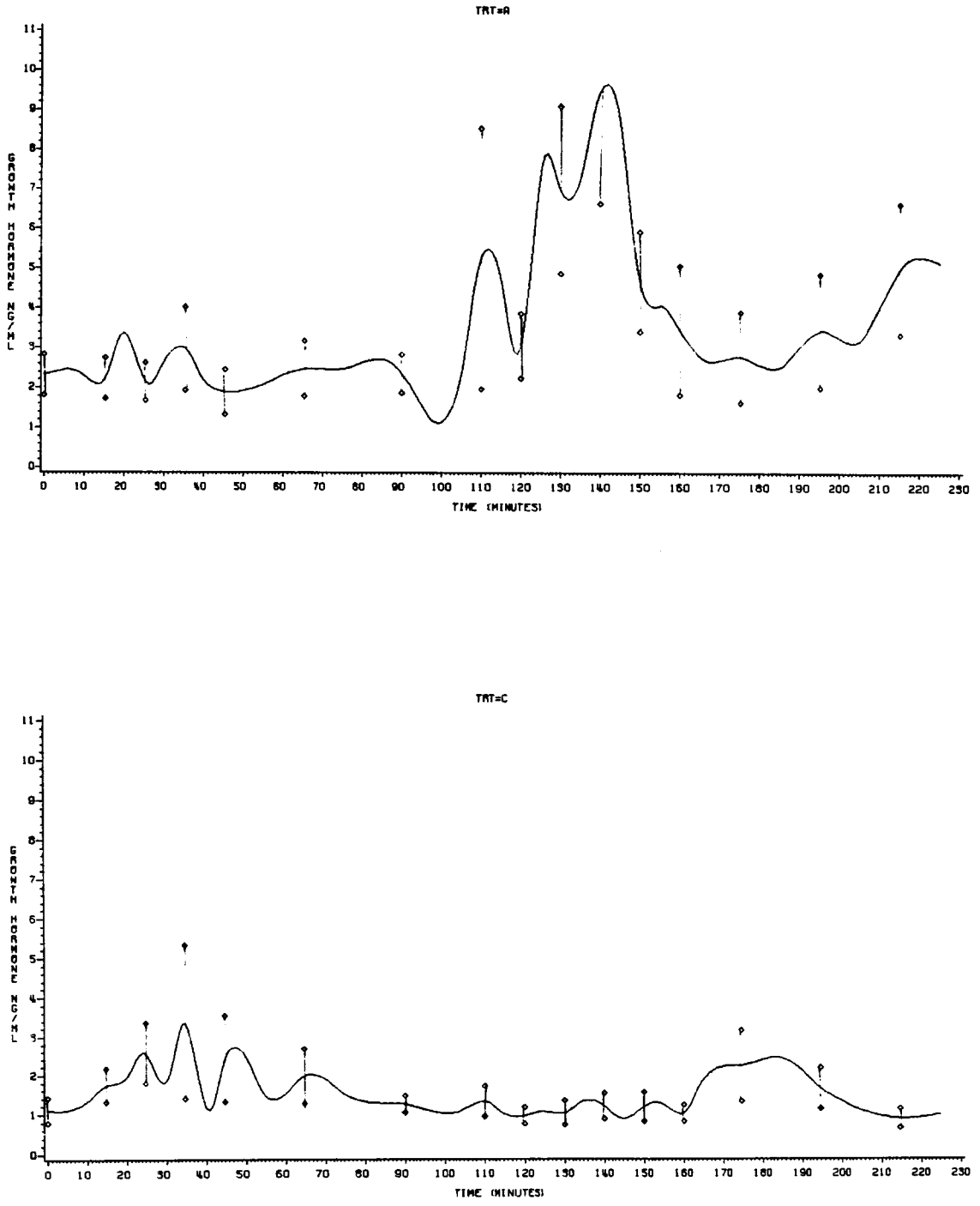

Figure 1: Trial 1 Mean Growth Hormone Secretion Versus Time

Individual sensitivity to stimuli appears to vary somewhat also, as can be seen by individual lamb responses (Appendices B and C). The GH response for Trial 1 can been seen in figure 1. A very rapid initial peak was noted in the 10-min period following arg injection. The second phase of the response was an enlarged, prolonged surge enduring for 30 min, with a peak at approximately 4-fold the basal level. The level of serum GH in treated lambs did not return to the initial baseline, and appeared to undergo a delayed surge toward the end of the sampling period. The presaline period (prior to 10 min) and the saline period (10 to 105 min) were not statistically different between control and arg-treated animals. The least squares mean overall GH level for controls was 1.54 ng/ml compared to 3.89 ng/ml for treated lambs.

Although time had no significant effect upon GH levels, a highly significant treatment by time interaction was noted ($P<.001$). This indicates that as time progressed, the effects of the two treatments produced differential responses in serum GH. Sex and treatment by sex interaction effects were not significant. A trend for treatment effect was noted ($P<.09$).

In this trial sex did not affect GH secretion. However, response to arg stimulus has been reported to be affected by

sex in man (Spitz et al., 1972; Merimee et al., 1966). Davis (1972) observed statistically higher plasma GH and insulin in female lambs compared to male lambs during saline infusion. He also noted a tendency toward increased response of ewe lambs to arg stimulus. Davis (1972), however, compared ram lambs to ewe lambs, whereas this study compared wethers to ewes. Since rams normally have higher plasma GH than wethers or ewes (Blunt, 1975), our results do not appear inconsistent with previous reports.

Total GH secretion was evaluated by integrating the area under the secretion curve. This total was then divided into three intervals; the presaline period, the saline period (following the administration of saline to all lambs), and the injection period (following the arg or saline injection). Large standard errors of the means resulted from tremendous individual variation in both basal GH and response to stimulus. Lamb within sex by treatment was used as an error term to test treatment, sex and interaction effects. Due to large standard error, mean hormone levels are often not significantly different (table 2). The postinjection treatment period tended to be higher in treated lambs ($P<.08$).

It is not surprising that total secretion throughout the sampling period was not higher in treated lambs compared to

TABLE 2 : TRIAL 1 MEAN TOTAL GROWTH HORMONE SECRETION[a]

Treatment	Total secretion	Presaline period	Saline period	Treatment period
Arginine	468±239	5.33±.059	266±67	642±168[b]
Control	300±85	2.03±.036	152±39	138±48

[a] ng/ml

[b] Means in the same column with different superscripts differ (P<.08).

controls, considering the 90 min pretreatment period incorporated into the total. We feel that this extended period of sampling to determine baseline GH was necessary, however, to ensure that fluctuations due to the initial stress of sampling would have subsided by the treatment period. In figure 1 small changes in GH are obvious in both treated and control lambs prior to 60 min. After that time, hormone levels appear to be stable. The reason for the rapid initial peak followed by a larger, sustained surge is unclear.

Insulin.

The least squares mean secretion curve for insulin appears in figure 2. Immediately following the 105-min arg injection insulin levels reached a peak of 2.07 ng/ml, then rapidly dropped, returning to baseline 30 min after the challenge. Peak insulin for treated lambs was 5-times greater than control levels. Serum insulin was similar for control and treated lambs except for the 30-min period following injection. The least squares mean overall insulin for controls was .39 ng/ml compared to .46 ng/ml for arg-treated lambs. This overall mean was not different across treatments. No sex or treatment by sex interaction effects were noted on serum insulin. Time and treatment by time interaction effects were observed ($P<.001$).

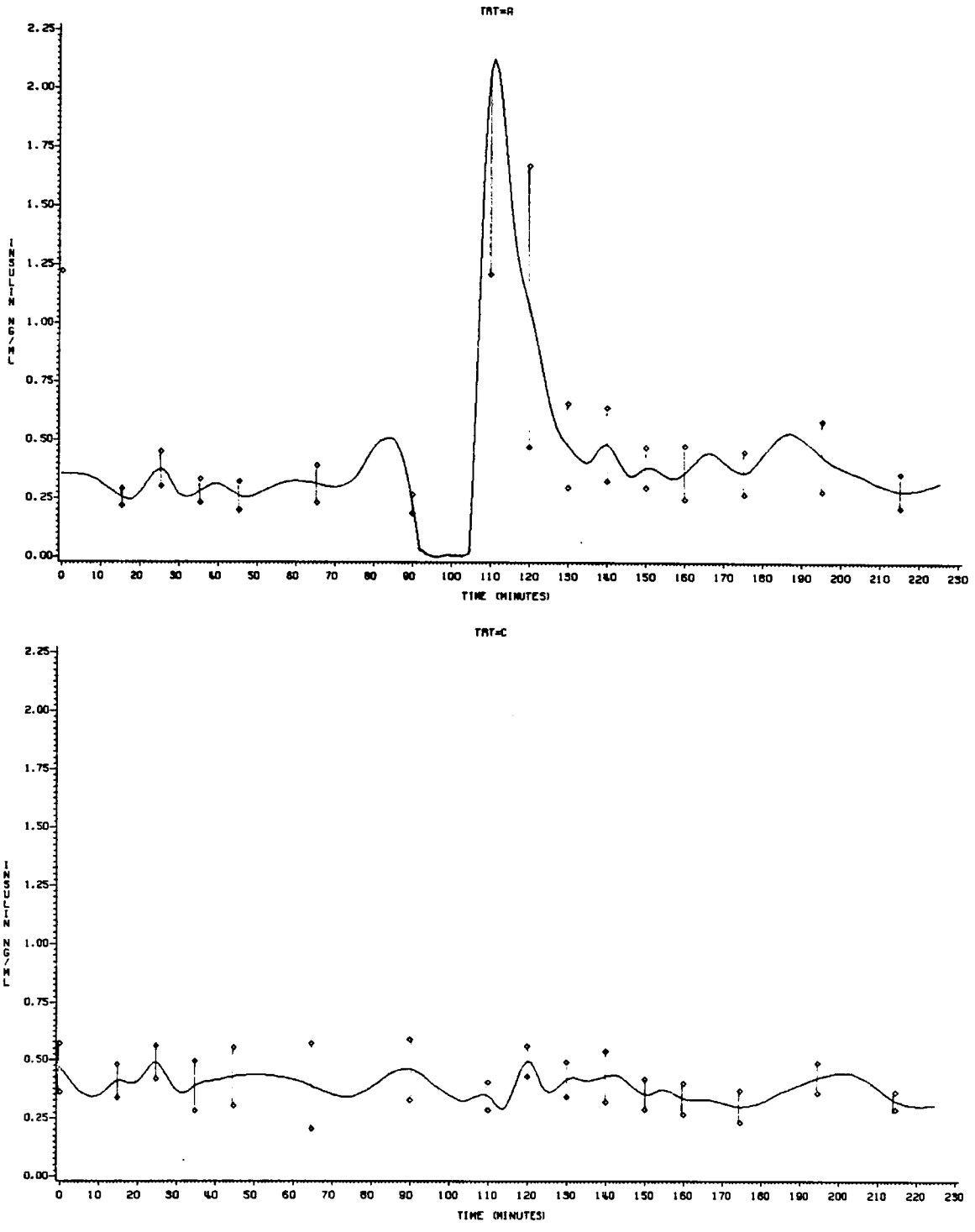

Figure 2: Trial 1 Mean Insulin Secretion Versus Time

Mean total insulin secretion and secretion during the presaline, saline, and treatment periods were analyzed (table 3). No significant treatment or sex effects were noted.

Serum insulin appears to drop to negligible levels immediately prior to the arg injection. This is due to experimental procedure difficulties. That is, in the excitement prior to administration of the injection several samples were missed, several tubes were dropped, and the remaining samples provided extremely low insulin levels. All treatments were administered on time, resulting in a very large transient surge of insulin. Again, inclusion of long periods prior to treatment and after the surge did not allow total secretion over the entire period to reach significance.

Trial 2

Growth Hormone.

In trial 2 a 20-ml dose of sterile physiological saline was administered to all lambs via jugular catheter following the 10-min blood sample. At 90, 150, and 210 min half the lambs received arg (.5 g/kg) while the remainder received an equivalent volume of saline. A switchback design was employed, so that 3 d after this sampling period, treatments were reversed and sampling procedure was repeated.

TABLE 3: TRIAL 1 MEAN TOTAL INSULIN SECRETION[a]

Treatment	Total secretion	Presaline period	Saline period	Treatment period
Arginine	108±33	.683±.009	32.6±5.9	76.3±25.1
Control	72±10	.678±.006	31.1±4.9	36.8± 5.4

[a] ng/ml

All serum samples were assayed together and data combined for statistical analysis. Figure 3 displays the least squares mean GH secretion curve. The initial peak at 30 min provides an excellent example of individual lamb variation. Reference to Appendix C, Trial 2, lamb 2204 indicates a tremendous surge peaking at 18.9 ng/ml which began prior to the initial saline injection. The only explanation which can be offered is a possible stress induced by beginning a new sampling period. Insulin, which is not influenced by stress, showed no comparable initial surge in this particular lamb.

The GH secretion pattern for arg-treated lambs shows a peak of 7.59 ng/ml 20 min after the first injection. Levels of GH declined, then peaked again (5.64 ng/ml) 1 h after the second arg injection (immediately prior to the third injection). No peak was noted in response to the third injection, but GH levels remained 2-times higher than controls for the remainder of the sampling period. No statistical difference was found between treatment groups during the presaline and saline periods.

The overall least squares mean GH for arg-treated lambs was 2.57 ng/ml compared to .86 ng/ml for control lambs. No difference in GH was observed due to sex or treatment by sex interaction using lamb within treatment by sex as an error term. Treatment, time and treatment by time interaction

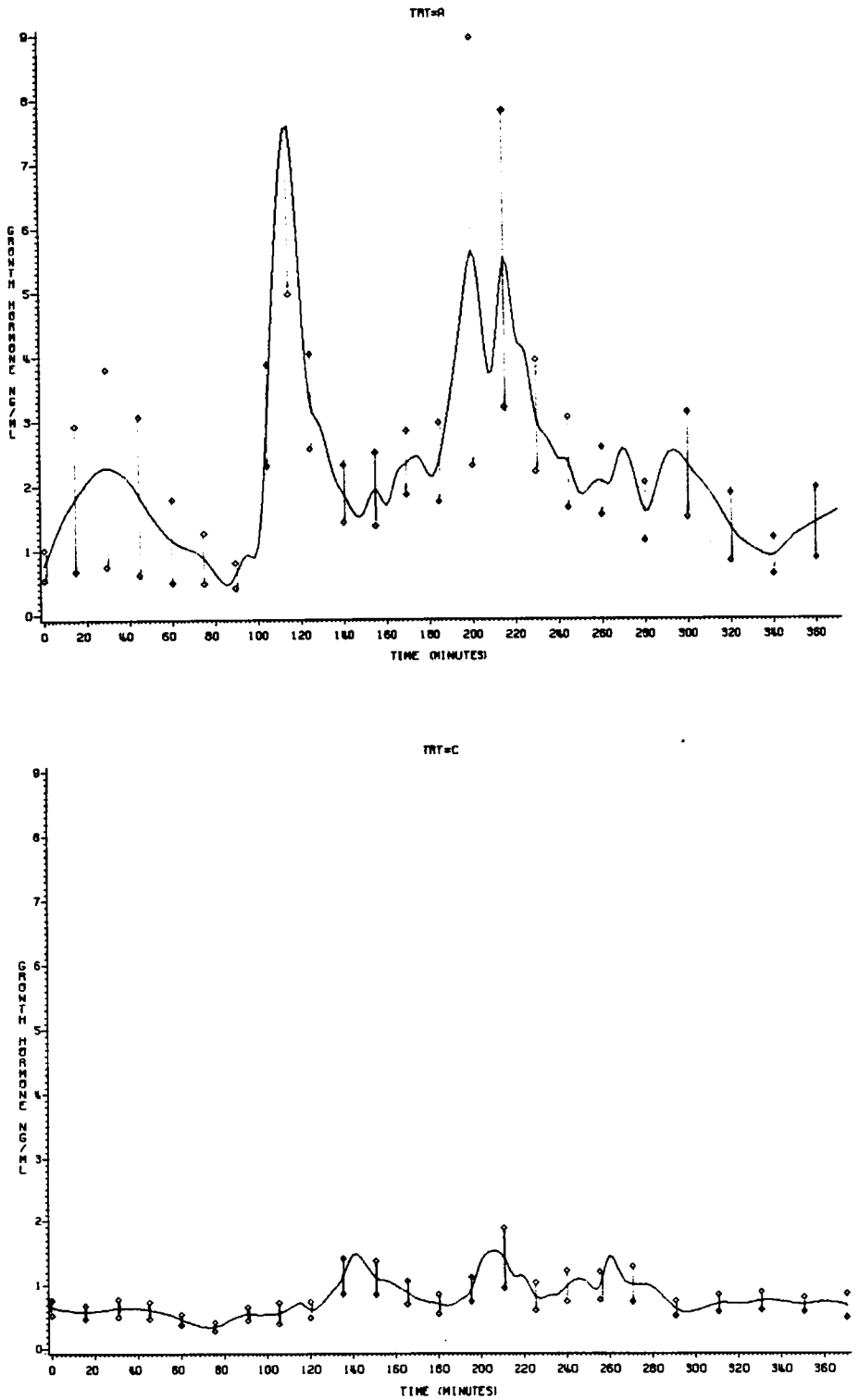

Figure 3: Trial 2 Mean Growth Hormone Versus Time

effects were all different (P<.005). Thus serum GH levels were different between treatments, changed over time, and changed as a result of the treatment as time progressed.

Total GH secretion from trial 2 was also calculated by integration. Secretion was divided into five intervals; presaline, saline, secretion between the first and second injections, between the second and third, and secretion following the third injection. Again very large individual differences are apparent. Statistical analysis of mean secretion (table 4) reveals a tendency for total GH secretion to be higher in arg treated lambs (P<.08). No difference in GH levels was observed prior to administration of treatments. Levels of GH in each interval between injections and after the final injection were higher in treated lambs, compared to controls (P<.05). No sex or interaction effects were seen.

The initial GH response to arg in trial 2 was very high, followed by a delay, then two peaks in rapid succession. Whether the double peak was due to the delayed response to the second injection immediately followed by an instant response to the third injection seems doubtful. Actually, the immediate surge is possible, as seen in the initial response.

TABLE 4: TRIAL 2 MEAN TOTAL GROWTH HORMONE SECRETION[ab]

Trt	Total secretion	Presaline period	Saline period	Inj1	Inj2	Inj3
Arginine	841±165[c]	2.6±.04	110±42	183±65[c]	175±45[e]	352±97[e]
Control	265±47	1.1±.03	36±9.9	28±6.5	58±8.2	134±21

[a] ng/ml
[b] Total = throughout sampling period.
Presaline = 30 min prior to saline.
Saline = 60 min period from saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = after 3rd treatment.
[c] Means in the same column with different superscripts differ (P<.08).
[d] Means in the same column with different superscripts differ (P<.01).
[e] Means in the same column with different superscripts differ (P<.05).

However, this leaves unexplained the delayed increase due to the second injection, unless time of injection occurred after full release of available GH and the double peak was due solely to the effect of the final injection.

Insulin.

Insulin levels from trial 2 are displayed in figure 4 as the least squares mean secretion curve. One serum insulin peak was observed in arg-treated lambs occurring immediately after the 90-min injection. The peak (1.76 ng/ml) was followed by a gradual decline to baseline levels by the end of the sampling period. Overall mean insulin was .91 ng/ml for arg-treated lambs and .53 ng/ml for controls. Testing the time and treatment by time interaction using residual as the error showed significant effects on insulin ($P<.001$). No significant treatment, sex, or treatment by sex interaction effects were found.

The summary of insulin statistical analysis, with division by interval, appears in table 5. Mean total secretion during the interval between the first two injections was higher for arg-treated lambs compared to controls ($P<.01$). The secretion during the second interval of the treatment period was not different between treatments. However, the

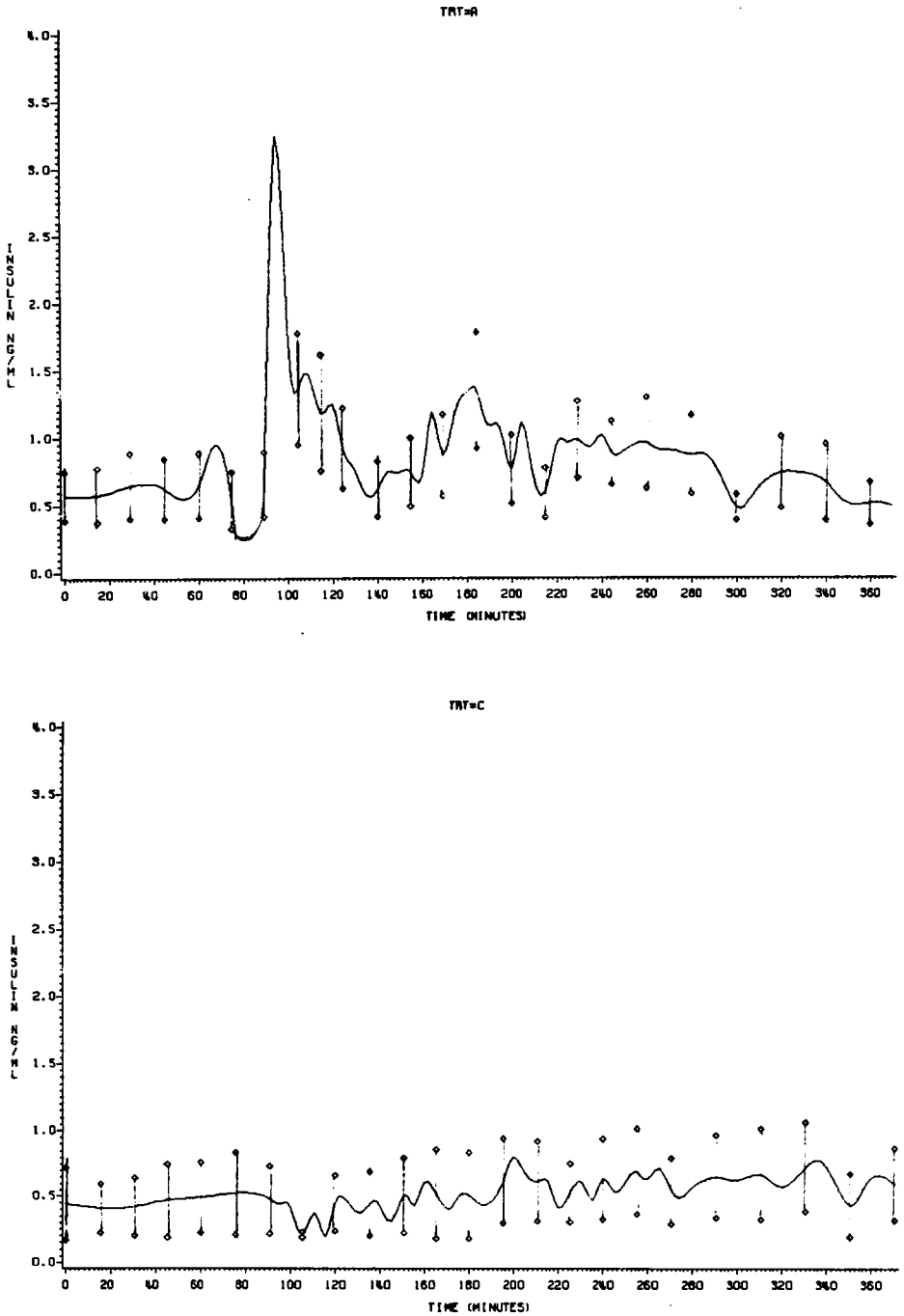

Figure 4: Trial 2 Mean Insulin Secretion Versus Time

TABLE 5: TRIAL 2 MEAN TOTAL INSULIN SECRETION[ab]

Trt	Total secretion	Presaline period	Saline period	Inj1	Inj2	Inj3
Arginine	253±83	.95±.01	36.8±20	70±19[c]	58±19	123±49[d]
Control	172±85	.78±.01	32.2±11	24±13	36±12	98±4

[a] ng/ml
[b] Total = throughout sampling period.
Presaline = 30 min prior to saline.
Saline = 60 min from saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = agter 3rd treatment.
[c] Means in the same column with different superscripts differ (P<.01).
[d] Means in the same column with different superscripts differ (P<.05).

secretion following the last injection was higher in treated lambs ($P<.05$). Hertelendy et al., (1970) reported a lag in insulin response to a second dose of arg. This seems to be the case here also.

The insulin secretory pattern was quite similar to that presented in trial 1 resulting from a single injection of arg. Serum insulin following the initial surge appeared to be slightly above baseline. Possibly the initial surge of insulin was sufficient to remove the increased blood amino acids from all three injections from circulation without the necessity of additional insulin secretion.

Trial 3

Growth Hormone.

Procedure relative to presaline and saline sampling was identical to trials 1 and 2. Following the 90, 105, 120 and 135 min samples half the lambs received arg (.5g/kg) while the remainder received a similar volume of saline. Switch-back design allowed each lamb to receive each treatment as in trial 2. Figure 5 displays the least squares mean GH secretion curve.

Elevated GH at time 0 is explained by the stress-related surge of lamb 2159 (Appendix C), which dropped to baseline levels after the first two samples were taken. One major

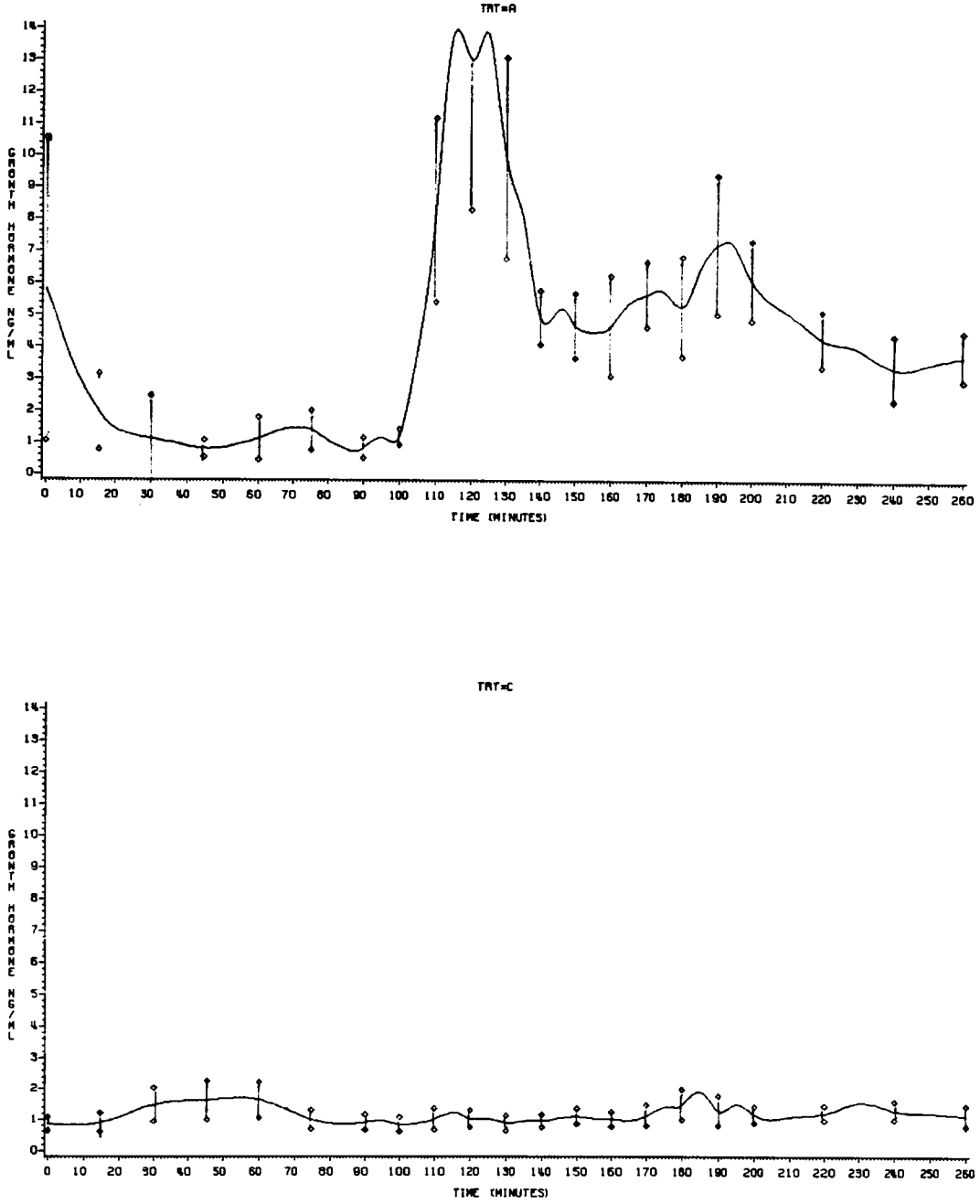

Figure 5: Trial 3 Mean Growth Hormone Secretion Versus Time

peak was observed in trial 3, with GH remaining near 13.5 ng/ml for a 15-min period beginning 20 min after the initial injection.

After the final injection at 135 min, serum GH declined to levels still five times higher than control baseline and remained elevated through the end of the sampling period. Control GH levels were not different from presaline and saline levels in treated lambs.

Overall least squares mean GH for arg-treated lambs was 5.25 ng/ml compared to the control lamb serum GH of 1.16 ng/ml. No significant effects on GH were observed due to sex or treatment by sex interactions. Treatment effects evaluated using lamb within treatment by sex mean square as an error term were different ($P<.005$). Time and treatment by time interaction effects tested using residual error were also significant ($P<.001$).

Statistical analysis of mean GH appears in table 6. This total is then divided into the respective intervals; presaline saline, and first, second, third and fourth treatment injection intervals.

Overall secretion was higher in treated lambs ($P<.08$). Secretion during each injection interval was also higher in treated lambs ($P<.05$). In addition, sex-related effects began to appear, apparently due to sex differences in capacity

TABLE 6: TRIAL 3 MEAN TOTAL GROWTH HORMONE SECRETION[ab]

Trt	Total secretion	Presaline period	Saline period	Inj1	Inj2	Inj3	Inj4
Arginine	1107±707[c]	7.72±.087	90±28	27±5.8[d]	150±45[e]	169±56[c]	631±71[d]
Control	454±67	1.77±.018	9.9±3	14±3.6	16±4.8	15±3.7	498±33

[a] ng/ml
[b] Total = throughout sampling period.
Presaline = 30 min period prior to saline.
Saline = 60 min period from saline injection to treatment.
Inj1 = after administration of trt (A, arginine .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = between 3rd and 4th treatment.
Inj4 = after 4th treatment.
[c] Means in the same column with different superscripts differ (P<.08).
[d] Means in the same column with different superscripts differ (P<.01).
[e] Means in the same column with different superscripts differ (P<.05).

to respond to large amounts of arg given in a short period of time. The first treatment injection interval showed a tendency toward a sex effect, with females secreting more GH. A significant treatment by sex interaction was observed ($P<.05$). This indicates that ewes and wethers differ in their ability to cope with the initial stimulus of arg. Although all the treatment intervals were significantly different, only the first interval was affected by sex and sex by treatment interactions.

The consistently elevated GH levels which occurred during the rapid administration of four doses of arg show a different pattern than that seen in trials 1 and 2. Since hormone levels do not return to baseline for at least 3 h after the initial injection, another mechanism is suggested. Perhaps very frequent arg stimulus results in increased secretion as well as initial release of GH. This would account for the elevated but stable GH seen in figure 5.

Insulin.

The least squares mean insulin secretion curve for trial 3 is plotted in figure 6. Following the injection at 90 min a short duration peak (1.46 ng/ml) was observed. The final injection was also followed by a peak at 1.19 ng/ml. Serum insulin never returned to baseline during the sampling period, unlike the previous trials. In fact, insu-

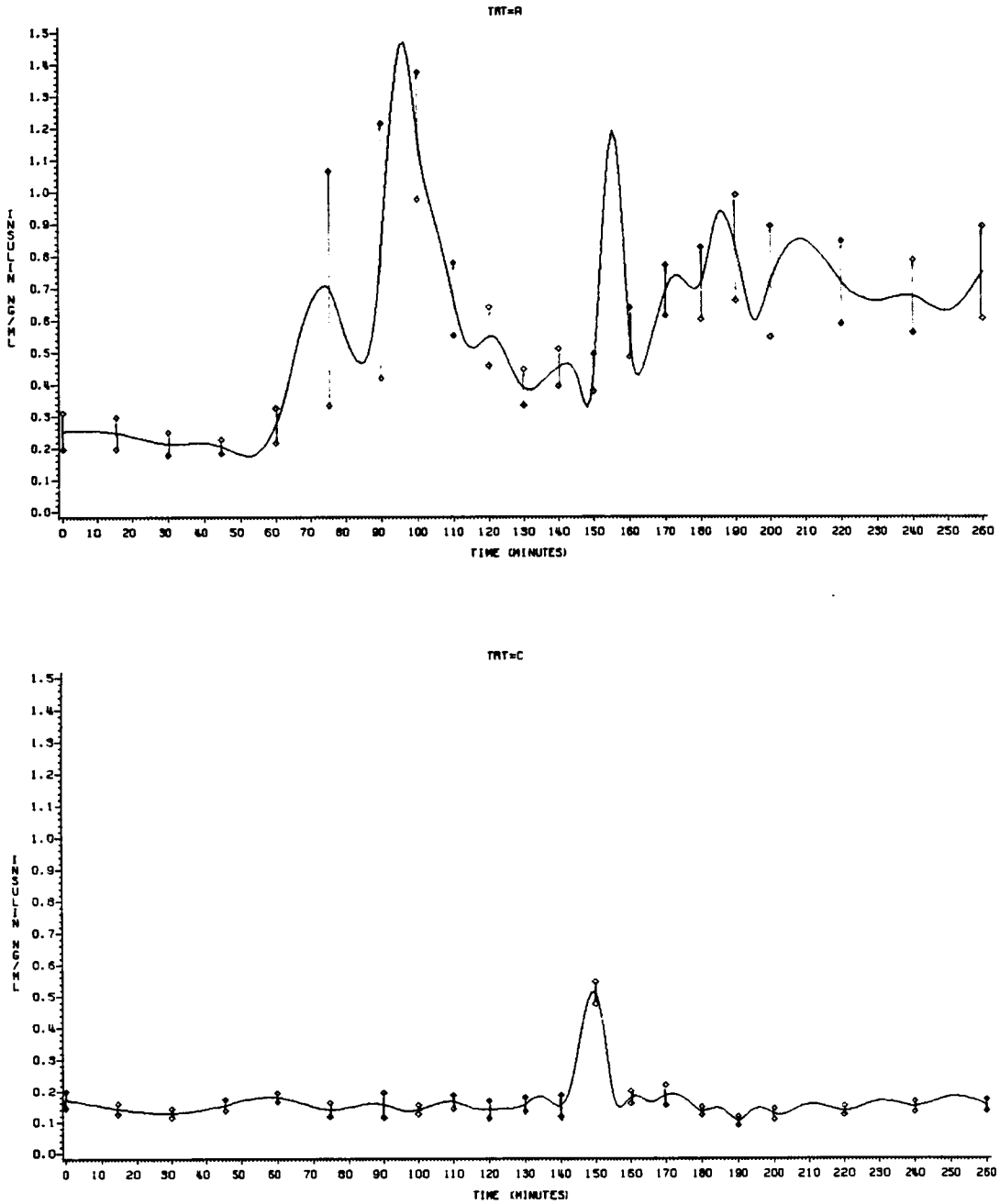

Figure 6: Trial 3 Mean Insulin Secretion Versus Time

lin remained elevated at about six times the basal level for 2 h after the second peak. Injection of four large doses of arg within 1 h stimulated insulin far above levels recorded in trial 2, where three doses were injected within 3 h. This suggests that rapid, repeated arg challenge still elicits insulin secretion at least under conditions of fasting.

Mean serum insulin in arg-treated lambs was .64 ng/ml compared to .15 ng/ml in controls. Effects of time and treatment by time interaction on insulin levels were observed ($P<.001$). Using the mean square for lamb within treatment by sex as an error term, treatment, sex, and their interaction were tested. Each was found to be significant (treatment, $P<.005$; sex, $P<.05$; treatment by sex interaction, $P<.06$). This indicates that when arg challenge is administered in large amounts in a short period of time, a differential ability to respond to this challenge is seen between ewe and wether lambs.

Statistical analysis of mean insulin secretion appears in table 7. Data are divided by interval, as previously described.

Total secretion and all secretion from treatment intervals were significantly elevated in treated lambs compared to controls ($P<.001$). The effects of sex and interactions were also evaluated. Sex and treatment by sex interaction ef-

TABLE 7: TRIAL 3 MEAN TOTAL INSULIN SECRETION[ab]

Trt	Total secretion	Presaline period	Saline period	Inj1	Inj2	Inj3	Inj4
Arginine	153±24[c]	.503±.002	28±8.6	18.4±3[c]	9.5±1.4[c]	6.5±.9[c]	86±5.4[c]
Control	40±4	.314±.001	11.2±.9	2.2±.3	2.3±.3	2.3±.3	20±5.9

[a] ng/ml
[b] Total = throughout sampling period.
Presaline = 30 min prior to saline.
Saline = 60 min period from saline injection to treatment.
Inj1 = after administration of trt (arginine, .5g/kg BW or saline control).
Inj2 = between 2nd and 3rd treatment.
Inj3 = between 3rd and 4th treatment.
Inj4 = after 4th treatment.
[c] Means in the same column with different superscripts differ (P<.001).

fects were different during the interval between the second and third injection, and between the third and fourth injection ($P<.05$). Sex becomes an important factor in insulin response, not during the initial or final response, but through the body of the secretion response. Several researchers (Fajans et al., 1972; Palmer et al., 1976) have described the initial insulin response to arg as the α cell component, while the later phase is due to β cell secretion. This, then, may indicate a differential sex control of β cell secretion, as the latter phases of response were different between sexes.

The pattern of elevated insulin levels due to rapid administration of arg indicates several surges and overall increased levels. This could be due to the presence of large amounts of plasma arg which continually stimulated insulin release to increase uptake. This is a reasonable explanation, especially compared with trial 2, where three doses of arg over 3 h allowed sufficient time for the available insulin to control plasma arg.

General comparison of control GH levels to values reported in the literature demonstrates wide variation. Explanations of this variation include the sampling method, animal age, and in particular, method of assay. Blunt (1975) reports GH in suckling lambs at 2 ng/ml following feeding.

Yearling ewes and wethers measured 1 to 2 ng/ml after feeding and 2 to 4 ng/ml after fasting. These values are consistent with those derived in the study. The overall mean control GH level in trial 1 was 1.54 ± .23 ng/ml, compared to .86 ± .23 ng/ml in trial 2 and 1.16 ± .23 ng/ml in trial 3. Overall control insulin in trial 1 was .39 ± .03 ng/ml, with .53 ± .02 ng/ml and .15 ± .02 ng/ml in trials 2 and 3 respectively. Interassay variation accounts for the difference between trials. Interassay variation is due to several factors. A limited amount of ^{125}I oGH or ^{125}I insulin is prepared at any given time. Antibody preparations may differ slightly from one assay to the next. While all samples from one trial were assayed with the same iodination stock, several iodinations were required to complete serum analysis.

Other researchers have reported baseline GH and insulin levels to be considerably higher than those reported here. Hertelendy et al. (1970) reported baseline GH at 9 ± 1 mug/ml, and insulin at 13 ± 2 uU/ml in ewe lambs. Davis (1972) showed similar GH levels but reported baseline insulin at 20 uU/ml in lambs. Basal insulin levels increase from 5 to 150 uU/ml after lambs are permitted to suckle, according to Blunt (1975). Mature sheep maintain insulin at 20 uU/ml in the prefeeding state.

Several other researchers have investigated the ovine hormonal response to arg challenge. Hertelendy et al. (1970) examined the GH response of sheep to arg infusion administered at two levels, and during prolonged and repeated infusion. Infusion (1 ml/min) of .5 g arg/kg BW increased GH from 9 to 124 ng/ml within 15 min. In our study, trial 1 was relatively similar in design except for the injection rather than infusion of arg. Our results indicated an increase in GH from 1.54 to 10 ng/ml within 15 min of injection. The injection resulted in a prolonged secretion which decreased to 2-times basal level in 60 min, then increased slightly until sampling ended 135 min after injection. The infusion study (Hertelendy et al., 1970), however, showed a decline in GH as rapid as the increase. This difference is probably due to the effect of mode of administration on the ability of the pituitary to respond with increased secretion. Slow infusion appears to permit the release mechanism to keep up with stimulation. The rapid decline suggests down regulation. Rapid injection, on the other hand, may overwhelm the secretory mechanism, resulting in a longer period of responsiveness to stimulation. Whether the arg alters production of GRF or somatostatin is not known. Total secretion data were not presented with the Hertelendy study. Comparison of total secretion under conditions of infusion or injection might prove interesting.

Davis (1972) also studied arg infusion, using mature ewes and young lambs. Infusion of .5 g arg/kg BW over 30 min in the ewes resulted in an increase from 9 to 40 ng/ml. Again, a very rapid decline in GH was observed in this study. These results indicated a peak at approximately 4-fold basal GH, which is less than half the increase noted both in our study and that of Hertelendy and coworkers (1970). Davis' results in female lambs were similar to those in ewes, while male lambs showed comparatively lower response.

Insulin increase in the Hertelendy study with a single arg infusion was from 13 to 77 uU/ml within 5 min with a gradual 75 min decline to baseline. The Davis study reported increased insulin from 20 to 60 uU/ml, with a similar decline. In our study, trial 1 insulin increased from .39 to 2.07 ng/ml, and had returned nearly to baseline levels within 20 min. This is similar in peak magnitude to the results of Hertelendy et al. (1970), but not in pattern. Again the mode of administration seems to account for the discrepancy in insulin-release pattern, as with GH.

Multiple injection trials 2 and 3 of our study can be only approximately correlated with published reports. Hertelendy et al. (1970) observed the effects of prolonged infusion (2h, .5 g arg/kg BW) and repeated infusion (two 15 min infusions of .25 g arg/kg BW each, separated by 105

min). The prolonged infusion study resulted in lowered GH and insulin, as would be expected compared to infusion of the same amount of arg in a 20- min period. The peak GH reached was 20 ng/ml, but GH release was sustained at that level throughout the infusion, then declined slowly. Plasma insulin increased throughout sampling to 145 uU/ml, reflecting the stimulus of amino acid infusion and increasing hyperglycemia. Repeated infusions at .25 g arg/kg BW resulted in comparable GH and insulin response to both infusions.

In our study trial 2 consisted of three .5 g arg/kg BW injections separated by 60 min. An 8-fold increase in GH (.86 to 7.59 ng/ml) occurred within 15 min of injection. Following the second injection, GH peaked again at 5.64 ng/ml, then slowly decreased. Correlating these results to those of Hertelendy and coworkers (1970) we see a similar pattern. Although the response to the second injection in our study did not reach the level of the first, as in Hertelendy's work, the 2 injections were separated by only 60 min as opposed to 105 min. This difference could indicate lack of complete recovery of the release mechanism within the 60 min period.

Trial 3, with four injections of .5 g arg/kg BW, can be approximately compared to continous infusion as described previously. However, it should be noted that in trial 3 we

used four times the amount of arg in half the time of Hertelendy's continuous infusion trial. Our results showed an increase in GH of 14 times baseline for 40 min then a decline to 5- times baseline for an additional 2 h. This is similar, given the methodology differences between the experiments.

Additional Comments

Lambs appeared to be in good condition through trial 1 (6/20/84) and trial 2 (9/4/84; switchback 9/7/84). Wether lamb 2173 went off feed 9/8 and became severely anorexic. A blood sample taken by Dr. Donna Matthews on 9/10 contained creatinine levels of 13.23 mg/dl and blood urea nitrogen (BUN) of 147 mg/dl. Normal ovine creatinine ranges from 1.2 to 1.9 mg/dl, while BUN varies from 8 to 20 mg/dl under normal conditions. Serum creatinine of 5 to 10 mg/dl is usually fatal in sheep (Goldston et al., 1981). Goldston and coworkers described the signs of renal failure in sheep. Elevated creatinine is a clinical sign of renal failure, appearing when the compensating renal mass is less than 20% of normal. Associated with this increase in creatinine is a severely reduced production and lifespan of erythrocytes, accounting for the anorexia. This lamb died foaming at the mouth, in convulsions on 9/11 and was necropsied by Dr. P.

Sponenberg, veterinary pathologist, on the following day. Gross examination revealed pale, swollen kidneys with ecchymoses scattered on the surface and extending into the cortex. Numerous pale lesions surrounded with bright red rings were noted on the surface of the liver. Histological findings included necrotic epithelium of many kidney tubules, and cortical hemorrhage of the adrenal gland. The final diagnosis was acute renal tubular nephrosis.

At this time all other animals appeared to be healthy. Blood samples were taken on 9/13 from all lambs to measure BUN (table 8) and creatinine (table 9). Meanwhile trial 3 proceeded on 9/11 and 9/13.

Ewe lamb 2186 went off feed 9/13, was examined and treated by Dr. Matthews, and died 9/14 foaming at the mouth, in severe convulsions. Necropsy by Dr. Sponenberg revealed findings similar to the previous case. Pinpoint hemorrhage mottling was observed on both kidneys and liver. Final diagnosis was acute tubular nephrosis and hepatic necrosis.

All surviving lambs appeared to be healthy. After seeing the results of the BUN and creatinine from 9/18, lambs 2245 and 393 were taken to the veterinary hospital for intensive study. The wether 2245 died on 9/20 in the same manner as the others with the addition of severe vomiting, an unusual event in ruminants, but a clinical sign of renal failure

TABLE 8: BLOOD UREA NITROGEN (BUN) LEVELS IN LAMBS TREATED WITH ARGININE[a]

	BUN (mg/dl)			
Lamb	9/10	9/13	9/18	10/10
2245[b]		81.4	245.0	--
2217		32.0	14.1	21.7
2215		13.1	10.9	21.8
395		50.6	17.5	25.0
2173[c]	147.0	--	--	--
2224		32.6	23.0	27.8
399		11.9	11.9	28.2
396		17.5	26.4	29.9
2204		32.5	50.1	49.4
393		32.6	32.5	27.7
2159[d]		16.4	8.5	27.7
2186[e]		214.0	--	--

[a]Trial 1 took place on 6/20; trial 2 on 9/4 and 9/7, trial 3 on 9/11 and 9/13.
[b]died 9/20, acute renal failure.
[c]died 9/11, acute renal failure.
[d]died 11/3, pneumonia, possible renal damage.
[e]died 9/14, acute renal failure.

TABLE 9: CREATININE LEVELS IN LAMBS TREATED WITH ARGININE[a]

	Creatinine (mg/dl)			
Lamb	9/10	9/13	9/18	10/10
2245[b]		6.43	12.7	--
2217		2.22	1.19	1.22
2215		1.13	1.42	1.4
395		2.96	2.35	1.59
2173[c]	13.2	--	--	--
2224		3.27	2.5	1.26
399		1.13	.84	1.21
396		1.04	2.45	1.39
2204		1.86	4.83	1.89
393		2.5	9.13	1.95
2159[d]		1.55	1.23	1.39
2186[e]		13.6	--	--

[a]Trial 1 took place on 6/20; trial 2 on 9/4 and 9/7; and trial 3 on 9/11 and 9/13.
[b]died 9/20, acute renal failure.
[c]died 9/11, acute renal failure.
[d]died 11/3, pneumonia, possible renal damage.
[e]died 9/14, acute renal failure.

(Goldston et al., 1981). Dr. Sponenberg diagnosed the cause of death as tubular nephrosis. The other lamb (393) recovered. All lambs were returned to Smithfield, Va. Blood analysis on 10/10/84 revealed no severely elevated creatinine. One additional lamb (2159) died spontaneously on 11/3/84 at Smithfield, and was autopsied 11/5. Too much autolysis had occurred for histopathology. Due to decomposition, kidney damage was evaluated grossly, but the extent of examination was limited. Kidneys appeared swollen and pale. Diagnosis was enzootic pneumonia. On 12/12/84 two lambs were slaughtered to observe the state of the kidneys. No gross or histopathological damage was found.

The intensive bleeding involved in trials 2 and 3 led to weight loss averaging 2.5 kg/lamb. Hematocrit values also decreased. After trial 2 average packed cell volume (PCV) was 23.08%. Values obtained following trial 3 averaged 19.83% PCV. Approximately 900 ml blood were removed from the experimental subjects during a 10-d period.

Chapter V

SUMMARY

Intravenous administration of arg is effective in elevating serum GH and insulin in lambs. Individual variation in basal GH levels was large, but the percentage increase above baseline in response to arg stimulus was quite similar. Due to individual variation in baseline and peak values, mean GH often did not reach significance. Trial 1 involved a single arg challenge and resulted in a single GH peak gradually tapering off to baseline. Insulin was not different between treatments. Trials 2 and 3 also showed a large GH response to the first injection of arg. Subsequent challenges resulted in smaller delayed peaks and persistence of elevated GH. The larger the total dose of arg, the more elevated the GH secretion was. Insulin was also increasingly elevated with increasingly higher doses of arg. The effect of lamb sex was not apparent until multiple doses of arg were administered in a short period of time. At this point, ewe lambs displayed higher GH levels than wethers. Insulin secretion was also affected by lamb sex. At high levels of arg the initial insulin surge was similar between sexes, but the later stages of the response show significant sex effects.

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Appendix A

COMPOSITION OF REAGENTS

Arginine. Add 400g Arg/l double distilled deionized water. Autoclave. Verify volume.

Citrate. Add 40g Sodium Citrate/l double distilled deionized water. Autoclave.

Saline. Add 9g NaCl/l double distilled deionized water. Autoclave.

TABLE 10: TRIAL 1 TOTAL GROWTH HORMONE SECRETION

			GH secretion by time interval[a] (ng/ml)			
Lamb	sex	trt	Total	Presaline	Saline	Injection
2245	W	A	833	.182	195	619
2217	E	A	878	.141	211	652
2215	W	C	350	.206	193	135
395	E	C	392	.154	184	192
2173	W	A	1892	.478	557	1286
2224	E	A	795	.300	190	575
399	W	C	274	.177	91	171
396	E	C	647	.287	220	398
2204	W	A	266	.132	114	139
393	E	A	308	.092	111	187
2159	W	C	659	.089	337	273
2186	E	C	128	.039	65	58
SEM[b]			239	.059	67	168
SEM[b]			85	.036	39	48

[a]Total = throughout the sampling period.
Presaline = 30 min period prior to any saline.
Saline = 60 min period from saline injection to treatment.
Injection = After administration of treatment (trt), either arginine (A, .5g/kg BW) or saline (C, .9% NaCl).

[b]Standard error of mean GH at each interval.

TABLE 11: TRIAL 1 TOTAL INSULIN SECRETION

			Insulin secretion by time interval[a] (ng/ml)			
Lamb	Sex	Trt	Total	Presaline	Saline	Injection
2245	W	A	53	.018	17.8	33.4
2217	E	A	44	.013	13.1	30.0
2215	W	C	57	.021	24.9	30.0
395	E	C	63	.026	27.0	33.9
2173	W	A	62	.017	25.9	36.6
2224	E	A	16	.005	14.8	24.4
399	W	C	104	.061	51.9	46.6
396	E	C	82	.037	36.0	42.4
2204	W	A	229	.062	48.5	174.2
393	E	A	162	.045	40.2	117.8
2159	W	C	128	.060	53.7	68.7
2186	E	C	79	.033	34.6	41.7
SEM[b]			33	.009	5.9	25.3
SEM[b]			10	.006	4.9	5.4

[a]Total = throughout the sampling period.
Presaline = 30 min period prior to any saline.
Saline = 60 min period from saline injection to treatment.
Injection = after administration of treatment (trt) either arginine (A, .5g/kg BW) or saline (C, .9% NaCl).

[b]Standard error of mean insulin at each interval.

TABLE 12: TRIAL 2 TOTAL GROWTH HORMONE SECRETION

			GH secretion by time interval[a] (ng/ml)					
Lamb	Sex	Trt	Total	Presaline	Saline	Inj1	Inj2	Inj3
2245	W	A	135	.011	31.4	41.4	40.2	104.0
2245	W	C	204	.044	4.7	22.1	37.1	49.2
2217	E	A	282	.040	68.4	83.2	78.0	137.6
2217	E	C	352	.062	8.3	57.5	63.1	123.5
2215	W	A	437	.030	16.0	216.3	86.1	111.9
2215	W	C	127	.021	11.5	14.7	45.0	51.5
395	E	A	1568	.043	61.5	576.4	227.7	692.8
395	E	C	351	.043	41.7	85.8	71.8	142.7
2173	W	A	906	.034	87.9	334.6	234.1	307.2
2173	W	C	695	.090	22.8	136.4	117.1	333.3
2224	E	A	380	.033	42.4	80.2	50.6	201.1
2224	E	C	237	.034	83.6	38.1	33.7	115.4
399	W	A	1086	.044	42.7	204.6	274.5	513.4
399	W	C	304	.430	53.1	39.0	80.9	130.8
396	E	A	821	.048	53.1	217.1	154.9	387.2
396	E	C	177	.028	21.9	23.1	13.9	112.1
2204	W	A	1364	.508	832.7	79.9	97.5	239.5
2204	W	C	268	.037	29.6	39.2	80.8	110.7
393	E	A	606	.126	88.2	201.7	147.1	140.5
393	E	C	275	.022	34.0	48.6	54.7	133.6
2159	W	A	2018	.140	77.6	209.4	606.6	1267.1
2159	W	C	54	.012	47.9	35.6	71.2	192.9
2186	E	A	493	.031	34.0	115.7	111.2	185.4
2186	E	C	133	.018	15.8	19.5	27.8	66.2
SEM[b]		A	165	.039	42.1	65.1	44.7	97.5
SEM[b]		C	47	.033	9.9	6.5	8.2	21.8

[a]Total = throughout the sampling period.
Presaline = 30 min prior to saline.
Saline = 60 min from saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C)
Inj2 = between 2nd and 3rd treatment.
Inj3 = after 3rd treatment.

[b]Standard error of mean GH at each interval.

TABLE 13: TRIAL 2 TOTAL INSULIN SECRETION

			Insulin secretion by time interval[a] (ng/ml)					
Lamb	Sex	Trt	Total	Presaline	Saline	Inj1	Inj2	Inj3
2245	W	A	55	.008	89.2	24.2	39.7	80.5
2245	W	C	71	.008	10.4	14.5	8.9	35.5
2217	E	A	315	.001	480.7	13.4	23.8	128.2
2217	E	C	57	.008	12.0	8.0	8.5	26.7
2215	W	A	101	.018	22.9	47.7	10.3	463.3
2215	W	C	682	.018	19.8	23.4	169.0	19.8
395	E	A	112	.004	4.7	145.7	145.5	405.7
395	E	C	950	.181	212.1	23.7	21.8	61.3
2173	W	A	999	.007	9.7	167.3	169.3	489.2
2173	W	C	47	.007	159.3	8.1	8.1	19.3
2224	E	A	407	.124	15.6	74.4	24.7	124.9
2224	E	C	37	.013	16.1	11.3	1.8	6.0
399	W	A	124	.033	18.1	27.8	24.7	47.9
399	W	C	98	.012	18.7	13.0	21.5	42.5
396	E	A	160	.016	16.0	34.0	40.8	66.1
396	E	C	90	.015	17.1	14.9	15.8	38.9
2204	W	A	287	.017	18.9	100.3	72.5	91.5
2204	W	C	89	.008	15.6	16.2	16.0	39.9
393	E	A	109	.012	14.4	34.0	22.8	35.3
393	E	C	67	.019	14.9	9.6	9.3	29.7
2159	W	A	751	.056	66.9	229.7	214.8	37.0
2159	W	C	92	.011	20.9	8.7	14.1	37.0
2186	E	A	235	.010	15.2	70.8	36.9	110.3
2186	E	C	34	.005	16.4	10.2	11.7	34.9
SEM[b]		A	83	.009	19.7	19.5	19.3	49.7
SEM[b]		C	85	.014	1.5	13.1	12.7	4.0

[a]Total = throughout the sampling period.
Presaline = 30 min period prior to saline.
Saline = 60 min period form saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = after 3rd treatment.
[b]Standard error of mean insulin at each interval.

TABLE 14: TRIAL 3 TOTAL GROWTH HORMONE SECRETION

			GH secretion by time interval[a] (ng/ml)						
Lamb	Sex	Trt	Total	Presaline	Saline	Inj1	Inj2	Inj3	Inj4
2245	W	A	580	.018	23.3	10.6	84.3	49.0	408
2245	W	C	418	.099	75.0	7.9	23.1	20.0	253
2217	E	A	1305	.025	31.9	8.4	477.2	197.9	537
2217	E	C	377	.102	112.1	8.2	19.2	15.4	184
2215	W	A	551	.034	31.4	10.9	62.8	45.2	222
2215	W	C	383	.026	209.6	11.8	38.4	32.1	241
395	E	A	2569	.053	58.8	10.6	408.9	655.8	1411
395	E	C	282	.033	94.8	17.9	3.1	3.9	170
2186	E	A	1450	.153	169.8	68.0	64.6	124.1	989
2186	E	C	674	.176	325.7	32.3	44.6	30.2	202
2224	E	A	1139	.207	292.9	38.8	58.1	40.0	663
2224	E	C	311	.034	47.3	11.7	14.8	26.7	203
399	W	A	8540	.040	44.8	31.3	98.4	82.1	8455
399	W	C	651	.153	67.4	6.7	3.8	4.2	385
396	E	A	682	.037	55.1	14.6	47.1	94.0	463
396	E	C	195	.019	34.9	7.0	7.8	6.2	135
2204	W	A	407	.013	25 2	13.0	106.2	86.4	173
2204	W	C	51	.038	17.6	1.4	3.0	3.9	16
393	E	A	637	.027	34.2	25.2	45.2	119.0	407
393	E	C	50	.005	11.8	1.9	2.2	2.3	30
2159	W	A	2586	.999	226.2	8.3	202.3	366.8	1279
SEM[b]		A	707	.087	28.4	5.8	45.7	56.2	719
SEM[b]		C	67	.018	30.9	3.6	4.8	3.7	33

[a]Total = throughout the sampling period.
Presaline = 30 min period prior to saline.
Saline = 60 min period from saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = between 3rd and 4th treatment.
Inj4 = following 4th treatment.
[b]Standard error of mean GH at each interval.

TABLE 15:TRIAL 3 TOTAL INSULIN SECRETION

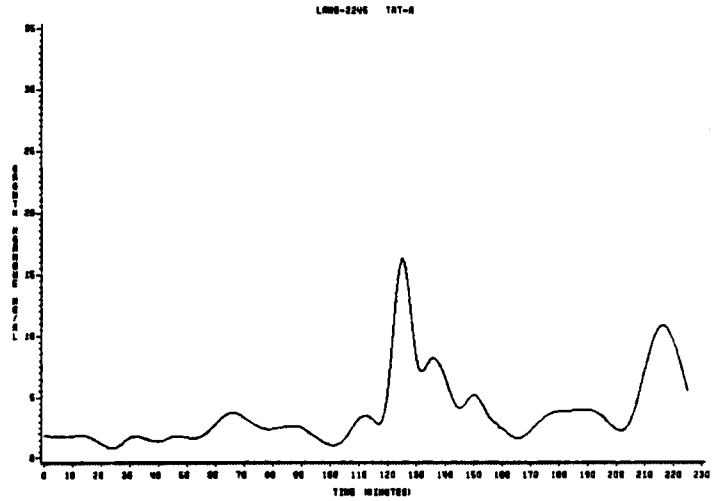
			Insulin secretion by time intervals[a] (ng/ml)						
Lamb	Sex	Trt	Total	Presaline	Saline	Inj1	Inj2	Inj3	Inj4
2245	W	A	142	.031	21.1	14.7	10.3	8.54	80.1
2245	W	C	28	.004	7.9	1.2	1.2	1.93	15.5
2217	E	A	81	.008	8.4	27.2	3.9	2.70	36.7
2217	E	C	28	.005	8.2	1.3	1.3	1.67	14.6
2215	W	A	138	.007	10.9	23.1	15.6	7.52	79.6
2215	W	C	50	.012	11.8	2.5	2.0	2.93	28.2
395	E	A	133	.007	10.6	24.5	10.4	6.82	79.5
395	E	C	70	.015	17.9	3.6	4.4	4.90	36.1
2224	E	A	122	.018	21.1	7.6	8.1	5.08	76.9
2224	E	C	26	.008	10.0	1.2	1.4	1.19	11.3
399	W	A	119	.019	17.5	14.4	6.8	5.08	70.9
399	W	C	53	.016	13.9	2.8	3.2	3.08	70.9
396	E	A	114	.009	15.9	16.2	7.1	5.53	66.8
396	E	C	39	.013	9.5	1.9	2.1	1.97	21.3
2204	W	A	360	.021	108.0	34.4	19.0	13.13	80.8
2204	W	C	39	.014	12.5	2.3	2.5	2.15	16.6
393	E	A	89	.007	28.0	5.3	3.5	3.41	47.9
393	E	C	26	.006	8.1	1.6	2.0	1.24	47.9
2159	W	A	251	.033	49.3	29.0	13.8	10.53	41.5
2186	E	A	131	.015	20.7	5.7	6.0	4.34	90.8
2186	E	C	41	.013	12.4	2.7	2.6	1.84	19.5
SEM[b]		A	24	.002	8.6	3.0	1.4	.94	5.4
SEM[b]		C	4	.001	.9	.3	.3	.3	5.9

[a]Total = throughout the sampling period.
Presaline = 30 min period prior to saline.
Saline = 60 min period from saline injection to treatment.
Inj1 = after administration of trt (A, arginine, .5g/kg BW or saline, C).
Inj2 = between 2nd and 3rd treatment.
Inj3 = between 3rd and 4th treatment.
Inj4 = after 4th treatment.
[b]Standard error of mean insulin at each interval.

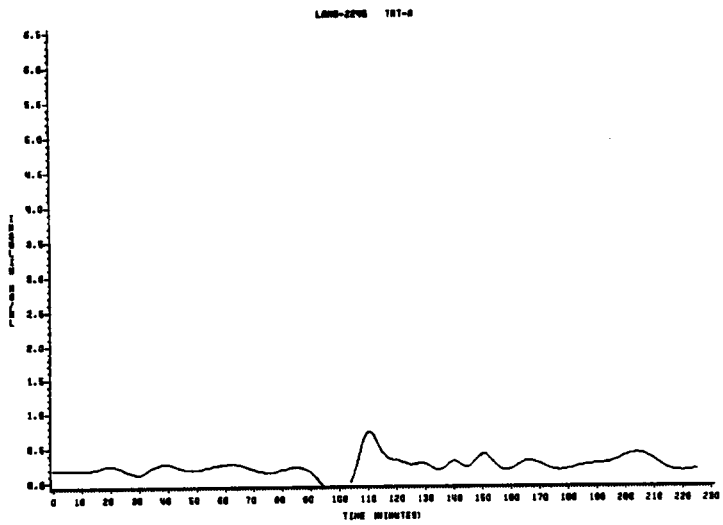
APPENDIX C

TRIAL 1

INDIVIDUAL LAMB RESPONSES



GROWTH HORMONE VS TIME



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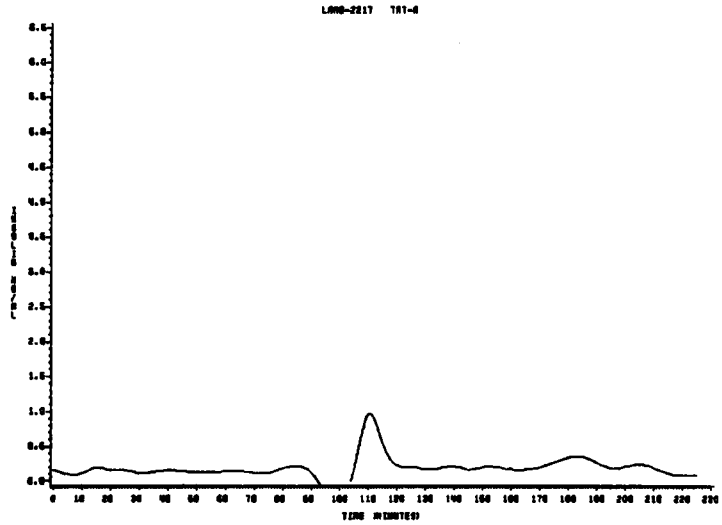

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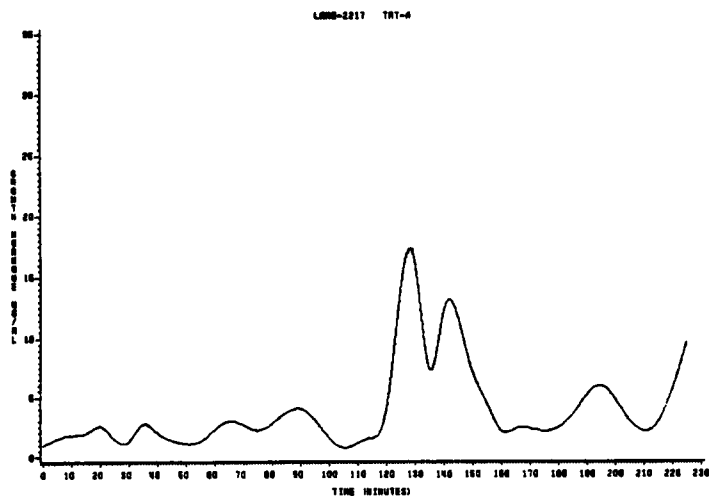

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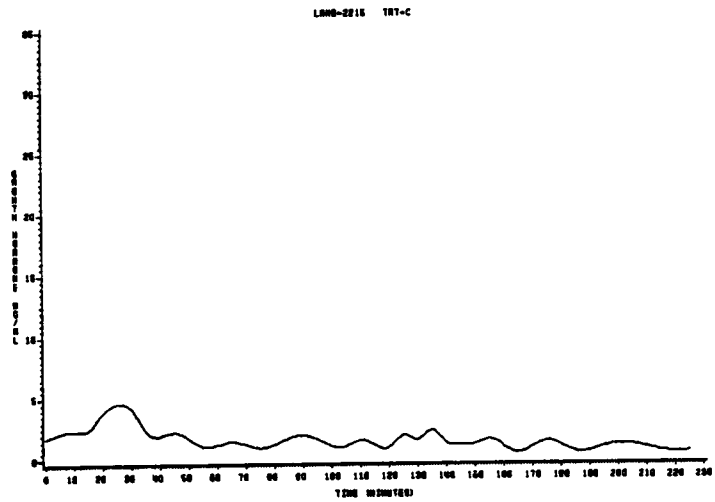

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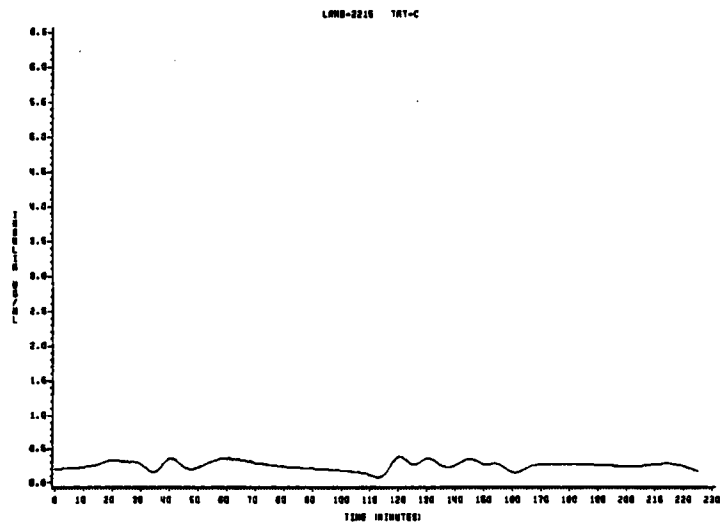

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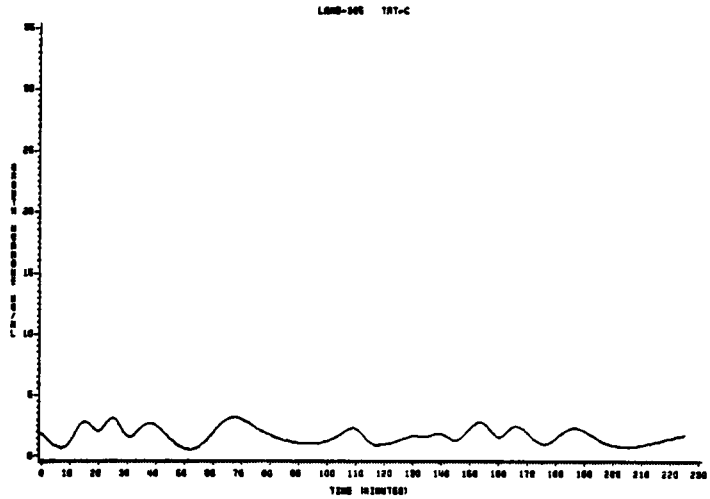

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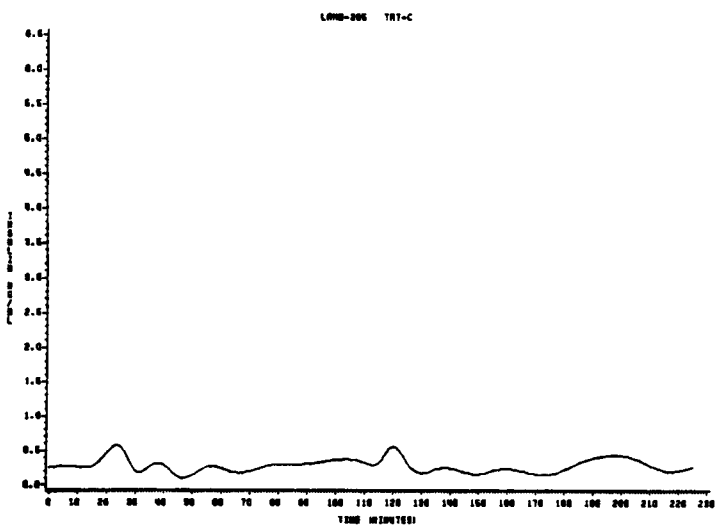

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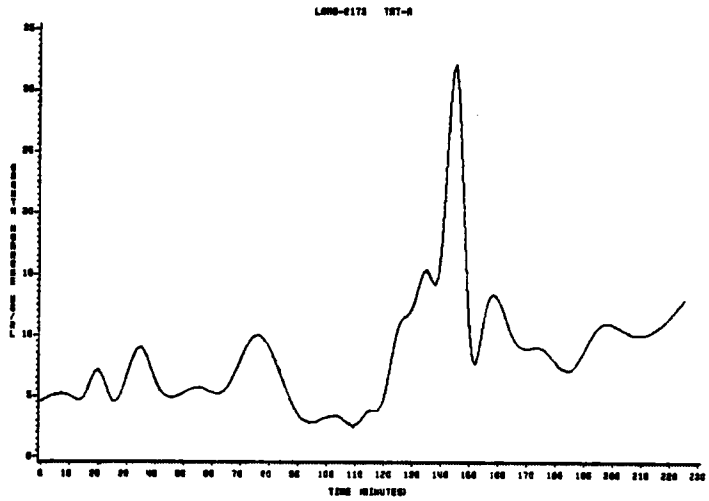
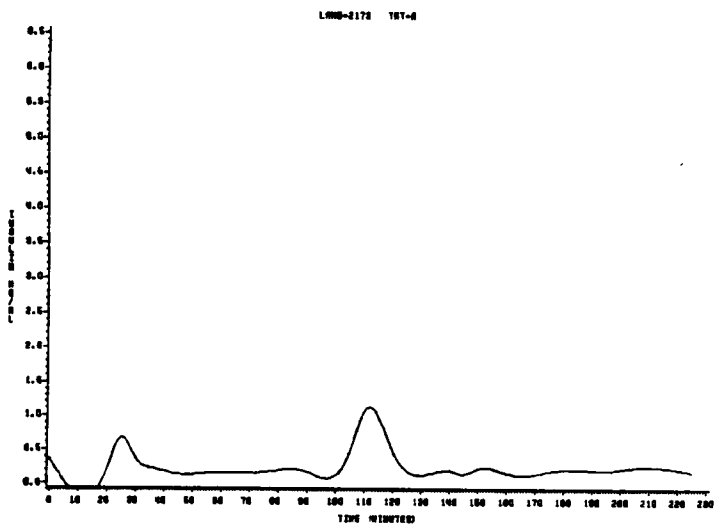


GROWTH HORMONE VS TIME



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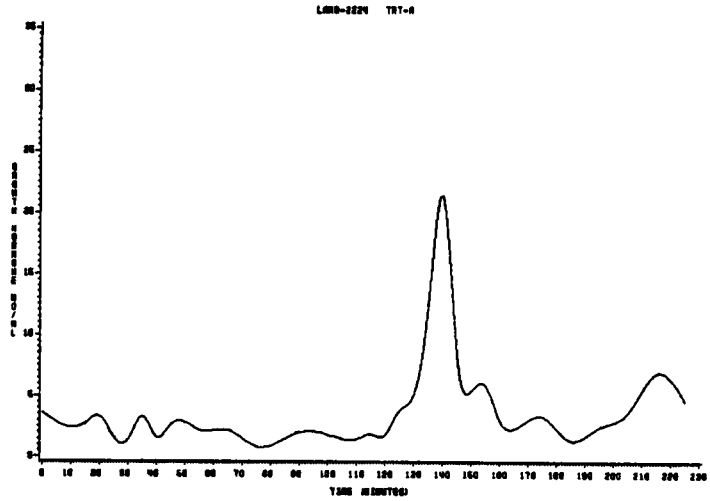
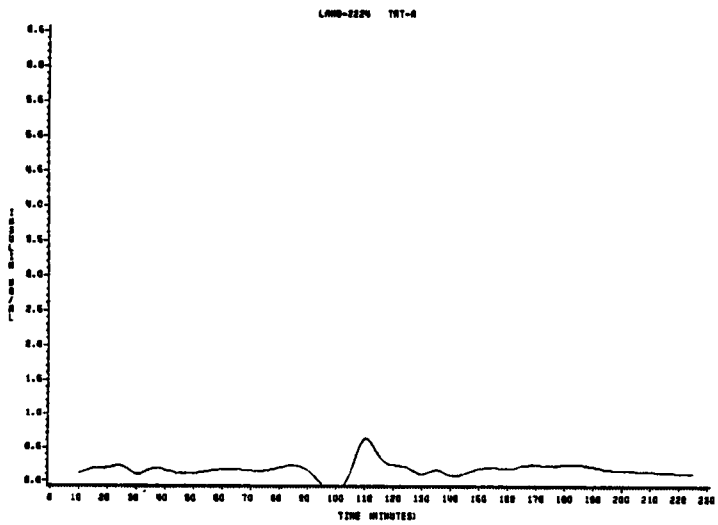
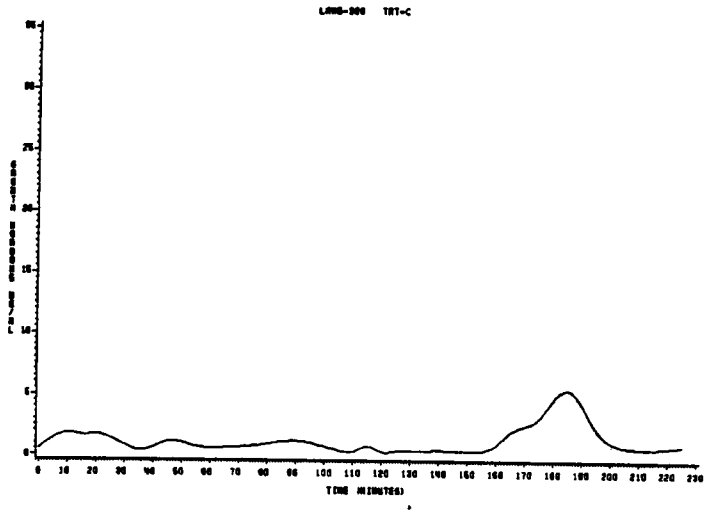


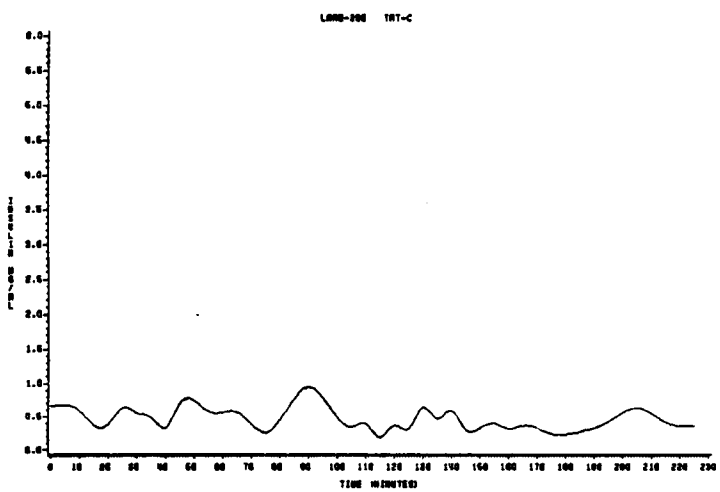
GROWTH HORMONE VS TIME



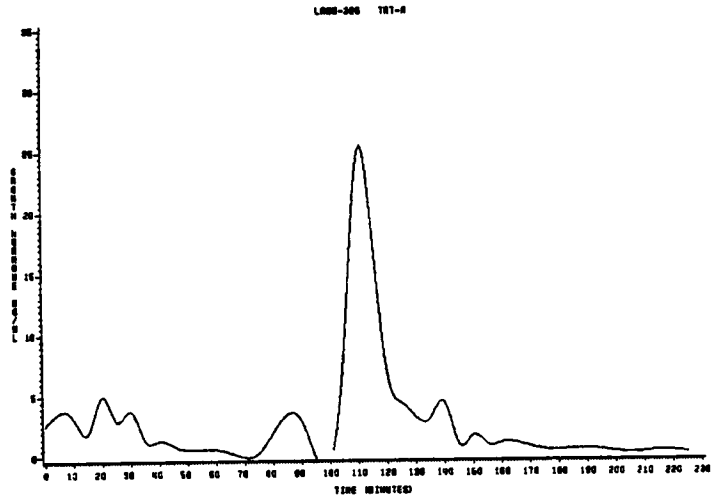
INSULIN VS TIME



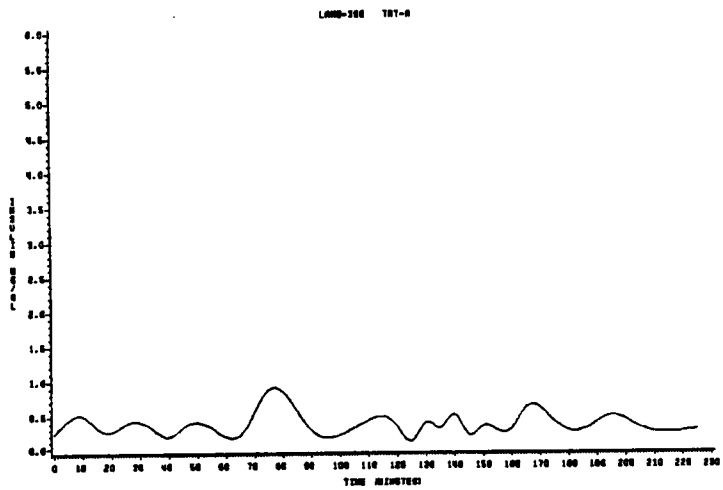
GROWTH HORMONE VS TIME



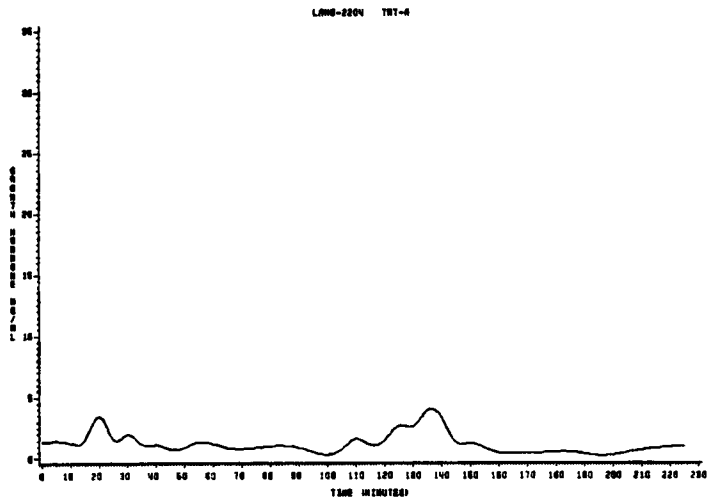
INSULIN VS TIME



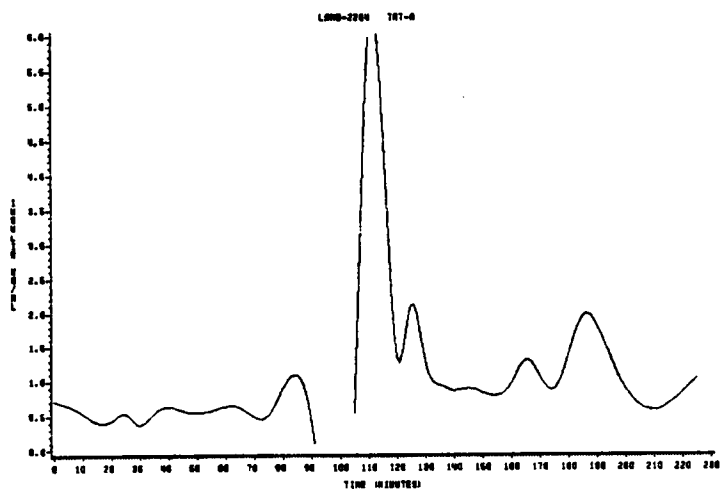
GROWTH HORMONE VS TIME



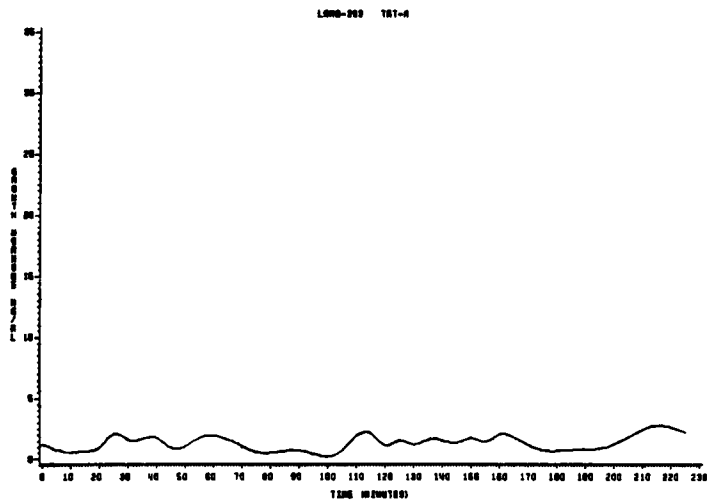
INSULIN VS TIME



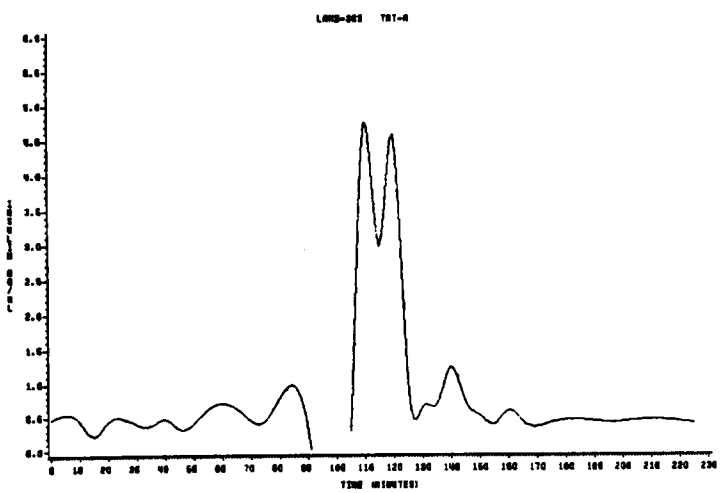
GROWTH HORMONE VS TIME



INSULIN VS TIME



GROWTH HORMONE VS TIME



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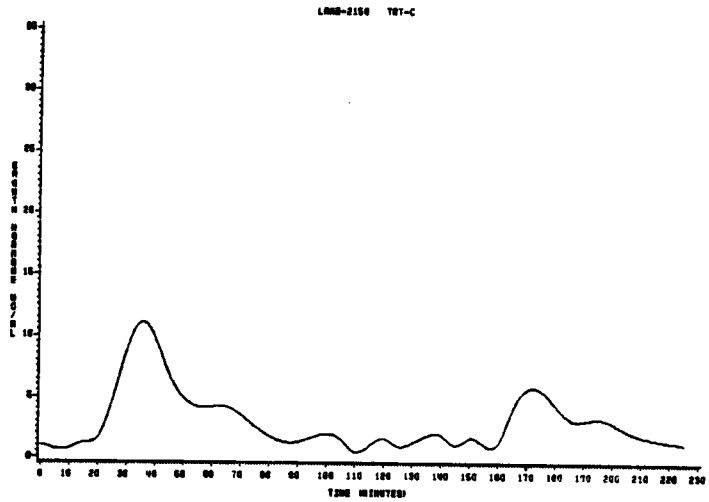
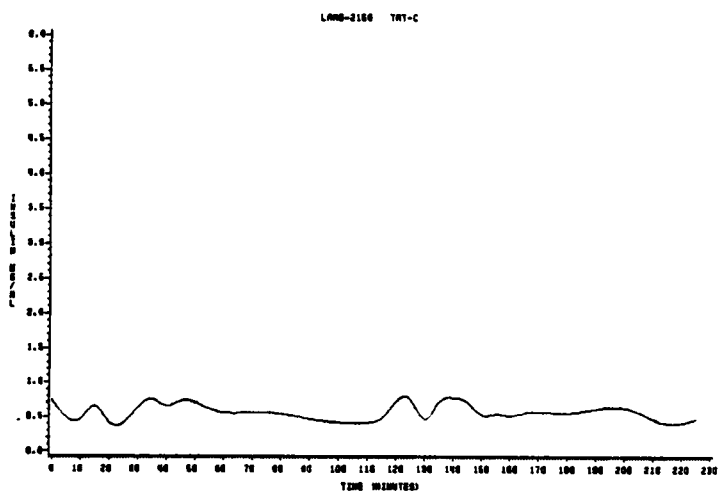
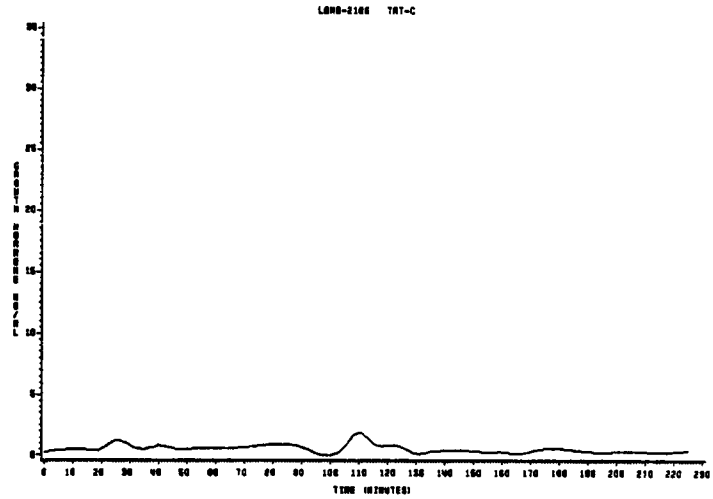


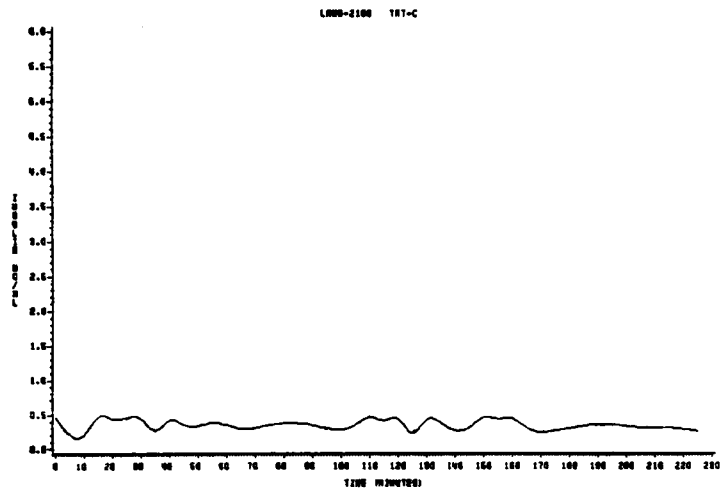
GROWTH HORMONE VS TIME



INSULIN VS TIME

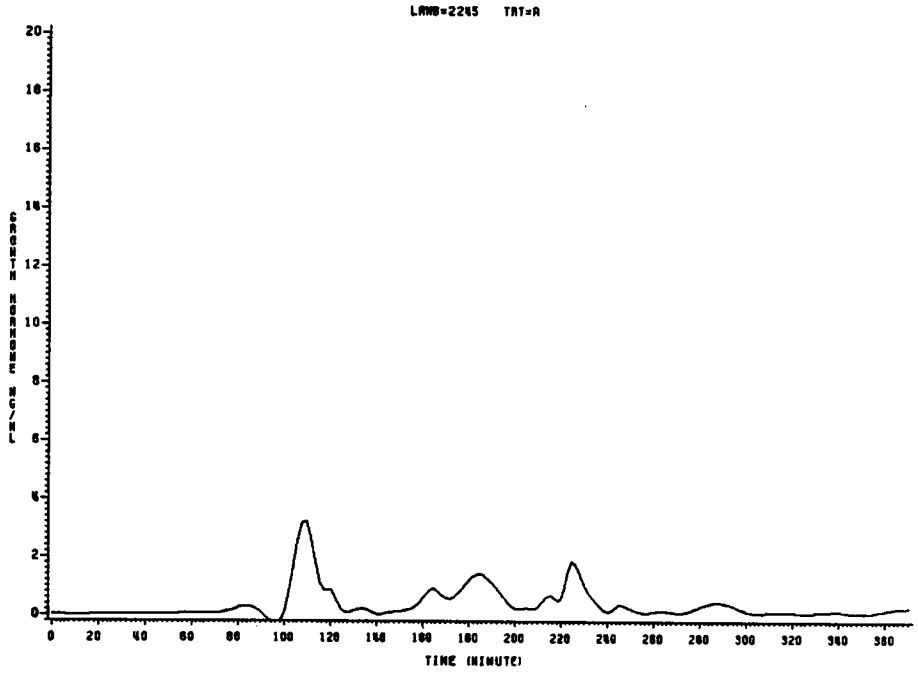


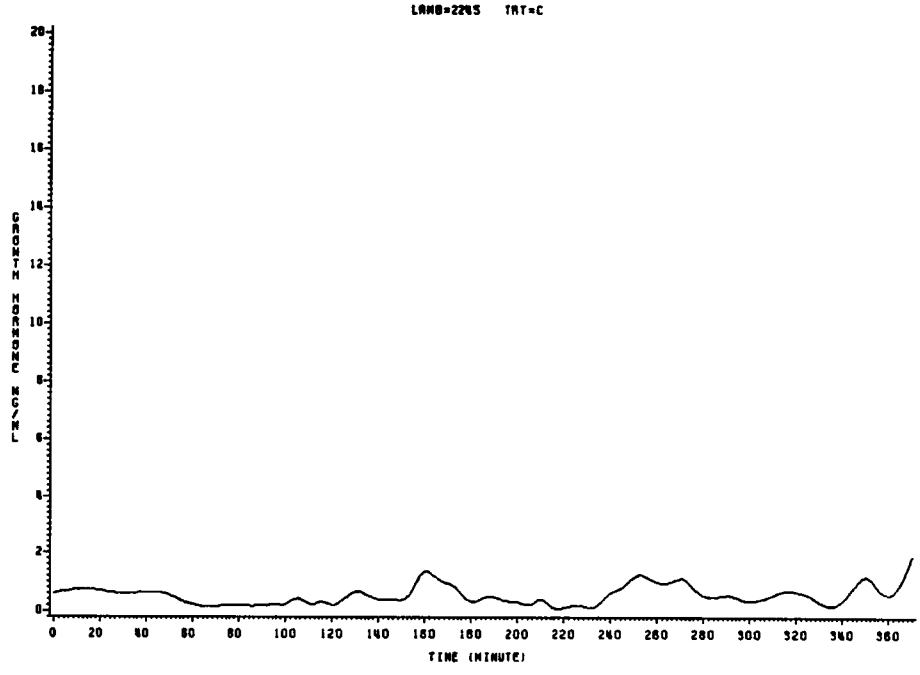
GROWTH HORMONE VS TIME



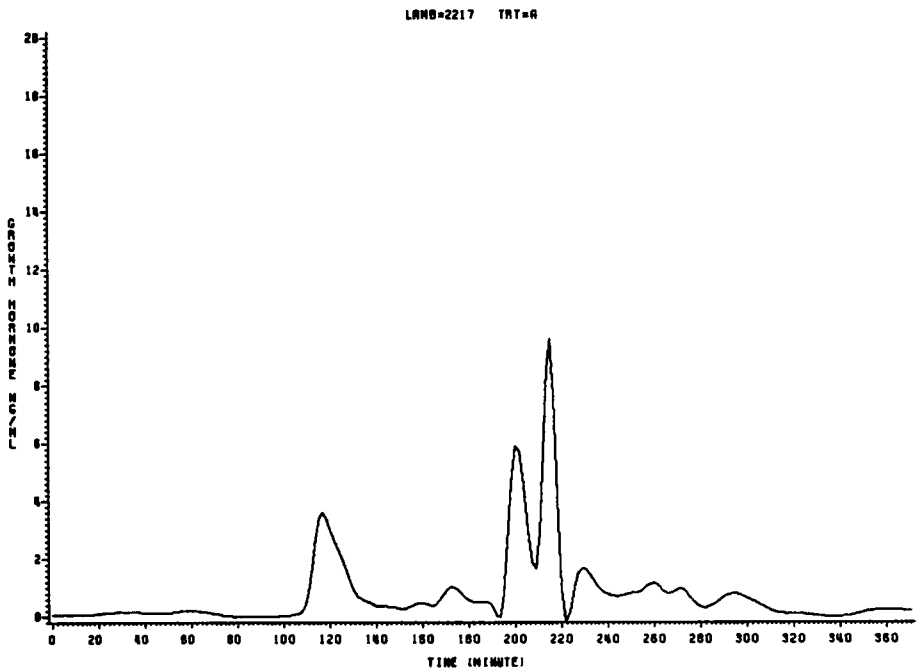
INSULIN VS TIME

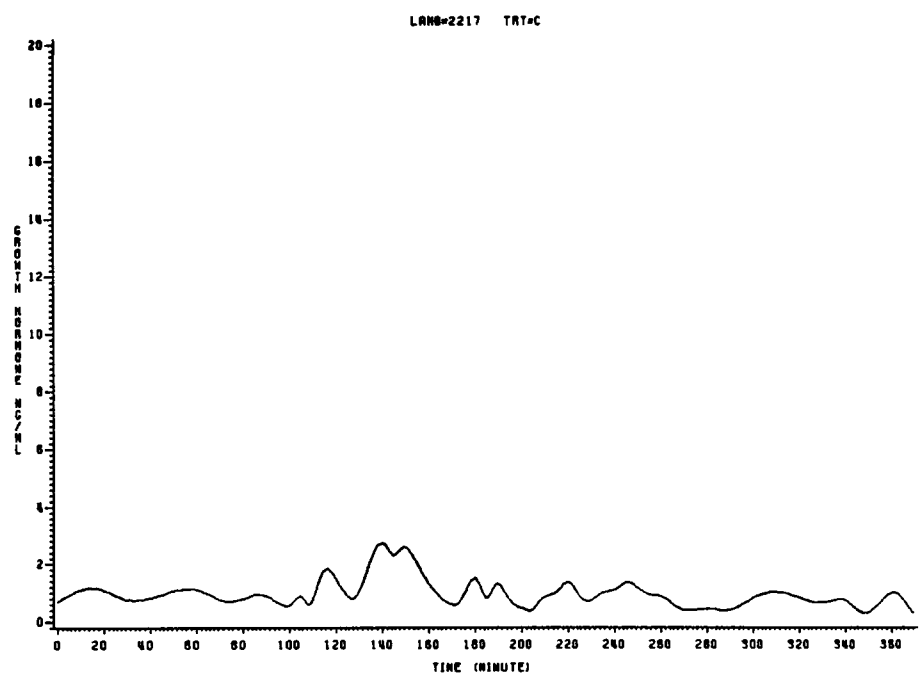
TRIAL 2





GROWTH HORMONE VERSUS TIME





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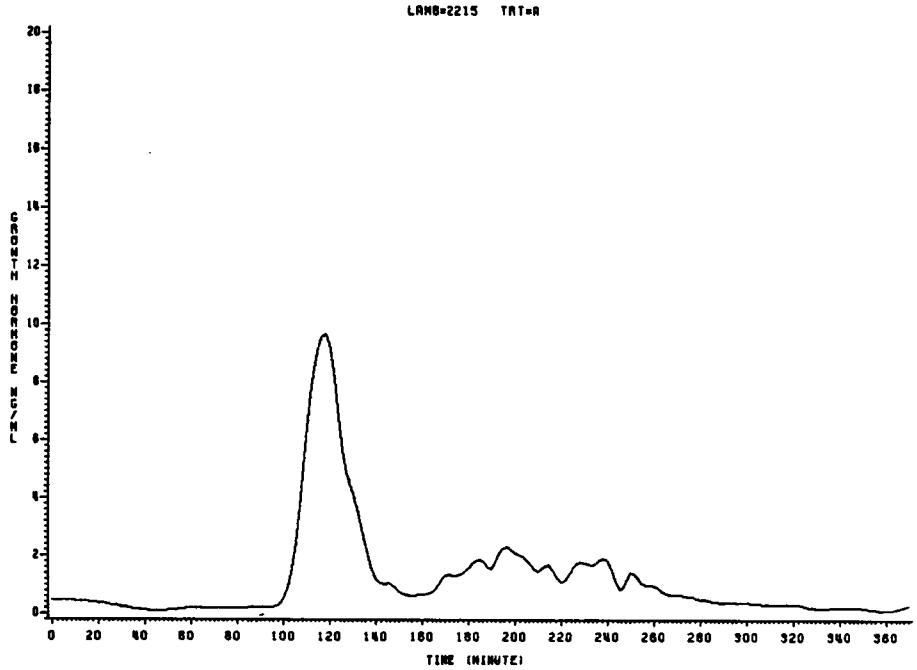

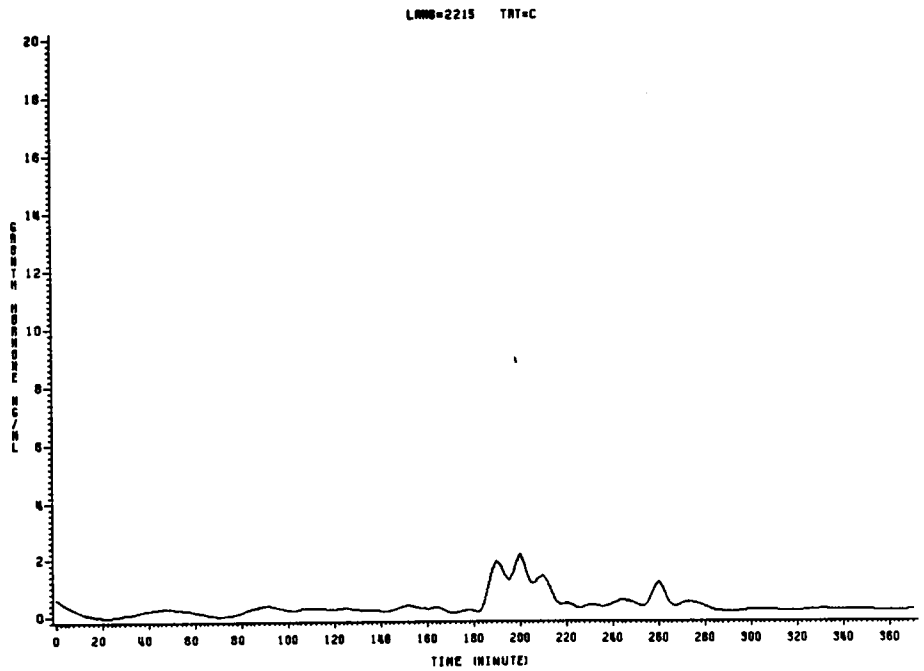

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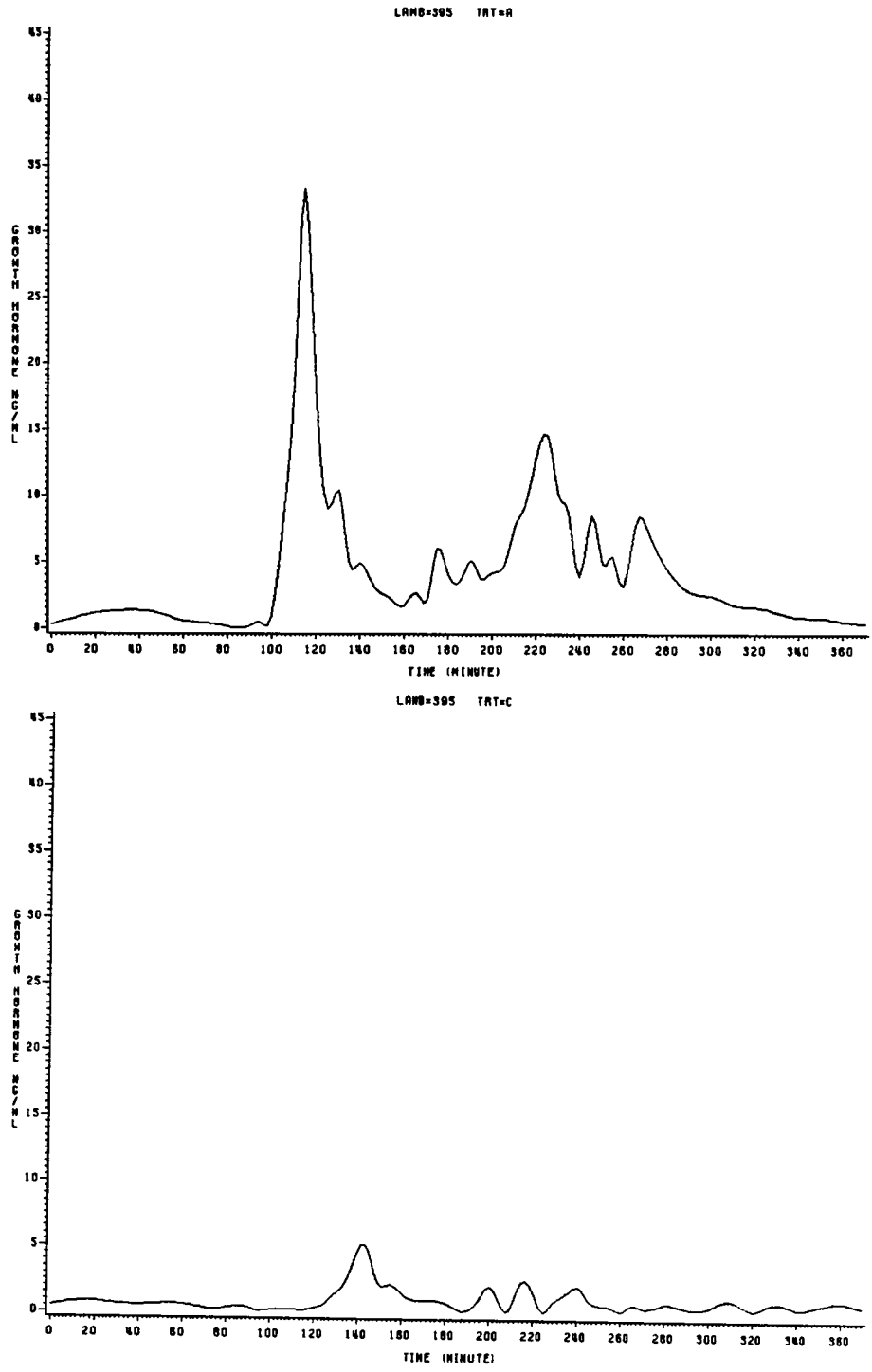

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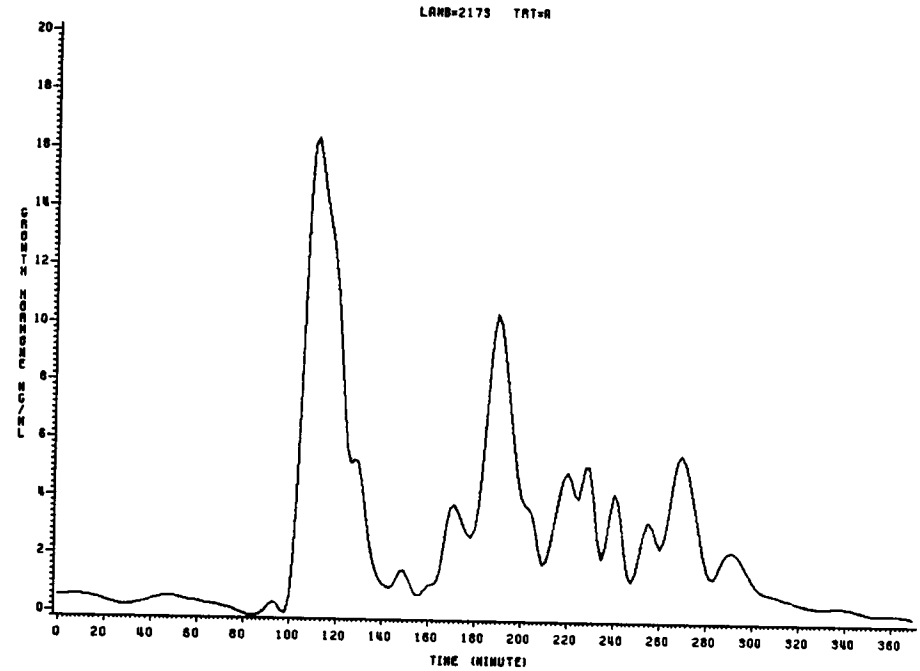

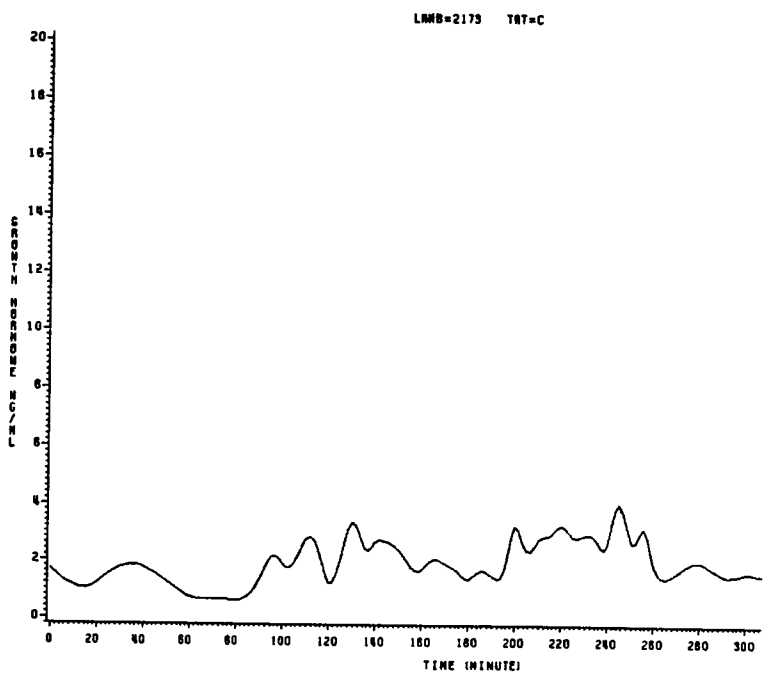

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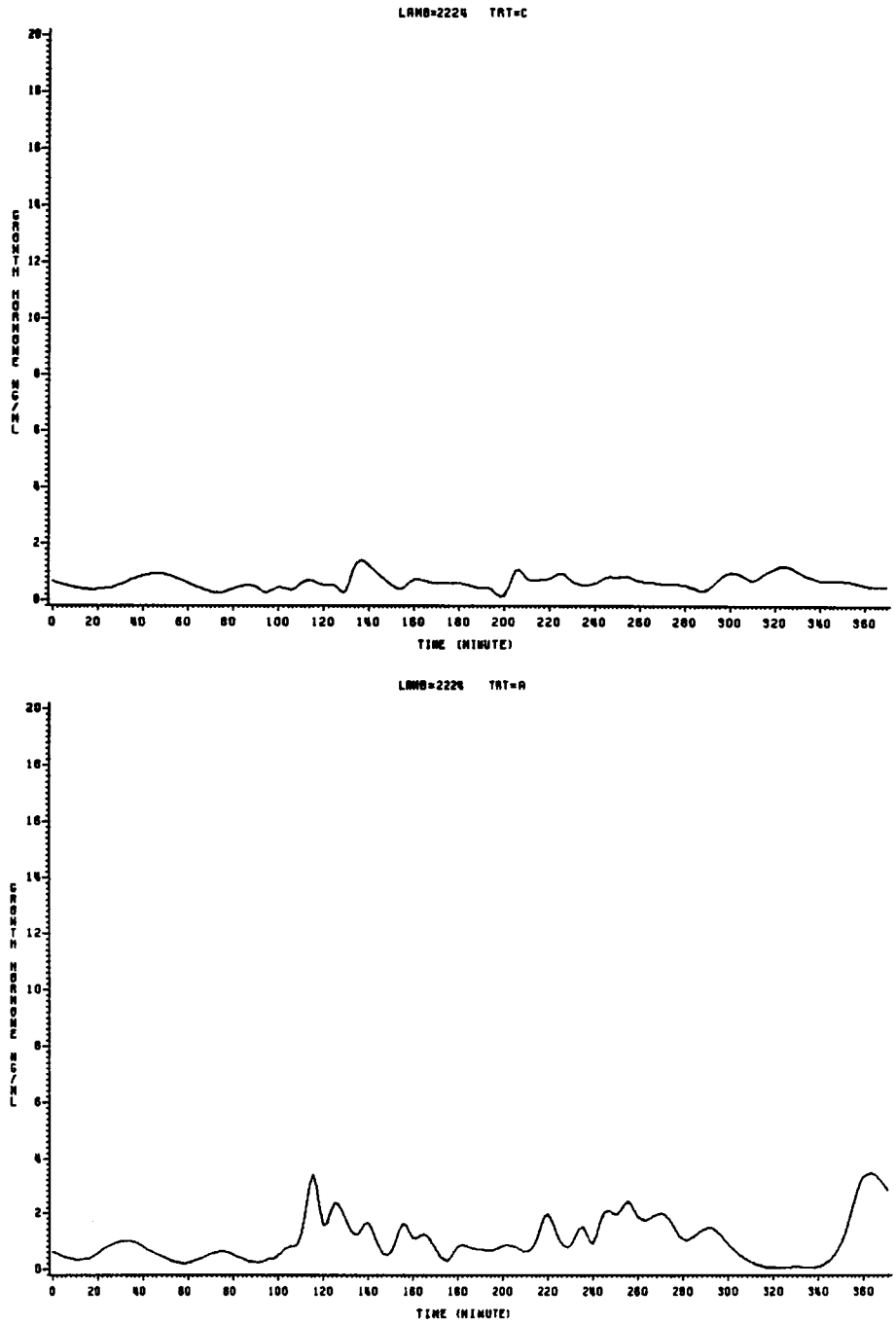

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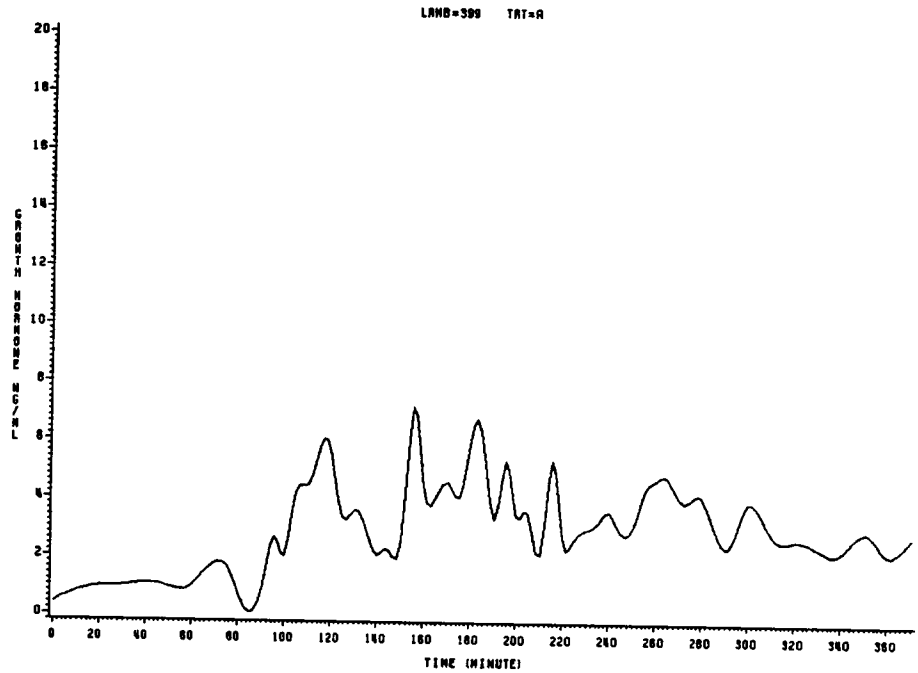

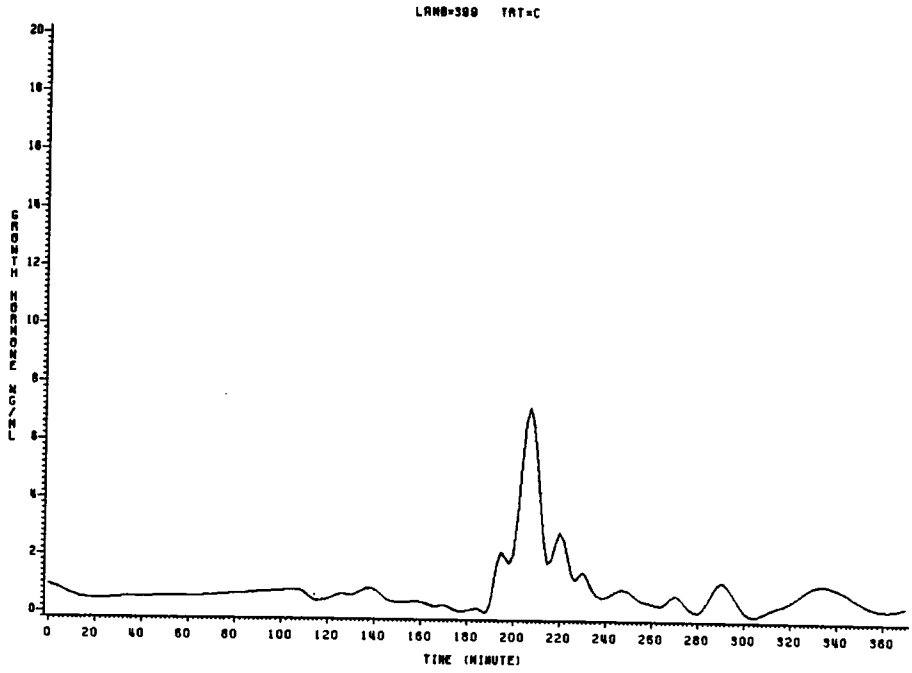

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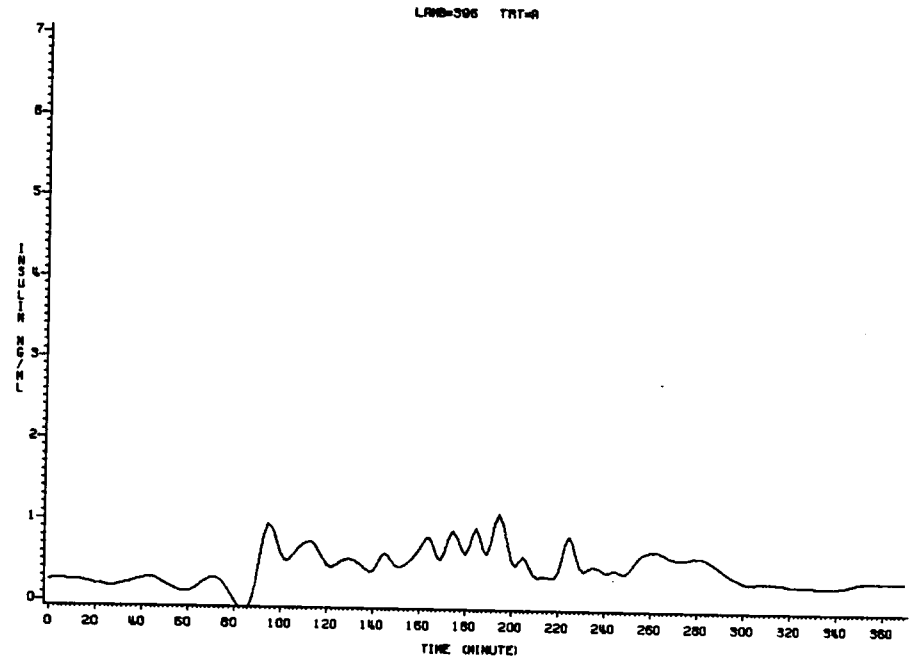

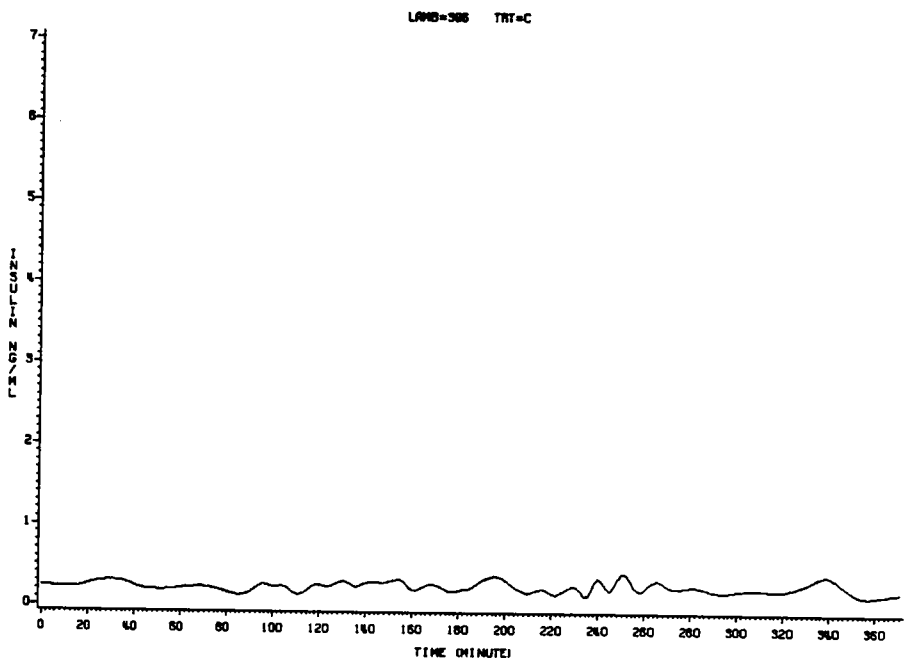

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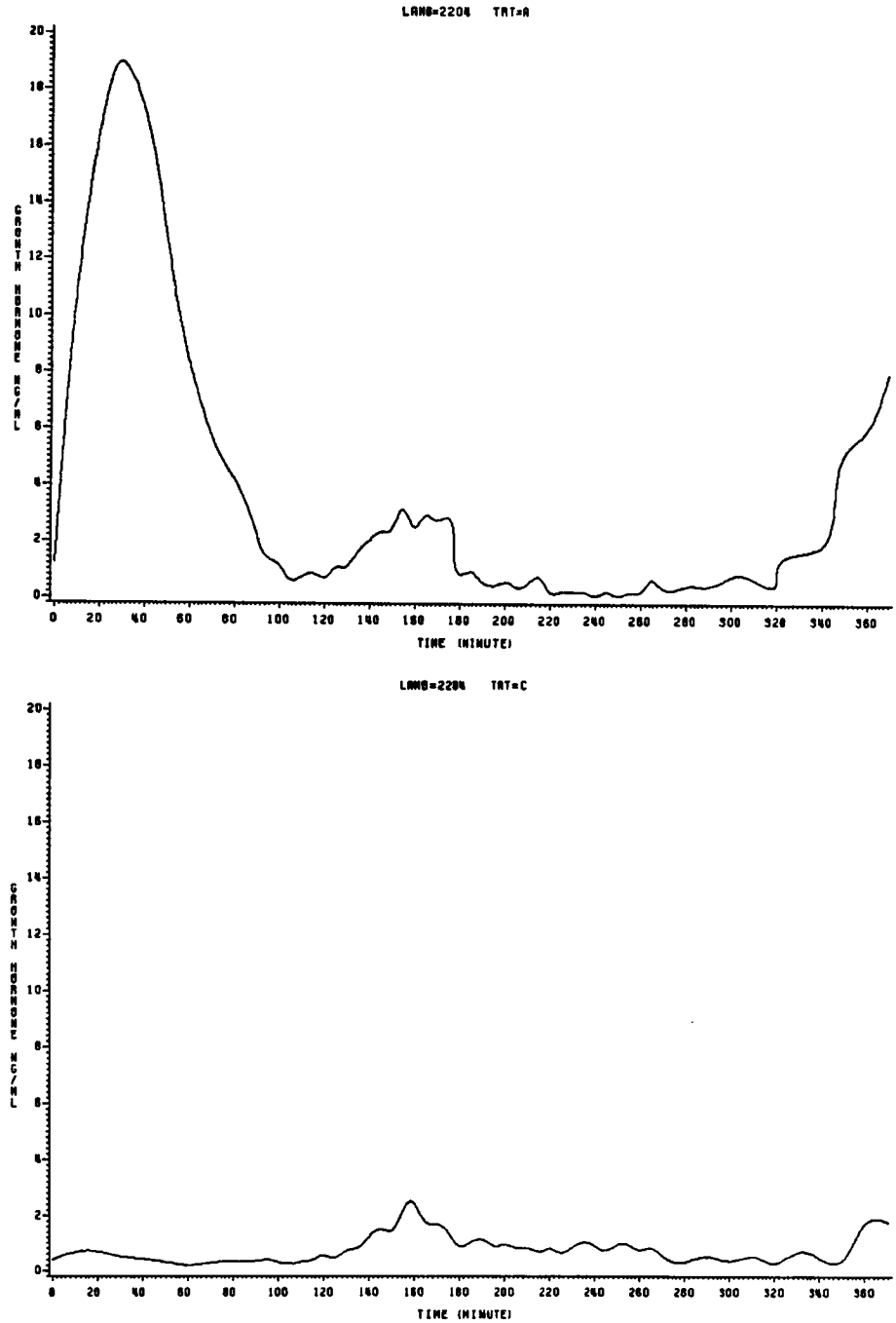

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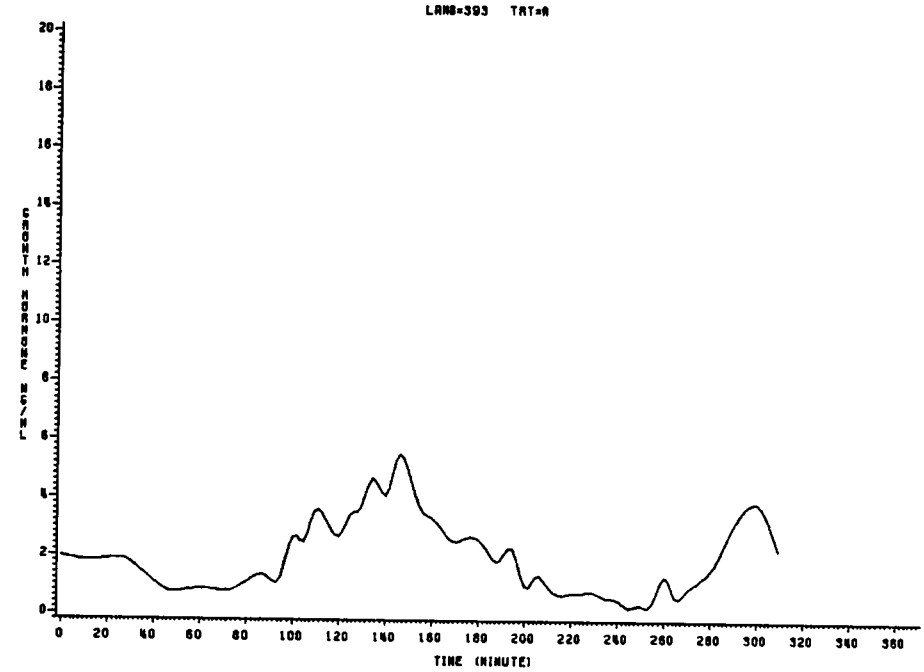

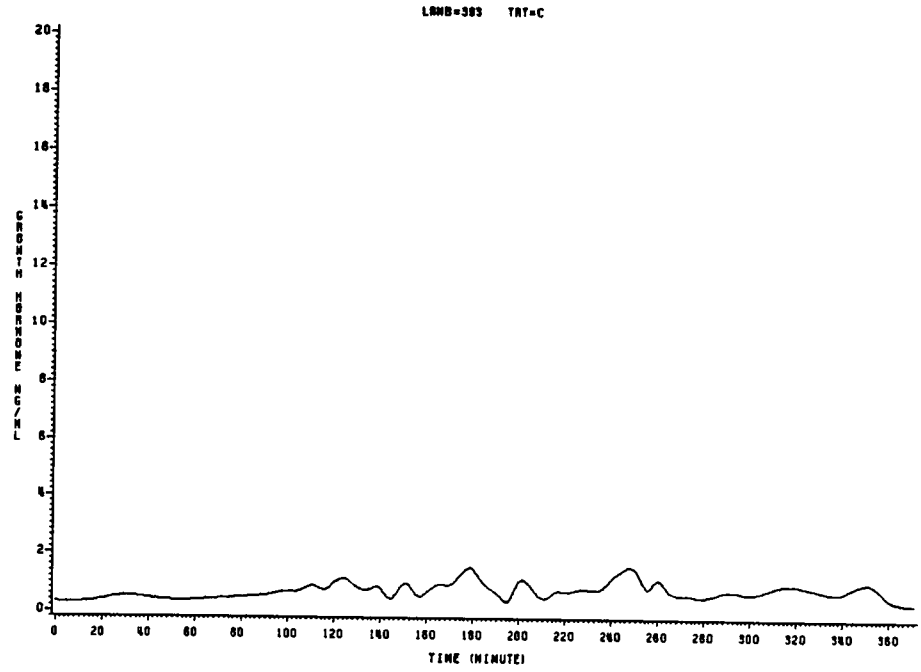

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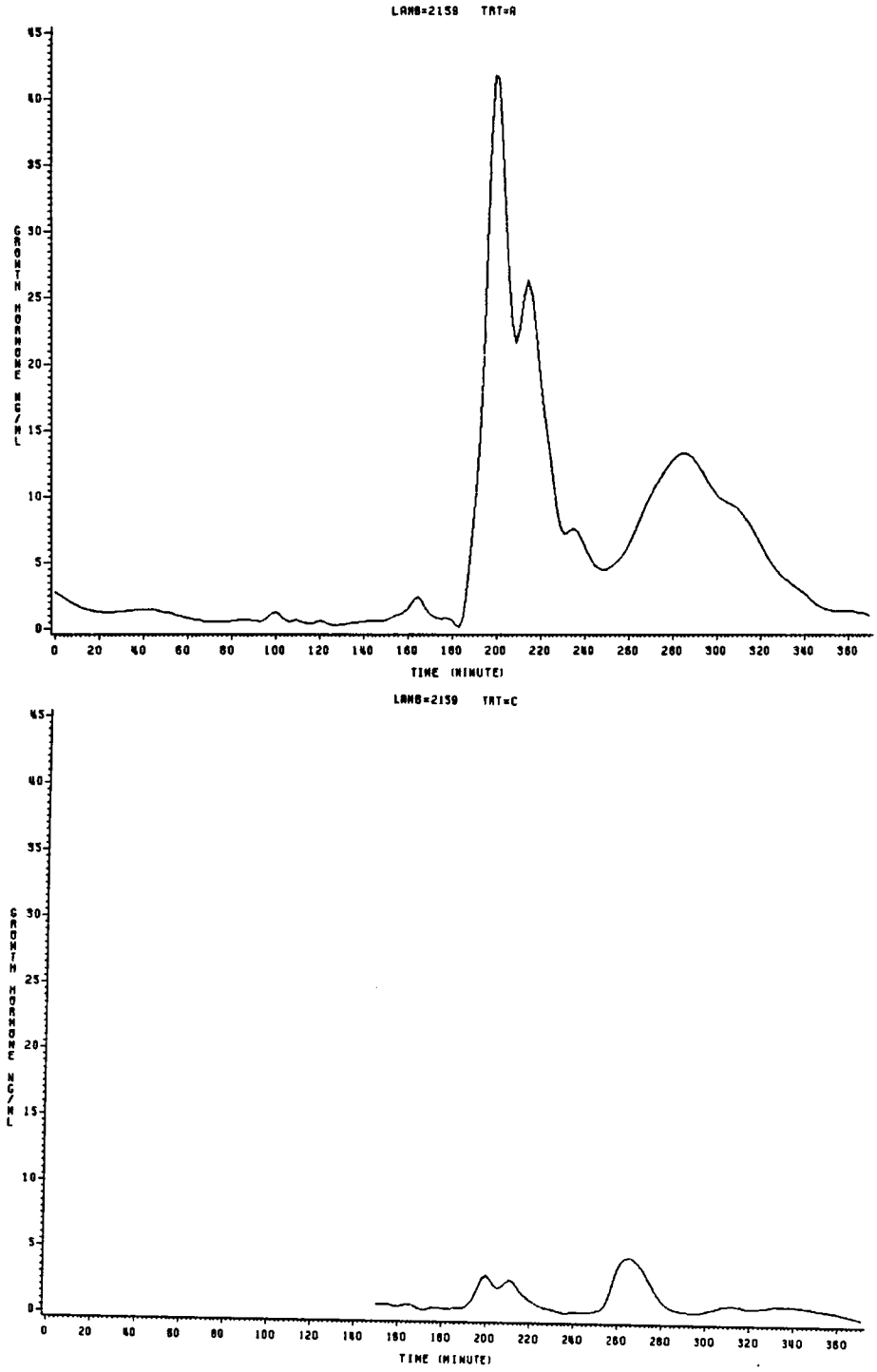

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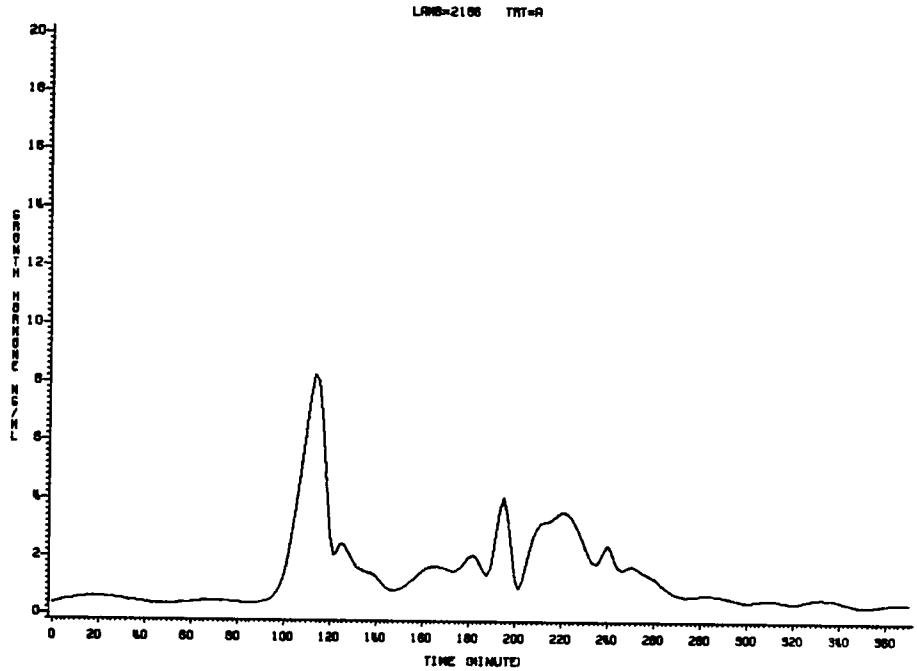
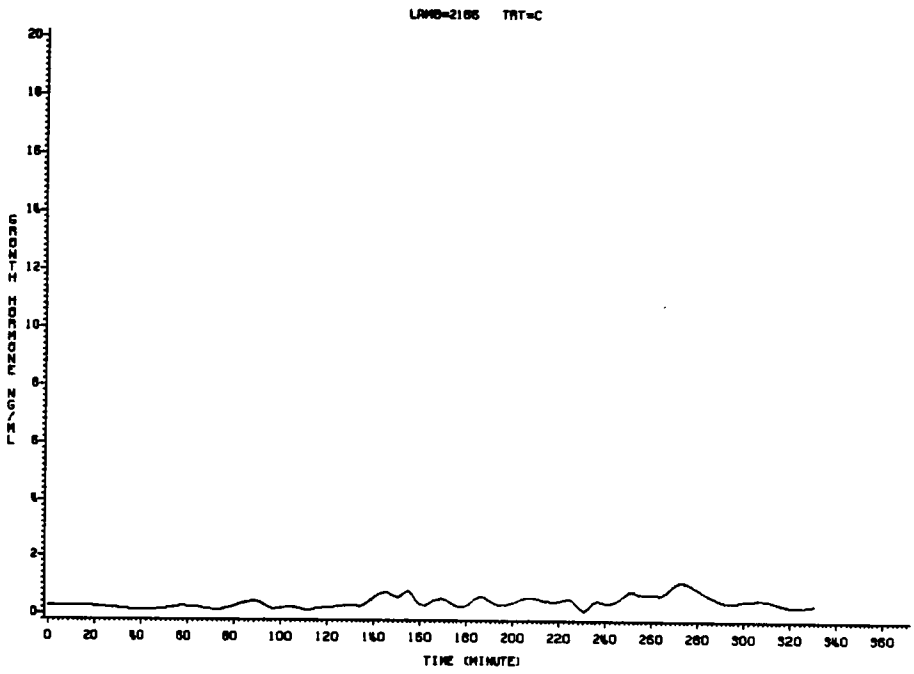
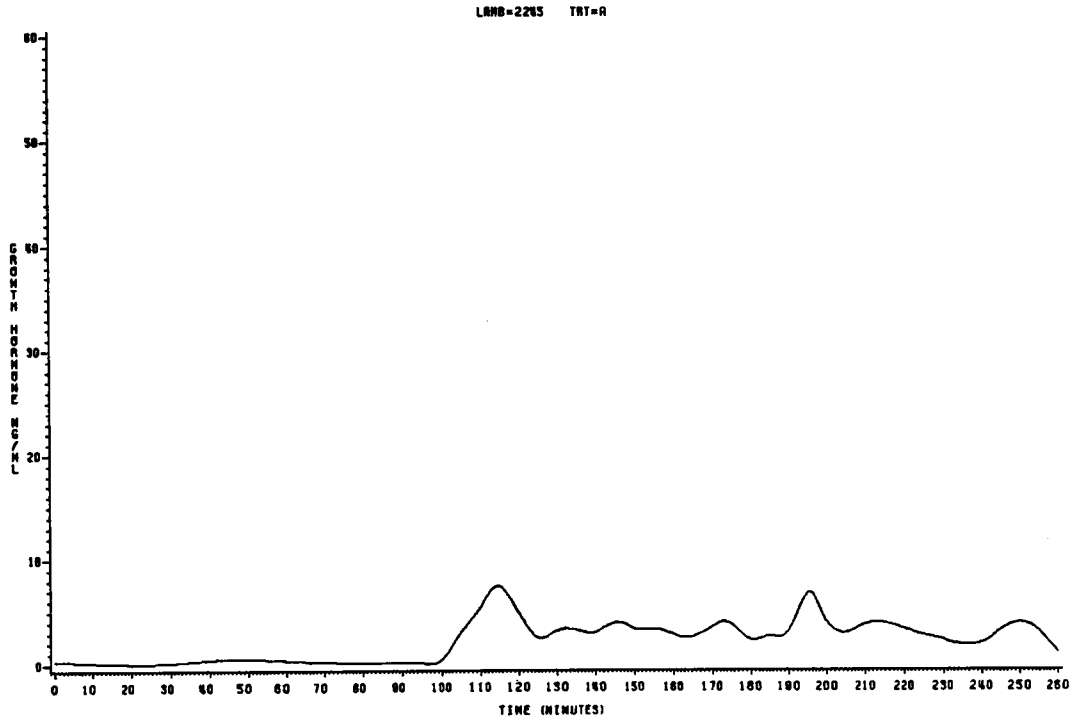
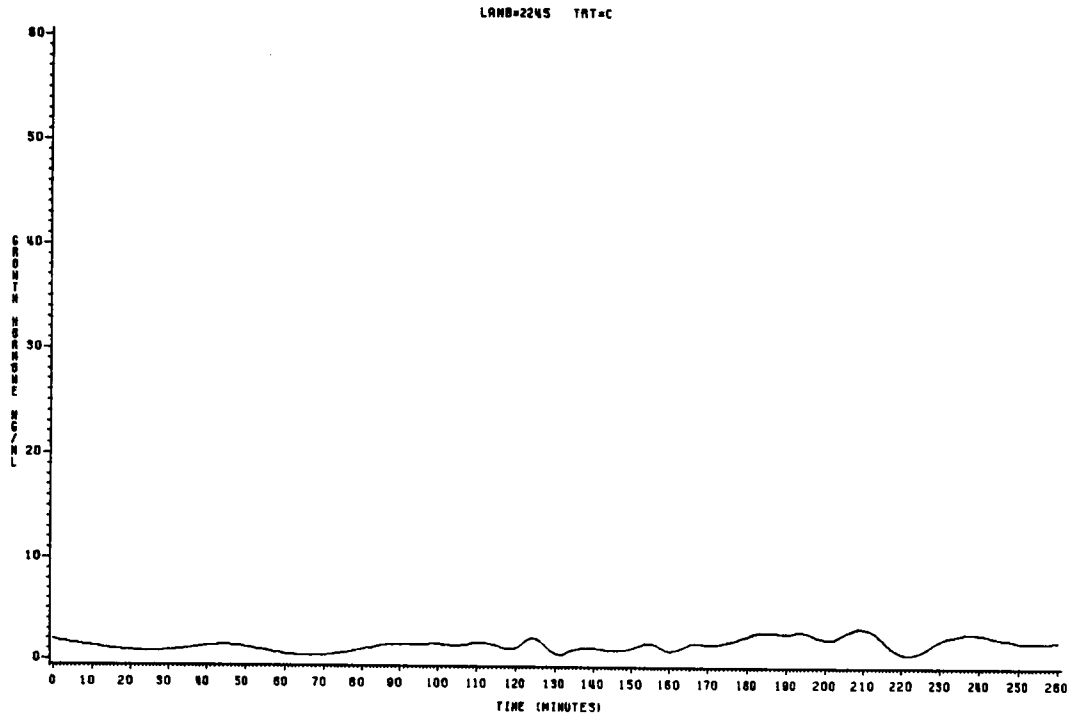




GROWTH HORMONE VERSUS TIME

TRIAL 3





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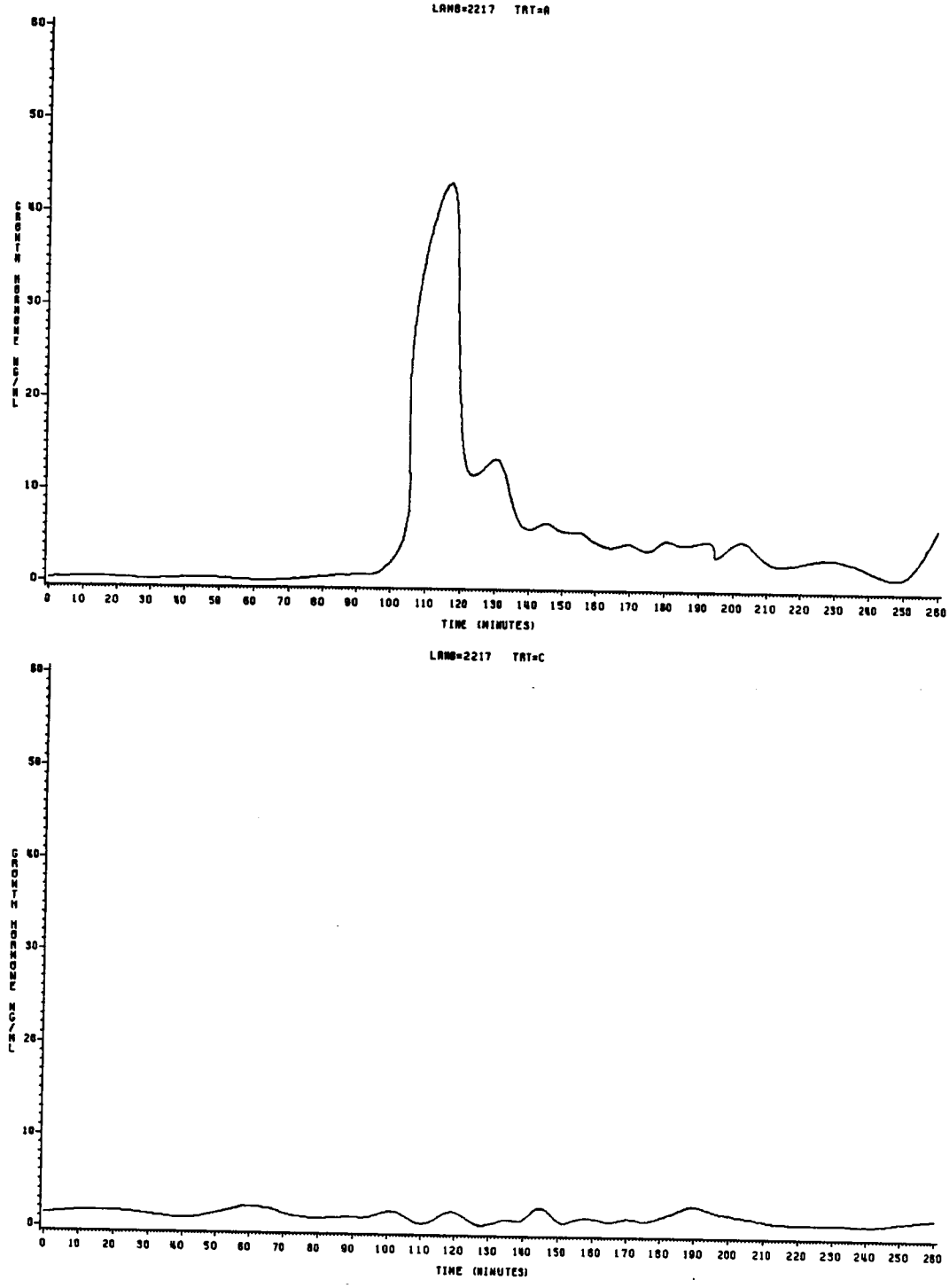

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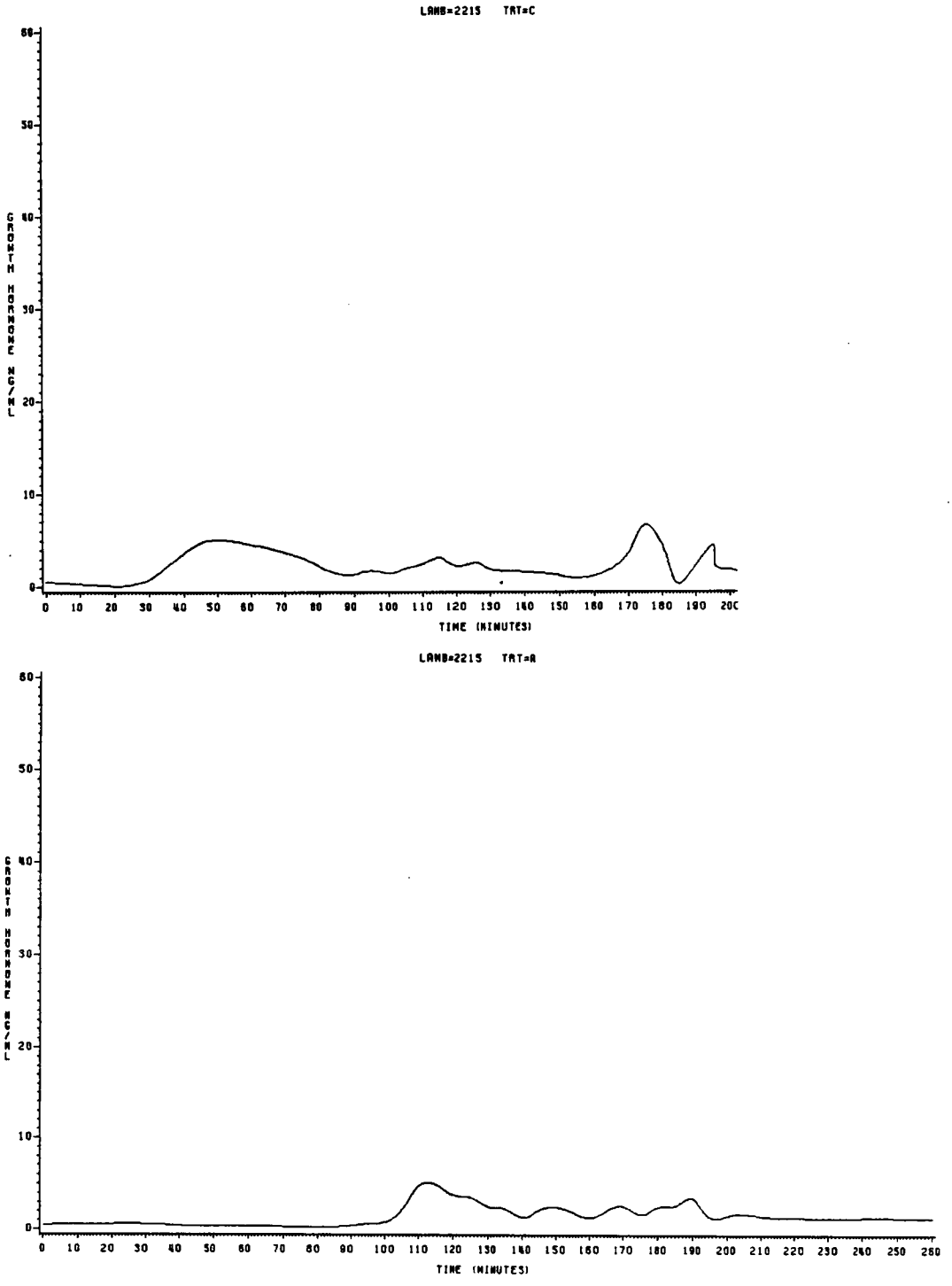

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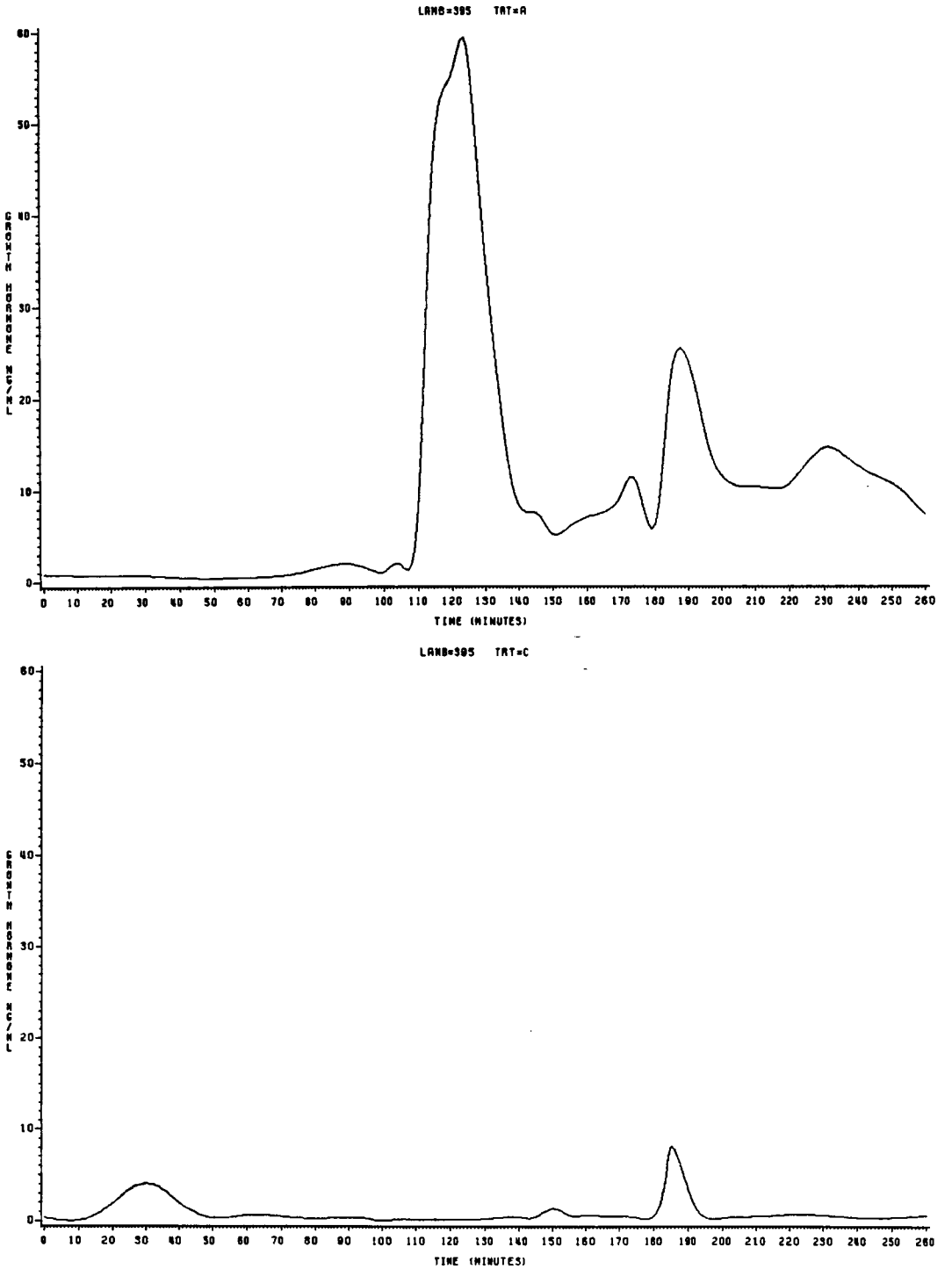

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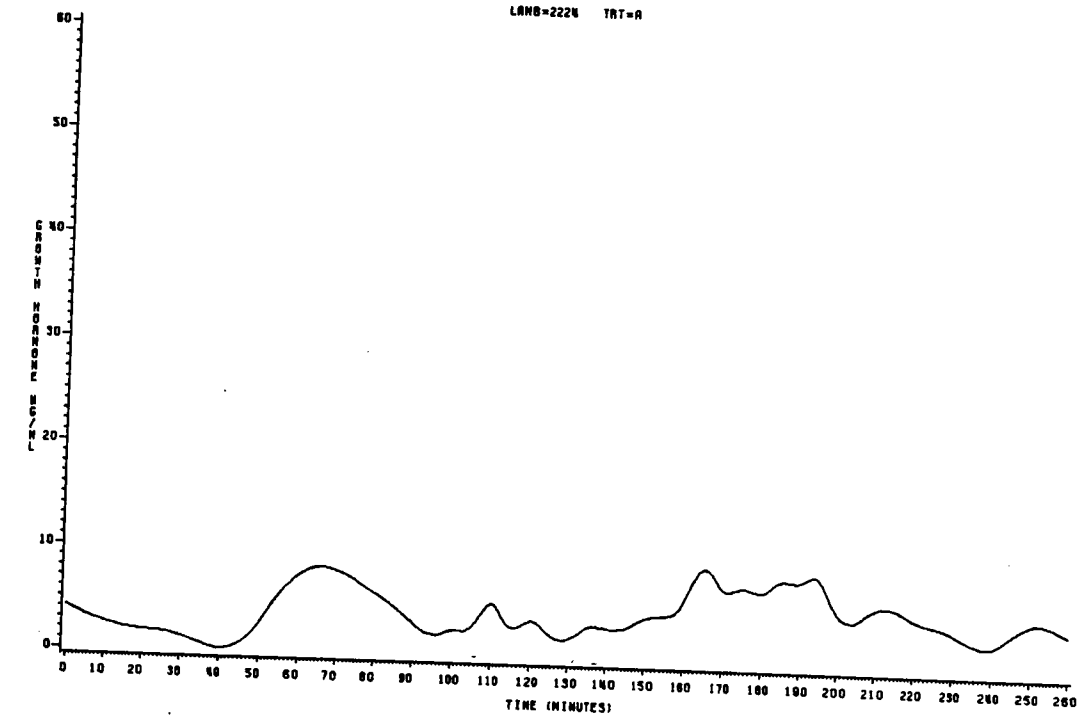

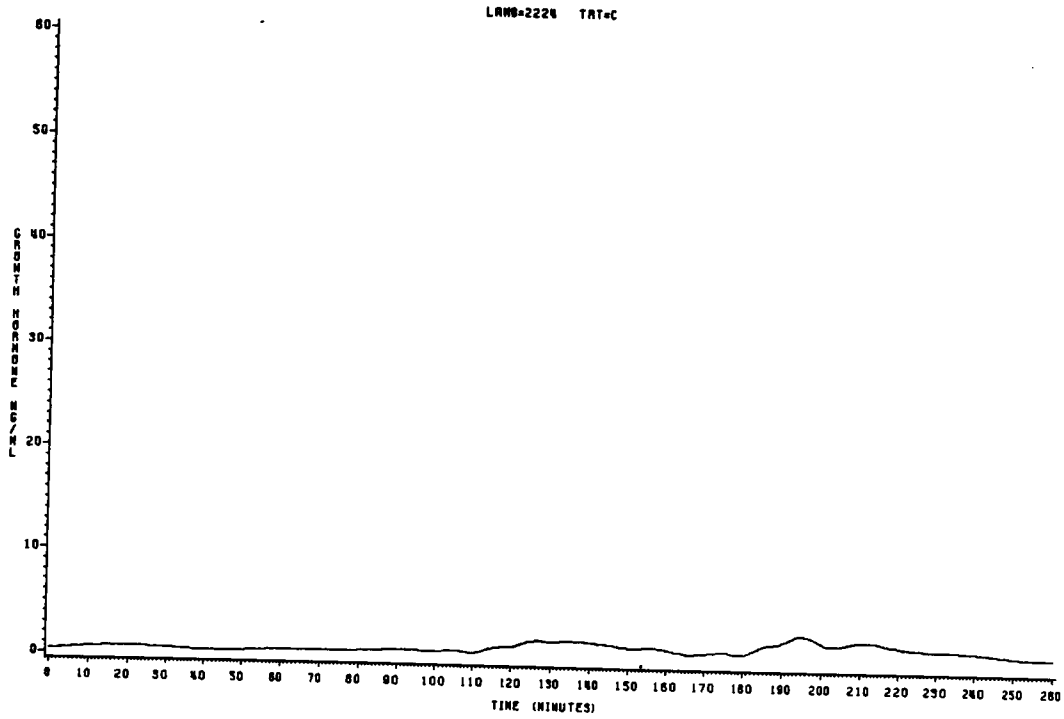

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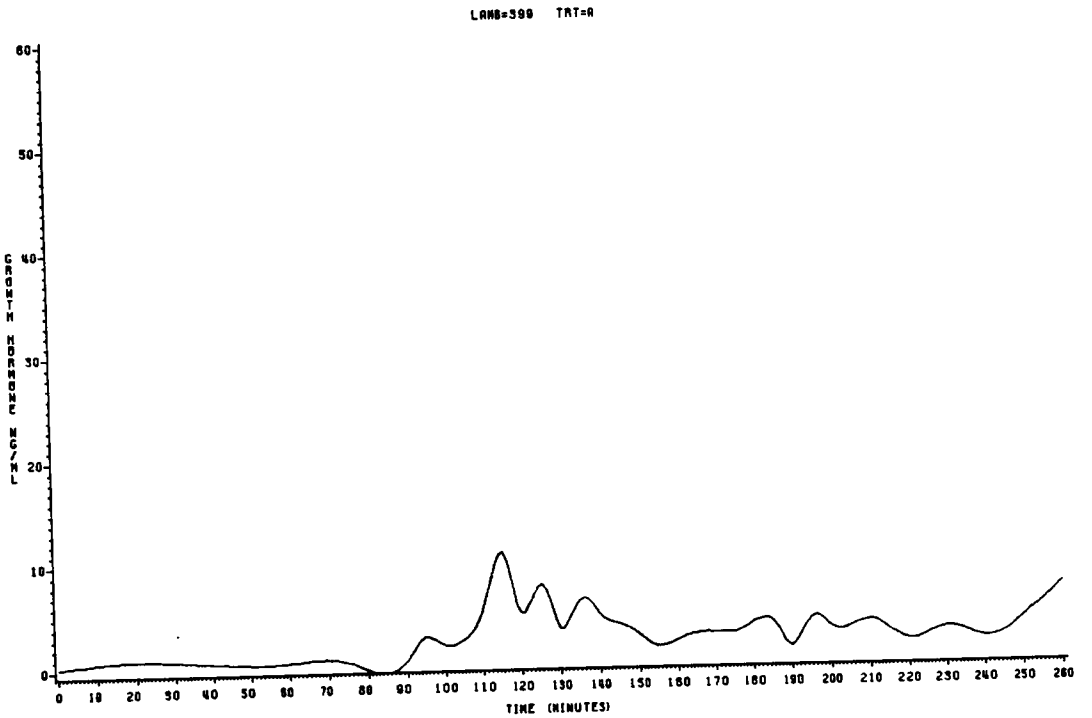

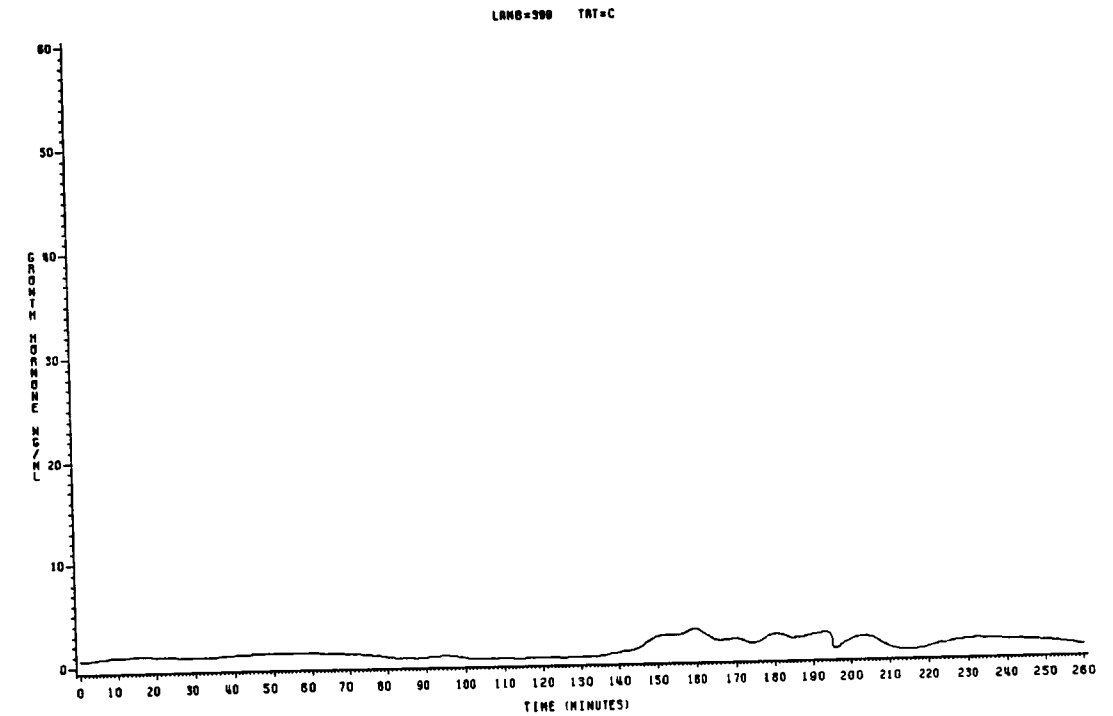

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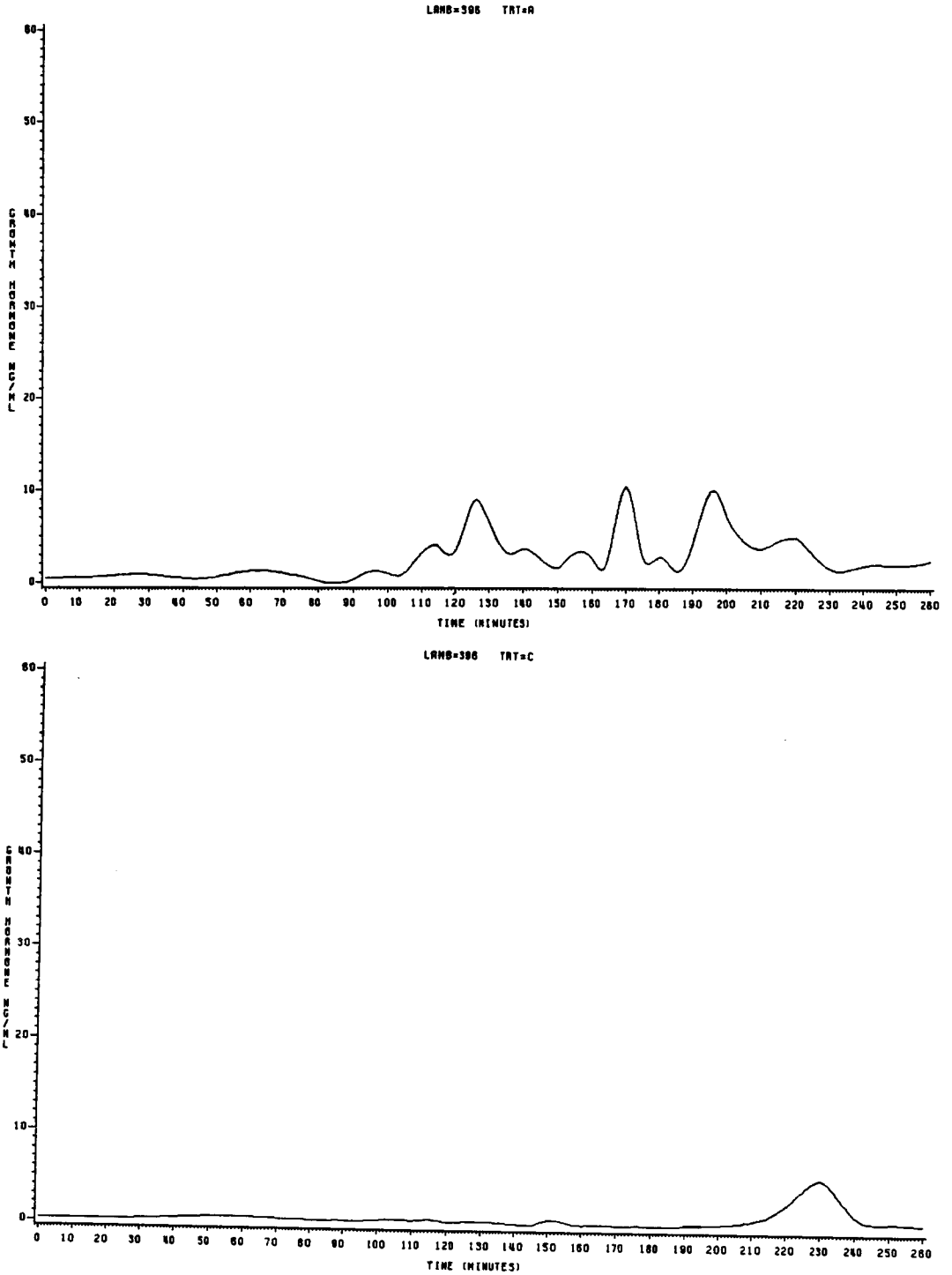

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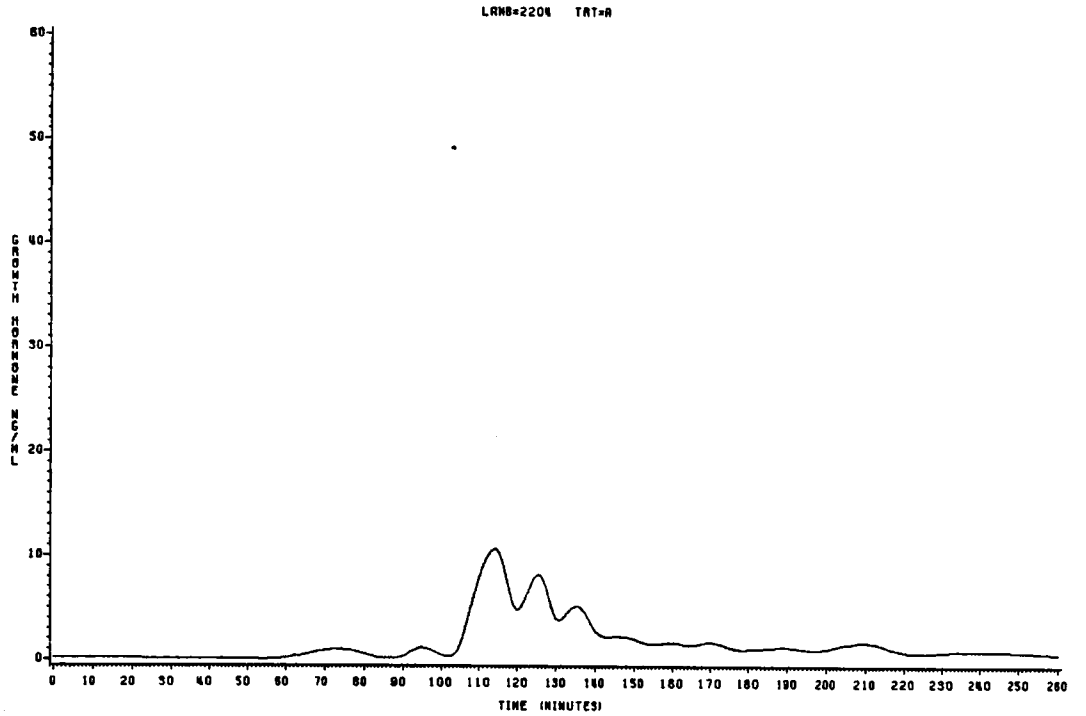

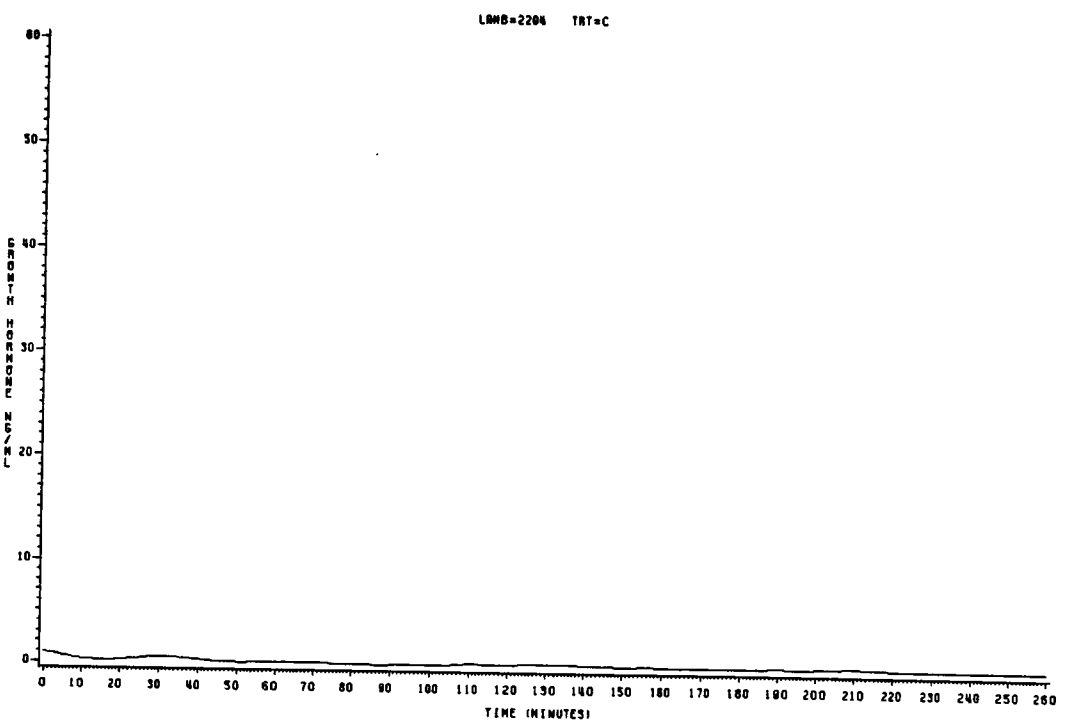

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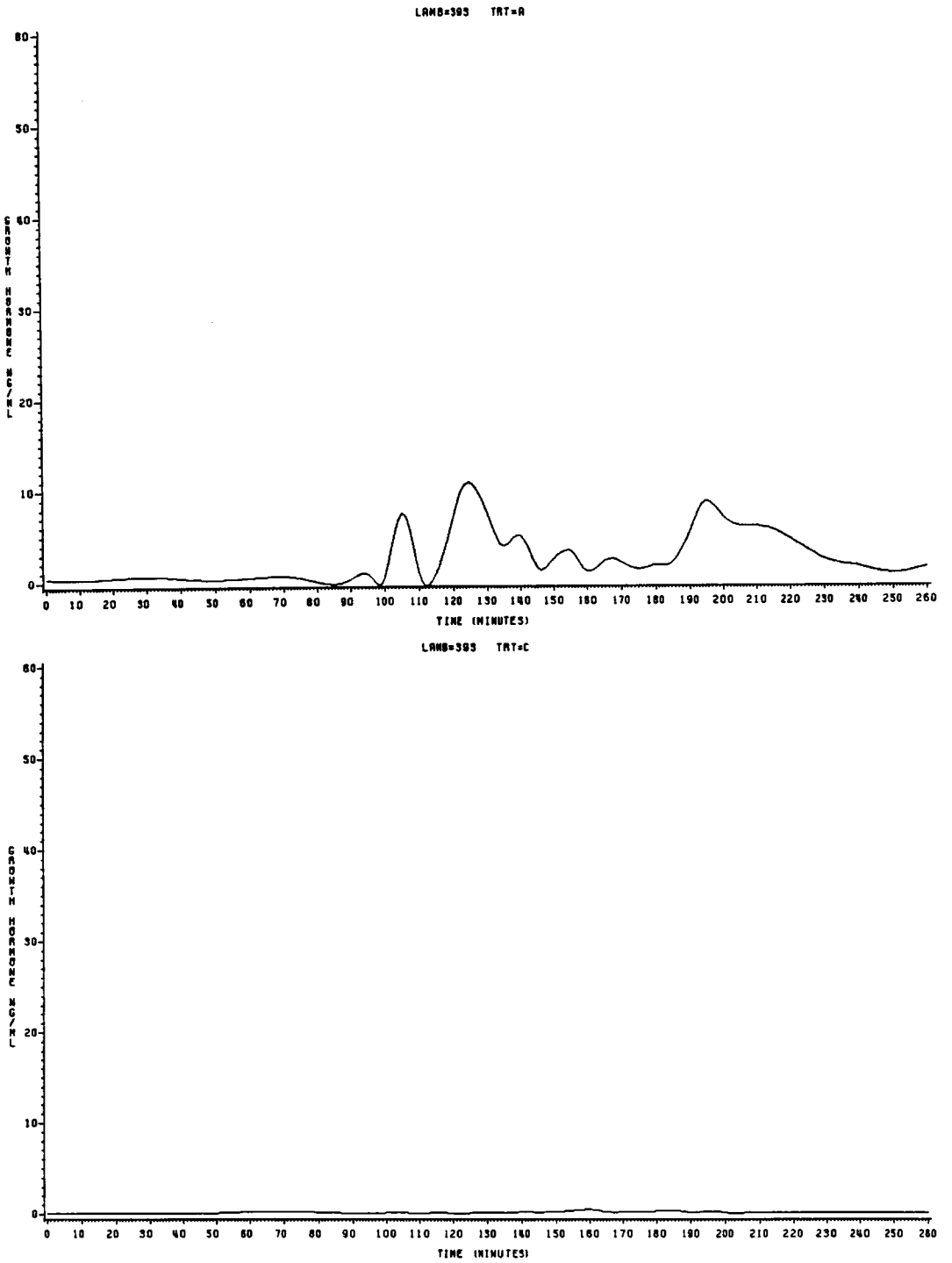

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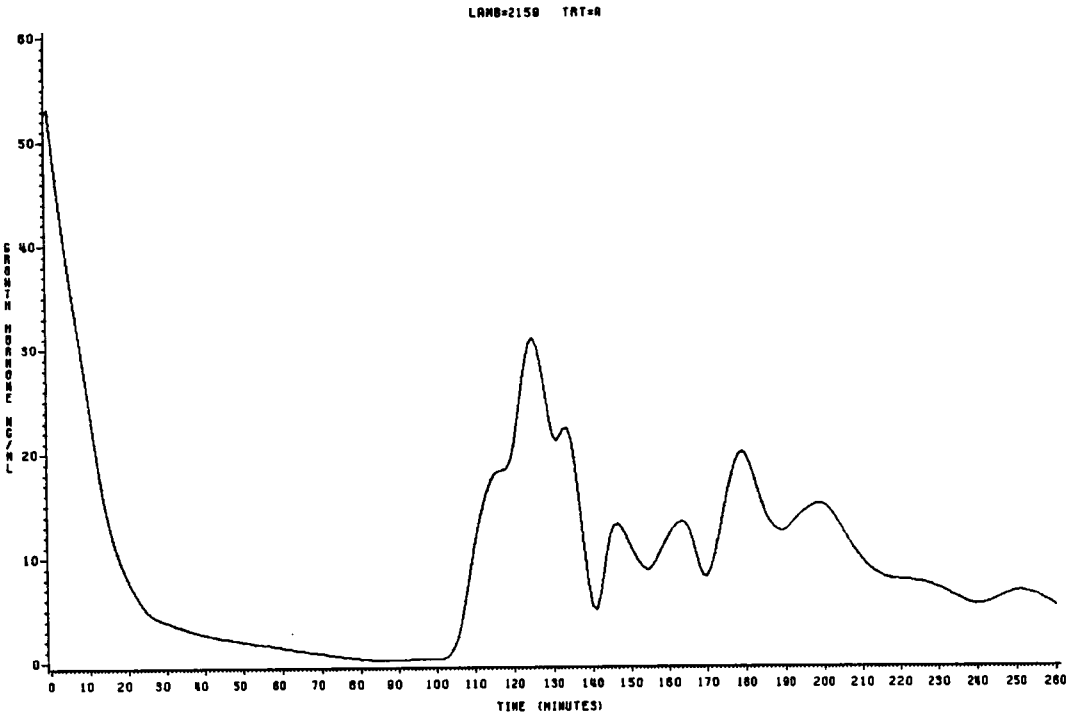
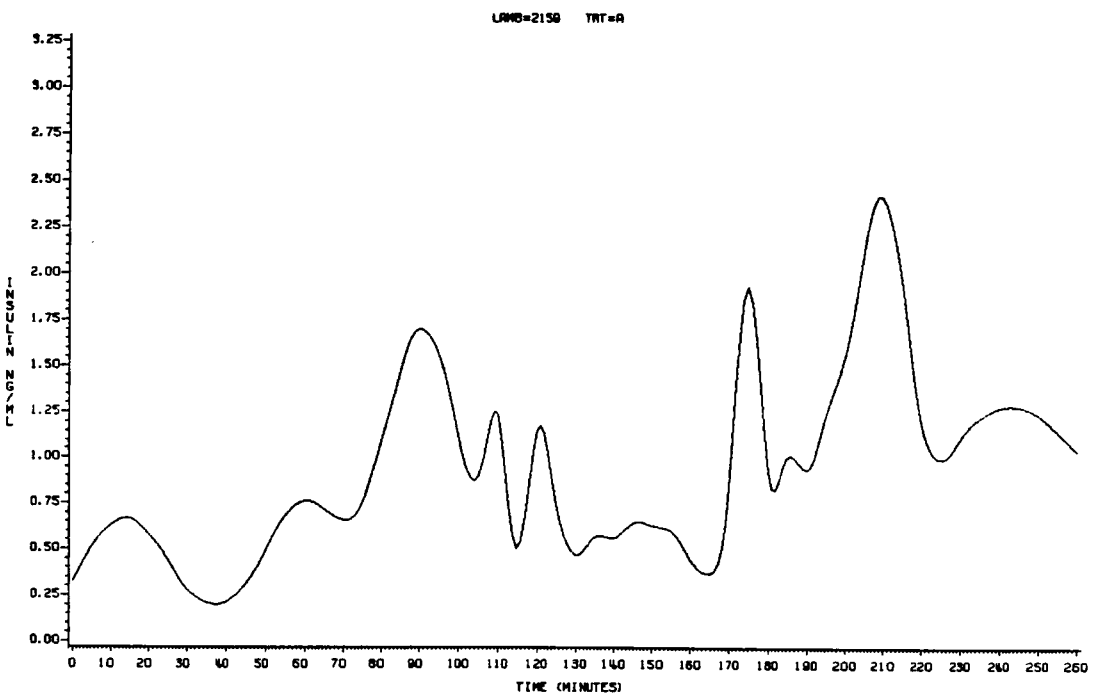


GROWTH HORMONE VS TIME



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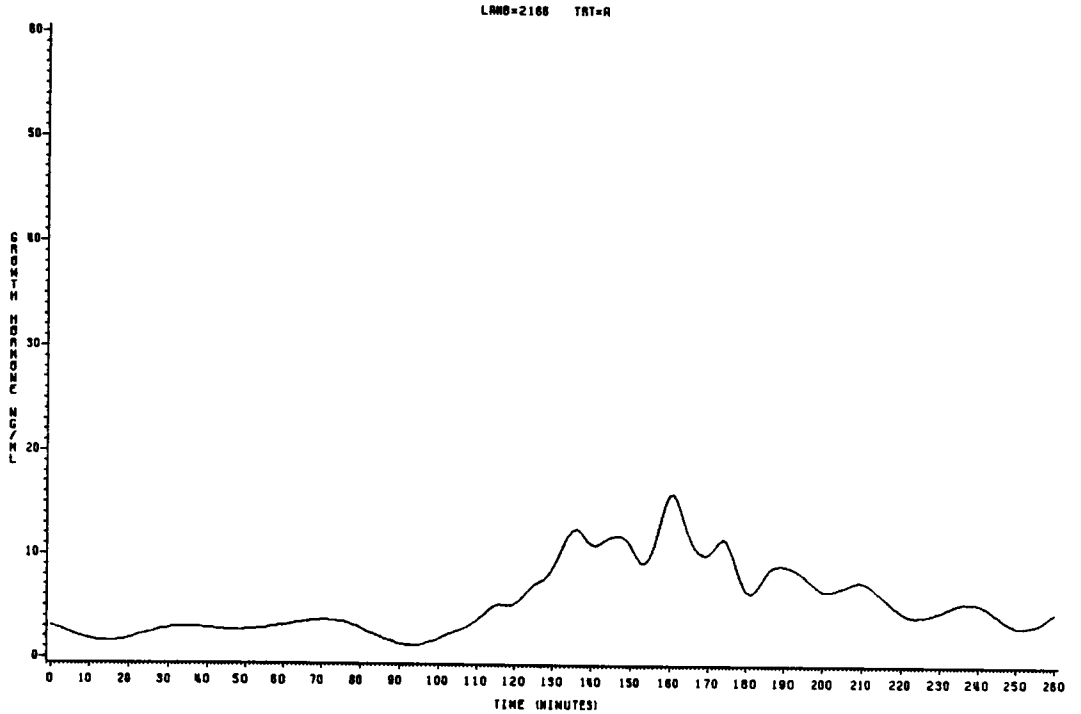

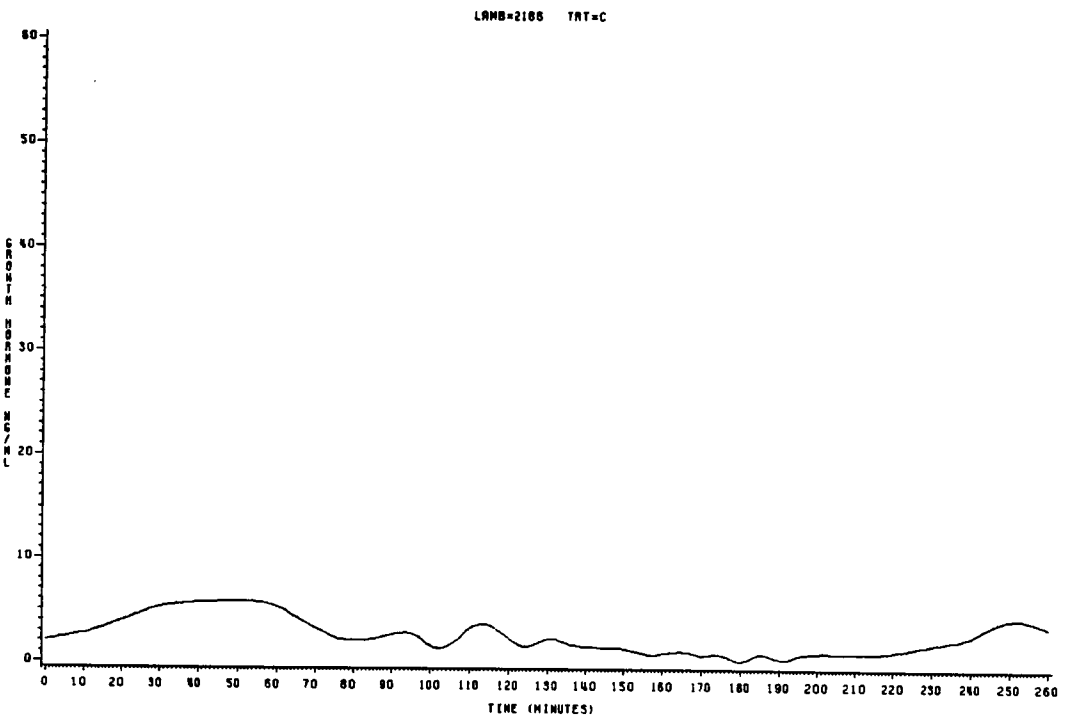

GROWTH HORMONE VERSUS TIME

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