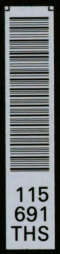

EFFECT OF DIETARY IODIDE ON PITUITARY AND THYROID HORMONE SECRETIONS IN HOLSTEIN HEIFERS

by

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ABSTRACT

EFFECT OF DIETARY IODIDE ON PITUITARY AND
THYROID HORMONE SECRETIONS IN HOLSTEIN HEIFERS

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The effect of various quantities of dietary iodide on development of the pituitary-thyroid axis was studied in dairy heifers. Heifers ranged in age from 11 to 15 weeks at the start of experiment and were randomly assigned to one of four treatment groups to be given 0, 50, 250 or 1250 mg supplemental iodide in the form of ethylenediamine dihydriodide for 25 weeks.

Supplemental iodide at a dose of 1250 mg per day decreased both basal tri-iodothyronine (T_3) and thyroxine (T_4) concentrations relative to comparable values for controls. Basal concentrations of thyrotropin (TSH) in serum of these heifers was greater than that of controls on day 78 of treatment.

Thyrotropin releasing hormone (TRH) was given on day -5, at 28 day intervals during treatment and on day 10 post-treatment. During iodide feeding period, TSH release by TRH was greater in heifers being fed 1250 mg iodide than in controls. However the increase in T_3 and T_4 in serum of these heifers in response to the TRH-induced increase of TSH were not different among groups.

Daily body weight gains of groups fed either 250 or 1250 mg iodide were less than that of controls during the first 21 and 77 days respectively, of the treatment. Prolonged decrease in daily weight gains of heifers fed 1250 mg supplemental iodide daily resulted in lower body weights for these heifers at 10 months of age.

Heifers fed 1250 mg dietary iodide had increased thyroid weights when expressed on a per 100 kg body weight basis. Iodide feeding did not affect either adrenocortical function or time of onset of puberty.

I conclude that the concentrations of iodide normally fed to dairy heifers in Michigan does not affect the development of their pituitary-thyroid axis.

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INTRODUCTION

The endocrine system regulates growth, reproduction and lactation of domesticated animals. Understanding the mechanism by which these events are controlled may allow manipulation of the endocrine system to achieve maximum expression of economically important traits in animals including dairy cattle.

Hillman in 1975 received a substantial number of complaints from Michigan dairy farmers concerning poor health and reduced milk production among cows in their herds and also reduced growth rate of their calves. Preliminary investigations indicated that 13 of 17 problem herds were fed iodide in excess of National Research Council requirements (unpublished observations). In some cases, daily iodide intake was 80 times the recommended requirement.

Iodide is often added to protein and mineral supplements, concentrates, trace mineralized salt and vitamin-mineral pre-mixes. Diets for cattle usually consist of combinations of these feedstuffs. In addition, other iodide compounds such as ethylenediamine dihydriodide are usually prescribed by veterinarians to treat or prevent footrot, fungal infections, and respiratory diseases. Thus, iodide intake could greatly exceed the recommended daily quantity. In addition, McCauley et al (1972) and Wallace (1975) described toxic reactions that could follow routine use of iodide compounds.

The purpose of this thesis was to investigate the effects of dietary iodide on development of the pituitary-thyroid axis of dairy heifers.

REVIEW OF LITERATURE

A. Embryology

I. Hypothalamus and Anterior Pituitary

The hypothalamus develops from the walls and floor of the diencephalon. A medial depression in the floor of the diencephalon forms the infundibular stalk. The anterior pituitary originates as an outgrowth from the roof of the embryonic pharynx. This diverticulum migrates upward toward the infundibular stalk. It retains its connection with the pharynx via a slender duct until the formation of the skull. With the disappearance of this duct, the diverticulum becomes an enclosed vesicle. The caudal portion of this vesicle contacts the infundibular stalk and develops into the pars intermedia. The rostal portion proliferates and becomes the pars distalis. The residue of the duct, at least in humans, forms the pharyngeal hypophysis which may contain adrenocorticotropin, prolactin and thyrotropin (Allen and Mahesh, 1976).

II. Thyroid

a. General

The thyroid is the earliest diverticulum arising midline on the pharynx between the first and second pairs of pharyngeal pouches. Thyroid primordial cells lying in the wall of this bilobed evagination penetrate the underlying mesenchyme and migrate caudad. During migration, the thyroid remains connected to the floor of the foregut via the thyroglossal duct. This duct later solidifies and disappears. At the point of origin of the thyroid gland, there remains a depression on the pharynx named the foramen caecum.

The thyroid moves caudad under the hyoid bone and laryngeal, cartilage, finally reaching its permanent position at the ventral and anterior surface of the trachea. As the thyroid proliferates laterally, the postbranchial bodies which arise from the fourth pharyngeal pouches become incorporated into the gland. Whether or not true thyroid tissue is formed by proliferation of these bodies is still unanswered (Patten, 1968, Langman, 1969). By this time, the thyroid has acquired its final shape, i.e. two lateral lobes linked by a medial isthmus.

b. Follicles

The initial primordial thyroid consists of a solid columnar cell mass arranging radially about a small lumen. As cells divide and get smaller, vascular mesenchyme which will develop into highly vascularized connective tissue facilitating the transport of thyroid hormones, seperate them into cords of cells. These epithelial cords then break up into nests of cells and form central lumens. Later in embryonic development, colloid, a major product of thyroid epithelial cells, starts to accumulate in the lumens (Valdes-Dapena, 1957).

B. Anatomy

I. Gross

a. Hypothalamus and Anterior Pituitary

The hypothalamus is located at the ventral medial portion of the brain. It forms the floor and lateral walls of the third ventricle and is delineated anteriorly by the optic chiasma and posteriorly by the mammillary bodies. It is attached to pituitary by the infundibular stalk.

The anterior pituitary is located in a deep depression in the sphenoid

bone called the sella turcica. It is separated from the intracranial cavity by the diaphrama sella which is penetrated by the infundibular stalk. The anterior pituitary is generally divided into three regions, i.e. pars intermedia which is attached to the posterior pituitary; pars tuberalis which surrounds the anterior and lateral surfaces of the infundibular stalk; and pars distalis which forms the major mass of the pituitary body.

The superior hypophyseal arteries which arise from the internal carotid arteries form the capillary network in the median eminence of the hypothalamus and infundibular stalk. From here, blood is collected and conducted via portal vessels to the sinusoids of the anterior pituitary. The venous return empties into the hypophyseal veins leading to the cavernous sinus. There is no evidence that sympathetic or parasympathetic innervation of the anterior pituitary play any part in regulating activity of this gland (Harris, 1955).

b. Thyroid

The thyroid gland is composed of spherical follicles which are lined with a single layer of epithelial cells and contain colloid in their central lumen. Thyroglobulin is the major component of this colloid.

An extensive network of capillaries surrounds each follicle and between these capillaries are lymphatic vessels which drain into the cervical lymph node. Blood is mainly supplied by the thyroid branch of the thyrolaryngeal artery. The caudal thyroid artery, if present, enters the caudal end of the lobe. The thyroid veins run parallel to the arteries and empty into the external jugular vein (Getty, 1975).

Both sympathetic and parasympathetic fibers innervate the thyroid gland. These nerve fibers run along and terminate mainly on blood vessels.

Although in some instances, they appear to make direct contact with follicular epithelial cells. Experiments wherein the thyroid was transplanted or denervated have shown that neural control has no effect on thyroid secretion (Turner and Bagnara, 1976).

II. Microscopic

a. Hypothalamus and Anterior Pituitary

Neurons in the hypothalamus have been categorized into two groups, i.e. magnocellular and parvicellular neurosecretory cells (Knigge and Silverman, 1974). The former group has large cell bodies and is located primarily in the supraoptic and paraventricular nuclei. These neurons are responsible for the synthesis of oxytocin and vasopressin. Parvicellular neurons which are relatively small and diffusely located are believed to synthesize and secrete releasing hormones into the hypophysial portal vessels in the infundibular stalk to influence anterior pituitary functions.

The anterior pituitary is composed of irregular cords of glandular cells which are intimately related to an extensive network of sinusoids. Recent development of immunohistochemical stains enables one to establish the cellular origin of various protein hormones produced in the anterior pituitary. Thyrotropin (TSH) is produced in thyrotrophs which are of polygonal shaped and have granules that are among the smallest in the pituitary (Bloom and Fawcett, 1975). The concentration of thyrotrophs is much greater in the medulla than in the cortex of the anterior pituitary gland (Jubb and McEntee, 1955).

b. Thyroid

Follicular epithelial cells vary in height according to the functional activity of the gland. In general, these cells tend to be cuboidal or low columnar in shape. An extensive endoplasmic reticulum, prominent Golgi

apparatus, numerous mitochondria and colloid droplets are the major features of this cell. There are also microvilli extending from the apical end of the cells into the lumen. The number of these microvilli increases following thyrotropin treatment (Turner and Bagnara, 1976).

In the interfollicular spaces and between follicular cells, there are lightly stained cells called parafollicular cells, or C-cells. These cells secrete calcitonin which decreases serum calcium concentrations.

C. SYNTHESIS AND SECRETION OF THYROID HORMONES AND THYROTROPIN

Thyroid hormone synthesis and secretion have been extensively reviewed by DeGroot and Stanbury (1975). A brief description follows.

I. Iodide Uptake

Dietary iodine is reduced to iodide before it is absorbed from the gastrointestinal tract into blood. Concentrations of inorganic iodide in plasma of cows is about 180 ng/ml (Convey et al. 1978). The epithelial cells of the thyroid have the greatest ability to concentrate iodide among all tissues of the body. The concentration gradient of iodide between thyroid and plasma can reach 20:1. Iodide transport activity of the thyroid gland is augmented by TSH and decreased when TSH secretion is suppressed. Although the salivary glands and gastric mucosa also actively transport iodide and establish a concentration gradient between cytoplasm and plasma, TSH does not alter iodide uptake by these glands. Iodide is excreted from the body primarily by the kidney but also via the mammary gland during lactation.

II. Thyroid Hormones

Thyroglobulin, the precursor of the thyroid hormones, is the major glycoprotein in colloid. The protein portion of thyroglobulin is formed at membrane bound polyribosomes on the endoplasmic reticulum and sugars are added both at the endoplasmic reticulum and Golgi apparatus. Newly formed molecules are then transported into colloid and iodination occurs just outside apical ends of the follicular cells. The iodination process requires peroxidase, H_2O_2 and tyrosine from thyroglobulin as an iodide acceptor. Radiographs of thyroid sections demonstrate protein bound iodine 15 to 20 seconds after intravenous administration of ^{131}I. Of iodide formed in the thyroid, 90 to 95% is bound to tyrosyl residues in thyroglobulin.

Iodination of tyrosine yields mono-iodotyrosine (MIT), which is further iodinated to form di-iodotyrosine (DIT). It has been suggested that the linear form of thyroglobulin is iodinated. After iodination, thyroglobulin develops a coiled secondary conformation and places some of the iodinated tyrosines close to one another. The coupling of two DIT yields thyroxine (3,5,3',5'-tetra-iodothyronine or T_4) and coupling of MIT and DIT yields tri-iodothyronine (3,5,3'-tri-iodothyronine or T_3). These couplings proceed at a much slower rate than does iodination of tyrosine.

The ratio of DIT/MIT (or T_4/T_3) in the thyroid is a function of availability of iodide. When the supply of iodide is plentiful, much thyroxine or DIT are found. With a low iodide diet, there is a greater probability of coupling of MIT and DIT, thus decreasing the ratio T_4 to T_3.

Under TSH stimulation, thyroglobulin reenters the follicular cells by endocytosis and intracellular droplets are formed. These droplets fuse with lysosomes to form phagosomes. Thyroid hormones are cleaved from thyroglobulin by proteolytic enzymes and are released into the blood.

In blood, more than 99% of serum thyroxine is bound to thyroxine binding globulin, albumin and prealbumin, whereas tri-iodothyronine is

weakly associated with binding globulin and albumin (Ingbar, 1963). This weak association with serum protein may be the reason for the high turnover rate of tri-iodothyronine. The biological half-life of tri-iodothyronine is about 1 to 2 days and that of thyroxine 6 to 7 days in man.

Sterling (1970) suggested that one third or more of tri-iodothyronine in blood may arise from peripheral conversion of thyroxine in organs such as liver and kidney. Reverse tri-iodothyronine (3,3',5'-tri-iodothyronine) which is also found in blood is synthesized by the thyroid and also produced by peripheral deiodination of thyroxine. However, no physiological function of reverse tri-iodothyronine has been demonstrated.

III Thyrotropin

Thyrotropin is composed of 2 peptides, an α subunit which is common to TSH and the pituitary gonadotropins and a β subunit in which hormonal specificity resides. Only non-covalent forces are involved in subunit-subunit interaction (Pierce et al. 1976). Immunohistochemical studies showed that both subunits are synthesized in the same cells in the pituitary (Baker et al., 1972). In normal pituitaries there is a large pool of free α subunit relative to β subunit, in addition to thyrotropin.

D. CONTROL OF TSH SECRETION

I. General

Grafe and Grunthal (1929) reported that dogs with massive diencephalic damage had a low metabolic rate. Houssay and coworkers (1935) demonstrated that hypothalamic lesions in the infundibulotuberal regions in toads led to reduced cell height of the thyroid epithelium. These studies provided early specific evidence that the anterior pituitary is at least partially controlled

by the central nervous system.

Green and Harris (1947) described the vascular portal system between the hypothalamus and pituitary. Scharrer and Scharrer in 1954 discovered that the hypothalamus was capable of secreting hormones. These observations formed the basis for the portal vessel chemotransmitter hypothesis. This hypothesis proposed that the hypophysiotropic hormones were synthesized by neurons in the hypothalamus, transported to and stored in the neural endings in the stalk median eminence, released into interstitial spaces around the portal capillary plexus and distributed throughout the anterior pituitary via the portal vessels.

II Thyrotropin Releasing Hormone

The earliest documented studies of thyrotropin releasing hormone (TRH) are those by Shibusawa and coworker (1956). Schreiber et al. (1961) demonstrated that incubation of a purified extract of bovine hypothalamus with rat pituitaries led to an increased release of thyrotropin into the medium. Five years later, Schally et al. (1966) reported that TRH is consisted of an equal molar ratio of 3 amino acids i.e. glutamate, histidine and proline. By the end of the decade, laboratories of Guilleman and Schally independently reported the amino acid sequence of TRH isolated from ovine and porcine hypothalami (Burgus et al., 1969 and Bowers et al., 1970). Subsequently, specific radioimmunoassays for TRH were developed and total hypothalamic TRH measured in hypothalami of various species. Demonstration of TRH in the hypophysial portal blood of rats (Wilber and Porter, 1970 and Oliver et al., 1973) and increased thyrotropin concentration in rats after infusion of TRH into the hypophysial portal vessel (Porter et al., 1971) provided further support to the portal vessel chemotransmitter hypothesis.

TRH has also been found in: 1) extrahypothalamic brain tissue of rats; 2) spinal cord of rats; 3) bovine, ovine and porcine pineal glands and; 4) cerebrospinal fluid of rats and humans (reviewed by Reichlin et al., 1976). In fact 80% of total brain TRH was found in extrahypothalamic areas. TRH has also been measured in serum and urine, but these findings may be artifact. For example,calculations based on TRH secretion rate, metabolite clearance rate and urinary excretion, estimate that the amount of TRH present in blood should be less than 5 pg/ml, which is below the level of sensitivity of TRH assays. Vagenakis et al. (1975) also suggested that measures of TRH in urine are probably not correct.

Mitnick and Reichlin (1972) reported that TRH formation continued in the presence of inhibitors of protein synthesis such as cycloheximide and puromycin, and that TRH synthesis required an ATP dependent soluble enzyme system. These observations led to a proposal that TRH was synthesized by a nonribosomal system mediated by an enzyme "TRH synthetase". The half-life of TRH in serum is very short, of the order of 3 to 7 minutes (reviewed by Sterling and Lazarus, 1977).

III. Neural Regulation of TRH Secretion

The neurosecretory neurons that synthesize and release TRH are believed to be capable of transforming a neural message into hormone output. They serve as the link between the central nervous system and anterior pituitary. Experiments in which various neurotransmitters were added to incubated hypothalamic fragments showed that both dopamine and norepinephrine increased and serotonin decreased release of TRH into culture medium (Grimm and Reichlin, 1973). Disulfiram, which inhibits the action of dopamine β hydroxylase to convert dopamine to norepinephrine, blocked the TRH response to dopamine, but not norepinephrine. Clonidine,

a noradrenergic receptor agonist, caused a significant increase of thyrotropin in serum (Annunziato et al., 1977). These studies support the idea of positive control of TRH secretion by noradrenergic neurons and negative control by serotoninergic neurons.

IV. Short Loop Feedback

In 1958, Halasz and Szentagothai first hypothesized the existence of a short-loop feedback in which anterior pituitary hormones antagonized their own synthesis and release. Motta et al. (1969) reported that thyrotropin given to thyroidectomized rats for 16 days lowered hypothalamic TRH concentration. The presence of thyrotropin in the stalk median eminence (Bakke and Lawrence, 1967) and subependymal network that drains blood from the anterior pituitary toward the hypothalamus (Torok, 1964) is consistent with the possibility of a short loop feedback of thyrotropin.

V. Feedback from Thyroid Hormones

The pituitary-thyroid axis is a classical example of a negative feedback system. The anterior pituitary secretes thyrotropin which stimulates the secretory activity of the thyroid. In turn, thyroid hormones regulate thyrotropin secretion through a direct action on the anterior pituitary (Reichlin, 1966). Inhibitors of protein synthesis such as puromycin, cycloheximide and actinomycin D prevent inhibition of thyrotropin release by thyroid hormones (Bowers et al., 1968). In addition, exogenous tri-iodothyronine decreased the thyrotropin response to TRH only if serum T_3 concentration remained elevated for at least 48 hours (Wartofsky et al., 1976). Collectively, these results suggest that the negative feedback effect of thyroid hormones on thyrotropin secretion requires a period during which protein synthesis takes place (Sterling and Lazarus, 1977).

E. MECHANISM OF THYROTROPIN ACTION

The mechanism of thyrotropin action on the thyroid function has been reviewed by Tong (1974). Thyrotropin binds to a receptor on the epithelial membrane of thyroid cells. Following binding, adenyl cyclase activity increases as does the intracellular concentration of cyclic adenosine monophosphate (cAMP). cAMP is believed to serve as a second messenger stimulating colloid endocytosis, release of thyroxine and tri-iodothyronine, glucose oxidation and synthesis of thyroidal RNA, phospholipid and protein. The question remains as to how so many responses of such diverse character can be triggered so rapidly in virtually simultaneous fashion.

F. EFFECT OF EXCESS IODIDE

The acute effect of excess iodide on thyroid function was first observed by Wolff and Chaikoff in 1948. Increasing doses of iodide caused a decrease in macromolecular organification. Excess iodide also causes a decrease in thyrotropin induced iodide concentrating activity (DeGroot and Stanbury, 1975). Formation of hypoiodous acid, tri-iodide and I_2 (Nagataki, 1974) and protein-iodide complexes (VanSande et al. 1975) were suggested as active agents that caused this acute inhibitory action of iodide.

Prolonged thyroid exposure to serum iodide concentrationa 100 times greater than normal could not maintain the acute effect of iodide for longer than 26 hours (Wolff et al., 1949). Nagataki et al. (1966) demonstrated that in rats given high doses of iodide (45 to 405 μg/day) for at least 10 days,

formation of thyroidal organic iodide was several times greater than that in controls fed 5 μg/day. However, concentration of serum iodothyronine and the disappearance rate of labeled thyroxine were not altered by excess iodide. Since the release rate of thyroid hormones did not increase, the thyroid must either increase storage of the increased amounts of organic iodide or secrete it in a nonhormonal form. In support of this view, Ohtaki et al. (1967) demonstrated increased release of nonhormonal iodide from the thyroid of patients on a high iodide diet. Braverman and Ingbar in 1963, showed that thyroid glands which have adapted to high iodide actually have a lower intrathyroidal iodide concentration than in controls. Nagataki et al. (1970) suggested that iodide treated rats decreased the reutilization of intra-thyroidal iodide derived from the deiodination of iodothyronine freed from thyroglobulin.

Of human patients exposed to excess iodide, few show evidence of goiters (Nagataki, 1974). Newton et al. (1974) also reported that Holstein bull calves fed up to 200 ppm iodide had normal thyroid weight, but had decreased weight gains and feed intake. However, Wallace in 1975 observed thyroid hypertrophy and adrenocortical hyperplasia in Georgia dairy herds fed an average of 107 mg iodide daily.

McCauley et al. (1974) demonstrated that beef and dairy herds in Minnesota, which were fed ethylenediamine dihydriodide (EDDI) for prevention of footrot, showed labored breathing, coughing, excessive nasal and lacrimal secretion, sluggishness, inappetence, salivation and fever. These symptoms gradually disappeared after the termination of EDDI treatment.

Swanson (1972) reported that milk production decreased in Holstein cows fed 100 mg iodide as KI beginning about 8 weeks prepartum. Daily milk yield of cows receiving supplemental dietary iodide averaged 27.8

kg/day compared with 31.6 kg/day for the controls. Increased iodide concentration in milk from cows fed 100 mg iodide was also observed. However, concentrations of plasma thyroxine, thyroxine disappearance rate and thyroxine secretion rate were not different between treatment groups.

MATERIALS AND METHODS

I. Experimental Animals

Forty Holstein heifers were purchased in Indiana. Heifers ranged in age from 11 to 15 weeks at the start of experiment. They were randomly assigned to one of four treatment groups to be given 0, 50, 250 or 1250 mg supplemental iodide daily for 25 weeks. Iodide was given in the form of ethylenediamine dihydriodide (EDDI) orally via drench. The day iodide treatment began was designed day zero.

Analyses of iodide content in the pelleted concentrate, alfalfa hay and drinking water revealed that all heifers were fed approximately 2.8 mg (0.51 pm) iodide daily at the onset of experiment. This quantity is about twice that (.25 ppm) recommended for growing heifers by the National Research Council (Jacobson et al. 1978). Heifers in the other three treatment groups were fed 32, 160 and 800 times the daily recommended requirement of iodide. The amount of iodide fed each group was constant during the treatment period. Therefore, iodide doses calculated on a body weight basis had decreased by approximately one half by the end of iodide feeding relative to that fed at the start of treatment (Table 1).

Five heifers in each group were randomly selected to be given thyrotropin releasing hormone (TRH, 15 μg/100 kg body weight) via jugular cannula at 5 days before the start of iodide treatment, 28 day intervals during treatment and 10 days post-treatment. The same five heifers from each group were used throughout. Those heifers given TRH were taken off iodide

Table 1. Doses of Iodide (mg/kg body weight/day) During Supplemental Iodide Feeding

Supplemental Iodide (mg/day)	Days of Treatment				
	20	48	76	104	160
0	.03 ± .002[a]	--	--	--	--
50	.4 ± .02	.3 ± .01	.3 ± .01	.3 ± .01	.2 ± .01
250	1.9 ± .06	1.7 ± .07	1.5 ± .06	1.3 ± .06	1.0 ± .04
1250	11.0 ± .48	10.0 ± .47	9.0 ± .46	7.8 ± .42	5.9 ± .31

[a] Values are mean ± SE

feeding after 172 days whereas the other five heifers from each group were fed iodide until the day of autopsy (≈ 190 days). This experiment took place between September 1976 and March 1977. Animals were housed in groups of 3 to 5 with controlled lighting (18L:6D) and temperature (10-15°C). Heifers were fed 1.8 kg pelleted concentrate daily and alfalfa hay ad libitum. All heifers were weighed 1 or 2 days prior to each TRH injection.

Jugular blood was collected via veni-puncture at weekly intervals from all heifers. Selected samples (see Results) from these bleedings were assayed for thyrotropin, thyroxine, tri-iodothyronine, progesterone and total corticoids. In addition, blood was obtained from jugular cannula at -30, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 and 240 min relative to TRH injection. Serum thyrotropin, thyroxine and tri-iodothyronine concentration of these samples were determined by radioimmunoassay.

Autopsy of these heifers was performed during a two week period beginning 12 days post-treatment. Thyroid, adrenal and pituitary glands were removed, trimmed and weighed. Since final body weights were not obtained from these animals at autopsy, body weights obtained on day 9 post-treatment were used to adjust thyroid, adrenal and pituitary weights for body weight differences.

II. Assays

Serum thyrotropin was assayed by double antibody radioimmunoassay (Appendix 1). Various dilutions of two pools of bovine sera containing low or high concentration of thyrotropin were assayed in each assay to ensure parallelism between standard sera and standard curves.

Thyroxine and tri-iodothyronine concentrations in serum were assayed using commercial radioimmunoassay reagents (Appendix 2 and 3, respectively).

Dilution curves of pooled bovine serum were parallel to both thyroxine and tri-iodothyronine standard curves. Known quantities of thyroxine or tri-iodothyronine added to bovine serum were quantitatively recovered (111.7% for thyroxine assay and 99.6% for tri-iodothyronine). Six to eight serum samples from a pooled bovine serum were assayed in each assay. Within and among assay coefficient of variation determined from 12 thyroxine assays were 5.5 and 4.5%. Comparable values for 7 tri-iodothyronine assays were 5.3% and 10.4%.

Selected serum samples were assayed for progesterone and total glucocorticoids by radioimmunoassay previously described by Louis et al. (1973) and Smith et al. (1972), respectively.

III. Statistical Analysis

Basal serum hormone concentrations prior to TRH, body weight gains, body weight and tissue weights were analyzed by one way analysis of variance. Changes in serum hormone concentration following TRH and basal hormone concentration over time were analyzed by split-plot analysis of variance for repeat measurements (Gill and Hafs, 1971). Significance of differences among treatment were determined by Dunnett's t test (Kirk, 1968). Onset of puberty data were analyzed using a Chi-square test of contingency table.

RESULTS

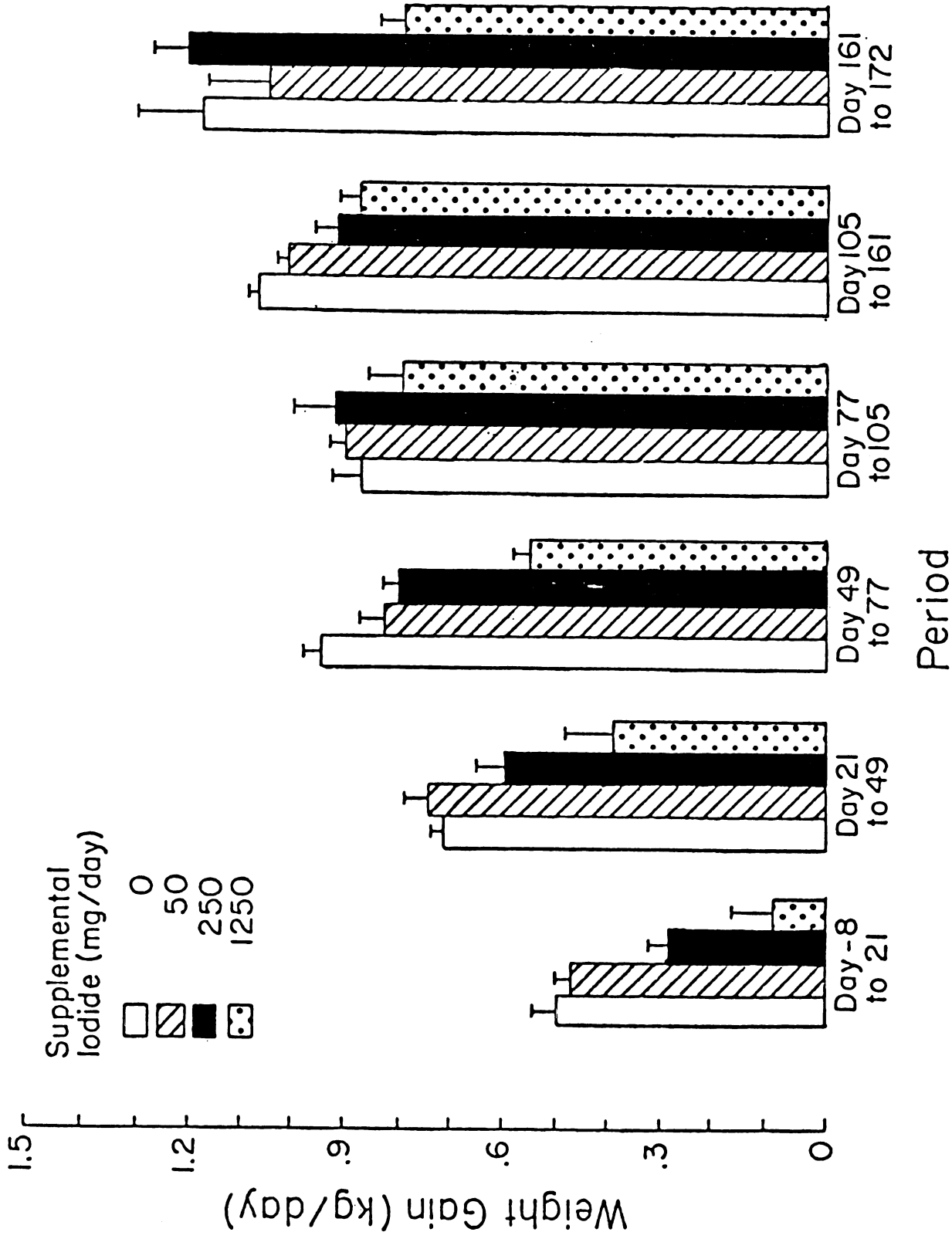
Two heifers from the group fed 1250 mg of iodide as EDDI died of bronchopneumonia during the treatment period. Data from these animals are excluded from results discussed below.

Those heifers that received TRH every four weeks were not fed iodide after day 162 (see Materials and Methods), whereas those not given TRH were fed iodide until autopsy ($\simeq$190 days). A within treatment comparison between heifers given TRH and those not given TRH revealed that differences in body weight, body weight gain, and endocrine gland weight adjusted for body weight were not different ($P > .10$). Therefore, values presented in Figure 1 and Table 2 represent means for all animals within each treatment group.

I. Body and Tissue Weights

Body weights of these heifers averaged 121 kg at 8 days prior to the onset of iodide feeding and differences among treatment groups were not significant ($P > .10$). After 21 days of feeding supplemental iodide, daily weight gains for heifers fed 250 and 1250 mg of iodide were (0.28 and 0.09 kg/day respectively) lower ($P < .0005$) than that of controls (0.50 kg/day) (Figure 1). This lower rate of gain persisted in heifers fed 1250 mg of iodide up to day 77. Between day 77 and 161 of treatment, heifers in all groups gained body weight at similar rates. During 10 days following cessation of treatment (day 162 to 172), heifers in the group fed 1250 mg iodide gained less rapidly than those in the other three groups. However, one way analysis of variance revealed no difference among groups ($P \simeq .18$).

Fig. 1. Daily weight gain of heifers during and after feeding supplemental iodide.


Weight Gain (kg/day)
1.5
1.2
.9
.6
.3
0
Supplemental Iodide (mg/day)
0
50
250
1250
Day -8 to 21
Day 21 to 49
Day 49 to 77
Day 77 to 105
Day 105 to 161
Day 161 to 172
Period

This prolonged decrease in daily weight gains of heifers fed 1250 mg supplemental iodide daily resulted in lowered ($P<.05$) body weights for those heifers on day 21, 49, 77, 105, 161 during treatment (data not shown) and on day 9 post-treatment (final body weight; Table 2) as compared to that of controls.

Thyroid, adrenal and pituitary weights of heifers at slaughter are reported in Table 2. One-way analysis of variance revealed no significant effect of dietary iodide on thyroid ($P \approx 0.20$) and adrenal ($P \approx 0.20$) weights unadjusted for body weight. Averaged over all groups, thyroids and adrenals weighed 21.7 g and 12.3 g, respectively.

Pituitaries from heifers fed 250 or 1250 mg of iodide daily averaged 1.1 g and 1.0 g, respectively, which was less ($P<.05$) than that of controls (1.4 g). In addition, there was a tendency for 50 mg of supplemental iodide to reduce pituitary weight relative to controls, however this difference was not statistically significant.

In view of the fact that heifers fed 1250 mg of supplemental iodide had lower final body weights than those in the control group. Thyroid, adrenal and pituitary weights were adjusted on a per 100 kg body weight basis (Table 2). Adjusted pituitary and adrenal weights were not different among treatment groups. However, analysis of variance revealed a difference ($P=.05$) between adjusted thyroid weights of control heifers (7.4 g/100 kg bw) and those heifers fed the highest dose of supplemental iodide (10.0 g/100 kg bw). The enlargement of the thyroid in our heifers was mainly due to accumulation of large amount of colloid (Sleight and Mangkoewidjojo, personal communication).

TABLE 2. Effect of Supplemental Dietary Iodide on Body, Thyroid, Adrenal Glands and Pituitary Weight[a]

Supplemental Iodide (mg/day)	Actual Weight				Adjusted Weight		
	Final Body Weight —kg—	Thyroid	Adrenal[b]	Pituitary	Thyroid	Adrenal[b]	Pituitary
		g				g/100 kg bw	
0	278 ± 5.6	20.4 ± 0.9	12.7 ± 0.4	1.4 ± 0.05	7.4 ± 0.4	4.6 ± 0.1	.49 ± .01
50	273 ± 7.8	19.7 ± 1.5	13.0 ± 0.8	1.2 ± 0.07	7.3 ± 0.5	4.8 ± 0.3	.43 ± .02
250	260 ± 8.2	24.8 ± 2.0	12.0 ± 0.4	1.1 ± 0.02*	9.7 ± 0.9	4.6 ± 0.1	.43 ± .02
1250	225 ± 12.4	21.4 ± 1.8	11.2 ± 0.8	1.0 ± 0.04*	10.0 ± 1.1**	5.1 ± 0.4	.46 ± .03

[a] Values are mean ± SE

[b] Pair weight

* Different from comparable controls; $P<.05$

** Different from comparable controls: $P = .05$

II Serum Hormonal Response to TRH injection

In each treatment group, same 5 heifers received periodic injections of TRH. Serum concentrations of TSH, T_4 and T_3 of these heifers relative to TRH are presented in Figure 2 to 8.

A. Thyrotropin (TSH)

(1) Prior to iodide feeding

Basal TSH concentrations were not different ($P>.10$) among the four groups prior to onset of iodide feeding and when averaged over all groups was 3.1 ng/ml (Figure 2). In addition, basal TSH was not affected by iodide feeding except on day 78 of treatment when serum TSH of heifers fed 1250 mg of iodide was greater ($P=.05$) than that of controls. Following TRH (Figure 3), TSH concentration increased ($P<.001$) in blood of heifers in all groups reaching highest concentration of 14 to 17 ng/ml within 15 minutes. By 240 min, TSH returned to values equal to basal concentrations. No difference in responses to TRH was detected among the four groups of heifers during this control period. The TSH secretory pattern induced by TRH was qualitatively similar after each TRH challenge. Therefore, each response during and following iodide feeding will not be graphically presented here. Rather, data will be presented as area under the TRH response curve (ng ml^{-1} min) as in Figure 4.

(2) During iodide feeding:

After 22 days of supplemental dietary iodide, amount of TSH released (ng ml^{-1} min) by TRH was greater ($P<.005$) in heifers fed 1250 mg of iodide as compared to that of controls (1713 vs 671, Figure 4). Even though heifers fed 250 mg of iodide daily tended to have an increased response to TRH on day 22 (857 ng ml^{-1} min), this increase was not significant.

Magnitude of TRH induced TSH release continued to be greatest

Fig. 2. Basal thyrotropin (TSH) concentration in serum of heifers 5 days before (PRE), during and 10 days after (POST) supplemental iodide feeding.

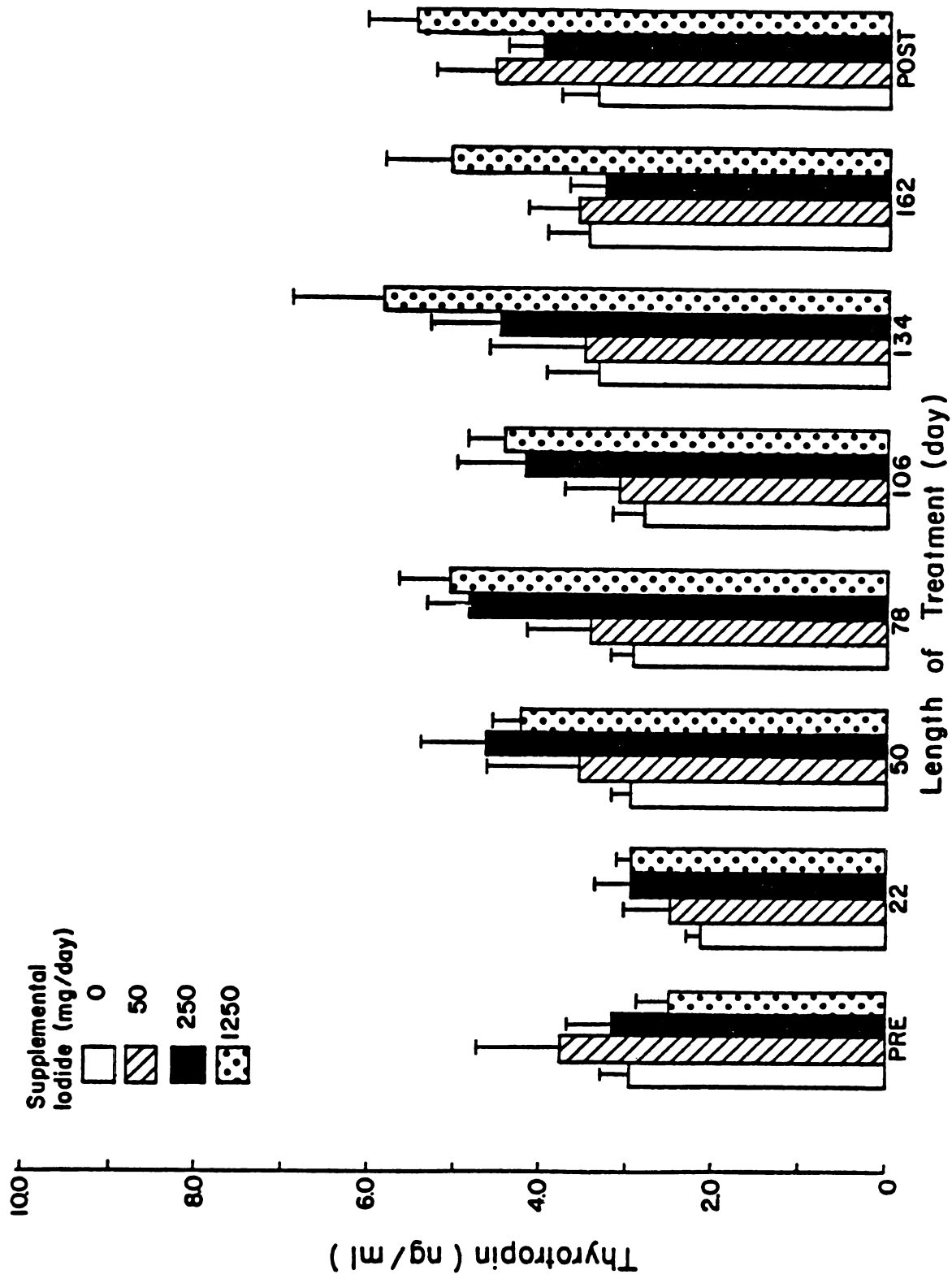

Thyrotropin (ng / ml)
100
80
60
40
20
0
Supplemental Iodide (mg/day)
0
50
250
1250
PRE
22
50
78
106
134
162
POST
Length of Treatment (day)

Fig. 3. Thyrotropin (TSH) response to thyrotropin releasing hormone (TRH) five days prior to beginning iodide feeding.

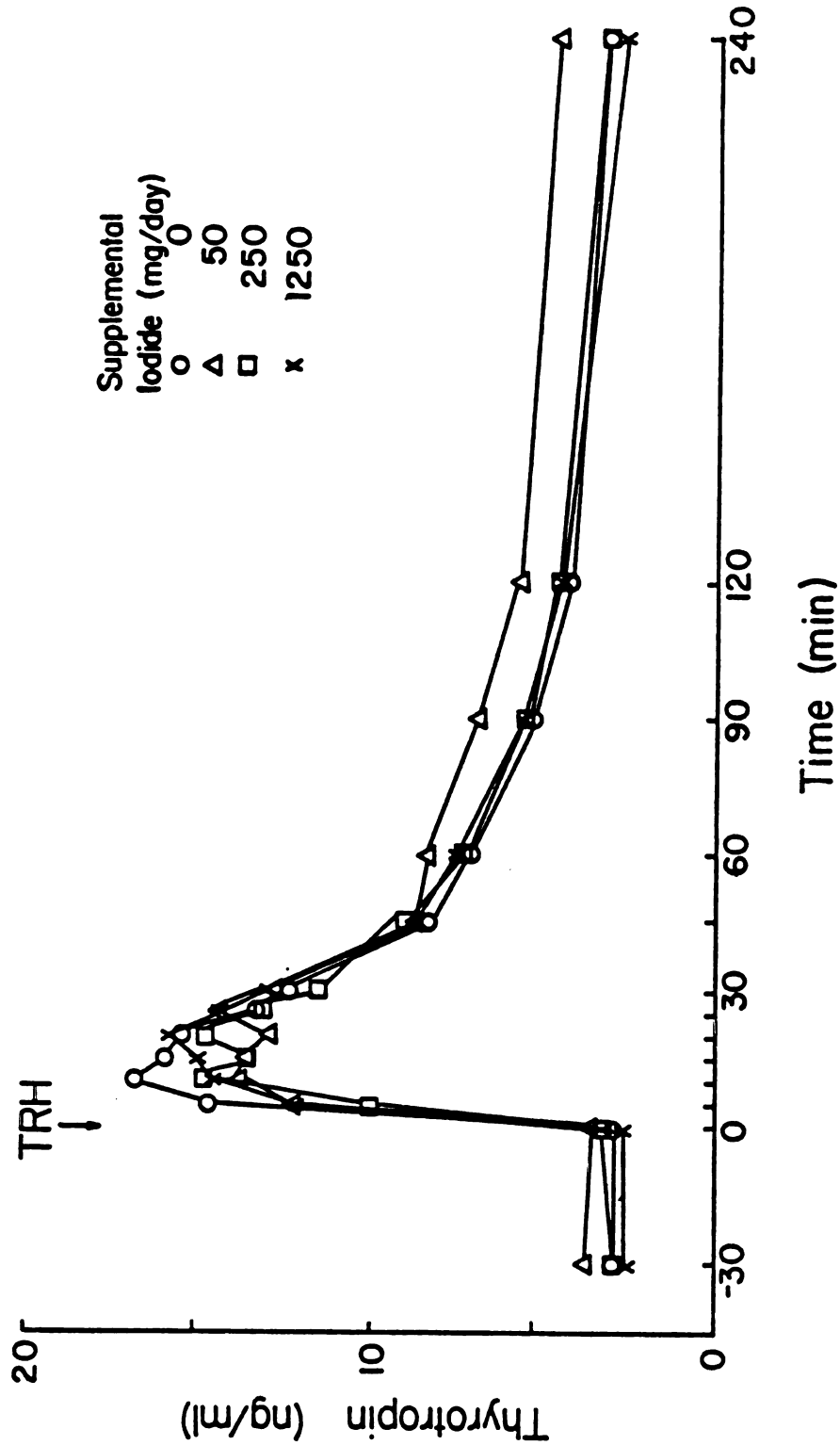
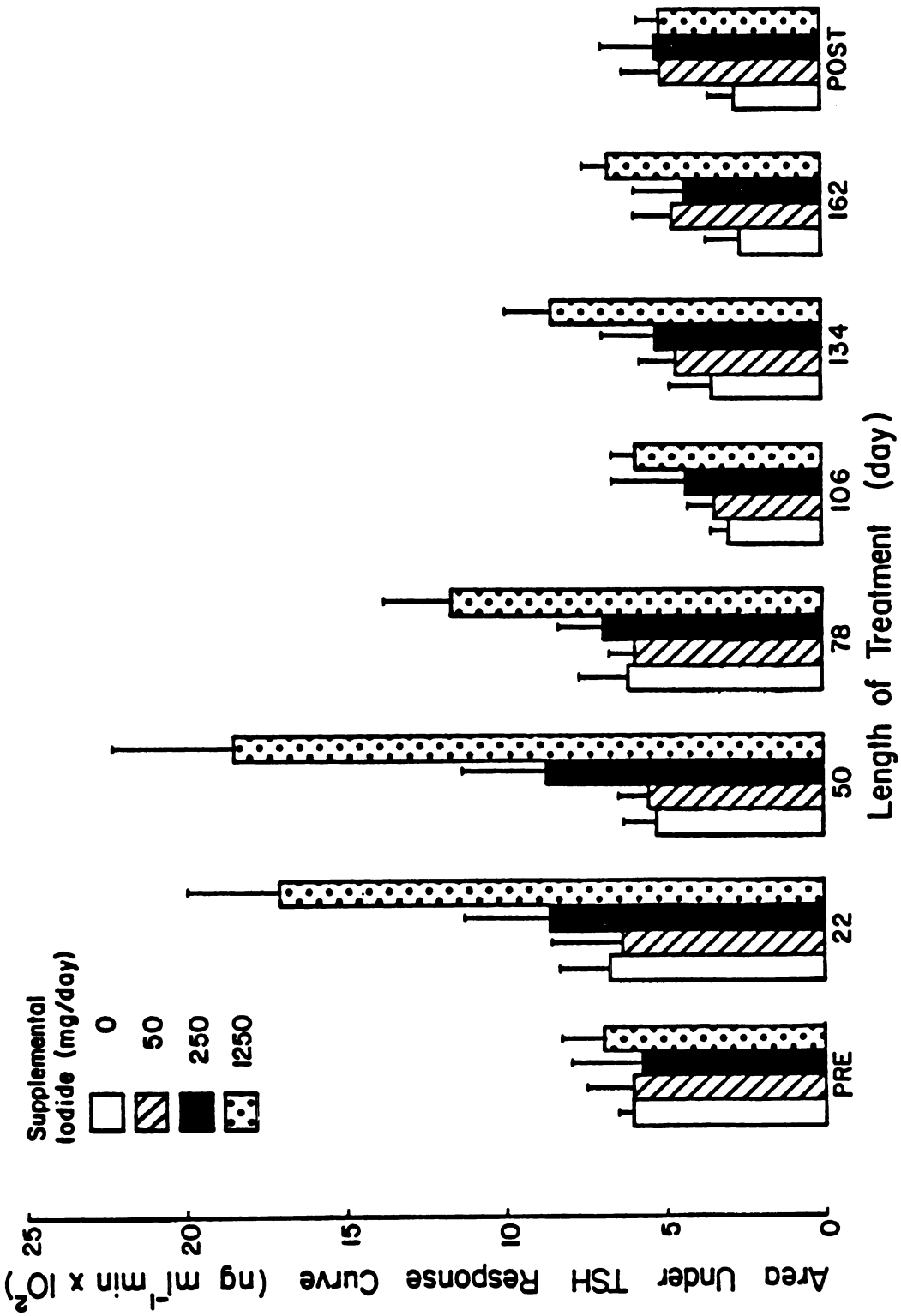

Thyrotropin (ng/ml)
20
10
0
TRH
-30
0
30
60
90
120
240
Time (min)
Supplemental Iodide (mg/day)
0
50
250
1250

Fig. 4. Thyrotropin (TSH) response to thyrotropin releasing hormone. Reported are areas under the TSH response curves (ng ml^{-1} min) 5 days before (PRE), during and 10 days after (POST) supplemental iodide feeding.


Area Under TSH Response Curve (ng ml⁻¹min x 10²)
0
5
10
15
20
25
Supplemental Iodide (mg/day)
0
50
250
1250
PRE
22
50
78
106
134
162
POST
Length of Treatment (day)

in heifers fed 1250 mg iodide throughout the entire treatment period. Interestingly, the TSH response to TRH gradually decreased with time in all four treatment groups (Figure4). In fact, there was a significant negative correlation between duration of treatment in days and area under TSH response curve (ng ml^{-1} min) within all groups except those fed 50 mg of iodide. Correlation coefficients were -.48 ($P<.01$), -.25 ($P \simeq .18$), -.37 ($P<.05$) and -.65 ($P<.001$) for heifers fed 0, 50, 250 and 1250 mg of iodide, respectively.

(3) Following iodide feeding:

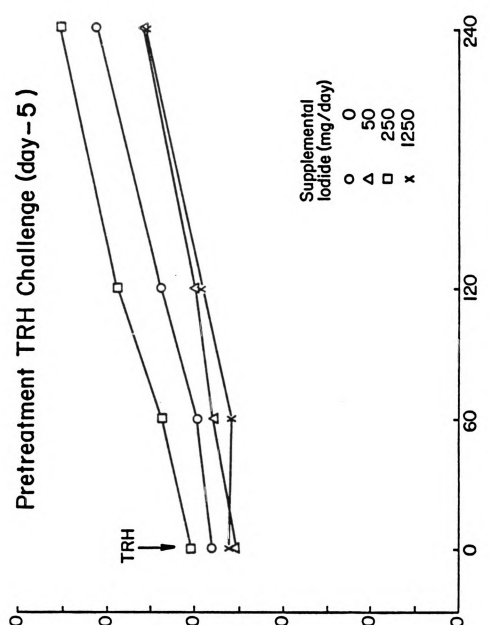
On day 10 after cessation of iodide feeding (Figure 2), basal concentration of TSH averaged over treatment was 4.4 ng/ml, and differences among groups were not significant. Although heifers which had been fed 50, 250 and 1250 mg of iodide daily for 162 days had a greater TSH response to TRH compared to controls, no statistical difference was detected ($P \simeq .17$; Figure 4).

B. Thyroxine (T_4)

(1) Prior to iodide feeding:

Changes in serum T_4 concentration after TRH are shown in Figure 5. Although basal serum T_4 concentrations (time 0) for heifers assigned to be fed 250 mg of iodide daily were greater and values for those assigned to be fed 50 and 1250 mg of iodide were less than that of controls, analysis of variance revealed no difference among groups ($P \simeq 0.17$). After TRH, T_4 increased linearly with time and had not reached a plateau at 240 minutes (Figure 5). Analysis of variance revealed a significant difference ($P<0.05$) in the T_4 responses to TRH among the four groups. Assuming that there should not be any difference in serum T_4 concentration prior to onset of iodide supplementation, all T_4 data collected during and after treatment

Fig. 5. Thyroxine (T_4) concentration in serum of heifers after thyrotropin releasing hormone (TRH) five days prior to beginning iodide feeding.


Pretreatment TRH Challenge (day-5)
T4 (ng/ml)
100
80
60
40
20
0
TRH
0
60
120
240
Time (min)
Supplemental Iodide (mg/day)
o 0
Δ 50
□ 250
x 1250

were adjusted by covariance for pretreatment differences. Actual data are presented in Figure 5 and 6.

The secretory pattern of T_4 after TRH shown in Figure 5 was qualitatively similar at each subsequent TRH challenge. In addition, there was no significant time by treatment interaction in T_4 response to TRH. For these reasons, only basal T_4 concentrations relative to duration of supplemental iodide feeding are presented in Figure 6.

(2) During iodide feeding:

After 22 days of feeding supplemental iodide, basal concentration of T_4 was not altered ($P \simeq .11$) when adjusted to pre-treatment values (Figure 6). However, on day 50, T_4 in serum of heifers fed 1250 mg iodide daily (32.1 ng/ml) was less ($P<.005$) than the comparable value for controls (49.7 ng/ml). Lower concentrations of T_4 in serum of heifers fed 1250 mg of supplemental iodide persisted throughout the entire feeding period.

(3) Following iodide feeding:

At 10 days after cessation of treatment (Figure 6), serum of heifers that had been fed the highest dose of supplemental iodide still had lower ($P<.01$) basal concentrations (ng/ml) of T_4 than did controls (45.9 vs 61.4).

C. Tri-iodothyronine (T_3)

(1) Prior to iodide feeding:

Average concentration (time 0) were not different among groups 5 days prior to onset of treatment (Figure 7). After TRH, T_3 increased linearly and reached peak values at 120 minutes. Analysis of variance revealed no difference in T_3 response to TRH among the four groups prior to start of treatment.

(2) During iodide feeding:

The pattern of change in T_3 concentration after TRH described in

Fig. 6. Basal thyroxine (T_4) concentration in serum of heifers 5 days before (PRE), during and 10 days after (POST) supplemental iodide feeding.

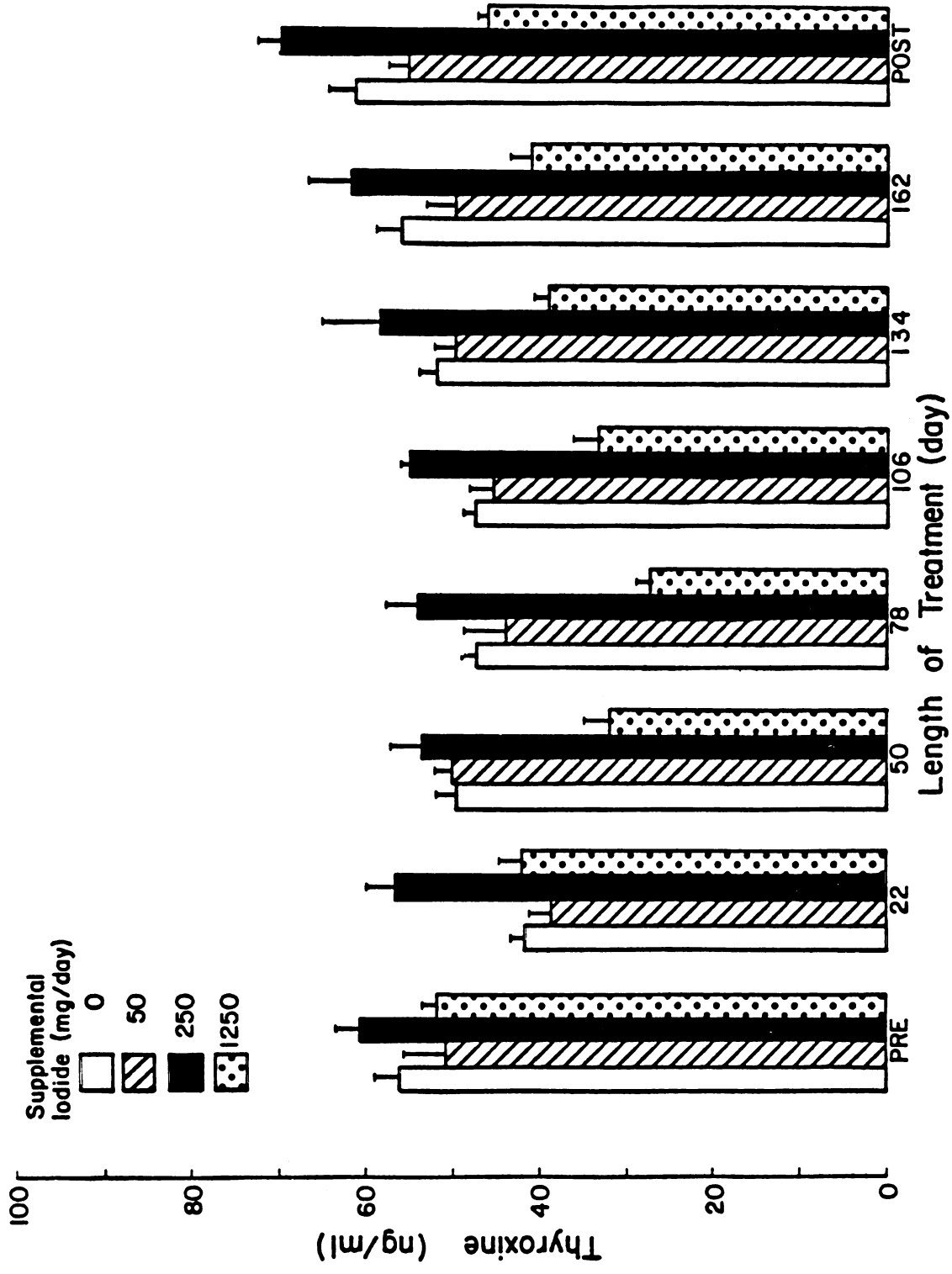

Thyroxine (ng/ml)
0
20
40
60
80
100
Supplemental Iodide (mg/day)
0
50
250
1250
PRE
22
50
78
106
134
162
POST
Length of Treatment (day)

Fig. 7. Tri-iodothyronine (T_3) concentration in serum of heifers after thyrotropin releasing hormone (TRH) five days prior to beginning iodide feeding.

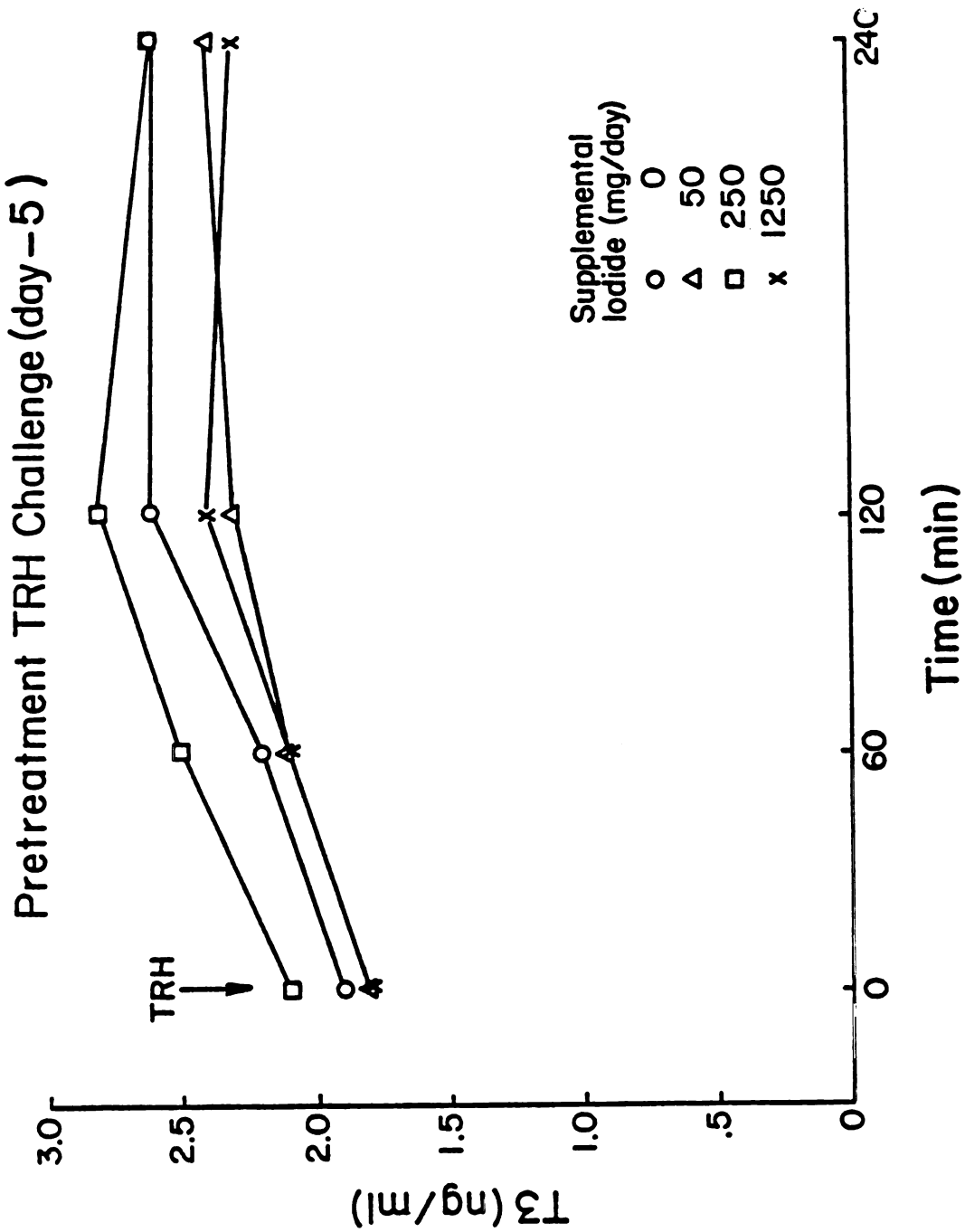

Pretreatment TRH Challenge (day −5)
TRH
T3 (ng/ml)
3.0
2.5
2.0
1.5
1.0
.5
0
0
60
120
240
Time (min)
Supplemental Iodide (mg/day)
0
50
250
1250

Figure 7 is qualitatively representative of changes during subsequent challenges. Additionally, no difference in magnitude of T_3 response to TRH among treatment groups was detected by any TRH challenge. Therefore, only basal T_3 concentrations relative to duration of treatment are presented in Figure 8.

After 22 days of supplemental iodide feeding, heifers fed 1250 mg iodide had a lower ($P<.01$) basal T_3 concentration than controls. This pattern continued throughout the treatment period. On day 78, heifer concentrations in all treatment groups showed lower concentrations of serum T_3. The cause of this observation was unknown.

(3) Following iodide feeding:

Groups of heifers that had been fed 1250 mg iodide had less ($P<.01$) T_3 in serum than did controls at 10 days post-treatment (Figure 8).

III. Effect of exogenous TRH on basal concentrations of thyrotropin, thyroxine and tri-iodothyronine.

Selected serum samples collected by venipuncture at various stages of this experiment were assayed for TSH, T_3 and T_4 to determine the effect of periodic injections of TRH on basal hormone concentrations. Hormone values revealed no evidence of either a direct TRH or TRH by supplemental iodide feeding effect on the pituitary-thyroid axis (data not shown).

IV. Total glucocorticoids

At 5 days prior to onset of treatment, total glucocorticoids concentration in serum averaged overall groups was 3.9 ng/ml (data not shown). Differences between groups were not significant ($P \simeq .48$). Total corticoids in serum of heifers after various duration of iodide feeding (Table 3) provided no evidence of altered adrenocortical function due to treatments ($P>.50$) although

Fig. 8. Serum tri-iodothyronine (T_3) concentration in serum of heifers 5 days before (PRE), during and 10 days after (POST) supplemental iodide feeding.

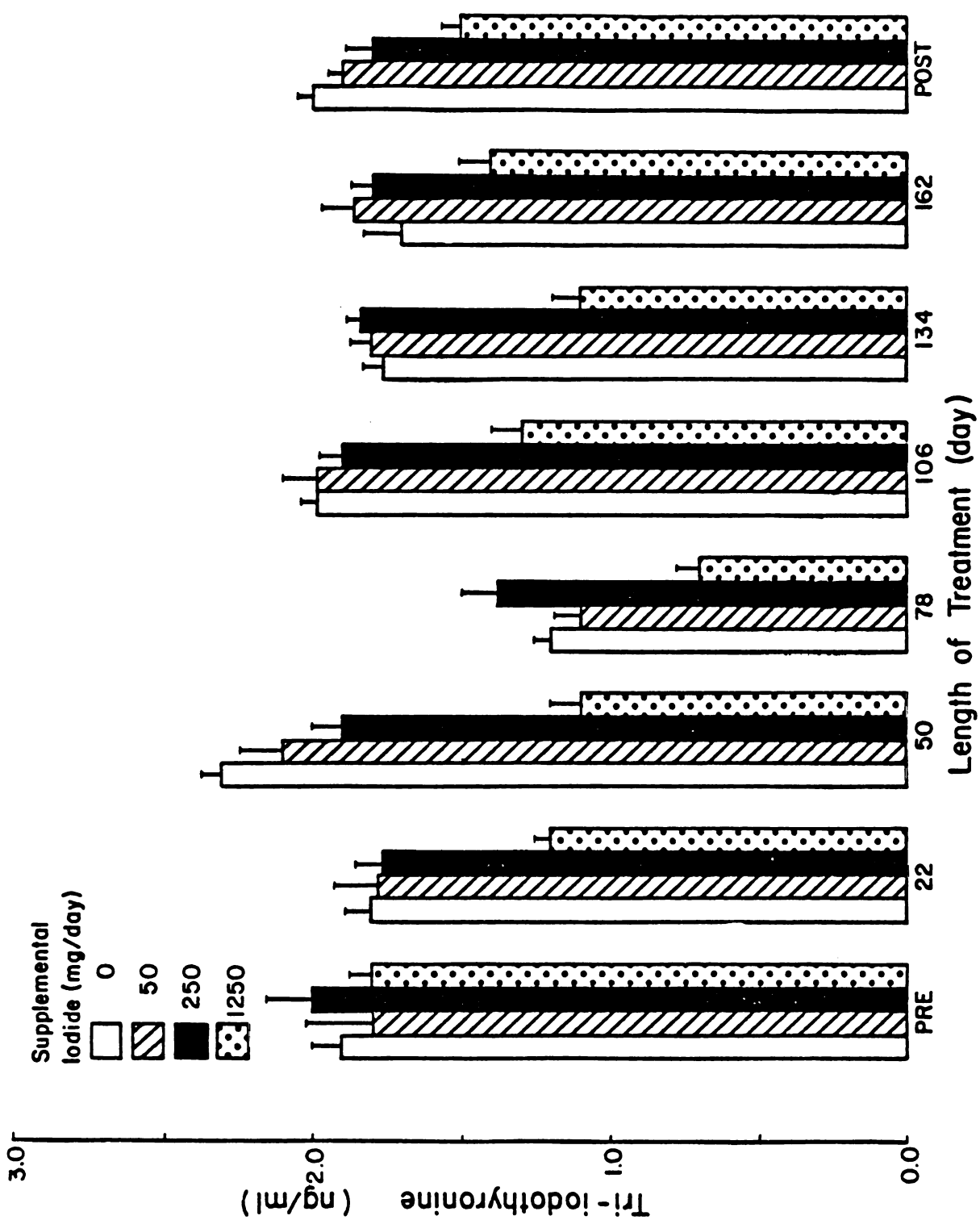

Supplemental Iodide (mg/day)
0
50
250
1250
Tri-iodothyronine (ng/ml)
3.0
2.0
1.0
0.0
PRE
22
50
78
106
134
162
POST
Length of Treatment (day)

TABLE 3. Serum Total glucocorticoid Concentrations (ng/ml) of Heifers During Treatment.

Supplemental Iodide (mg/day)	Duration of Treatment (Days)							
	8	16	37	58	79	100	121	AVG
0	3.2	5.4	5.9	2.5	6.2	3.0	5.4	4.5
50	3.4	3.1	4.6	2.9	8.8	4.2	6.2	4.7
250	3.3	1.8	3.2	3.1	5.3	4.1	5.0	3.7
1250	2.3	2.7	5.0	2.6	5.6	3.7	2.7	3.5
AVG	3.1	3.3	4.7	2.8	6.5	3.8	4.8	4.1

Standard Error Due to Treatment: $\pm$ .58

Standard Error Due to Time: $\pm$ .45

Standard Error Due to Each Cell: $\pm$ .89

differences between day of sampling were significant (P<.005).

V. Age at puberty

Selected serum samples from all heifers were assayed for progesterone to determine time of onset of puberty. Animals were considered to have reached puberty if serum progesterone values were equal to or above 1.0 ng/ml i.e. had functional corpora lutea. Two heifers fed 50 mg of supplemental iodide were later discovered to be free-martins at autopsy and were therefore dropped from this aspect of the experiment. By day 142, one heifer from each treatment group had developed a functional corpus luteum. At the end of the experiment, 7 of 8, 3 of 10 and 1 of 8 heifers from groups fed 50, 250 and 1250 mg of iodide as EDDI respectively, had exhibited behavioral estrus. These data were not different from those of controls (5 out of 10 heifers, P>.05).

VI. Normal endocrine pattern of pituitary-thyroid axis in heifers

Hormone data (Table 4) from heifers (n=5) which served as the controls in this experiment provided us an opportunity to evaluate TSH and thyroid hormone changes in normal growing heifers between 3 and 9 months of age under conditions of controlled photoperiod and temperature. In these heifers, basal TSH concentration remained constant (P>.05) during this period averaging 3.1 ng/ml, while serum T_3 and T_4 concentrations fluctuated greatly (P<.0005) over time. We did note that the TSH response to TRH decreased with age. However, the increase in serum T_3 and T_4 concentrations that occurred between 0 and 120 min after TRH was similar at each TRH challenge.

TABLE 4. Basal and Thyrotropin Releasing Hormone Induced Increase in Thyrotropin, Tri-iodothyronine and Thyroxine[a]

Age (month)	Thyrotropin		Tri-iodothyronine		Thyroxine	
	Basal[b]	Area[c] Under Curve	Basal[b]	Δ[d]	Basal[b]	Δ[d]
3	3.0 ± 0.3	606 ± 43	1.9 ± 0.10	0.72 ± 0.07	56.0 ± 3.1	11.6 ± 1.6
4	2.2 ± 0.1	671 ± 166	1.8 ± 0.09	0.82 ± 0.12	41.7 ± 1.4	9.4 ± 1.5
5	3.0 ± 0.2	521 ± 106	2.3 ± 0.07	0.70 ± 0.09	49.7 ± 2.4	12.7 ± 2.5
6	3.0 ± 0.2	611 ± 157	1.2 ± 0.06	0.72 ± 0.10	46.9 ± 1.6	9.5 ± 1.5
7	2.9 ± 0.3	289 ± 64	2.0 ± 0.06	0.78 ± 0.06	47.2 ± 1.4	9.6 ± 2.7
8	3.4 ± 0.4	343 ± 136	1.8 ± 0.07	0.94 ± 0.09	51.9 ± 2.1	13.1 ± 2.0
9	3.5 ± 0.5	252 ± 108	1.7 ± 0.13	0.94 ± 0.15	56.3 ± 2.9	12.5 ± 1.6

[a]Values are means ± SE.

[b]Basal values are ng/ml.

[c]Area under TSH response curve are in ng ml^{-1} minute.

[d]Δ = change in hormone concentration from 0 to 120 minutes.

DISCUSSION

Our observation that daily weight gains are reduced in heifers fed 1250 mg supplemental iodide daily agrees with results previously reported by Forbes et al. (1932) and Newton et al (1974). Our heifers fed 1250 mg supplemental iodide daily weighed on the average 225 kg at ten months of age which was less than that of controls (278 kg). Newton et al. (1974) observed a decrease in feed consumption and feed efficiency in Holstein bull calves fed 736 mg supplemental iodide daily. Feed consumption and feed efficiency were not measured in the present investigation.

Wallace (1975) observed adrenocortical hyperplasia in lactating dairy cows estimated to be receiving an average of 107 mg iodide per head per day. In addition, Newton et al. (1974) described adrenal enlargement in calves consuming 100 times the recommended quantities of dietary iodide. The latter authors suggested that adrenal hypertrophy may result from a stress imposed by the feeding of high iodide diets. However, our measures of adrenal weights at slaughter and also total glucocorticoids in serum during the iodide feeding period revealed no effects of iodide treatment on adrenal function. Adrenal weights of our control heifers was similar to those reported by Sorenson et al. (1959) for heifers of comparable age and body weight (4.2 g/100 kg bw).

Due to the small number of animals in each of our treatment groups, we did not detect any effect of excess iodide on age at which onset of puberty occurred. In the present study, body weights of these heifers averaged 257 kg at first estrus which is slightly less than that (280 kg) reported by Sorenson et al. (1959). The latter authors suggested that body weight is an important factor controlling age of first estrus. With underfeeding,

onset of puberty is delayed until the proper body weight is attained. If their hypothesis held true in this investigation then heifers fed 1250 mg iodide in our study which had an average body weight less than that of controls would have been expected to reach puberty at an older age than the controls. Unfortunately, the small number of animals involved precluded reaching a definitive conclusion regarding this measure.

Anterior pituitary weight of all animals in the present study averaged 0.46 g/100 kg bw which was slightly lower than that (0.50 g/100 kg bw) reported by Desjardins and Hafs (1968) for Holstein heifers of comparable age. Failure to detect differences in anterior pituitary weight per unit body wt among treatment groups is consistent with the general belief that iodide exerts its effect not on the pituitary, but directly on the thyroid.

Thyroid weight of our control heifers averaged 7.4 g/100 kg bw which was comparable to values (7.1 to 7.4 g/100 kg bw) reported by Sorenson et al. in 1959 for heifers of similar age and body weight. In agreement with our observation that excess iodide cause increased thyroid weight in heifers, others have observed increased thyroid weight in chicks (Wheeler and Hoffman, 1949), horses (Baker and Lindsey, 1968), humans (Wolff, 1969) and lactating cows (Wallace, 1975). However, Newton et al. 1974 reported that feeding excess amounts of iodide increased or did not change thyroid weights of Holstein bull calves in three trials. Iodide goiter is more prevalent in women than in men (Suzuki, et al. 1965). If a similar sex difference exists in the bovine species, it might explain differences in results obtained by Newton et al. 1974 with bulls and our results with heifers.

Basal T_3 and T_4 concentrations in serum of heifers fed 1250 mg iodide remained lower than that of controls throughout the treatment period. Since excess dietary iodide does not increase peripheral metabolism of

T_4 in rats (Nagataki et al. 1966) and humans (Vagenaki et al. 1973), the decrease in serum T_3 concentrations we observed may reflect decreased generation of T_3 from T_4 by peripheral monodeiodination, secondary to a decrease in serum T_4 concentration.

Basal concentrations of T_3 and T_4 in serum of heifers fed 1250 mg iodide decreased with time with the lowest levels observed on day 78 of the treatment period. On that day, basal TSH concentrations in these heifers were higher than that of controls. Negative feedback of T_3 and T_4 on TSH secretion is believed to be mediated by induction of a protein inhibitor of TSH release (Schally and Redding, 1967, Bowers et al. 1968, and Vale et al. 1968). Decreased T_3 and T_4 concentrations in blood probably reduced the production of this protein. Both endogenous and exogenous sources of TRH could then elicit a greater release of TSH from the pituitary.

Others in our laboratory (Padmanabhan and Convey, unpublished data) demonstrated that T_3, at 1 ng/ml, added to primary bovine pituitary cell culture inhibited basal release of TSH. On the other hand, 5 μg of T_4 was required to exert a similar degree of inhibition. Since the ratio of T_3 and T_4 is about 1:30 in bovine blood, T_3 may be the key thyroid hormone exerting negative feedback control of TSH release in cattle. Larsen and Frumess (1977) reported that in rats TSH release was suppressed by an increase in either T_3 or T_4 concentration in blood. However, T_4 may have been deiodinated to T_3 in sufficient quantities to exert this effect. Silva et al. (1978) reported that intrapituitary T_4 monodeiodination provided one half of the T_3 occupying nuclear T_3 receptors in the pituitary of rats. Their findings provided another mechanism by which TSH secretion could be altered by changes in either serum T_3 or T_4 concentration.

Apparently, negative feedback control of TSH secretion is not tightly

coupled to serum thyroid hormone concentrations. For example, in the present study, increased serum TSH concentrations in heifers fed 1250 mg supplemental iodide were not detected until T_3 and T_4 concentrations in these heifers had decreased to 58% and 59% of controls, respectively. In addition, Hopkins et al. (1975) did not observe any change in TSH concentrations in thyroidectomized sheep until serum T_4 decreased to 27% of presurgery values. These observations are all contradictory to the commonly held belief that feedback control of TSH secretion responds to slight changes in the amount of T_3 and T_4 in serum. A possible explanation for this phenomenon is that the carrying capacity of serum proteins may have changed. A majority of T_3 and T_4 in blood are bound to binding globulin and albumin (Ingbar, 1963). The rest are free and constitute the biologically active forms of thyroid hormones which are involved in the negative feedback control of TSH release from the pituitary. If concentrations of thyroid hormone binding proteins decreased, concentrations of free T_3 and T_4 in the blood would then increase and may reach that of controls despite a decreased in total (bound and free) T_3 amd T_4 concentrations in serum. Serum levels of TSH comparable to that of controls would be maintained under conditions described above.

TRH stimulates TSH release in cattle at least in part by a direct action on the anterior pituitary (Kesner et al., 1977b). TSH was increased to peak concentrations in serum of our heifers at 15 min after TRH and declined thereafter. This decrease was not likely due to the increasing T_3 and T_4 concentration caused by TRH induced TSH release because T_3 and T_4 concentrations were not increased in serum until 30 min after TRH (Convey et al., 1978). In humans, an oral dose of T_3 was more effective in inhibiting TRH induced release of TSH than was T_4 (Wenzel et al., 1975)

and continued high concentrations of T_3 in serum did not suppress TSH response to TRH for at least 48 hours (Wartofsky et al., 1975).

Enhancement of the TSH response to TRH under conditions when basal T_3 and T_4 concentrations were decreased was clearly demonstrated in the present study. Snyder and Utiger (1972) observed TSH response to TRH in hypothyroid patients as compared to euthyroid patients. This response could be normalized by slightly increasing serum T_3 and T_4 concentrations. They also observed a negative correlation between basal serum thryoid hormone concentrations and magnitude of TSH response to TRH in normal humans, even though concentration of T_3 and T_4 were within the normal range during treatment.

The amount of TSH released by TRH differed among treatment groups by as much as 3.5 fold during the iodide feeding period. However the resulting increase in T_3 and T_4 did not differ among treatment groups. These results suggest that the smallest quantity of TSH released by 15 μg TRH per 100 kg bw could cause a release of T_3 and T_4 equal to that caused by the largest TSH release (on day 50, 522 vs 1845 ng ml^{-1} min; Figure 4). There may be an intrinsic mechanism to limit the amount of T_3 and T_4 which can be released from the thyroid in response to TSH. Such a mechanism would prevent marked fluctuation of the thyroid hormones in blood.

Kahl et al. (1977) reported that plasma T_3 and T_4 concentrations in growing Holstein heifers between 10 and 14 weeks of age averaged 1.0 and 42 ng/ml, respectively. These values were lower than those obtained in the present investigation (1.9 ng/ml and 55 ng/ml, respectively). In additon, Kahl et al. (1977) detected a gradual increase in basal T_3 concentrations

in heifers between 6 and 22 weeks of age, an observation which was not confirmed in the present study.

Based on the results in the present experiment and those of previous investigations, we can suggest the series of events that may have taken place in the pituitary-thyroid axis during supplemental iodide feeding.

First, high iodide diets caused a dramatic increase in serum iodide concentration in these heifers. Then, iodide would be quickly trapped by the thyroid gland resulting in an abrupt increase in its intrathyroidal concentration. This increase probably induced formation of a protein-iodide complex of the type described by VanSande et al. (1975) which then inhibited thyroid hormone secretion at the level of thyroid adenylate cyclase (Burke, 1970) and at sites distal to generation of cAMP (Pisarev et al. 1971 and Yamamoto et al. 1972) in the pathway of TSH action. Thus, rate of iodide uptake, iodination of thyroglobulin and proteolysis of thyroglobulin would all be decreased.

In our control heifers, we estimate that iodide concentration in serum averaged 180 ng/ml based on measures made by Convey et al. (1978). If we assume a concentration gradient of iodide between thyroid and serum of 20:1 (DeGroot and Stanbury, 1975), then intrathyroidal concentration of iodide would be approximately 3.6 μg/ml. Normally, when iodide intake increases, the thyroid adjusts by inhibiting further uptake of iodide effectively establishing a new thyroid to serum concentration gradient but maintaining a normal intrathyroidal concentration of iodide i.e. 3.6 μg/ml. However, in the present experiment, the concentration of iodide in serum of these heifers probably reached levels higher than that inside the thyroid. Using data reported by Convey et al. (1978), we estimate that dietary iodide at 800 times daily requirements would bring the concentration of serum

iodide in heifers to approximately 5.7 μg/ml. Iodide in blood would then enter the thyroid follicle cells via passive diffusion. Intrathyroidal concentration of iodide would be maintained at concentrations high enough to cause continuous production of inhibitor, thus preventing these animals from escaping the effects of high serum iodide.

Suppression of iodide transport and iodination of thyroglobulin probably decreased numbers of DIT and MIT per molecule of thyroglobulin. This would account for the decrease in serum T_3 and T_4 concentrations we observed here.

Jubiz et al. (1977) performed an experiment similar to ours but utilized humans. Four normal subjects were given approximately 1080 mg iodide daily for 11 weeks. Gradual decreases in serum T_3 and T_4 concentration preceded an increase in basal TSH concentration. Two to three weeks after this initial increase in TSH, basal T_3, T_4 and TSH returned to their respective pretreatment concentrations. These authors suggested that adaptation of the human thyroid to chronic intake of high level of dietary iodide results from an increase in TSH secretion caused by decreasing thyroid hormone concentrations. This theory may be applicable to our heifers. Increased concentrations of serum TSH in heifers fed 1250 mg iodide was observed on day 78 of treatment. However, the duration of the adaptation period is probably longer in these heifers. Since at the end of the treatment period (day 163), their serum T_4 and T_3 level remained lower (Figure 6 and 8) and their basal TSH tended to be higher than that of controls (Figure 2).

The time required for our heifers to recover from these iodide effects apparently depended on the quantity of iodide fed. If one studies the basal T_3, T_4 and TSH concentration in heifers fed 160 times recommended requirement

of iodide (250 mg), one will see that while the two thyroid hormones showed no particular secretory pattern, basal TSH peaked at day 78 and then gradually returned back to control levels before the end of treatment period. Even though these hormone values were not statistically different from those of controls, the trend suggests that the larger the quantity of iodide fed to animals, the longer it takes them to recover completely from the effect.

The recovery is also hindered by the poor health of heifers receiving 800 times the daily requirement of dietary iodide (1250 mg). In the first part of the treatment period, these heifers developed typical signs of iodism: coughing, lacrimal and nasal secretion, hypertherma, excessive salivation and open mouth breathing. Two heifers in this treatment group contracted bronchopneumonia and failed to respond to medications and they died. These clinical findings were comparable to that reported by McCauley et al. (1972). The rest of heifers in this treatment group recovered from all these symptoms of iodism. In the last month of the treatment period, one could not distinguish these heifers from the controls by their physical appearance. These animals may have finally adapted to high concentrations of serum iodide.

An alternative explanation as to why these heifers recovered from the adverse effect of iodide is the change in amount of iodide fed to these heifers per body weight. Since a constant quantity of iodide was fed to animals in each treatment group, as the body weight of the heifers increased, dose of iodide per body weight decreased and may have dropped below the threshold level for iodism and its inhibitory effect on the thyroid gland. The present investigation does not permit us to conclude which mechanism is responsible for the recovery of these heifers.

The preliminary investigation by Hillman and recent surveys conducted

by this department indicated only a few dairy herds in Michigan received up to 80 times the National Research Council requirement of iodide daily. This quantity of iodide, based on this investigation, will not affect development of the pituitary-thyroid axis in growing dairy heifers. However, Haggard et al. (1978) did observe decreased immune response in these heifers at levels of supplemental iodide as low as 50 mg daily. Since no beneficial effect of excess iodide was observed in the present and other investigations, it is the opinion of this author that feeding dietary iodide of quantities exceeding the National Research Council requirement to dairy heifers should not be recommended. Periodic monitoring of dietary iodide levels and awareness of the adverse effect of excess dietary will prevent iodide from becoming an epidemic problem in the dairy industry.

SUMMARY AND CONCLUSIONS

The effects of feeding supplemental iodide on pituitary and thyroid function in Holstein heifers were studied. These heifers averaged 11 to 15 weeks of age when assigned to be fed 0, 50, 250 or 1250 mg of iodide in the form of ethylenediamine dihydriodide for 162 days.

Daily weight gain of heifers fed 250 or 1250 mg of supplemental iodide daily were less than for control heifers for at least 21 and 77 days after onset of feeding respectively. Thyroid weights, when adjusted for body weight differences, were greater in heifers fed 1250 mg supplemental iodide daily. Pituitary weights were unaffected by iodide feeding. Serum total glucocorticoid concentration in serum and adrenal weights were similar among treatment groups. Failure to detect an effect on adrenal function does not support the idea that feeding iodide at concentrations greater than recommended by the National Research Council imposes a stress on growing heifers.

Concentrations of T_3 and T_4 in serum of heifers fed 1250 mg supplemental iodide daily gradually decreased with time reaching lowest concentrations of 32.7 ng/ml and 1.2 ng/ml, respectively, on day 78 of iodide feeding. Concentration of both thyroid hormones increased thereafter but remained lower than that of controls throughout. Basal TSH concentrations in all heifers were not different throughout the treatment period with the single exception of day 78 when TSH concentration in serum of heifers fed 1250 mg of iodide were higher than for controls. These observations are contradictory to the belief that feedback control of TSH secretion responds to slight changes in thyroid hormone concentrations in serum.

TSH release by TRH in heifers fed 1250 mg supplemental iodide daily was greater than that of controls beginning on day 22 of treatment and continuing throughout the entire iodide feeding period. However, the increases in serum T_3 and T_4 that occurred in response to the TRH-induced increase in serum TSH were not different among treatment groups.

These data suggested that acute release of TSH from the thyrotrophs may be under a mechanism different from the one that regulates basal TSH concentrations in blood. Much smaller decreases in thyroid hormone concentrations will enhance the responsiveness of thyrotrophs to TRH. The amount of TSH released after TRH challenges induced maximum release of thyroid hormone from all treatment groups.

Both T_3 and T_4 concentrations in heifers which received 1250 mg of dietary iodide remained depressed 10 days after the cessation of the feeding. However, TSH responses to TRH were no longer statistically different among groups. A period longer than 10 days period may be needed before these heifers could recover from the effect of excess dietary iodide.

Small numbers of animals involved in this investigation did not permit us to detect any change in age at which heifers came into puberty.

We conclude that iodide fed at 800 times the recommended daily requirement decreased daily weight gain in heifers. This may be due to the enlargement of the thyroid and decreased release of tri-iodothyronine and thyroxine. TRH-induced release, but not basal levels of TSH was affected by the marginal decrease in thyroid hormones. Excess iodide in the diet did not delay onset of puberty in these heifers.

APPENDIX I

Radioimmunoassay for Serum Thyrotropin

Serum thyrotropin was assayed by a double antibody radioimmunoassay.

I. First Antibody

Antiserum obtained from rabbits was supplied by Dr. S.L. Davis, University of Idaho, Moscow, ID. It was diluted 1 : 400 with 0.05 M EDTA in pH 7 phosphate buffered saline (0.05M EDTA-PBS) and frozen at -20° C. Before using the antiserum was further diluted to 1 : 100,000 with 1 : 400 normal rabbit serum in 0.05M EDTA-PBS and incubated with 40 ng of NIH-LH-S11 and 40 ng of NIH-FSH-S9 per ml of final working antiserum at 4° C for 24 hours under continuous stirring.

II. Iodinated Hormone

2.5 μg of Highly purified (Dr. J.G. Pierce) bovine TSH was dissolved in double distilled deionized water (1μg/μl) was predispensed into 1 ml serum vials. 25 μl of 0.5M phosphate buffer was also added to each vial before they were snap frozen and stored at -60° C until use. 15μg of Chloramine-T (1μg/μl in PBS), 1 mCi of carrier free Na ^{125}I (Catalog # IMS-300, Amersham) provided a suitable condition for iodinating thyrotropin. Oxidative reaction was terminated with 125 μg sodium metabisulfite (2.5 μg/μl in PBS), 100 μl of 2% bovine serum albumin in PBS (2% BSA-PBS) and 100 μl transfer solution containing 16% sucrose and 1 mg potassium iodide were added to the reaction vial before transfering to a Biogel P-60 column (0.8 x 20 cm) coated with 2% BSA-PBS. One ml fractions were

collected in disposable test tubes containing 1 ml of 2% BSA-PBS. The most radioactive fraction corresponding to the labeled bovine thyrotropin was used in the radioimmunoassay. The labeled hormone was diluted to approximately 20,000 cpm/100 μl with 1% BSA-PBS on the day before usage.

III. Second Antibody

Antirabbit gamma globulin (anti-RGG) was obtained from ponies injected with rabbit gamma globulin emulsified in Freund's adjuvant. Anti-RGG was diluted with 0.05M EDTA-PBS to appropriate concentration on the day of use.

IV. Assay Procedure

NIH-TSH-B4 (National Institute of Health) was used as assay reference standard. Varying volumes of serum samples or standards were pipetted into disposable culture tubes (12 x 75 mm) and 1% BSA-PBS was added to bring the volume to 500 μl. 200 μl of diluted antiserum, 100 μl of I^{125}-bTSH and 200 μl of diluted anti-RGG were added to assay at 24 hour interval. The contents of each test tube were well mixed after addition of each reagent. The assay was incubated at 4° C at all times and 72 hours after the addition of anti-RGG, 3 ml of cold PBS was added to each test tube. The assay was then centrifuged at 2800 RPM for 30 min. Supernatant fluids were discarded and the pellets were counted in a gamma counter.

V. Quality Control

Dilution curves of two pools of bovine sera containing low or high concentration of thyrotropin were assayed in each assay to ensure parallelism between standard sera and standard curves.

APPENDIX 2

Radioimmunoassay for Serum Thyroxine

Serum thyroxine was assayed using commercial radioimmunoassay reagents (Radioassay System Laboratories, Inc., Carson, CA). Assays were carried out according to the instruction provided. Standard points of 5, 10, 30 and 60 ng/ml were added whereas 320 ng/ml was omitted. 25 μl of either standard or serum was diluted with 200 μl of diluent buffer (1% normal rabbit serum in 0.1M phosphosaline buffer with 0.01 M EDTA, pH 7.5). 100 μl of 8-Anilino-1-Naphthalene-Sulfonic Acid (1.5 mg/ml) which dissociate thyroxine from binding proteins, was added to each assay tube. Then 100 μl of anti T_4 serum and 100 μl of radioactive labeled hormone were added. The assay was incubated at 37° C for 2 hours before the addition of second antibody (100 μl). The assay was then stored at 4° C and 16 to 20 hours later, 3 ml of cold PBS was added to each test tube. The assay was centrifuged at 2800 RPM for 30 min. Supernatant fluids were discarded and pellets were counted in a gamma counter.

Six to eight serum samples from a pooled bovine serum were assayed in each assay as quality control.

APPENDIX 3

Radioimmunoassay for Serum Tri-iodothyronine

Serum tri-iodothyronine was assayed using commercial radioimmunoassay reagents (Radioassay System Laboratories, Inc., Carson, CA). Assays were carried out according to the instruction provided. Standard points of 0.25, 0.75, 1.5 ng/ml were added whereas 8 ng/ml was omitted. 200 μl of either standard or serum was diluted with 600 μl of diluent buffer (1% normal rabbit serum in 0.1 M phosphosaline buffer with 0.01 M EDTA, pH 7.5). 100 μl of 8-anilino-1 Naphthalene-Sulfonic Acid (1.5 mg/ml) which dissociate tri-iodothyronine from binding proteins, was added to each assay tube. Then 100 μl of anti T_3 serum and 100 μl of radioactive labeled hormone were added. The assay was incubated at 37° C for 2 hours before the addition of second antibody (100 μl). The assay was then stored at 4 °C and 16 to 20 hours later, 3 ml of cold PBS was added to each test tube. The assay was centrifuged at 2800 RPM for 30 min. Supernatant fluids were discarded and pellets were counted in gamma counter.

Six to eight serum samples from a pooled bovine serum were assayed in each assay as quality control.

BIBILOGRAPHY

Allen, M.B. Jr. 1976. Embryology and anatomical connections of the pituitary. In: The Pituitary, a Current Review. (M.B. Allen and V.B. Mahesh, ed.) Academic Press.

Annunziato, L., G. DiRenzo, G. Lombardi, F. Scopacasa, G. Schettini, P. Preziosi and U. Scapagnini. 1977. The role of central noradrenergic neurons in the control of thyrotropin secretion in the rat. Endocrinology 100:738.

Baker, B.L., J.G. Pierce and J.S. Cornell, 1972. The utility of antiserums to subunits of TSH and LH for immunochemical staining of the rat hypophysis. Am. J. Anat. 135:251.

Baker, H.J. and J.R. Lindsey. 1968. Equine goiter due to excess dietary iodide. J. Amer. Vet. Med. Ass. 153:1618.

Bakke, J.L. and N. Lawrence, 1967. Thyrotropin (TSH) in the rat stalk median eminence. 2:315.

Bloom, W. and D.W. Fawcett, 1975. A textbook of histology. 10th ed., W.B. Saunders Company, Philadelphia.

Bowers, C.Y., K.L. Lee and A.V. Schally, 1968. Effect of actinomycin D on hormones that control the release of thyrotropin from the anterior pituitary glands of mice. Endocrinology 82:303.

Bowers, C.Y., A.V. Schally, F. Enzmann, J. Boler and K. Folkers, 1970. Porcine thyrotrophin releasing factor is (pyro-Glu-His-Pro-NH_2). Endocrinology 86:1143.

Braverman, L.E., and S.H. Ingbar, 1963. Changes in thyroidal function during adaptation to large doses of iodide. J. Clin. Invest. 42:1216.

Burgus, R., T. Dunn, D. Desiderio and R. Guillemin. 1969. Structure moleculaire du facteur hypothalamique hypophysiotrope TRF d'origine ovine: Mise en evidence par spectrometre de masse de la sequence PCA-His-Pro-NH_2. C.R. Acad. Sci. Paris 269:1870.

Burke, G. 1970. Effect of iodine on thyroid stimulation. J. Clin. Endocrinol. Metab. 30:76.

Convey, E.M., L.T. Chapin, J.W. Thomas, K. Leung and E.W. Swanson. 1978. Serum thyrotropin, thyroxine, and tri-iodothyronine in dairy cows fed varying amounts of iodine. J. Dairy. Sci. 61:771.

DeGroot, L. and J.B. Stanbury, 1975. The Thyroid and its Diseases. 4th Ed., John Wiley and Sons, New York.

Desjardin C. and H.D. Hafs. 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Animal Sci. 27:472.

Forbes, E.B., G.M. Karns, S.I. Bechdel, P.S. Williams, T.B. Keith, E.W. Callenbach and R.R. Murphy. 1932. The value of iodine for livestock in central Pennsylvania. J. Agr. Res. 45:111.

Getty, R. 1975. Sisson and Grossman's The Anatomy of the Domestic Animals. 5th Edition. W.B. Saunders Company, Philadelphia.

Gill, J.L. and H.D. Hafs. 1971. Analysis of repeated measurements of animals. J. Animal Science 33:331.

Grafe, E. and E. Grunthal. 1929. Uber isolierte beeinflussung des gesamtstoffwechsels vom zwischenhirn aus. Klin. Wochenschr. 8:1013.

Green, J.D. and G.W. Harris. 1947. The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol. 5:136.

Grimm, Y. and S. Reichlin. 1973. Thyrotropin releasing hormone (TRH): neurotransmitter regulation and secretion by mouse hypothalamic tissue in vitro. Endocrinology 93:626.

Halasz, B. and J. Szentagothai. 1958. Control of adrenocorticotropic function by direct influence of pituitary substance on the hypothalamus. Acta Morph. Acad. Sci. Hung 9:251.

Harris, G.W. 1955. Neural control of the pituitary gland. Edward Arnold Ltd.

Hopkins, P.A., A.L.C. Wallace and G.D. Thorburn. 1975. Thyrotropin concentrations in plasma of cattle, sheep and foetal lambs as measured by radioimmunoassay. J. Endocrinol. 64:371.

Houssay, B.A., A. Biasotti and R. Sammartino. 1935. Modifications functionnelles de l'hypophyse apres les lesions infundibulotuberiennes chez le crapaud. Compt. Rend. Soc. Biol. Fil. 120:725.

Ingbar, S.H. 1963. Observations concerning the binding of thyroid hormones by human serum prealbumin. J. Clin. Invest. 42:143.

Jacobson, N.L. subcommittee chairman. 1978. Nutrient requirements of dairy cattle, 5th revised ed. National Research Council, National Academy of Sciences, Washington, D.C.

Jubb, K.V. and K. McEntee. 1955. Observations on bovine pituitary gland. II Architecture and cytology with special reference to basophil cell function. Cornell Vet. 45:593.

Jubiz, W., S. Carlile and L.D. Lagerquist. 1977. Serum thyrotropin and thyroid hormone levels in humans receiving chronic potassium iodide. J. Clin. Endocrinol Metab. 44:379.

Kahl, S., T.R. Wrenn and J. Bitman. 1977. Plasma tri-iodothyronine and thyroxine in young growing calves. J. Endocrinol. 73:397.

Kesner, J.S., V. Padmanabhan and E.M. Convey. 1977. Effect of T_4 and thyrotropin releasing hormone on hormone secretion from bovine pituitary cell cultures.

The Endocrine Society 59th Annual Meeting Abstract p. 288.

Kirk, R.E. 1968. Experimental Design: Procedures for the Behavioral Sciences. Wadsworth. Belmont, California.

Knigge, K.M. and A.J. Silverman. 1974. Anatomy of endocrine hypothalamus. In: Handbook of Physiology Sec. 7. Vol. IV. Part I. (W.H. Sawyer and E. Knobil, ed.) Waverly Press Inc., Baltimore.

Langman, J. 1963. Medical Embryology. 2nd Ed. Williams and Wilkins Company Baltimore.

Larsen, P.R. and R.D. Frumess. 1977. Comparison of the biological effects of thyroxine and tri-iodothyronine in the rat. Endocrinology 100:980

Louis, T.M., J.N. Stellflug, H.A. Tucker and H.D. Hafs. 1974. Plasma prolactin, growth hormone, luteinizing hormone and glucocorticoids after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 147:128.

McCauley, E.H., D.W. Johnson and I. Alhadji. 1972. Disease problems in cattle associated with rations containing high level of iodide. Bov. Practitioner 7:22.

Mitnick, M., and S. Reichlin. 1972. Enzymatic synthesis of thyrotropin releasing hormone (TRH) by hypothalamic "TRH Synthetase". Endocrinology 91:1145.

Motta, M., N. Sterescu, F. Piva and L. Martini. 1969. The participation of "short" feedback mechanisms in the control of ACTH and TSH secretion. Acta Neurol. Psychiat. Belg. 69:501.

Nagataki, S., K. Shizume and K. Nakao. 1966. Effect of chronic graded doses of iodide on thyroid hormone synthesis. Endocrinology 79:667.

Nagataki, S., K. Shizume and K. Nakao. 1970. Alterations in intrathyroidal iodine metabolism in response to chronic iodide administration. Endocrinology 87:1218.

Nagataki S. 1974. Effect of excess quantities of iodide. In: Handbook of Physiology. Sec. 7 Vol. IV (R.O. Greep and E.B. Astwood, ed.). Waverly Press Inc. Baltimore.

Newton, G.L., E.R. Barrick, R.W. Harvey and M.B. Wise. 1974. Iodine toxicity, physiological effects of elevated dietary iodine on calves. J. Animal Sci. 38:449.

Ohtaki, S., S. Moriya, H. Suzuki and Y. Horiuchi. 1967. Nonhormonal iodide escape from the normal and abnormal thyroid gland. J. Clin. Endocrinol. Metab. 27:728.

Oliver, C., R.L. Eskay, R.S. Mical and J.C. Porter. 1973. Radioimmunoassay for TRH and its determination in hypophysial and portal and peripheral plasma of rats. Program Meet. Am. Thyroid Assoc., 49th (Abstr.)

Patten, M.B. 1968. Human Embryology. 3rd Ed. McGraw-Hill Book Company. New York.

Pierce, J.G. 1974. Chemistry of thyroid-stimulating hormone. In: Handbook of Physiology Sec. 7. Vol. IV., Part I. (W.H. Sawyer and E. Knobil, ed.). Waverly Press Inc., Baltimore.

Pisarev, M.A., L.J. DeGrootand R. Hati. 1971. KI and imidazole inhibition of TSH and cAMP induced thyroid iodine secretion. Endocrinology 88:1217.

Porter, J.C., W. Vale, R. Burgus, R.S. Mical and R. Guillemin. 1971. Release of TSH infused directly into a pituitary stalk portal vessel. Endocrinology 89:1054.

Reichlin, S., R. Saperstein, I.M.D. Jackson, A.E. Boyd, III and Y. Patel. 1976. Hypothalamic Hormones. In: Annual Review of Physiology (E. Knobil, ed.) 38:389.

Schally, A.V., C.Y. Bowers, T.W. Redding and J.F. Barrett. 1966. Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus. Biochem. Biophys. Res. Comm. 25:165.

Schally, A.V. and T.W. Redding. 1967. In vitro studies with thyrotropin releasing factor. Proc. Soc. Exp. Biol. Med. 126:320.

Scharrer, E. and B. Scharrer. 1954. Hormones produced by neurosecretory cells. Recent Prog. Horm. Res. 10:183.

Schreiber, V., A. Eckertova, Z. Franc, J. Koci, M. Rybak and V. Kmentova. 1961. Effect of a fraction of bovine hypothalamic extract on the release of TSH by rat adenohypophyses in vitro. Experientia 17:264.

Shibusawa, K., S. Saito, K. Nishi, T. Yamamoto, K. Tomizawa and C. Abe. 1956. The hypothalamic control of the thyrotroph-thyroidal function. Endocrinol. Jpn. 3:116.

Silva, J.E., T.E. Dick and P.R. Larsen. 1978. The contribution of local tissue thyroxine monodeiodination to the nuclear 3,5,5'-tri-iodothyronine in pituitary, liver and kidney of euthyroid rat. Endocrinology 103:1196.

Smith, V.G., E.M. Convey and L.A. Edgerton. 1972. Bovine serum corticoid response to milking and exteroceptive stimuli. J. Dairy Sci. 55:1170.

Snyder, P.J. and R.D. Utiger. 1972. Inhibition of thyrotropin response to thyrotropin-releasing hormone by small quantities of thyroid hormones. J. Clin. Invest. 51:2077.

Sorenson, A.M., W.H. Hough, D.T. Armstrong, R.W. Bratton and W. Hansel. 1959. Causes and prevention of reproductive failures in dairy cattle. I. The influence of underfeeding and overfeeding on growth and development of Holstein heifers. Cornell Univ. Agr. Expt. Sta. Bull. 936.

Sterling, K. 1970. The significance of circulating triiodothyronine. Recent Prog. Horm. Res. 26:249.

Sterling, K., and J.H. Lazarus. 1977. The thyroid and its control. In: Annual Review of Physiology (E. Knobil, ed.) 39:349.

Suzuki, H., T. Higuchi, K. Sawa, S. Ohtaki and Y. Horiuchi. 1965. Endemic coast goitre in Hokkaido, Japan. Acta Endocrinol. 50:161.

Swanson, E.W. 1972. Effect of dietary iodine on thyroxine secretion rate of lactating cows. J. Dairy Sci. 55:1763.

Tong, W. 1974. Actions of thyroid-stimulating hormone. In: Handbook of Physiology. Sect. 7., Vol. III. (R.O. Greep and E.B. Astwood, ed.) Waverly Press Inc. Baltimore.

Torok, B. 1954. Lebendbeobachtung des hypophysenkreislaufes an hunden. Acta Morphol. Acad. Sci. Hung. 4:83.

Turner, C.D. and J.T. Bagnara. 1976. General Endocrinology. 6th Edition. W.B. Saunders Company, Philadelphia.

Vagenakis, A.G., P. Downs, L.E. Braverman, A. Burger and S.H. Ingbar. 1973. Control of thyroid hormone secretion in normal subject receiving iodide. J. Clin. Invest. 52:528.

Vagenakis, A.G., E. Roti, J. Mannix and L.E. Braverman. 1975. Problems in the measurement of urinary TRH. J. Clin. Endocrinol. Metab. 41:801.

Valdes-Dapena, M.A. 1957. An atlas of fetal and neonatal histology. J.B. Lippincott Co. Philadelphia.

Vale, W., R. Gurgus and R. Gullemin. 1968. On the mechanism of action of TRF: Effects of cycloheximide and actinomycin on the release of TSH stimulated in vitro by TRF and its inhibition by thyroxine. Neuroendocrinology 3:34.

Van Sande, J., G. Grenier, C. Willems, and J.E. Dumont. 1975. Inhibition by idoide of the activation of the thyroid cyclic 3',5'-AMP system. Endocrinology 96:781.

Wallace, C.E. 1975. Iodine toxicity in cattle. Geo. Vet. 27:25.

Wartofsky, L., R.C. Dimond, G. L. Noel, A.G. Frantzand J.M. Earll. 1976. Effect of acute increase in serum triiodothyronine on TSH and prolactin response to TRH, and estimates of pituitary stores of TSH and prolactin in normal subjects and in patients with primary hypothyroidism. J. Clin. Endocrinol. Metab. 42:443.

Wenzel, K.W., H. Meinhol and H. Schleusener. 1975. Different effects of oral doses of triiodothyronine or thyroxine on the inhibition of thyrotrophin releasing hormone (TRH) mediated thyrotrophin (TSH) response in man. Acta Endocrinol. Copenhagen 80:42.

Wheeler, R.S. and E. Hoffman. 1949. Goiterogenic action of iodide and etiology of goiters in chicks from thyroprotein fed hens. Proc. Sco. Exp. Biol. Med. 72:250.

Wilber, J.F., and J.C. Porter. 1970. Thyrotropin and growth hormone releasing activity in hypophyseal portal blood. Endocrinol 87:807.

Wolff, J and I.L. Chaikoff. 1948. Plasma inorganic iodide as a homeostatic regulator of thyroid function. J. Biol. Chem. 174:555.

Wolff, J., I.L. Chaikoff, R.C. Goldberg and J.R. Meier. 1949. The temporary nature of the inhibitory action of excess iodine on organic iodine synthesis in the normal thyroid. Endocrinology 45:504.

Yamamato K., T. Onaya, T. Yamado and M. Kotani. 1972. Inhibitory effect of excess iodide on thyroid hormone release as measured by intracellular colloid droplets. Endocrinology 90:986.