ENDOCRINE STUDIES IN DAIRY ANIMALS

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INTRODUCTION

The increase in knowledge of both the origin and mechanisms of action of the various endocrine secretions on specific target tissues during the past decade has changed considerably the views regarding a suitable description of a hormone. The secretions of the posterior pituitary gland are no longer thought to be produced in this area of the brain but originate in the hypothalamus and are stored and released from the posterior pituitary gland. Likewise, there is evidence that the hypothalamus secretes a substance which regulates the activity of the anterior pituitary gland.

Many of the names ascribed to the various endocrine secretions suggest a specific target organ for each of the endocrine secretions. However, with the advent of increased knowledge, it is now recognized that while the actions of a given secretion may be elicited mainly on one organ or type of tissue, the actions at the cellular level are many and diverse. Hormones are believed to alter the permeability of the cell membrane, and in some way affect the cellular enzyme systems. Generally speaking, hormones elicit both a direct and indirect action on most of the cells of the mammalian body. Consequently, the role of the various endocrine secretions on lactating animals is not well understood.

Some aspects of the role of crude glandular extracts on lactating animals have been known for many years. With the advent of improved isolation and purification techniques, without the subsequent loss of biological activity, the actions of each of the hormones affecting milk production have been studied by endocrinologists in all parts of the world.

Until recently, only crude growth hormone preparations were available to study the galactopoietic effects of supplementary growth hormone on lactating animals. The most common contaminants of such preparation are thyrotropin and prolactin. Moreover, all research previously reported involved the use of injected growth hormone preparations which have a relative short-time physiological response.

Very little is known about factors affecting the rates of secretion of the various hormones affecting milk production. The effects of stress on endocrine secretion rates is of current interest. Nutritional stress during the rearing period has been shown to affect growth and subsequent lactation, reproduction, and longevity of dairy heifers.

The research reported here represents a further attempt to obtain additional information on: (1) the effect of plane of nutrition on endocrine secretion rates, (2) the effects of a highly purified growth hormone preparation, as compared to a crude growth hormone preparation on lactation response, and (3) the galactopoietic effects of implanted bovine and porcine growth hormone pellets administered to lactating cows.

REVIEW OF LITERATURE

Introduction

With the recognition of inner secretory organs as primary regulators in the maintenance of homeostasis, the volume of literature on the physiological effects of combined and individually administered hormones to intact and extirpated animals has grown and continues to increase. Only in the past decade have the individual protein hormones of the anterior pituitary been elucidated, and already the literature concerning the effects of these agents on lactation has grown voluminous. Unfortunately, however, there is a paucity of information concerning the role of homologous and heterologous glandular secretions and the levels, ratios, and synergisms of these on intermediary metabolism, especially that of the mammary epithelial tissues in the synthesis of the various milk constituents. Although definite synergistic relationships exist for the optimal secretion(s) of several hormones (17, 136, 145), only growth hormone (GH), thyroxine and those hormones known to affect pituitary secretion will be discussed.

Ovarian-Pituitary Synergism and Lactation

Turner and associates (60, 61, 62, 74) were the first to develop the concept that estrogens act through the anterior pituitary to stimulate mammary growth and lactation. Subsequent research by these workers (9, 86, 136) and other research groups (22, 41, 48, 52, 55, 114) using an array of research techniques involving hypophysectomy, thyroidectomy, and/or gonadectomy with subsequent replacement therapy has further shown

this to be a meritorious theory. It is well known that the secretions of the pituitary gland are carried in the venous and arterial systems to their respective target organs.

The initiation of milk secretion in mammals is dependent upon the continued effects of growth hormone, ovarian steroids, follicle stimulating hormone (FSH), luteinizing hormone (LH), adrenocorticotrophic hormone (ACTH), and lactogenic hormone (prolactin)(35). Progesterone, in the rat, is formed by the corpus luteum under the influence of prolactin; estrogen is secreted from the follicle through the stimulation of FSH and LH in optimal proportions. The liberation of prolactin appears to be under inhibitory nervous influences. This inhibitory mechanism has been shown by Haun and Sawyer (71) to reside in or to project through the basal tuberal hypothalamus. It was suggested by these authors that since this very same hypothalamic site controls the discharge of LH, which supports the hypothesis by Everett (45), the same humoral substance(s) transversing the hypophyseal portal system to stimulate LH discharge also acts to inhibit the release of prolactin. The importance of sheep prolactin, GH and triiodothyronine in restoring lactation in the hypophysectomized goat has recently been shown by Cowie et al. (36).

Following the initial work by deFremery (58) demonstrating that udder growth and development could be induced in virgin goats by anointing the udder with a salve containing estradiol benzoate, Folley et al. (55) were successful in bringing both virgin goats and dairy heifers into lactation using injections of synthetic estrogens. Subsequent studies involving estrogen injections and implants in a variety of domestic animals have confirmed these early findings. Folley and

associates (52) found stilbestrol or hexostrol implants to be an easier and more effective method of stimulating the initiation of lactation than the use of ointments. Oral administration of diethlstilbestrol to lactating cows was ineffective in altering the yield or composition of milk, while subcutaneous implantation of this compound led to a striking and prolonged increase in milk solids. Moderate size injections of diethylstilbestrol in an oily solution gave essentially the same results as did the implants. Increasing the size of the injections resulted in a rise in milk solids accompanied by a rapid fall in milk yield.

It is well known that in the intact cow the increase in blood estrogens and progestins during advanced pregnancy results in partial cessation in milk production when administered in high amounts (22, 48, 52, 55, 114). However, moderate to small exogenous or endogenous sources of estrogen will increase milk production (22, 41, 52, 86, 136, 142), change the composition of the milk (52) and increase feed efficiency (22). This would suggest a change in anterior pituitary secretion rate of thyroid stimulating hormone (TSH). However, Pipes et al. (104) found that estrogen and progesterone administered in amounts comparable to those normally secreted during the first two-thirds of pregnancy and sufficient to stimulate mammary growth and development of heifers, do not affect thyroidal activity. However, when estrogen alone was administered in sufficient quantities to initiate lactation there was evidence of increased thyroid activity.

The prolactin content of the rat pituitary gland has been shown by Reece and Turner (114) to be higher following the administration of estrogen. These findings were confirmed by Hymer et al. (76) using

electron microscope analysis of anterior pituitary glands from normal and estrogen-injected (50, 100, and 400 μg. estrone over a five day period) mature female rats. The low dosages gave rise to greater increases in pituitary prolactin content than larger levels. The inhibitory mechanisms of milk secretion due to increased estrogens and progesterone are not well understood; however, recent work by Bernstein (11) indicates hypertrophy of the adrenal gland when high concentrations of estrogens are found in the portal blood. There are indications that the estrogens cause epithelial hyperplasia in many tissues and that estrogens alone increase thyroid activity. Therefore, it is reasonable to assume that the increased feed efficiency for milk production reported by Browning et al. (22) was a manifestation of increased rates of metabolism of mammary secretory tissues as a result of an enhanced secretion rate of thyroid hormones, mediated via the anterior pituitary. The possibility that estrogens likewise stimulate the anterior pituitary to secrete GH can not, however, be completely excluded.

Effects of Anterior Pituitary Extracts on Lactation

Several workers (1, 74, 96, 111, 118) have shown that hypophysectomizing laboratory animals during lactation resulted in cessation of lactation, while others (60, 62) have shown that lactation can be initiated or maintained in hypophysectomized animals by the administration of a crude anterior pituitary extract, thus eliciting a galactopoietic role for the anterior pituitary secretions.

Cotes et al. (34) established that the most potent galactopoietic hormone of the anterior pituitary was GH. Azimov and Krouze (7) in 1937 indicated that injection of anterior pituitary extracts (presumably rich in GH) into lactating cows resulted in a temporary increase in both total milk production and fat percent. Sykes et al. (129, 130) reported that daily injections of 500 mg. of crude anterior pituitary extract in lactating cows for periods up to 33 days resulted in increases in percent fat, lactose and solids not-fat. Asimoff and associates (4) made daily injections of 30 to 40 mg. of an alkaline anterior pituitary extract into lactating cows, over a period of one to three days, with the result that a daily increase in milk yield of one to three liters was obtained. The increase lasted for only two to four days, while in a subsequent study (5), using similar techniques with cows in early lactation and on pasture, the increase in milk yield was not followed by a decrease. These findings were later confirmed by Folley and Young (49, 50, 51) of England.

Sykes et al. (130) showed injections of crude anterior pituitary extracts to give substantial (50-100 percent) increases over initial milk production and fat percent, while a prolactin preparation increased milk volume without an appreciable change in fat percent. The authors concluded that the small percent change so obtained may have been from a thyrotropin contaminant. Less pronounced increases in milk production were obtained by Donker and Petersen (39) using an unspecified amount of preparation. Increases amounting to 12.4, 19.5, and 15.9 percent for 2, 4, and 8 day comparison periods, respectively, were obtained. The average increase from two single intramuscular injections, with

seven cows, was 11.6 percent compared to a 9.1 percent average increase for two single injections for a four-day period. Multiple intramuscular injections over a four-day period, however, gave rise to a 36.4 percent average increase as compared to an average increase of 21.2 percent for two multiple injections. An increase of 28.8 percent was obtained over a four-day period when injected four days consecutively.

Brumby and Hancock of New Zealand (23) subsequently established that continuous subcutaneous injections of GH to identical twin cows both at peak production and during the declining phase of lactation resulted in a marked increase in both milk and butterfat production. It was also established that the efficiency of production was increased with no apparent change in either blood or milk composition from the GH injections. Anterior pituitary extracts appear to have a blood glucose elevating effect when injected into non-ruminants. Folley and associates (53, 54) suggested that the diabetogenic fraction and galactopoietic fraction of the anterior pituitary are one and the same. It has since been shown that purified growth hormone preparations injected into rats and dogs increase the cellular demands for insulin and (26) produce characteristic diabetic symptoms in these animals when administered over extended periods of time. Although Shaw et al. (122) reported that the depression in milk yield resulting from fasting could be prevented or corrected by daily injections of GH, this hormone does not appear to be diabetogenic in ruminant animals. Chung et al. (28) reported a 50 percent increase in milk fat production and a 20 percent increase in blood glucose following daily injections of 100 mg. GH for a period of 6 days to lactating cows. In a subsequent publication (122)

these workers attributed the blood glucose elevating effect of the GH preparation to the TSH contaminant.

Very little is known about the role of growth hormone at the cellular level; however, it has been suggested that the action of GH is to alter mitochondrial permeability. Graymore et al. (63) showed that the mitochondria of growth hormone-treated rats have a lower phosphorus/oxygen ratio due to an increase in the rate of oxygen utilization rather than to a decrease in the rate of phosphorylation. Although thyroxine likewise uncouples oxidative phosphorylation (133), thyroxine does not affect oxygen uptake. GH when injected into rats (63) gives rise to an increased rate of oxidation of betahydroxybutyrate possibly by altering the mitochondrial permeability and thus permitting increased cellular metabolic activity. The galactopoietic effect, therefore, of GH may be due to the increased availability of mammary tissue substrates for the net synthesis of milk fat and protein.

Effect of Thyroid Hormones on Lactation

The thyroid hormones--thyroxine and triiodothyronine--and thyroprotein have been shown to stimulate milk secretion in lactating dairy cattle (15, 16, 57), with an increased milk fat percent but with no change in the percent solids non-fat (113). The magnitude and duration of this lactational response is believed dependent upon the inheritance of optimal secretion rates of the various hormones influencing mammary epithelial proliferation, intensity of milk secretion and milk removal (137). Thyroxine directly influences milk secretion by increasing heart rate, blood circulation and cellular metabolism of the mammary

milk secreting cells. Thyroxine is presumed to act indirectly to stimulate secretion of lactogenic hormone (99) and growth hormone (24, 108) as evidenced by increased growth rates. The synergistic effect between growth hormone and thyroxine may be due to sensitization of similar secretory cells of the pituitary or it may be that thyroxine sensitizes the target tissues to growth hormone. Geschwind et al. (59) compared the effects of injected thyroxine and growth hormone on liver polyploidy of hypophysectomized rats and found that either hormone, acting alone, stimulated polyploidization but the combination of both hormones synergistically produced a liver cell picture dominated by higher polyploid classes.

Thyroxine has been shown to be the limiting hormone for intensity of milk production (136). Premachandra and Turner (109), using nine lactating dairy cows at peak production, injected thyroxine daily at a level 50 percent above their individual thyroxine secretion rates and obtained lactational responses varying from 12.1 to 67.9 percent with a mean increase of 27.6 percent. In this study the initial injection of thyroxine was sufficient to give an immediate rise in the thyroxine body pool to a level it would eventually reach after a variable period of time. Prolonged exogenous sources of thyroxine cause varying degrees of atrophy of the thyroidal secretory cells. Thyroxine withdrawal results in a precipitous fall in milk yield due to subnormal levels of thyroprotein until endogenous thyroxine secretion is re-established (110). Swanson and Hinton (126) suggested a more gradual withdrawal of thyroprotein in order to overcome the effects of reduced endogenous thyroxine

secretion rate. The utility of feeding thyroxine and thyroprotein for lactation is described elsewhere (15, 127, 135).

Factors Affecting Thyroid Activity

Goitrogens: The antithyroid compounds may be broken down into two principal categories, the thiocarbamide derivatives such as thiourea, thiouracil, and propylthiouracil, and compounds possessing an amino-benzene ring. These latter compounds include the sulfa drugs, para-aminobenzoic acid, para-aminosalicylic acid and related compounds.

The thioureas, sulfonamides, and related compounds are believed to be goiterogenic by interfering with the iodination of tyrosine (66, 91). Thiocyanate, perchlorate, periodate, and their derivates produce goiter by interferring with the uptake of iodide by the thyroid gland and by causing the release of iodide already in the gland at the time of their administration (67, 68, 144). Goitrogens have been extensively employed in I^{131} uptake studies to prevent the uptake of circulating radio-iodide.

Temperature: It has long been known that thyroid hormones regulate the rate of metabolic processes. However, only recently (due to improved techniques) have the effects of environmental temperatures on thyroxine secretion rate of cattle been elucidated. Johnson and Ragsdale (79) varied the environmental temperatures of Holstein, Brown Swiss and Jersey heifers from 35° to 95° F. and found the I^{131} release rate to be negatively correlated with temperature. As the environmental temperature was increased from 35° to 80° F. there was a gradual decrease in thyroid I^{131} release rate, however, above 80° F. a precipitous

fall in thyroid I^{131} activity was noted. These results were confirmed by Thompson et al. (132) using 10 Holstein heifers exposed for 48 days to controlled low (40-65° F.) temperatures and for 72 days under controlled high (75-90° F.) temperatures. Heat production (kcal/cwt$^{.72}$ per hr.) was highly correlated with serum protein bound iodine and thyroxine secretion rate. Pipes et al. (105) using a total of 114 observations on dairy cattle reported that on the average the secretion rate during the summer was only 30 percent of the winter secretion rate. Mixner et al. (98) using dairy cows and a total of 48 observations found thyroid secretion rates to be highest in the spring and lowest in the fall. A considerable lag between temperature phase and thyroid secretion rate was noted. It is generally believed that thermal changes either augment the rate of thyroxine utilization in the peripheral tissues, thereby evoking a feed-back mechanism via the hypothalamus, or activate a nervous reflex operating through the hypothalamus to regulate the release of pituitary TSH. Elucidation of the exact mechanism in cattle awaits the development of a satisfactory microassay technique for TSH.

Individual Variation: Age and breed have been shown by Johnson and Ragsdale (78) to affect thyroid activity. Under carefully controlled environmental conditions it was shown that thyroid I^{131} release rate of Jersey calves was approximately twice that of Holstein and Brown Swiss calves with the latter intermediate to the Jersey and Holstein calves. Pipes et al. (105) found considerable individual variation in thyroid activity among dairy cattle. A total of 67 estimates of thyroxine secretion rate in winter ranged from 1 mg. to 10 mg. per 1,000 lb. body weight with a mean of 1.7 mg. Since these animals were all low milk

producers it is reasonable to assume even greater individual differences among breeds would have been obtained had low and high producers been compared. Mixner et al. (98) using 48 observations found that the thyroid secretion rate per 100 lb. of body weight were highest at the beginning of lactation and lowest at the ninth month of lactation. However, the trend was for the blood thyroxine level to be lowest at the beginning of lactation and highest at six months of lactation.

Other Factors

Vandersall et al. (139) compared thyroidal I^{131} release rates of heifers fed legume-grass silage and alfalfa hay free choice and obtained higher I^{131} release rates from those animals fed the legume-grass silage. The authors suggested that some unidentified factor(s) in certain forages apparently exerts an influence on thyroidal activity. This, coupled with depression in milk production reported by Vandersall et al. (139) when lactating animals were switched from alfalfa hay to legume-grass silage, may have been due to a stress condition brought about by changing rations.

Effect of Adrenocorticotropic Hormone and Glucocorticoids on Milk and Fat Production

Roy (117) reported that a crude preparation of ACTH under certain conditions exerted galactopoietic activity, whereas Cotes et al. (34) observed a depressing effect on milk production. Shaw and associates (121, 122) reported ACTH to have a definite depressing effect on milk volume coupled with decreased milk fat production but increased fat percent and blood glucose level. The ACTH used by Shaw et al. (122)

was a highly purified preparation while the crude preparation used by Roy (117) may have contained some GH contaminant.

Cortisone acetate or hydrocortisone acetate has been shown to be essential for the initiation of milk secretion in hypophysectomized, oophorectomized virgin rats and hypophysectomized, gonadectomized immature male rats (30). Chung (30) found these compounds to have a depressing effect on milk production when administered in large doses to lactating cows. Turner (138) obtained variable increases with low levels (40 mg./100 lb. body weight) when a synthetic glucocorticoid (Medicorten) was injected into heifers.

Effect of Plane of Nutrition on the Endocrine System

Growth hormone and thyroxine are primary regulators of growth as evidenced when animals are either hypophysectomized and/or thyroidectomized. The type of growth which takes place following removal of these glands is very similar to that reported when swine and poultry are subjected to severe undernutrition (94, 107). Similar skeletal changes, although temporary, have been noted with dairy animals when subjected to limited energy intakes during their rearing periods. Results of research by Reid (116), Swanson and Spann (128), Bonnier and Hansson (18, 19), and Crichton et al. (37, 38) indicate that varying the plane of nutrition of dairy heifers during the rearing period affects growth, subsequent lactation, reproduction and longevity.

Crichton et al. (37, 38) found that late maturing characteristics such as live-weight and heart girth size were affected most by continuous restricted feeding, while height and length which are early maturing

characteristics were affected least. Hansson et al. (69) found as much as 130 lbs. difference in body weight in favor of heifers reared on the high plane of nutrition over those reared on a medium plane. However, these researchers reported that by the second calving this advantage had disappeared.

It appears that heifers reared on a low plane of nutrition and brought to full feed at time of parturition make rapid body weight gains during the first lactation. A few workers (14, 116, 128) have reported decreased milk production during the first lactation with heifers reared on a very low plane of nutrition. Hansson et al. (69), however, using identical twin heifers reported higher total milk yield from the first through the sixth lactations from animals receiving only 51 percent of the Scandinavian standard during rearing. Reid (116) reported that animals receiving 65 percent of Morrison's standard during rearing produced almost as much milk as those receiving higher levels and that these animals out produced all other groups in subsequent lactations.

Most of the studies dealing with very high levels of nutrition during rearing have no record of mammary tissue examination. However, in the work that has been done, less mammary secretory tissue development and more fat development have been observed (128). In experiments involving sheep, the plane of nutrition definitely affected both size and weight of udder; however, no determination of amount of secretory tissue or fat was made (141).

From recently accumulated knowledge on the mode of action of anterior pituitary and thyroid secretions, especially with regard to

lactation and growth, it would appear that intrinsic hormonal factors are involved. Baird et al. (8) reported greater amounts of growth hormone per pound of body weight in the pituitary glands of swine when rapidly grown than in their counterparts grown more slowly. Pipes et al. (106) recently showed that female mice restricted to 3/4 full feed had thyroid secretion rates decreased 33 percent, while body weights were unaffected. Reducing the feed intake to one-half normal further reduced the thyroid secretion rate to 41 percent; however, a decrease in body weight was observed. While these studies indicated that the pituitary-thyroid axis was sensitive to plane of nutrition, Armstrong et al. (3) found no significant differences between growth hormone and thyrotrophic hormone content of pituitary glands of Holstein heifers reared on either 60, 110, or 140 percent of Morrison's standards.

Immunological Investigation of Bovine Pituitary Growth Hormone

The standard biological methods for the assay of bovine GH are not sufficiently sensitive for its quantitative estimation in body fluids. The most popular biological methods are based either on the increased width of the epiphysial cartilage of the tibia, or on increased body weight of hypophysectomized rats following the administration of GH (43, 64). More recently an assay method has been used based upon a "sulfation factor" in serum (2). This method is based upon the <u>in vitro</u> uptake of sulfate by cartilage from hypophysectomized rats. Although a highly significant linear relationship between the concentration of "sulfation factor" activity of sera and $\log_{10}$ of human GH administered has been shown, no such relationship has been reported for bovine GH.

Furthermore, the "sulfation factor" activities of prolactin, ACTH, TSH, FSH, and LH, which are common contaminants of anterior pituitary extracts, have not been definitely demonstrated.

More recently, Greenspan et al. (65) developed a method for the assay of human GH utilizing a specific antibody labeled with I^{131}. While this method has a high degree of precision and can detect as little as 0.5 μg./ml. of GH in serum, it has the disadvantage in that labeling the antibody with I^{131} is very tedious, and nonspecific antigens found in the serum are reported to interfere with the assay.

Following the demonstration of the specific antigenecity of human GH preparations by several workers (70, 73, 102, 112), a variety of immunological procedures were proposed for assaying this hormone in blood serum. Only recently, however, have high antibody titers to bovine GH been demonstrated (72).

Early reports by Elberg and Li (42) and Morrison et al. (101) indicated that their purified bovine GH preparations were only weakly antigenic. More recently, Hayashida and Li (72) obtained an antibody titer of 1.0 μg./ml. in a rabbit using 80 mg. of purified bovine GH preparation in Bayol-Arlacel adjuvant. Wallace (140) obtained an antibody titer to ovine GH of 0.004 μg./ml. using singular subcutaneous injections of 0.4 mg. of ovine GH in Ramon's adjuvant in rabbits followed by a singular intravenous injection of 5 mg. ovine GH in saline. Although ovine GH and bovine GH have been found to be similar in structure (varying only in the amino acid sequence) and indeed immunologically similar (73, 87), the highest antiserum titer thus far reported in the literature for bovine GH is 1 μg./ml. (73). Titers of much higher magnitude, however, have been reported for human GH.

EXPERIMENTAL PROCEDURE

Growth Hormone Injections and Implants[1]

Care and Treatment of Animals

The specific objective of the present experiments was to determine the effect of administering supplementary GH on the performance of lactating dairy cattle having a propensity for high production. Twelve cows were used to evaluate the galactopoietic effects of three levels of GH implants and 15 cows were used to compare the galactopoietic effects of injecting commercial and NIH-purified GH preparations on milk production, composition of milk, incidence of mastitis, changes in blood cell counts and blood glucose level. Both the injected and implanted experimental cows were selected from the Oklahoma State University dairy herd on the basis of age, level of milk production, and health. Only those cows commencing their second, third, or fourth lactations having a propensity to produce in excess of 50 lbs. of milk per day at their peak lactational period, as determined from 10 day consecutive milkings taken prior to starting the cows on the experiments, and free from clinical mastitis were used in these experiments.

Each of the 12 implanted cows received each of three levels of GH, 0, 500, and 1,000 mg. (estimated to contain 0, 300, and 600 Armour units,

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[1]All growth hormone preparations obtained gratis from Armour Pharmaceutical Company, Kankakee, Illinois.

respectively) in a sequence determined by a balanced Latin square design. The duration of each period was six weeks. The GH[2] in a pelleted form was implanted subcutaneously in the mid-thoraic area approximately three inches ventral to the spinal column. The site of implantation was clipped free of hair, cleaned with soap and water, swabbed with 70 percent alcohol, and anethesized with 1.0 percent procaine. An incision approximately 1.5-2.0 inches long was made lateral to the 7th and 9th rib. The GH pellets were placed under the skin and the area closed with sutures. The area was swabbed with tincture of iodine following the implantation and once daily for a few days thereafter. The sutures were removed approximately two weeks following implantation.

Each cow implanted with GH was fed individually twice daily a constant amount of the same kind of feed (Table I) consisting of a palatable grain concentrate, alfalfa hay and sorghum silage throughout the experiment. The feed was alloted according to Morrison's standards (100) based upon 10 day preliminary milk yields and three consecutive daily body weights taken two weeks prior to placing the animals on the experiment and again immediately before the first experimental period. Additional weights were taken at weekly intervals on two consecutive days. A two-week pre-experiment period was allowed for each animal to become

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[2]Lot No. R-038-268 (bovine origin) containing 250 mg. growth hormone per tablet, assaying 75% of standard before tableting and estimated to contain 150 ± 25% Armour units per tablet.

Lot No. 759-008 (porcine origin) containing 500 mg. GH per tablet, assaying 37.5% of standard before tableting, and estimated to contain 150 ± 25% Armour units.

TABLE I

AVERAGE PERCENT[a] CRUDE PROTEIN AND ESTIMATED TOTAL DIGESTIBLE NUTRIENTS[b] OF FEED FED TO COWS RECEIVING GROWTH HORMONE IMPLANTS

Ingredient	Number of Samples	Crude Protein	Estimated TDN
		(%)	
Grain Concentrate[c]	2	14.62	72.45
Alfalfa Hay	4	17.15	52.07
Sorghum Silage	2	4.14	23.25

[a]Percent on "as fed" basis.

[b]Estimated TDN from Morrison (100) based upon crude protein analysis.

[c]Concentrate consisted of 50% ground milo, 25% rolled barley, 10% wheat bran, 5% dried molasses, 8% soybean oil meal (50%), 1% trace mineralized salt, and 1% dicalcium phosphate.

TABLE III

AVERAGE PERCENT[a] CRUDE PROTEIN AND ESTIMATED TOTAL DIGESTIBLE NUTRIENTS[b] OF FEED FED TO COWS RECEIVING GROWTH HORMONE INJECTIONS

Ingredients	Number of Samples	Crude Protein	Estimated TDN
		(%)	
Grain Concentrate[c]	3	14.63	72.79
Alfalfa Hay	18	17.77	52.06
Sorghum Silage	2	4.14	23.25

[a]Percent on "as fed" basis.

[b]Estimated TDN from Morrison (100) based upon crude protein analysis.

[c]Concentrate same as in Table I.

accustomed to their respective rations. Feed refused by each animal was weighed back daily and recorded. All cows were milked twice daily throughout the experiment.

The 15 cows receiving GH injections were assigned to a randomized block experimental design consisting of three treatments and five blocks. The cows were injected intramuscularly for 10 consecutive days with either 50 mg. of Somar[3] or a semi-purified GH[4] preparation in saline, or saline alone. The cows were fed individually twice daily the same kind of feed according to the 1956 National Research Council (NRC) recommended requirements (upper level) for lactating dairy cattle based upon three day-composite body weights and 10-day preliminary milk yields. Additional two-day body weights were taken at weekly intervals throughout the experimental period. The rations consisted of a grain concentrate, alfalfa hay, and sorghum silage (Table II). Refused feed was weighed back and recorded daily for each animal.

Methods of Evaluating Animal Response

Blood samples were taken from each animal on two consecutive weeks prior to placing the animal on the experiment and every two weeks during the experimental periods. Blood obtained from the jugular vein in citrated tubes was immediately placed in an ice bath and all blood determinations were performed the same day. Blood glucose was determined on protein-free

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[3]Somar (Armour Lot No. M108) containing approximately 0.1 to 0.2 units TSH per mg.

[4]NIH-purified growth hormone (Lot No. R50109) containing less than 0.014 units TSH per mg.

filtrates by the method of Benedict (13). Total and differential white blood cell counts were made using standard procedures (131).

The milk yield at each milking was recorded and two-day composite milk samples were taken each week during the experimental periods for the determination of total solids and fat content using the Mojonnier (92) and Babcock (6) procedures, respectively.

Observations on the incidence of mastitis of each animal were made using "Breed" smears from 18-hour incubation Hotis tubes. Milk samples for this purpose were collected at weekly intervals prior to and during the experimental periods. Leucocyte counts and identification of mastitis-producing organisms were made. A strip-cup examination of the milk from each quarter prior to each milking and observations of physical changes of the udder were also made. Additional milk samples were taken for the detection of mastitis producing organisms cultured from Hotis tubes when mastitis was suspected.

Growth Hormone Bioassay

Growth hormone preparations were measured by the increase in width of the tibial epiphysial cartilage in young hypophysectomized rats, as described by Greenspan et al. (64). A standard assay curve was constructed utilizing a total of 24 rats divided into 6 groups of 4 rats each. Graded levels (0, 5, 25, 100, 175, and 250 µg./day) of a NIH-GH preparation (see footnote 4, page 21) were injected intraperitoneally into each of the four rats of each group daily for four consecutive days. Each group, receiving one of the above levels, represented one point on the standard curve.

Each of two different lots of growth hormone pellets (see footnote 2, page 19) were assayed by comparing the average width of the epiphysial cartilage of 4 rats receiving the equivalent in estimated activity of 100, 150, or 200 µg. per day of the GH with that of rats given purified GH as a standard. Analysis by the method of Least Squares yielded the following equation for the standard curve: $Y = 182.205 + 61.614 \log X$, where Y equals the response of the cartilage plate in micra and X equals the total dose of GH in µg. which compares favorably with that of Greenspan et al. (64). The mean response curve based upon the width of the proximal epiphysial cartilage of tibia of hypophysectomized rats is presented graphically in Figure 1. When the mean response of the rats given the GH tablets was compared to the standard curve, based on the response of rats to the NIH-purified material, it was observed that the bovine GH tablet possessed a biological potency approximately one-tenth the potency of the porcine GH tablet (Table III) at the time the assay was performed.

TABLE III

MEAN CARTILAGE PLATE RESPONSE OF HYPOTHYSECTOMIZED RATS AND ASSAY VALUE OF BOVINE AND PORCINE GROWTH HORMONE PREPARATIONS

Source of Growth Hormone[a]	Estimated Daily Dose[b]	Cartilage Plate Response[c]	Assay Value From Standard Curve
	µg.	micra	µg.
Bovine	100	237.6	7.94
	150	271.4	28.18
	200	283.7	44.67
Porcine	100	336.9	323.60
	150	303.1	91.20
	200	346.1	457.10

[a] See footnote 1, page 18.

[b] Estimated daily dose, µg./day, injected intraperitoneally over a four day period.

[c] Mean cartilage plate response of eight rats per dosage level.

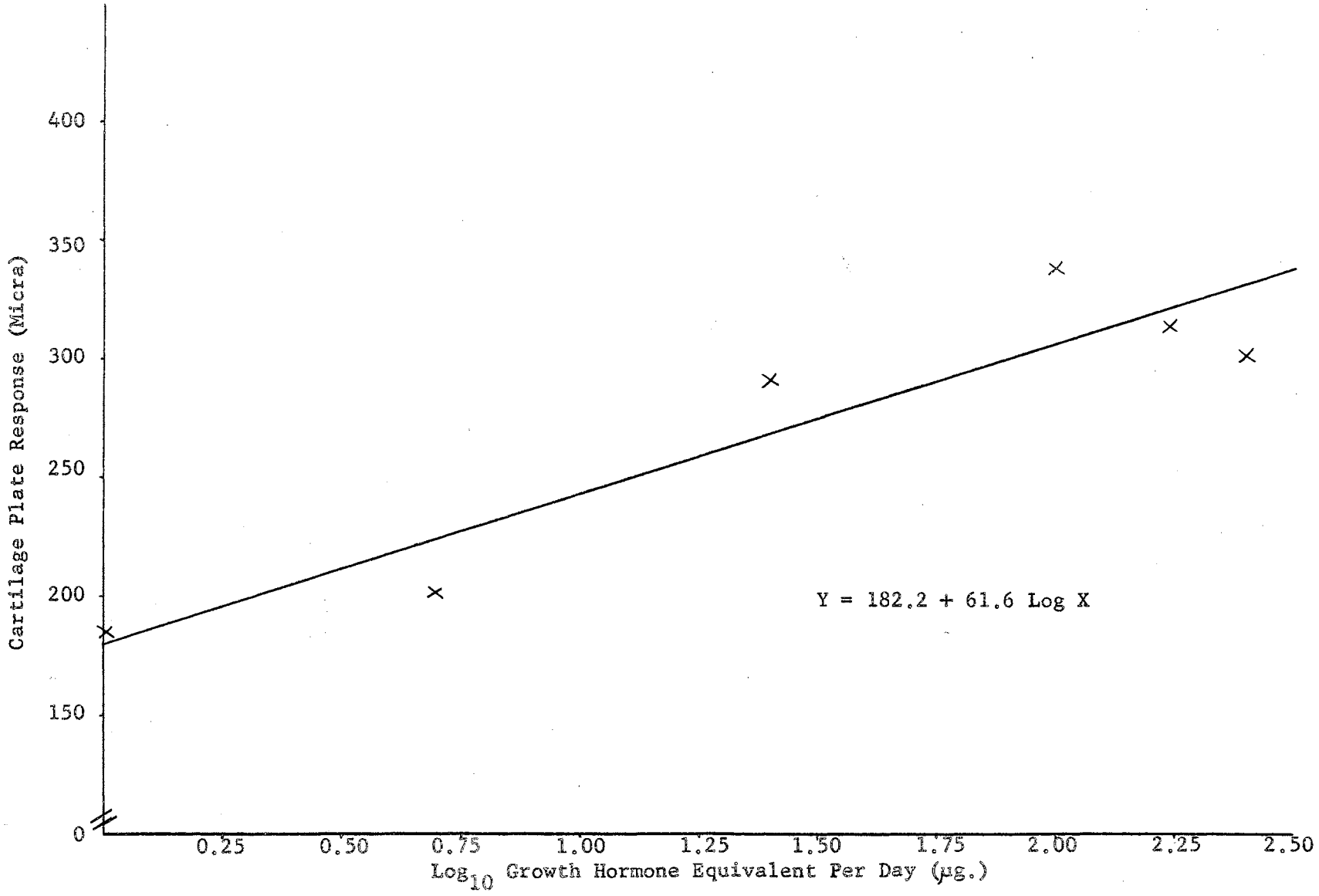

Figure 1. Effect of Pituitary Growth Hormone on the Width of Proximal Epiphseal Cartilage of Tibia of Hypophysectomized Rats. The Dose-Response Curve.

Effect of Level of Nutrition on Hormone Secretion Rates

Selection and Treatment of Animals

Thirty female Holstein calves were used to study the effect of three levels of nutrition on growth and development, and to determine the effect of different levels of nutrition during the developmental period on the secretion of GH and thyroxine from birth to twelve months of age. The calves were selected from the Oklahoma State University dairy herd on the basis of sex, size, health, and absence of abnormalities. All calves were fed colostrum for 3-4 days following birth, followed by whole milk feeding at the rate of 10 percent of initial weight for 14 days and at the rate of 8 percent for an additional 10 days. The calves were weaned abruptly from milk at 28 days of age. All calves were fed to appetite a palatable alfalfa hay-grain mixture (preliminary ration, Table IV) containing 80 percent grain and 20 percent dehydrated alfalfa meal until two months of age at which time the animals were assigned to treatment groups according to a randomized block design. The experiment consisted of three treatments and ten blocks. The calves within each block were assigned at random to their respective treatment groups, i.e., 75, 100, or 125 percent of the 1956 National Research Council's requirements (NRC) for normal growth. All rations were adjusted at bi-weekly intervals throughout the experimental period based on two-day body weights of the respective animals.

From two to six months of age all calves were fed a ration consisting of 0.1 alfalfa hay and 0.9 milk-grain pelleted concentrate (experimental calf ration, Table IV) according to their assigned treatment

level. Dried skimmed milk was added to the ration in order to increase palatability.

The 75 percent, 100 percent, and 125 percent groups were fed two times, three times, and four times daily, respectively, as a means of attaining a higher level of intake for the higher plane groups and to partially offset the difference in energy utilization among the different groups. From 6 months to 12 months of age all animals were fed twice daily a ration (Table V) at different levels with free access to water. From 2 to 12 months of age, the animals had access to an exercise lot approximately 14 hours each day.

Methods of Evaluating Animal Response

The progressive growth and development of each animal was determined by two-day body weights taken bi-weekly and skeletal measurements using a calibrated grid taken at monthly intervals. The age at which sexual maturity was reached was determined by observing the first noticeable onset of estrus. Observations for incidence of estrus were made twice daily (15 minute periods) beginning at 8 months of age. The animals were not bred during the course of this study.

Blood was collected from the jugular vein at 5-month intervals (2, 7, and 12 months of age). The plasma was extracted and frozen until such time that it could be assayed for concentration of GH. Thyroxin secretion rate of each animal was determined at 12 months of age using the method as described by Mixner and Lennon (97), with one important modification. All thyroxine secretion rates were calculated using the total amount of protein bound iodine (PBI) after thyroxine injection, rather than using total PBI minus control PBI. This method involved

TABLE IV

COMPOSITION OF PRELIMINARY AND EXPERIMENTAL CALF CONCENTRATE RATIONS

Ingredient	Non-pelleted Preliminary Ration[a]	Pelleted (3/16 in.) Experimental Ration[b]
	%	
Cubed Corn	24.0	30.0
Alfalfa Leaf Meal	20.0	20.0
Crimped Oats	24.5	12.5
Wheat Bran	8.2	5.0
Corn Distiller Solubles	5.5	10.0
Soybean Oil Meal (44%)	11.5	6.0
Dried Molasses	4.0	5.0
Dried Skimmilk	---	10.0
Trace Mineral Salt	1.0	1.0
Dicalcium Phosphate	1.0	0.4
Aurofac 10 (Chlortetracycline)	0.3	0.1

[a]Fed from one to two months of age.

[b]Fed from two to six months of age. Total digestible nutrients and digestible protein were 70.28% and 15.41% (dry matter basis) respectively.

TABLE V

COMPOSITION OF EXPERIMENTAL CONCENTRATE RATION[a] FED TO HEIFERS FROM SIX TO TWELVE MONTHS OF AGE

Ingredient	Pelleted (1/2 inch) Experimental Ration[b]
	(%)
Ground Milo	50.0
Ground Barley	25.0
Wheat Bran	10.0
Soybean Meal (50%)	8.0
Molasses (Beet Strap)	5.0
Dicalcium Phosphate	1.0
Trace Mineralized Salt	1.0

[a]Total digestible nutrients and digestible protein were 73.89% and 11.86% dry matter basis, respectively.

[b]Fed with alfalfa hay containing 65.07% total digestible nutrients and 18.35% digestible protein (dry matter basis).

calculating the secretion rate as the product of the plasma protein bound iodine (PBI) level, the thyroxine turnover rate and the thyroxine volume of distribution following singular intravenous (jugular) injection of L-thyroxine at the rate of 5 mg. per 100 lb. body weight for small calves and 25 mg. per 100 lb. body weight for larger animals. The dry ash method described by Brown et al.(21) and modified by Lennon and Mixner (85) was used for determining PBI in blood plasma. All statistical analyses were calculated as outlined by Steel and Torrie (125).

Immunological Studies With Bovine Growth Hormone

Experimental Objectives

The primary objective of this study was to develop an improved immunological assay procedure for estimating bovine GH levels in bovine blood serum. The initiation of this study commenced prior to publications by Trenkle et al.(134) describing a cell lysis technique for estimating bovine GH and that by Wallace (140) describing the hemagglutination technique for estimating GH in ovine serum.

Although immunological assay procedures for bovine GH have been reported, they leave much to be desired in that they lack both precision and specificity. The cell lysis technique developed by Trenkle et al.(134) produced variable results due to non-specific cellular lysing. The hemagglutination technique used in assaying growth hormone employing bisdiazotized (124) or tannic acid treated erythrocytes (40) have been of limited use due to the poor or unpredictable stability of the cells and the unpredictable and uncontrollable variation in the sensitivity among different lots of sensitized erythrocytes. Inasmuch as the

complement components are extremely labile and the hemagglutination method has been shown to be 20-50 times more sensitive than the complement fixation test (83) it seemed desirable to approach this problem through an improved hemagglutination procedure.

Erythrocytes possessing soluble antigens or haptens attached to their surface have proven to be a sensitive reagent for the detection of antigens in biological fluids (103). Erythrocytes which have been stabilized by treating with formaldehyde become very adherent and require severe agitation to obtain a homogenous suspension for agglutination reactions. When prepared in the usual manner these suspensions are reported to agglutinate spontaneously in the presence of traces of formalin. McKenna (95) recommended prolonged washing and dialysis to free the cells of loosely bound formaldehyde.

Erythrocytes treated with formaldehyde by the method of Ingraham (77) can be dispersed by manual shaking and are not agglutinated by traces of formaldehyde following several washings with saline. The method of treating the erythrocytes with formaldehyde in this study was a modification of the Ingraham method in that different buffer systems were used and the cells were subjected to two concentrations (4% and 10%) formaldehyde in 0.85% NaCl, and 2 percent bovine albumin replaced normal rabbit serum for dilution purposes. By using the aforementioned concentrations of formaldehyde the spontaneous coagulation of the erythrocytes described by Ingraham (77) was avoided.

Production of Antisera

The antiserum used in this study was prepared in the following manner. Young albino female rabbits weighing approximately 2 to 4 lb.

each were injected with a total of 40 to 80 mg. of NIH-purified bovine GH[5] suspended in Freund's adjuvant. Each rabbit was injected initially, via a subcutaneous route, with 1 mg. of the antigen suspended in Freund's complete adjuvant[6] followed by weekly subcutaneous injections of 2 to 5 mg. antigen suspended in Freund's incomplete adjuvant. This procedure was routinely followed until an antiserum titer (as determined by the precipitin ring test) reached approximately 1 μg./ml. This usually required a total of 60 to 80 mg. antigen. The rabbits were challenged with 2 to 5 mg. antigen in Freund's incomplete adjuvant[7] at bi-monthly intervals and bled via cardiac puncture 8 to 10 days later. The sera were either stored at 5° C. and used within a few days or frozen and stored until use. In each case complement was destroyed by heating to 56° C. for 30 minutes.

The quantitative interaction between the antiserum and growth hormone, prolactin, FSH, LH, gamma globulin and albumin were tested using the Ouchterlony double-diffusion method employing 1 percent Agar-Agar #3 in buffered saline (pH 7.6) 1: 10,000 (w/v) merthiolate. Thirty-forty ml. of agar were poured into each petri dish. The wells were made using a No. 7 cork borer.

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[5]NIH-purified growth hormone (Armour Lot #R50109) containing less than 0.014 ± 0.004 units/mg. TSH and less than 0.1 units/mg. prolactin.

[6]Bacto-adjuvant, Complete Freund. Difco Laboratories, Detroit 1, Michigan.

[7]Bacto-adjuvant, Incomplete Freund. Difco Laboratories, Detroit 1, Michigan.

Hemagglutination Technique for Quantitatively Assaying Growth Hormone

Preparation of Reagents

Salines buffered at pH 7.6, 7.0, and 6.4 were prepared from glass distilled water in the following manner: pH 7.6 - 0.85 percent NaCl, 0.013 M KH_2PO_4 and 0.087 M $Na_2HPO_4 \cdot 12H_2O$; pH 7.0 - 0.85 percent NaCl, 0.039M KH_2PO_4 and 0.061 M $Na_2HPO_4 \cdot 12H_2O$; and pH 6.4 - 0.85 percent NaCl, 0.073 M KH_2PO_4 and 0.026 M $Na_2HPO_4 \cdot 12H_2O$. Reagent grade (40 percent) formalin diluted to 10 percent and 3 percent by volume in pH 7.0 buffered saline and cooled to 5° C., was used in preserving the erythrocytes. Buffered citrate (pH 3.9-4.1) was prepared using 0.5 M sodium citrate and 0.5 M citric acid in a ratio of 2.0 : 1.7 and added to the blood collection tubes immediately before use (0.1 ml./ml. of whole blood).

Preparation of Erythrocytes

Citrated rabbit blood, collected via cardiac puncture, was washed 4 times with 40 volumes of 0.85 percent pH 6.4 buffered saline. Removal of the wash solution was accomplished by centrifugation at 3,000 g. After washing, the erythrocytes were resuspended in an equal volume of pH 7.0 buffered saline, chilled to 5° C. and poured into 4 volumes of cold 3 percent formalin and rolled at approximately 60 r.p.m. for 24 hours at 5°C. The cells were then washed once using 40 volumes of pH 7.0 buffered saline, resuspended in an equal volume of buffered saline and poured into 4 volumes of cold 10 percent pH 7.0 buffered formalin. The cells were again rolled for 24 hours, then washed 4 times using 40

volumes of pH 7.0 buffered saline. Once treated with formalin, separation of erythrocytes from the wash solution was easily accomplished by centrifugation at 2,000 g.

The formalin-treated erythrocytes (FTE) were then resuspended to make a 10 percent solution using pH 7.0 buffered saline. Tannic acid, (1 : 2,000 w/v), was added (v/v) to the 10 percent solution of FTE and rolled for 10 minutes at room temperature. The unabsorbed tannic acid was removed by washing 4 times with 40 volumes of pH 7.0 buffered saline and finally resuspended to 10 percent using normal saline containing 2 percent bovine albumin and 1 : 10,000 w/v merthiolate. This preparation has been used satisfactorily for several months when kept under refrigeration (5°C.).

Coating of FTTE with Antigen

One mg. of purified bovine GH was added to each 10 ml. of 10 percent formalin, tannic acid treated erythrocytes (FTTE) and rolled in polyethylene bottles at 5° C. for 24 hours. The antigen-absorbed FTTE were then washed free of unabsorbed antigen using pH 6.4 buffered saline and extended to 2.5 percent v/v in pH 6.4 buffered saline containing 2 percent bovine albumin and 1 : 10,000 merthiolate. The titers of the antigen absorbed FTTE were checked daily and when low titers appeared the FTTE were re-absorbed with antigen. The same antigen supernatant has been used successfully to coat FTTE several times when stored at 5° C.

Hemagglutination-Inhibition Reaction

The hemagglutination reactions were carried out in agglutination trays. The antisera to be used were titrated against an equal volume

of antigen-absorbed FTTE and diluted to approximately one-half the titer. Antisera titers usually ranged from 1/100 to 1/200. Antisera dilutions of from 1/50 to 1/100 were routinely used. These titers were checked daily and when found unsatisfactory the FTTE were reabsorbed with antigen. Serial double dilutions of NIH-bovine GH in 2 percent bovine albumin in 0.85 percent saline with concentrations ranging from 5.0 μg./ml. to 0.00125 μg./ml. were reacted routinely along with dilutions of blood plasma against which the degree of agglutination was compared. The blood plasma were routinely diluted in 0.85 percent saline containing 2 percent bovine albumin as follows: 1/2, 1/4, 1/6, 1/8, 1/10.

Each well of the agglutination tray received 0.1 ml. of the diluted antigen or blood plasma to be assayed and 0.1 ml. of 1/100 diluted antisera. The trays were then rotated for several minutes to mix and then left to stand 30 minutes at room temperature. Two drops (approximately 0.1 ml.) of 2.5 percent antigen absorbed FTTE were then added to each well. The trays were again swirled to allow thorough mixing, left undisturbed at room temperature, and read at three hours. Control wells containing 2 percent bovine albumin in 0.85 percent saline, antisera, and antigen absorbed FTTE were routinely used as were wells containing antigen and/or antisera and normal FTTE. The range of agglutination from 0 to 4 was recognized. The relative GH potencies of unknown sera were obtained by dividing the concentration of the standard (μg.) first showing complete agglutination by the dilution of unknown sera showing similar agglutination. All agglutination reactions were carried out in duplicate and when discrepencies occurred the reactions were repeated.

RESULTS AND DISCUSSION

Galactopoietic Response to Implanted Growth Hormone

The average daily milk production for the cows implanted with 0, 300, and 600 Armour units of growth hormone was 41.5, 40.4, and 42.5 lb., respectively (Table VI). Differences among groups in production were not statistically different ($P > 0.05$) as determined by Duncan's multiple range test (125) using adjusted treatment means (Table VII). Moreover, the significant difference ($P < 0.05$), as determined by the multiple range test (Table VIII) between adjusted treatment means for pounds of total solids when comparing the high and low level implanted groups was not deemed to be of any real importance in view of the lack of consistency with the other data on milk yield and composition. Likewise, there were no significant differences ($P > 0.1$) among groups with respect to total fat, body weight changes, blood glucose level, total leucocytes, or incidence of mastitis.

Implantation abcesses were observed following implantation. On several occasions, approximately two weeks following implantation, these cysts were opened and the contents therein expelled. In no case was there evidence of GH pellets left intact. Invariably these cysts were walled off forming a pouch as evidenced by the increased connective tissue surrounding the implantation site. Observations with rats (119) indicate that GH can stimulate connective tissue growth at the site of application. Although not examined

TABLE VI

RESPONSE OF DAIRY COWS TO IMPLANTATION WITH GROWTH HORMONE

Level A.U.[a]	Av. TDN Intake (lb/day)	Av. Milk Production (lb/day)	Fat Test (%)	Total Solids (%)	Total SNF (%)	Gain in Body Wt. (lb/6wk.)	Blood Glucose (mg/100ml.)	Av. Total WBC Count (1,000/mm^3)
None	26.7	41.5	3.7	12.1	8.3	29	53	10.1
300	26.7	40.4	3.7	12.5	8.7	30	54	10.0
600	26.4	42.5	3.7	12.2	8.5	27	53	10.0

[a] A.U. = Armour units.

TABLE VII

ANALYSIS OF VARIANCE ON MILK PRODUCTION FROM COWS IMPLANTED WITH GROWTH HORMONE

Source of Variation	Degrees of Freedom	Mean Square	F
Cows (Sequences)	11	433871.1	39.3[a]
Periods Within Squares	8	132138.5	11.9[a]
Direct Effects (Unadj.)	(2)	25398.0	2.3
Residual Effects (Adj.)	(2)	36142.3	3.3[b]
Residual Effects (Unadj.)	(2)	10812.3	0.9
Direct Effects (Adj.)	(2)	50728.2	4.6[b]
Error	12	11060.3	

5% Multiple Range Test for Adj. Treatment Means

	C	A
B	129.74 (169.83)[c]	75.85 (161.94)
A	53.89 (161.94)	

[a]Significant at the one percent level.

[b]Significant at the five percent level.

[c]Numbers in parentheses, least significant range.

TABLE VIII

ANALYSIS OF VARIANCE ON TOTAL SOLIDS FROM COWS IMPLANTED WITH GROWTH HORMONE

Source of Variation	Degrees of Freedom	Mean Square	F
Cows (Sequences)	11	5123.2	36.5[a]
Periods Within Squares	8	1444.3	10.3[a]
Direct Effects (Unadj.)	(2)	378.5	2.7
Residual Effects (Adj.)	(2)	507.7	3.6[b]
Residual Effects (Unadj.)	(2)	162.8	1.2
Direct Effects (Adj.)	(2)	723.5	5.2[b]
Error	12	140.3	

5% Multiple Range Test for Adj. Treatment Means

	C	A
B	23.69[b] (19.12)[c]	11.58 (18.23)
A	12.11 (18.23)	

[a]Significant at the one percent level.

[b]Significant at the five percent level.

[c]Numbers in parentheses, least significant range.

histologically, it was assumed that both hyperplasia and hypertrophy of the surrounding connective tissue occurred as a result of exposure to antigenic substance(s) thus provoking a hypersensitive response and local inflammation.

Folley (56) obtained small but nonsignificant galactopoietic responses from both cows and goats injected with GH preparations, and noted that while there was a high degree of variation in response among animals, a greater galactopoietic response was obtained with small doses of the hormone as compared to large doses. This author suggested that the experimental animals were refractory to the galactopoietic actions of GH particularly in the latter stage of lactation. However, Brumby et al. (23) and Shaw et al. (122) obtained marked increases in milk and fat production during the declining phase of lactation in cattle. Although the cows used in the present study were implanted during the early part of lactation, the possibility that the higher levels of GH rendered the animals refractory to a galactopoietic response should not be overlooked, particularly in view of the levels used and the fact that a portion of the hormone was of porcine origin. The hypothesis of an acquired refractoriness to a growth hormone response could explain both the small lactational response and the formation of implantation cysts. It would appear (Tables XVII, XVIII, XIX) that there was no appreciable difference in the galactopoietic response resulting from implantation of the bovine and procine growth hormone material. The porcine GH preparation was used for the fourth group (square) of cows. The biological activity of the implanted material was assayed using the method of Greenspan et al. (64)

following the experiment. There was noticeable loss of biological activity only in the bovine pelleted material. There was no noticeable loss of biological activity in the other GH preparations during storage at approximately 5° C. over a period of several months.

Galactopoietic Response to Injected Growth Hormone Preparations

The cows injected with 50 mg. per day of either NIH-purified or the impure (Somar) GH preparation produced more milk than the control animals during a 10 day injection period and for a few days thereafter. The average daily milk production for 14 days (10 day injection period plus 4 days post injection) was 42.0, 36.1, and 34.5 pounds, respectively, for the Somar, NIH-GH and saline treated groups (Table IX). The average daily increase in milk production for the three respective treatment groups was 7.6, 3.36, and -4.35 percent. Similar, but less pronounced differences were noted with respect to pounds of fat produced. The Somar-treated group produced significantly ($P < 0.01$) more milk than either of the other two groups (Table X). However, there was no significant difference ($P > 0.05$) between treatment means with respect to pounds of milk of the group receiving the highly purified NIH-GH and the saline-treated group. However, with respect to pounds of fat produced, the Somar group exceeded that of both the NIH-GH and saline groups ($P < 0.01$) and the group receiving the NIH-purified GH produced significantly ($P < 0.01$) more fat than the saline-treated group (Table XI).

The galactopoietic response effected by small daily injections of GH confirms the results of Fawns et al. (46), Donker and Petersen (39), Cotes et al. (34), Shaw et al. (122), and Brumby et al. (23), and

TABLE IX

AVERAGE DAILY MILK AND FAT PRODUCTION, FEED INTAKE, AND BODY WEIGHT CHANGE OF EXPERIMENTAL COWS INJECTED WITH GROWTH HORMONE PREPARATIONS

Treatment	Milk		Fat		SNF		TDN Intake	Body Weight Gain
	Prelim	Treat.	Prelim.	Treat.	Prelim.	Treat.		
	(lb./day)		(lb./day)		(lb./day)		(lb./day)	(lb./day)
A (Somar)[a]	39.0	42.0	1.35	1.70	4.59	5.26	24.0	0.6
B (NIH-GH)[b]	34.9	36.1	1.17	1.27	4.06	4.29	22.3	0.7
C (Saline)	36.0	34.5	1.15	1.11	4.01	3.84	23.9	1.3

[a]Somar (Armour Lot No. M108) containing approximately 0.1 to 0.2 units TSH per mg.

[b]NIH-purified growth hormone (Lot No. R50109) containing less than 0.014 units TSH per mg.

TABLE X

ANALYSIS OF COVARIANCE ON POUNDS OF MILK FROM COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Source of Variation	df	xx	xy	yy	y Adjusted for x			
					df	SS	MS	F
Total	14	228867.8	270418.4	332086.3				
Blocks	4	194338.4	220504.1	250586.2				
Treatments	2	9065.2	15242.2	30624.2				
Error	8	25464.2	34672.1	50876.0	7	3666.4	523.8	
Treatments Plus Error	10	34529.4	49914.3	81500.1	9	9345.9		
Treatments Adjusted					2	5679.5	2839.7	5.42[b]

5% Multiple Range Test for Treatments

	A	B
C	105.06 (35.51)[c]	22.38 (34.28)
B	82.68 (34.28)	

1% Multiple Range Test for Treatments

	A	B
C	105.06 (53.42)[c]	22.38 (50.66)
B	82.68 (50.66)	

[a]Treatments: A - Somar
B - NIH Growth Hormone
C - Control

[b]$P < 0.05$

[c]Numbers in parentheses, least significant range.

TABLE XI

ANALYSIS OF COVARIANCE ON POUNDS OF FAT FROM COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Source of Variation	df	xx	xy	yy	y Adjusted for x			
					df	SS	MS	F
Total	14	16877.3	23568.6	38484.9				
Blocks	4	10338.4	12338.3	15218.8				
Treatments	2	2541.6	5880.1	14287.8				
Error	8	3997.3	5350.2	8978.3	7	1817.2	259.6	
Treatments Plus Error	10	6538.9	11230.3	23266.1	9	3978.5		
Treatments Adjusted					2	2161.2	1080.6	4.16[b]

5% Multiple Range Test for Treatments

	A	B
C	73.95 (14.44)[c]	23.12 (13.94)
B	50.85 (13.94)	

1% Multiple Range Test for Treatments

	A	B
C	73.95 (21.73)[c]	23.12 (20.60)
B	50.85 (20.60)	

[a]Treatments: A - Somar
B - NIH Growth Hormone
C - Control

[b]$P < 0.1$

[c]Numbers in parentheses, least significant range.

Hutton (75). However, these results indicate, as one might expect, a synergistic galactopoietic response of thyroid stimulating hormone. Thyroid hormones are known to influence milk secretion directly by increasing heart rate, circulation of blood and general cellular metabolism; and indirectly by stimulating the secretion of prolactin (99). The synergistic response elicited is probably due to increased sensitization of pituitary secretory cells and/or the target organs of their secretions.

The differences among treatment means with respect to percent fat approached significance ($P < 0.01$). Differences among groups were not significant ($P > 0.10$) with respect to total solids, body weight change, blood glucose level, total leucocytes or incidence of mastitis. These results are in agreement with those of Shaw et al. (122) that the fat content of the milk is not altered by the daily administration of GH. It would appear that the small increase (3.5 to 4.0) in percent fat noted in the group receiving Somar was due to the TSH contaminant. The alteration in milk fat content without a significant change in blood glucose as a result of the GH treatment is not conflicting inasmuch as milk fat is synthesized in the mammary tissues from pools of free fatty acids and glycerol (88).

Considerable variation was noted in the degree of galactopoietic response elicited among cows within a given treatment (Figures 2, 3, and 4). This observation strengthens the hypothesis put forth by Turner et al. (136) that the lactational response of a given lactation is dependent upon the rates of secretion of the various endocrine glands that supply the hormones necessary for the growth of the udder and the maintenance of the intensity of milk secretion.

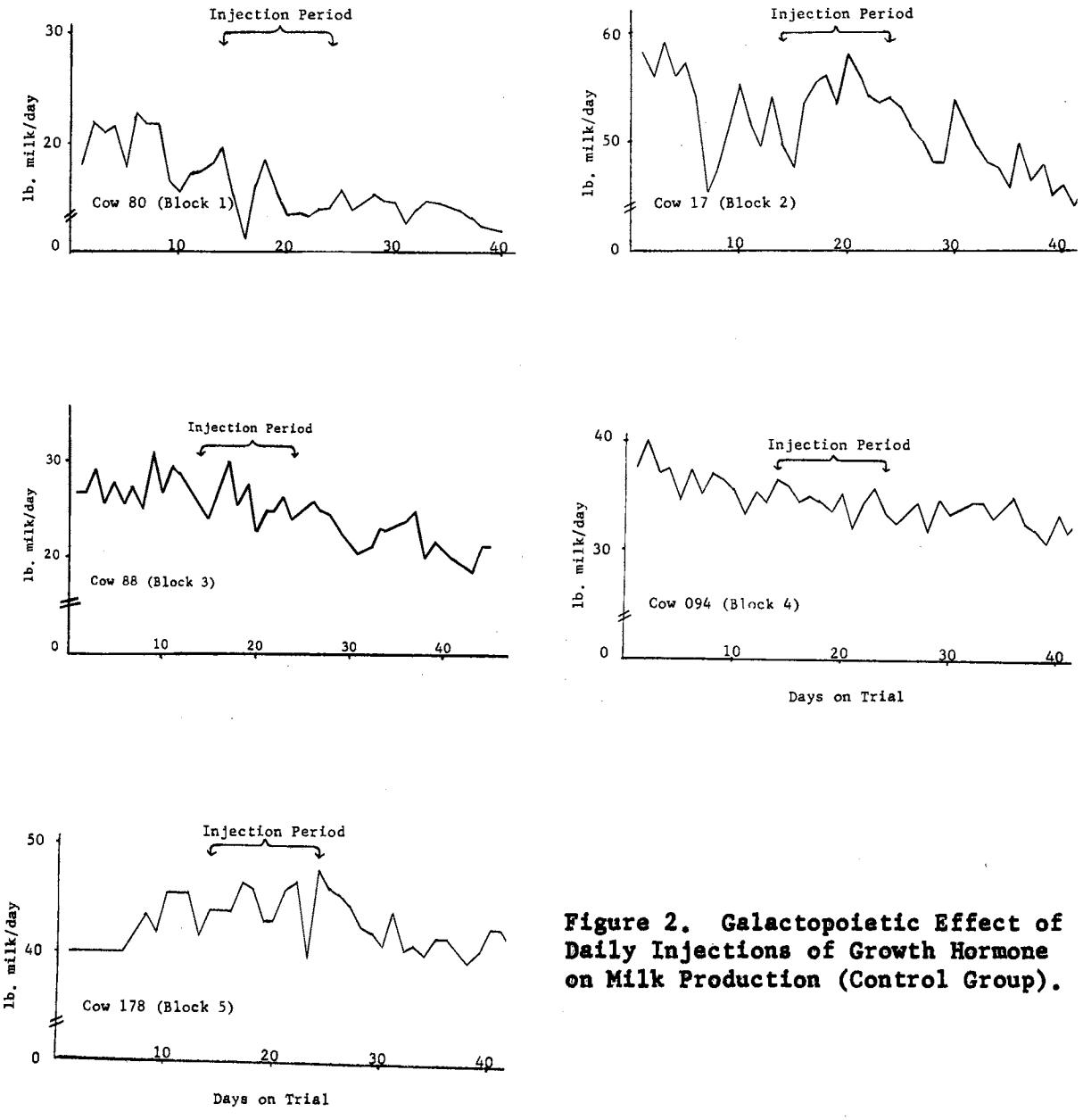


Figure 2. Galactopoietic Effect of Daily Injections of Growth Hormone on Milk Production (Control Group).

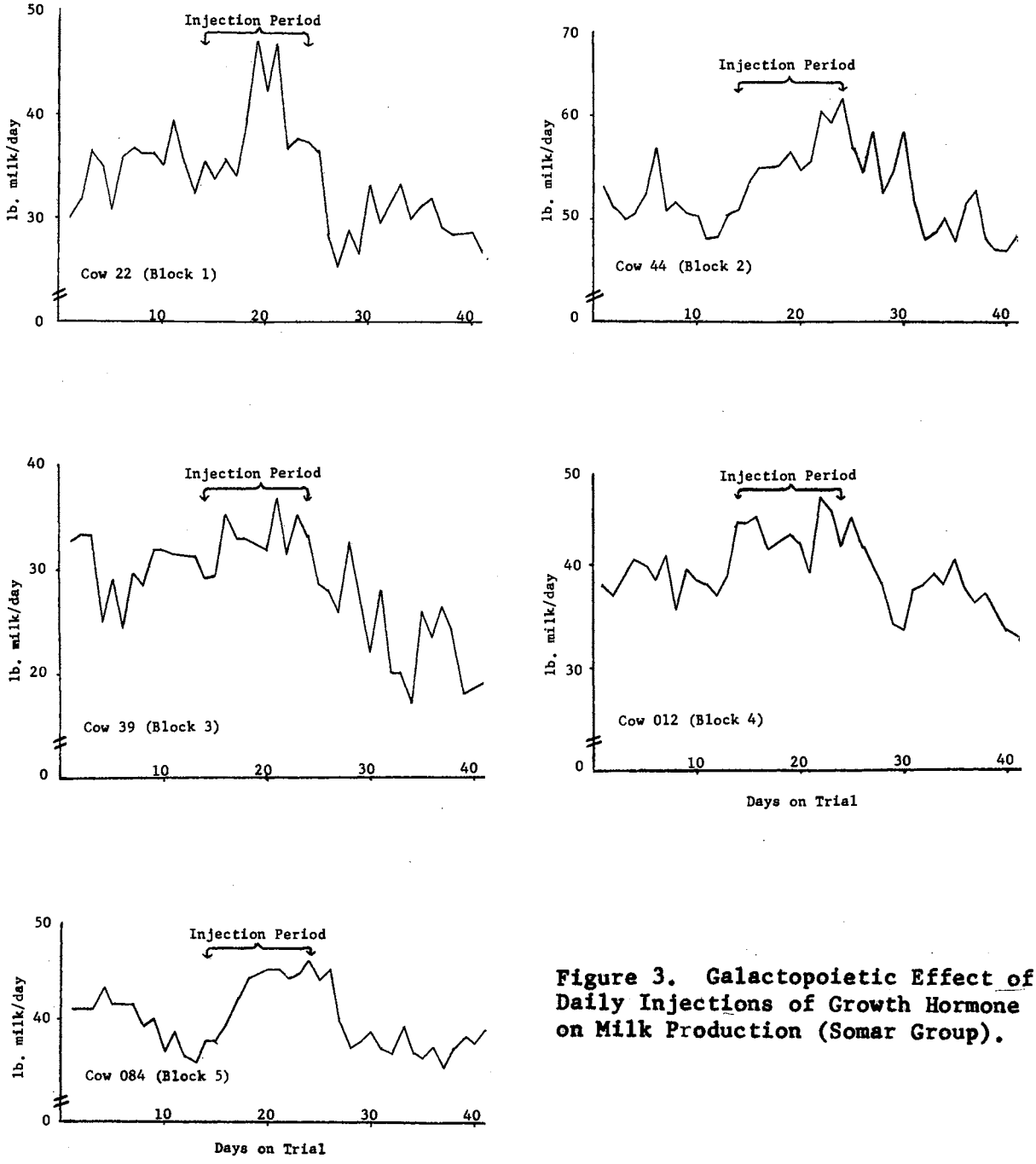


Figure 3. Galactopoietic Effect of Daily Injections of Growth Hormone on Milk Production (Somar Group).

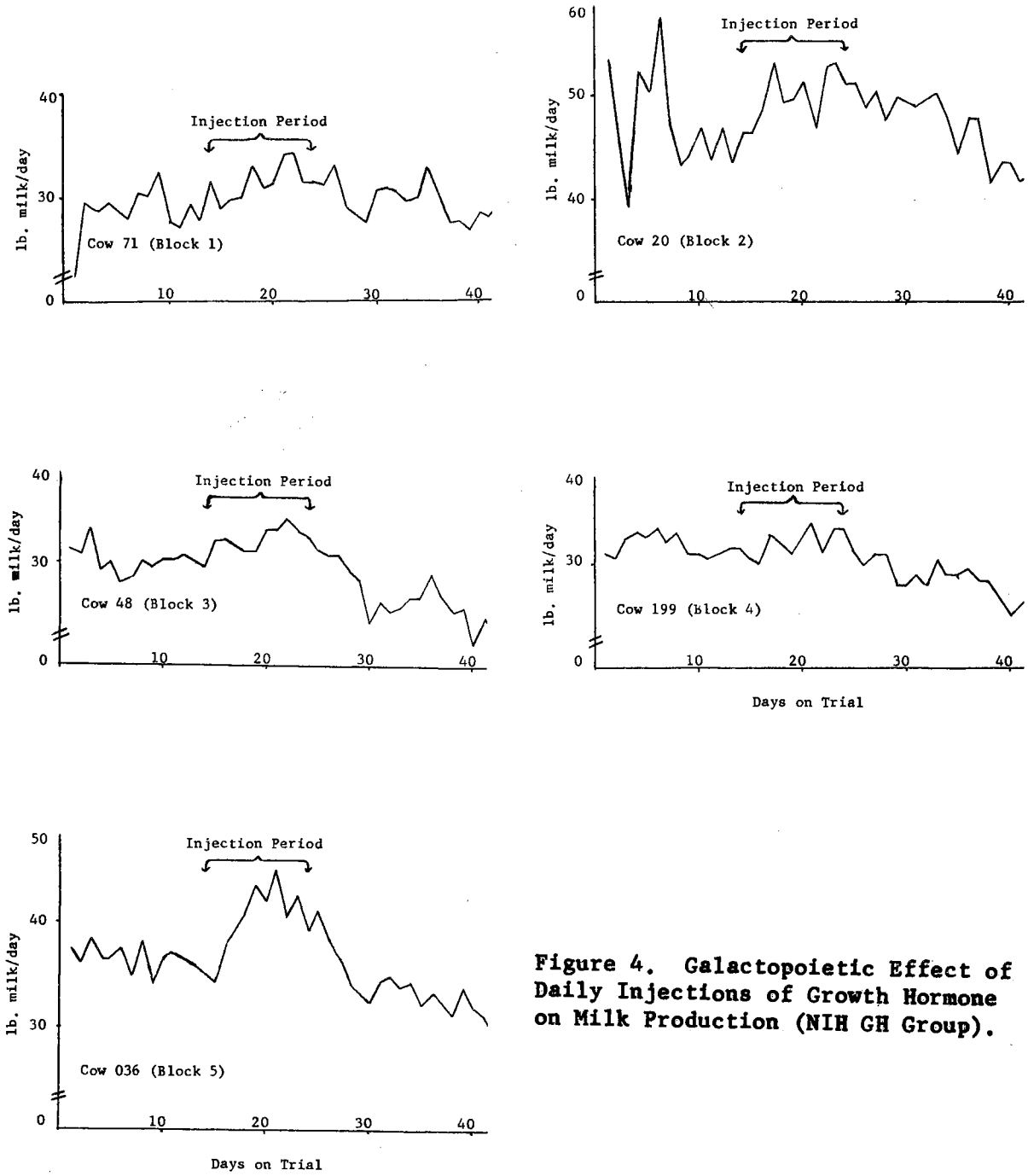


Figure 4. Galactopoietic Effect of Daily Injections of Growth Hormone on Milk Production (NIH GH Group).

Effect of Energy Intake During Rearing on Body Growth of Holstein Heifers

Growth is an ambiguous term covering many cellular processes including reproduction, increase in dimensions, gain in weight, linear increase, gain in organic mass, cellular hypoplasia, and cellular hypertrophy. It appeared desirable, therefore, to use several criteria of growth manifestations in this phase of the study.

TABLE XII

AVERAGE BODY WEIGHTS AND MEASUREMENTS AT 6 MONTHS OF AGE OF HOLSTEIN HEIFERS REARED ON THREE LEVELS OF ENERGY INTAKE

	Level of Energy Intake (% of N.R.C. Standard)		
	75	100	125
Av. Body Weight Gain (lb.)[a]	0.70	1.21	1.45
Body Weight (lb.)	226.18	301.56	337.00
Circ., Heart Girth (in.)	42.32	45.90	47.95
Circ., Barrel (in.)	50.87	56.89	58.50
Length, Withers to Pin Bone (in.)	30.08	33.86	34.92
Length, Withers to Hip Crest (in.)	20.83	21.69	22.20
Height, Sole to Rump (in.)	38.03	39.65	40.24
Height, Sole to Withers (in.)	36.73	38.15	39.05

[a]From 2 to 6 months of age.

Later maturing characteristics such as body weight and heart girth appeared to be affected most by restricting the energy intake while height and length which are early maturing were affected least. The average daily body weight gain for the 125 percent group (1.45 lb./day) for the first 6 months of age is in close agreement with the Beltsville growth standards for Holsetin heifers (93). Similarly, the 1.70 lb./day body weight gain for the 125 percent group from 6 months to 12 months approximates the daily gain recommended by the Beltsville growth standards

(Figure 5). It would appear, therefore, that although the TDN intakes from the skimmilk-fortified ration fed at 100 percent N.R.C. were in keeping with those of the National Research Council, the lower biological value of the grain ration as compared to whole milk, failed to produce daily body weight gains comparable to those obtained with milk fed calves. The lower body weight gain of the 100 percent group from 6 months to 12 months of age may be explained on the basis of higher roughage intake during this period.

TABLE XIII

AVERAGE BODY WEIGHTS AND MEASUREMENTS OF HOLSTEIN HEIFERS REARED ON THREE LEVELS OF ENERGY INTAKE AT 12 MONTHS OF AGE

	Levels of Energy Intake (% of N.R.C. Standard)		
	75	100	125
Av. Body Weight Gain (lb./day)[a]	0.86	1.30	1.70
Body Weight (lb.)	381.71	535.84	641.48
Circ., Heart Girth (in.)	50.90	55.35	60.79
Circ., Barrel (in.)	59.68	67.24	71.73
Length, Withers to Pin Bone (in.)	37.72	44.13	44.68
Length, Withers to Hip Crest (in.)	26.69	27.20	29.13
Height, Sole to Rump (in.)	43.62	45.79	47.56
Height, Sole to Withers (in.)	42.68	45.12	46.46

[a]From 6 to 12 months of age.

The amount of TDN required subsequent to 2 months of age to produce a given size animal under the three levels of energy intake is shown in Figure 6. It would appear from these data that the 125 percent group required slightly less TDN to attain a given weight than the 100 percent group, whereas the 75 percent group was considerably less efficient in converting dietary energy into body growth. For example, to produce a 300 lb. animal approximately 540, 450, and 420 lb. of TDN were required

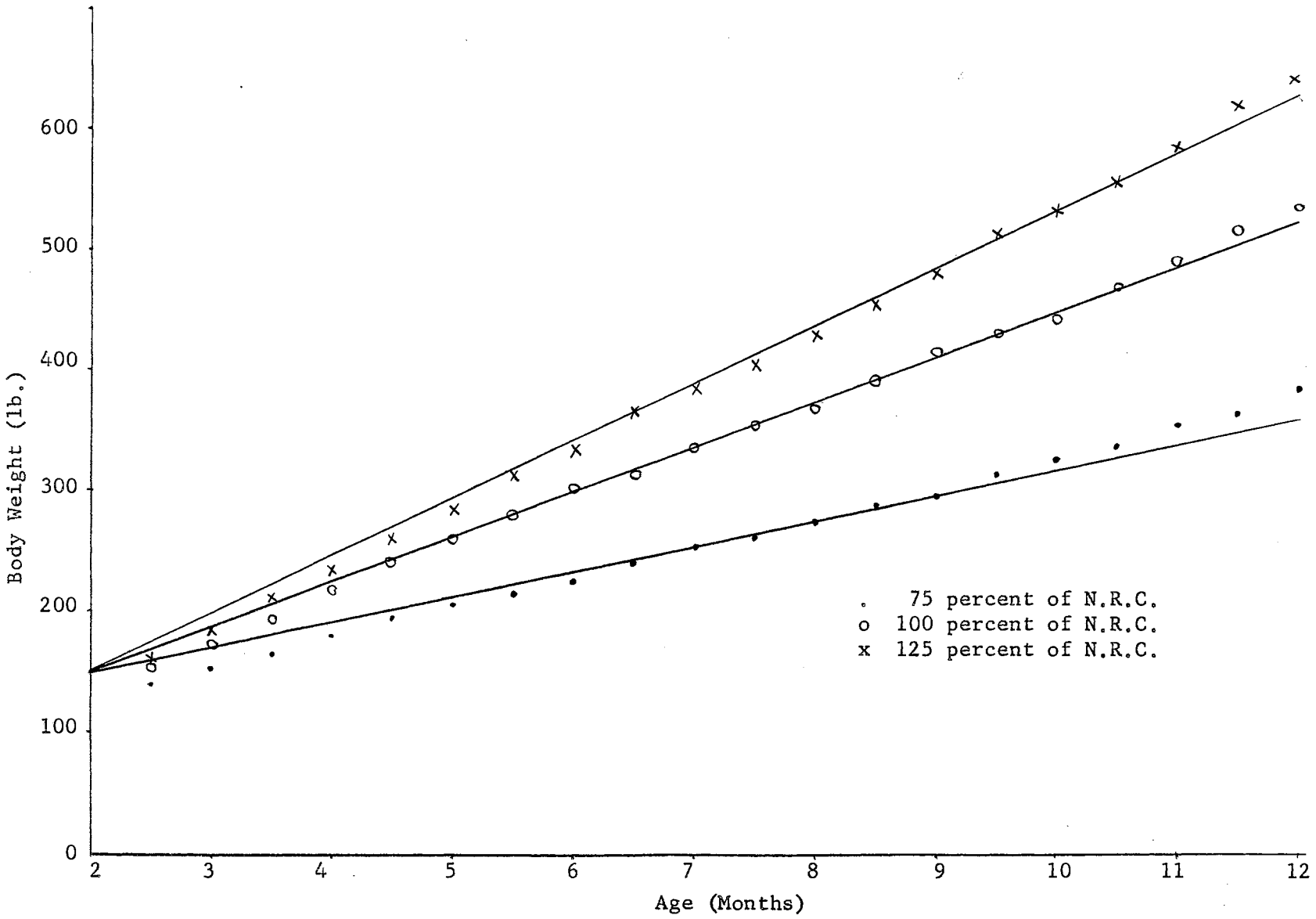


Figure 5. Growth-Response Curves of Holstein Heifers Maintained on Three Levels of Energy Intake.

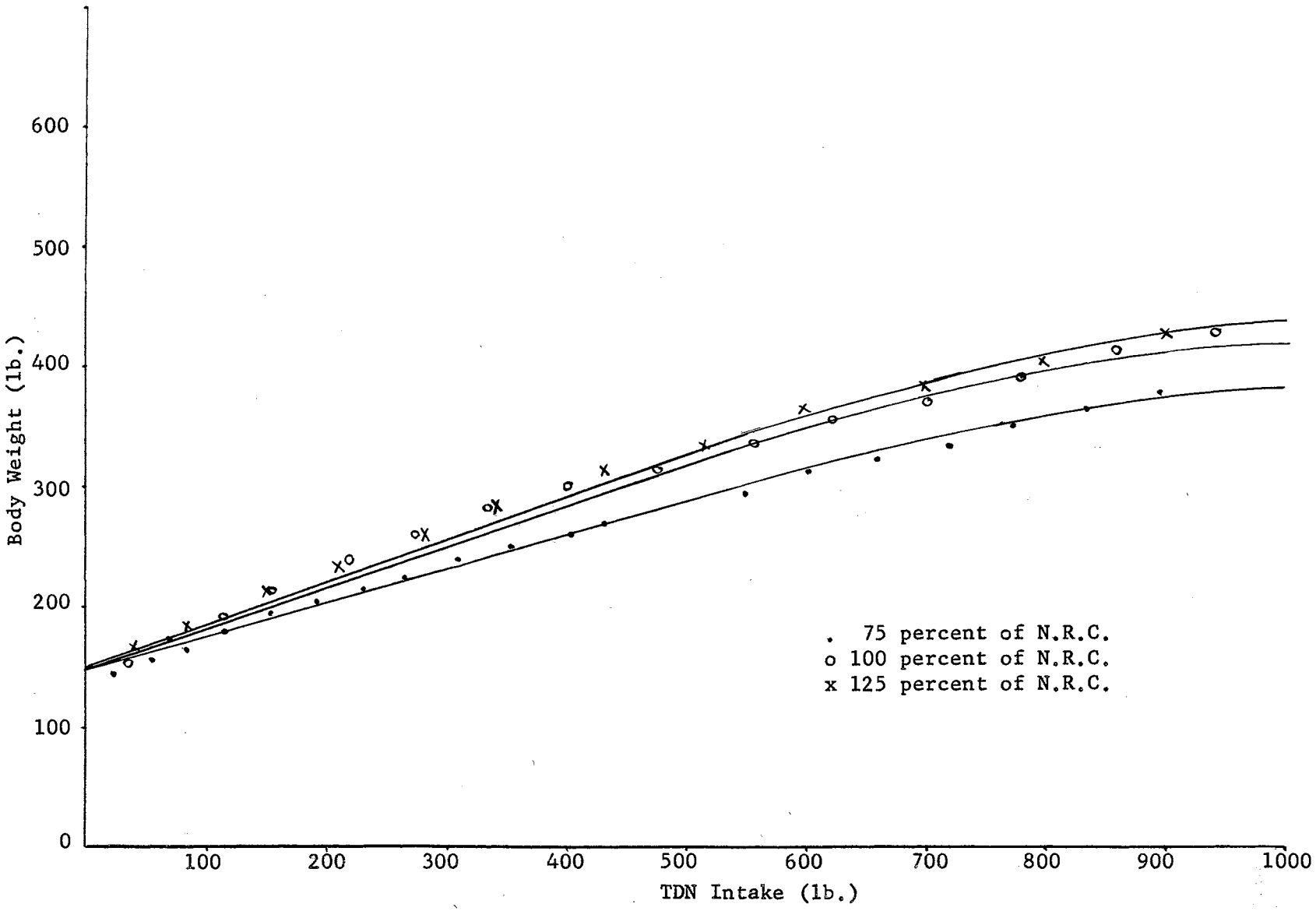


Figure 6. Effect of TDN Intake on Body Weight of Holstein Heifers Maintained on Three Levels of Energy Intake (TDN Intake Subsequent to 2 Months of Age).

for the 75, 100, and 125 percent groups, respectively. A somewhat different picture develops, however, when body height and length are plotted against TDN intake subsequent to 2 months of age (Figure 7 and 8). Inasmuch as both height and length are early maturing characteristics, two general formulas were used to classify these two distinct stages of growth:

$$(\text{Body Height})_i = \Sigma\frac{(\text{Height of Withers}) + (\text{Height of Rump})}{2}$$

$$(\text{Body Length})_i = \Sigma\frac{(\text{Length, Withers to Pins})+(\text{Withers to Hip Crest})}{2}$$

The respective values when plotted against TDN intake coupled with the energy intake data of the tables indicate: (a) that body length occurs at a faster rate than body height and, (b) that varying the level of energy intake from 75 to 125 percent of N.R.C. during the rearing period has little effect upon body length or height. The effect of three levels of energy intake on body growth from 2 to 12 months of age is illustrated in Figures 5, 9, and 10.

The level of energy intake appeared to have no affect on age at puberty, as judged by the onset of first heat, when comparing the 125 percent and 100 percent groups of heifers. All of the animals receiving 125 percent of N.R.C. requirements reached puberty by 12 months of age, two had reached puberty by 11 months of age, and one had reached puberty by 10 months of age. In the group fed at 100 percent of N.R.C. requirements, 9 out of 10 animals reached puberty by 12 months of age and one showed evidence of silent heat; one animal reached puberty by 9 months of age and one had reached puberty by 11 months of age. Contrarily, only one of the heifers maintained at 75 percent of N.R.C. requirements

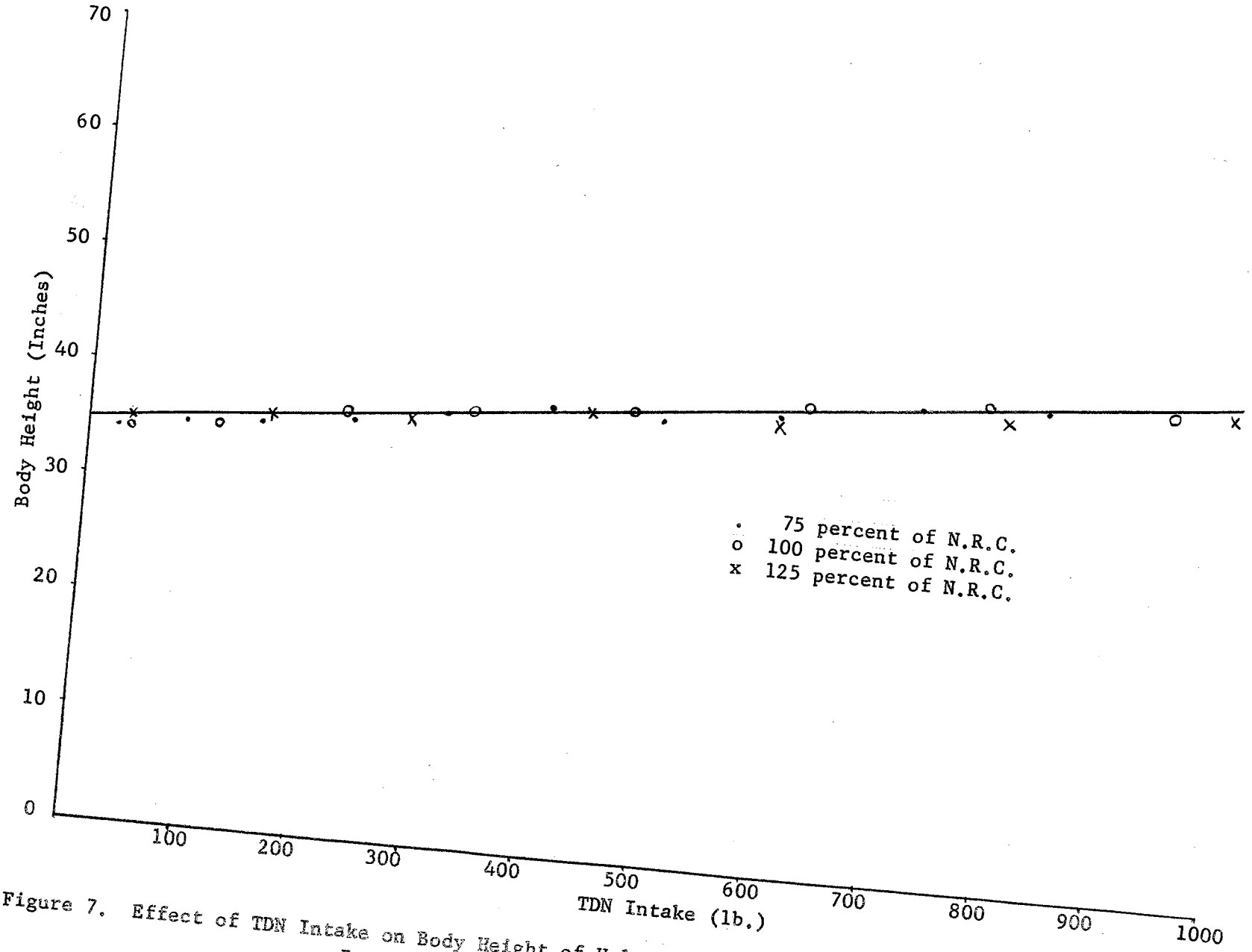


Figure 7. Effect of TDN Intake on Body Height of Holstein Heifers Maintained on Three Levels of Energy Intake (TDN Intake Subsequent to 2 Months of Age).

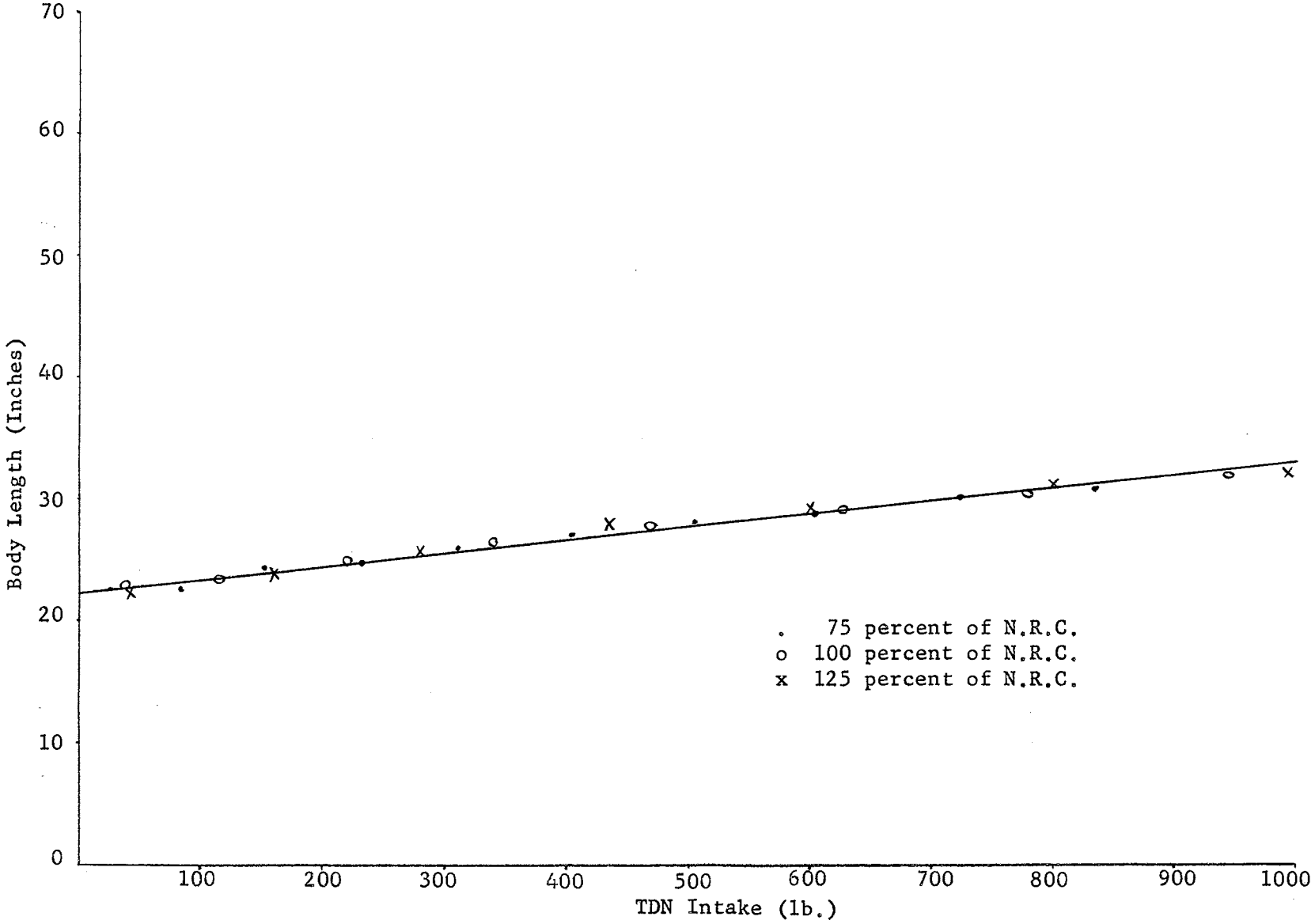


Figure 8. Effect of TDN Intake on Body Length of Holstein Heifers Maintained on Three Levels of Energy Intake (TDN Intake Subsequent to 2 Months of Age).

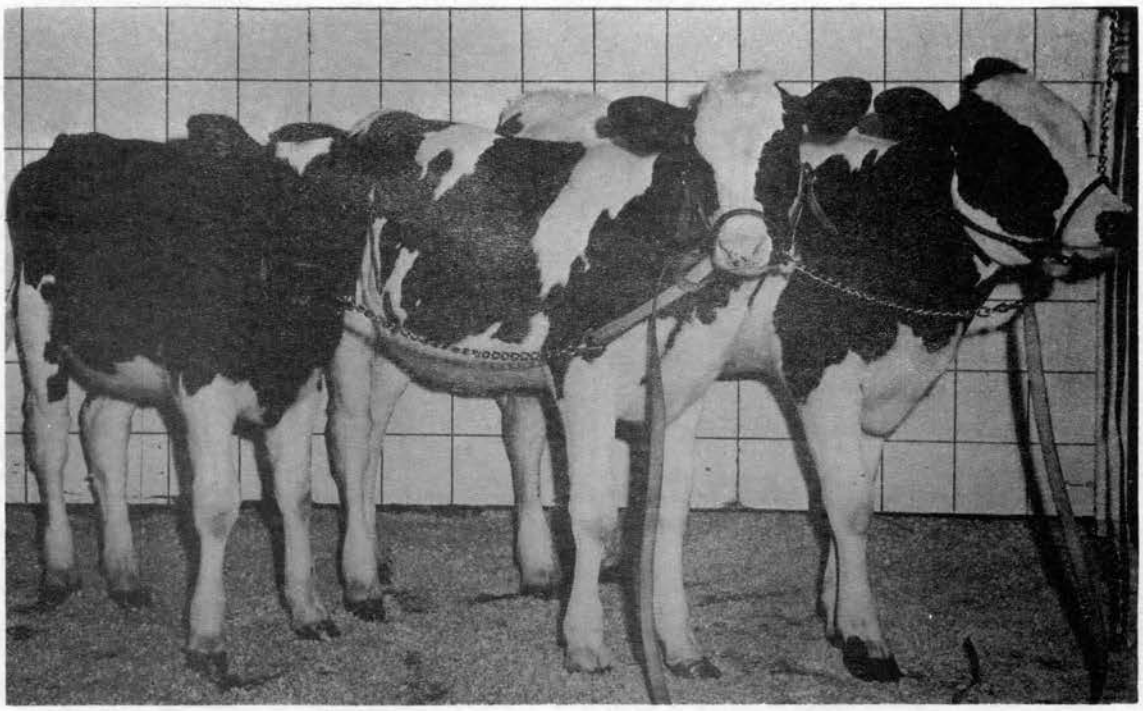


Figure 9. Calves 4, 2, and 427 at 6 Months of Age After Receiving 75, 100, and 125 Percent of N.R.C. Requirements Respectively From 2 to 6 Months of Age.

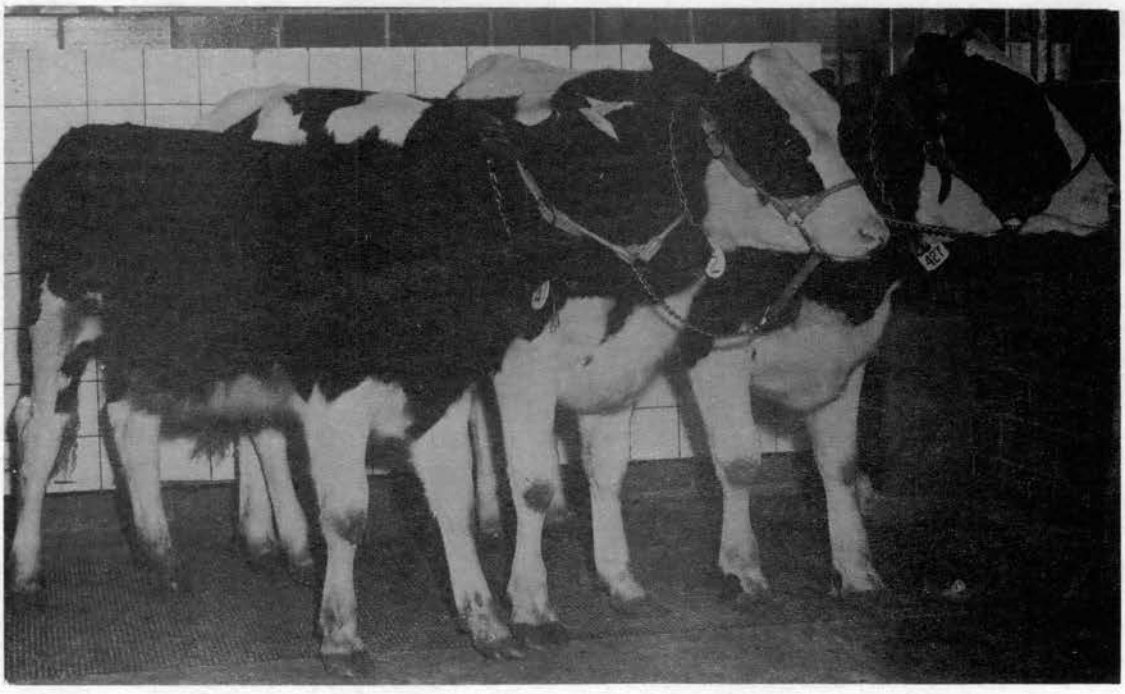


Figure 10. Calves 4, 2, and 427 Receiving 75, 100, and 125 Percent of N.R.C. Requirements Respectively From 6 to 12 Months of Age. Photo Taken at 12 Months of Age.

had reached puberty by the time the experiment had terminated and this was at approximately 12 months of age.

Effect of Energy Intake During Rearing on Thyroid Secretion Rate of Holstein Heifers

The data summarizing the effect of rearing Holstein heifers on three levels of energy intake (75, 100, and 125 percents of N.R.C. requirements) on the thyroid secretion rate (TSR) are presented in Table XIV. The mean TSR per 100 kg. body weight and the mean TSR per 100 kg. body weight raised to the 0.75 power (metabolic size) along with TTR, ETT, TVD, and mean normal PBI are classified according to treatment groups.

TABLE XIV

EFFECT OF THREE LEVELS OF ENERGY INTAKE ON THE THYROID ACTIVITY OF HOLSTEIN HEIFERS[a]

Energy Intake[b] (%)	TVD[c] (%)	ETT[d] mg.	TTR[e] Per Day	TSR/100 kg. B.W. Per Day[f] mg.	TSR/100 kg.$^{.75}$ B.W. Per Day[g] mg.
75	35.06	2.63	0.49	1.04	3.36
100	50.02	7.35	0.43	0.70	2.80
125	47.9	3.94	0.42	0.72	3.03

[a]Average of 10 animals.

[b]Percent of National Research Council's recommendations.

[c]Thyroidal volume of distribution, percent of body weight; standard error = 10.41.

[d]Extra thyroidal thyroxine, mg.

[e]Thyroxine fractional turnover rate per day.

[f]Thyroxine secretion rate, mg. per 100 kg.

[g]Thyroxine secretion rate, mg. per 100 kg. body weight$^{.75}$ per day; standard error = 0.26.

The mean daily TSR/100 kg. body weight was 1.04, 0.70, and 0.72, respectively, for the groups receiving 75, 100, and 125 percent of N.R.C. requirements. These differences were significant ($P < 0.10$). However, when the TSR per day was adjusted to metabolic size the differences among treatment means were not significantly different ($P > 0.10$). Likewise, there was no significant difference among treatment groups with respect to total volumes of distribution (TVD), extra-thyroidal thyroxine (ETT), thyroxine fractional turnover rate (TTR), or mean normal protein bound iodine (PBI).

TABLE XV

ANALYSIS OF VARIANCE ON TSR[a] PER 100 KG. BODY WEIGHT OF 12 MONTH OLD HOLSTEIN HEIFERS

Source of Variation	Degrees of Freedom	Mean Square	F
Total	28	0.092	
Blocks	9	0.181	4.3[b]
Treatments	2	0.124	2.9[c]
Error	17	0.042	

[a]Thyroxine secretion rate, mg./100 kg. body weight per day.

[b]Significant at the five percent level.

[c]Significant at the ten percent level.

TABLE XVI

ANALYSIS OF VARIANCE ON TSR[a] PER 100 KG. B.W. $^{0.75}$ OF 12 MONTH OLD HOLSTEIN HEIFERS

Source of Variation	Degrees of Freedom	Mean Square	F
Total	28	1.36	
Blocks	9	2.82	4.29[b]
Treatments	2	0.76	1.16
Error	17	0.65	

[a]Thyroxine secretion rate, mg./100 body weight $^{0.75}$ per day.

[b]Significant at the five percent level.

The standard error based upon residual mean squares of the tables, were calculated to be 0.07 and 0.26, respectively, for TSR/100 Kg. B.W. (Body Weight) and TSR/100 B.W.$^{0.75}$ which are slightly higher than those reported by Mixner et al. (98), using this same method of determining thyroxine secretion rate in mature cattle. There was no significant differences between treatment means with respect to TTR, TVD, ETT, or mean normal PBI. A difference in TVD of L-thyroxine would indicate that not all of the injected L-thyroxine was distributed from the plasma into the body fluid compartment or space at the time the blood samples were collected. The standard error for the other determinations was 0.04, 10.41, 0.67, and 44.62, respectively, for TTR, TVD, PBI, and ETT. Since ETT was calculated as the product of PBI (μg./100 ml.) and TVD, the standard error associated with ETT is associated with the product of the respective population standard deviations. The standard error

error associated with each of TVD, PBI, and ETT is higher than those reported by Mixner et al. (98) and are sufficiently high to limit the usefulness of this procedure for estimating thyroid activity. The TSR was calculated as the product of TTR and ETT. The standard error of TSR increased when adjusted for metabolic size (TSR per 100 kg. body weight$^{.75}$).

The action of the thyroid hormones has been shown to be at the peripheral cellular level. Although the exact mechanism of action is unknown, it is reasonable that the requirements for iodothyronines at the cellular level are a function of cellular activity and metabolism. While it has been known for some time that these hormones, when converted to their respective acetic acid analogues, promote oxygen uptake and uncouple oxidative phosphorylation in rat mitochondria (133), the mechanism involved is yet to be elucidated. Recent results by Lee et al. (84) have been enlightening in that thyroxine stimulates the levels of activity of both 6-phosphogluconate and glucose-6-phosphate dehydrogenase. The thyroxine effect can be reversed in hypophysectomized rats by discontinuing the administration of thyroxine. The possibility exists therefore, that the hormones either influence the enzymes directly or indirectly by altering the permeability of the cell membrane thereby increasing the substrate concentration to the enzyme systems. With an increased activity of these enzyme systems one would expect increased mobilization of carbohydrates and, consequently, increased rates of formation of the Krebs-cycle intermediates. It is therefore reasonable to assume, based upon the "feedback hypothesis" that the cellular demands for thyroid hormones depend upon the demands for needed metabolites coupled with

the availability of suitable enzyme substrates. Therefore, it may be conjectured that the TSR of a given animal is not a function of simple body weight, but a function of energy metabolism and surface area which, according to Brody (20), are proportional to the square of linear size.

Inasmuch as the external surface area per unit weight and basal metabolic rate decline with increasing body weight, it would appear that TSR per unit of surface area more closely represents the true rate when comparing the TSR of animals where surface area may be affected by treatments. Pipes et al. (106) found that reducing the feed intake to three-fourths full feed significantly lowered the TSR of mice without affecting body weight. These findings do not disprove the above hypothesis since lowering the food intake would, in effect, also lower the rate of metabolic processes.

IMMUNOLOGICAL STUDIES WITH BOVINE GROWTH HORMONE

Production of Antisera

The titer of antiserum as determined by the precipitin ring test and/or by FTTE titration obtained from rabbits previously injected with multiple doses of NIH-purified GH varied considerably among donor rabbits. The highest concentration of antiserum obtained from any one rabbit reacted with as little as 0.0345 μg./ml. of GH with the precipitin ring test.

One antiserum obtained early in this study against NIH-purified GH (lot No. R50109, containing less than 0.1 units per mg. prolactin) gave rise to three distinct ouchterlony precipitin zones, two of which appear to be similar and one dissimilar. Absorbing this antiserum with an equal volume of prolactin (10.0 μg./ml.) precipitated out all antibody activity to bovine GH. The loss of immunological activity against GH of the antiserum following absorption with prolactin suggests that either GH and prolactin are immunologically similar, or that they are bound together in sera forming a homologous molecule. It would appear, therefore, that the purity of antigen with respect to prolactin content is of importance in obtaining a suitable antiserum for the immunological estimation of GH content in blood. Furthermore, the presence of high concentrations of prolactin in unknown test sera might limit the usefulness of any immunological assay procedure for GH.

The interaction of antisera to NIH-purified GH (lot No. R491114) gave rise to one distinct precipitin zone on Ouchterlony plates (Figure 11). Using Ouchterlony plates to study the interaction of antisera to prolactin, FSH, LH, and gamma globulin, a negative cross reaction was found between the antisera and all of these antigens. It may be assumed that the above antisera were free of both prolactin and gamma globulin contaminants since there were no cross reactions between these two antigens and the antisera (Figures 12 and 13). These antisera were used in all blood determinations reported herein.

Hemagglutination-Inhibition Reaction Employing Unformalinized Erythrocytes

The hemagglutination-inhibition reaction employing tannic acid and protein treated erythrocytes described by Read (112) was employed with one important modification - bovine erythrocytes were utilized in place of sheep erythrocytes. These erythrocytes were obtained from a 3 month old Ayrshire calf at slaughter and preserved in Alsever's solution until use.

Although the bovine GH-antigen absorbed readily onto the bovine erythrocytes, the instability of the preparation coupled with non-specific hemagglutination when used to attempt inhibition of agglutination produced such unpredictable results that the procedure was abandoned as an assay method for bovine GH in blood sera or plasma.

Hemagglutination-Inhibition Employing Formalin-Tannic Acid Treated Erythrocytes

Attempts to combine the protein-antigen (bovine GH) onto bovine erythrocytes (via the method outlined under procedure) were unsuccessful.

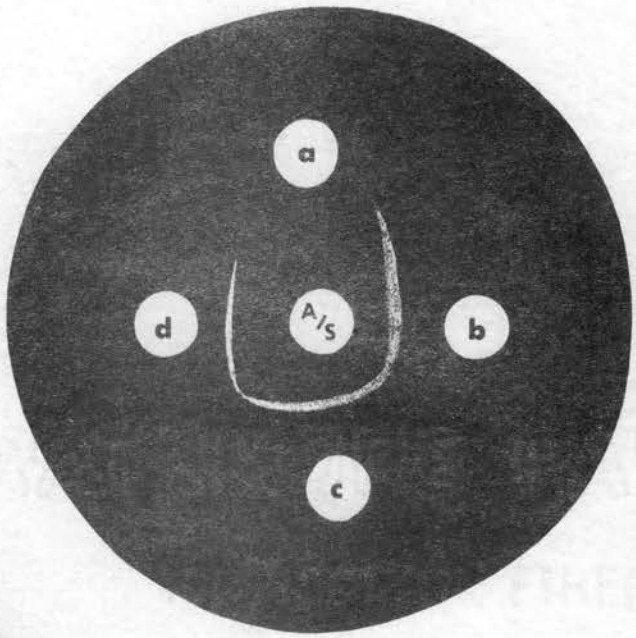


Figure 11. Interaction of antisera (A/S) to purified GH with diluted antigen. The GH concentrations shown are 0 ⟶ (a), 5.0 (b), 2.5 (c), and 1.25 (d), μg./ml. in saline.

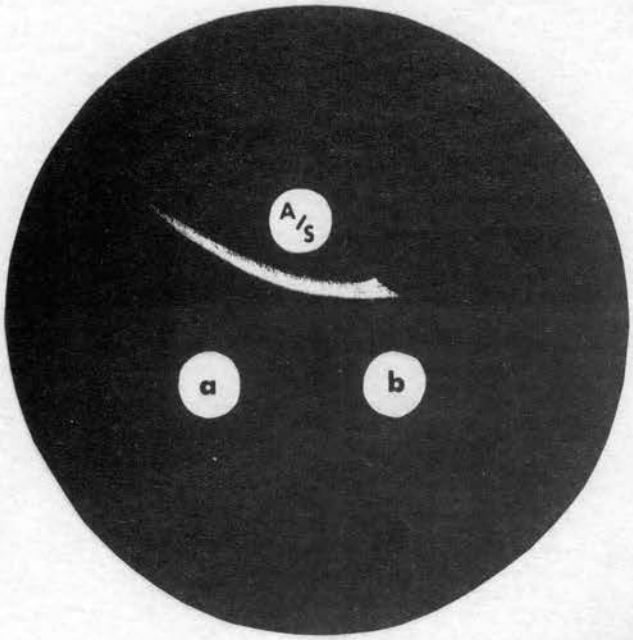
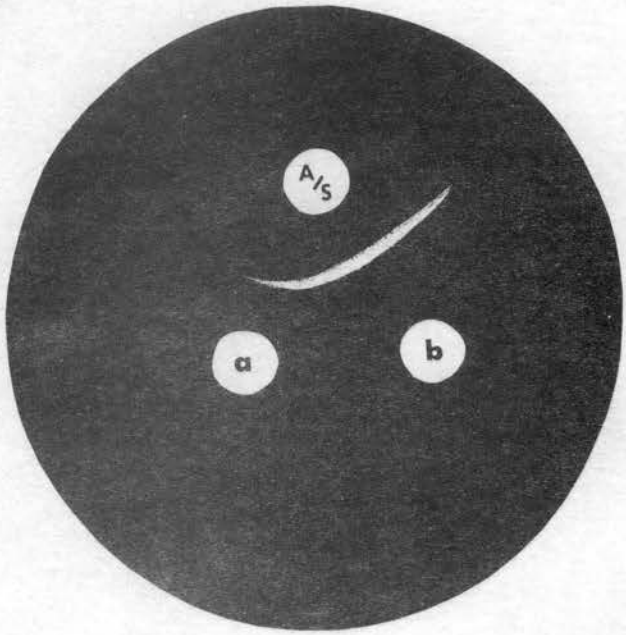


Figure 12. Interaction of antisera (A/S) to purified GH with 5.0 μg./ml. GH in saline (a) and 10% gamma globulin (b). ⟵

Figure 13. Interaction of antisera (A/S) to purified GH with 100 ⟶ ug./ml. prolactin in saline (a) and 5.0 μg./ml. GH in saline (b).



However, rabbit cells (when obtained from a young rabbit) were capable of combining small quantities of antigen. By this method it was possible to detect as little as 0.03 µg. of GH per ml. of calf blood plasma. Sera from these same animals, however, were found to be of no value due to spontaneous agglutination. All reactions were carried out in duplicate and compared to a GH standard as outlined under procedures. Occasionally the end points were indistinct or duplicate end points varied widely. In these cases the analyses were repeated with satisfactory results.

TABLE XVII

EFFECT OF ENERGY INTAKE ON THE GROWTH HORMONE CONTENT OF BLOOD PLASMA OF 7 MONTH OLD HOLSTEIN HEIFERS

Block	Level of Energy Intake (% of N.R.C. Standard)		
	75	100	125
	µg./100 ml.	µg./100 ml.	µg./100 ml.
1	93.6	93.6	93.6
2	124.8	93.6	93.6
3	93.6	156.0	31.2
4	156.0	93.6	195.0
5	93.6	195.0	124.8
6	124.8	156.0	62.4
7	156.0	78.0	156.0
8	156.0	156.0	--- [a]
9	156.0	--- [b]	78.0
10	156.0	78.0	124.8
Mean	131.0	122.2	106.6

[a] Sample clotted, value estimated for statistical analysis as outlined by Steel and Torrie (125).

[b] Animal taken off experiment at 5 months of age; value estimated for statistical analysis as mentioned above.

The results of determining the GH concentration in the plasma of 28 Holstein heifers are reported in Table XVI. The mean GH potencies for the 75, 100, and 125 percent groups were found to be 131.0, 122.2, and 106.6 ug./100 ml. of plasma, respectively. The differences among treatment means were not significant ($P > 0.10$). However, there appeared to be a tendency for the animals on the high level of energy intake to have lower plasma concentrations of the hormone than the low energy intake group. This might well be expected since the high plane animals were larger and, therefore, were physiologically older at the time they were tested.

Armstrong and Hansell (3) similarly found no significant differences between the GH content of pituitary glands of Holstein heifers reared on either 60, 110, or 140 percent of Morrison's standard for growing animals. The GH potencies reported above are in close agreement with those reported by Wallace (140) for mature sheep and Trenkle et al. (134) for one year old beef heifers.

SUMMARY

Twelve Holstein cows were used to evaluate the effect of three levels of growth hormone (GH) implants, and 15 cows were used to compare the effects of injecting a commercial grade of the hormone with a highly purified (NIH) GH-preparation. The average daily milk production for the cows implanted with 0, 300, and 600 Armour units of GH was 41.5, 40.4, and 42.5 lb., respectively. The differences among adjusted treatment means were not significantly different ($P > 0.05$). Likewise, there were no significant differences ($P > 0.1$) among groups with respect to total fat, body weight changes, blood glucose level, total leucocytes, or incidence of mastitis.

The average daily milk production for the cows injected with 50 mg. per day of either NIH-purified or the commercial GH preparation was 36.1 and 42.0 lb., respectively, whereas the control group produced an average of only 34.5 lb. per day over a 14-day production period. The differences among treatment means were highly significant ($P < 0.01$), as were differences among groups with respect to pounds of fat, when comparing the commercial GH treated group with all other groups. No significant differences were noted among treated groups with respect to pounds of solids not-fat, body weight changes, blood glucose level, total leucocytes, or incidence of mastitis.

The mean daily thyroid secretion rate (TSR) per 100 kg. body weight was 1.04, 0.70, and 0.72 mg. per day, respectively, for the Holstein

heifers reared on 75, 100, and 125 percent of N.R.C. energy requirements. However, when the TSR per day was adjusted to metabolic size the differences among treatment was not significantly different.

The average GH potencies of blood plasma obtained from Holstein heifers receiving 125, 100, and 75 percent of N.R.C. requirements were found to be 106.6, 122.2, and 131.0 μg. per 100 ml. of blood plasma, respectively, at 7 months of age. These differences were not significantly different ($P > 0.10$). The higher blood GH levels and TSR of the group maintained on 75 percent of standard is believed due to the differences in physiological age, as opposed to chronological age, among the three groups of animals.

Restricting the energy intake of Holstein hiefers during the rearing period affected body weight and heart girth to a greater extent than height and length. The group fed at 125 percent of standard was considerably more efficient than the 75 percent group in converting dietary energy into body growth. The age at puberty was delayed considerably in the group fed at 75 percent of standard, whereas the age at which the other groups reached puberty appeared typical for Holstein heifers.

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A P P E N D I X

TABLE XVIII

MILK PRODUCTION BY TREATMENTS AND SQUARES (GROUPS) OF COWS IMPLANTED WITH GROWTH HORMONE

	Treatments			
Squares	1	2	3	Total
1	4,247	4,062	4,474	12,783
2	5,011	5,216	5,597	15,824
3	5,918	5,613	5,878	17,409
4	5,729	5,447	5,493	16,669
Total	20,905	20,338	31,442	

TABLE XIX

TOTAL SOLIDS BY TREATMENTS AND SQUARES (GROUPS) OF COWS IMPLANTED WITH GROWTH HORMONE

	Treatments			
Squares	1	2	3	Total
1	522.7	509.8	550.7	1,583.2
2	604.5	641.0	686.6	1,932.1
3	691.3	663.8	689.3	2,044.4
4	705.3	671.8	690.6	2,067.7
Total	2523.8	2537.3	2617.2	

TABLE XX

TOTAL FAT BY TREATMENTS AND SQUARES (GROUPS) OF COWS IMPLANTED WITH GROWTH HORMONE

	Treatments			
Squares	1	2	3	Total
1	159.1	152.5	166.4	478.0
2	177.7	195.7	205.9	579.3
3	229.6	203.5	208.8	641.9
4	212.6	203.5	208.8	624.9
Total	779.0	755.2	789.9	

TABLE XX I

ANALYSIS OF VARIANCE ON PERCENT TOTAL SOLIDS FROM COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Source of Variation	df	xx	xy	yy	y Adjusted for x		
					df	SS	MS	F
Total	14	17.057	21.208	35.085				
Blocks	4	2.414	2.802	4.923				
Treatments	2	0.195	5.444	12.839				
Error	8	14.447	12.962	17.323	7	5.694	0.8134	
Treatments Plus Error	10	14.643	18.406	30.162	9	7.026		
Treatments Adjusted					2	1.332	0.6669	1.221

[a]Treatments A - Somar
B - NIH Growth Hormone
C - Control.

TABLE XXII

ANALYSIS OF VARIANCE ON PERCENT FAT FROM COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Source of Variation	df	xx	xy	yy	df	y Adjusted for x SS	MS	F
Total	14	6.137	7.374	14.964				
Blocks	4	3.012	2.491	2.957				
Treatments	2	0.369	1.152	4.212				
Error	8	2.757	3.731	7.795	7	2.745	0.392	
Treatments Plus Error	10	3.127	4.883	12.007	9	4.3799		
Treatments Adjusted					2	1.634	0.817	2.084

[a]Treatments A - Somar
B - NIH Growth Hormone
C - Control.

TABLE XXIII

ANALYSIS OF VARIANCE ON POUNDS SOLIDS NOT-FAT FROM COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Source of Variation	df	xx	xy	yy	df	y Adjusted for x SS	MS	F
Total	14	2731.8	3464.59	4718.21				
Blocks	4	2225.15	2600.89	3057.52				
Treatments	2	213.31	460.51	1023.69				
Error	8	293.41	403.19	636.99	7	82.99	11.85	
Treatments Plus Error	10	506.73	863.69	1660.69	9	-173.37		
Treatments Adjusted					2	-256.36	-128.18	-10.811

[a]Treatments A - Somar
B - NIH Growth Hormone
C - Control.

TABLE XXIV

DIFFERENTIAL BLOOD COUNTS AND BLOOD GLUCOSE LEVELS OF COWS IMPLANTED WITH GROWTH HORMONE[a]

Treatment	None	300 Armour Units	600 Armour Units
Total Leucocytes	10,829	10,042	10,100
Neutrophils			
Segmented	27	29	24
Band	3	3	3
Eosinophils	9	6	7
Basophils	1	0	1
Lymphocytes	56	56	57
Monocytes	6	7	5
Blood Glucose, mg.%	53	54	53

[a]Treatment averages.

TABLE XXV

DIFFERENTIAL BLOOD COUNTS AND BLOOD GLUCOSE LEVELS OF COWS INJECTED WITH GROWTH HORMONE PREPARATIONS[a]

Treatment	Somar	NIH-Growth Hormone	Saline
Total Leucocytes	9,947	11,007	10,501
Neutrophils			
Segmented	23	19	16
Band	9	5	8
Eosinophils	7	7	10
Basophils	0	0	0
Lymphocytes	57	63	61
Monocytes	5	6	5
Blood Glucose, mg.%	49	48	48

[a]Treatment averages.

TABLE XXVI

ANALYSIS OF VARIANCE ON TTR[a] PER DAY (MG.) OF 12 MONTH OLD HOLSTEIN HEIFERS

Source of Variation	Degrees of Freedom	Mean Square	F
Total	28	0.033	
Blocks	9	0.073	4.9456[b]
Treatments	2	0.007	0.4898
Error	17	0.013	

[a]Total Thyroidal Thyroxine, mg. per day.

[b]$P < 0.005$.

TABLE XXVII

ANALYSIS OF VARIANCE ON MEAN NORMAL PBI (µG.)[a] OF 12 MONTH OLD HOLSTEIN HEIFERS

Source of Variation	Degrees of Freedom	Mean Square	F
Total	28	10.34	
Blocks	9	23.02	5.0445[b]
Treatments	2	2.43	0.5326
Error	17	4.56	

[a]Mean Normal Protein Bound Iodine Expressed as µg./100 mL blood.

[b]$P < 0.005$.

VITA

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