FACTORS INFLUENCING BOVINE PROLACTIN AND GROWTH HORMONE

Dissertation for the Degree of Ph. D.
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VALDIN G. SMITH
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This is to certify that the

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Factors Influencing Bovine
Prolactin and Growth Hormone Release

presented by

V.G. Smith

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Dairy Science

Major professor
E.M. Convey

Date September 25, 1974

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ABSTRACT

FACTORS INFLUENCING BOVINE PROLACTIN AND GROWTH HORMONE RELEASE

By

Valdin G. Smith

Experiments were conducted *in vivo* and *in vitro* to investigate the control of prolactin and growth hormone (GH) release in the bovine. Ten lactating Holstein cows were randomly assigned to receive subcutaneously either 5 ml of 50% ethanol (controls) or 80 mg of ergocryptine in 5 ml of 50% ethanol on two consecutive days. The effect of ergocryptine on prolactin and GH release from bovine pituitary cell cultures was also investigated. Serum prolactin concentration in both groups of cows on the day before treatment, increased from an average of 14-16 ng/ml at 5 min before milking to approximately 30 ng/ml at 10 min after the start of milking ($p < 0.05$). On the 2 days of treatment prolactin concentration (ng/ml) in cows treated with ethanol averaged 20 and 35 (day 1) and 17 and 27 (day 2) at 5 min before and 10 min after the start of milking respectively ($p < 0.05$). Comparable averages for cows treated with ergocryptine were 1.3 and 1.4 (day 1) and 1.1 and 1.1 (day 2). Following ergocryptine treatment serum prolactin concentration was decreased within 2 hr and remained suppressed for at least 5 days

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after treatment. But average serum GH concentration (4 ng/ml) was not affected by either ergocryptine treatment or stimuli associated with milking. Incubation of pituitary cell cultures with 0.01 to 10 ug ergocryptine/ml TC medium 199, reduced prolactin concentration approximately 60%, ($p < 0.001$) but did not affect ($p > 0.05$) media GH concentration. Therefore, ergocryptine decreases serum prolactin concentration in cattle perhaps by a direct action on the anterior pituitary.

Cell cultures prepared from anterior pituitaries of cows, steers and a bull were incubated for 2 hr with thyrotropin releasing hormone (TRH) at 72 hr or 96 hr of culture. Media prolactin concentration was increased ($p < 0.01$) following addition of TRH to 72-hr pituitary cell cultures of cows. Prolactin concentration averaged -23, 799, 1966, 1926 and 1976 ng/ml after 0, 0.01, 0.1, 1.0 and 10 ng TRH/ml TC medium 199, respectively, when expressed as the difference in quantity released before and after TRH treatment. Comparable results were obtained with pituitary cell cultures from additional cows and from steers and a bull. Thyrotropin releasing hormone also increased ($p < 0.05$) media GH concentration from 72-hr pituitary cell cultures but not from those treated at 96 hr.

Neither baseline prolactin concentration nor TRH-induced prolactin release from cell cultures was affected by triiodothyronine (T_3) or thyroxine (T_4) at concentrations of 0.1 or 1.0 ug/ml medium. But 5 and 50 ug T_4/ml medium decreased ($p < 0.05$) spontaneous release of prolactin and

the quantity of prolactin released by TRH. Prolactin concentration in the media averaged 161, 119.5 and 82.5 ng/ml after treatment with 0, 5 and 50 ug T_4/ml respectively. When these cultures were subsequently treated with TRH, prolactin released into the media averaged 261, 222 and 171.5 ng/ml respectively ($p < 0.05$). These results suggest that TRH releases prolactin in cattle at least in part by a direct action on the anterior pituitary and T_4 at high doses may inhibit spontaneous release of prolactin and the quantity releasable by TRH.

The effect of concentrations of progesterone and estradiol, that approached physiological levels, on serum concentrations of prolactin and GH was also investigated. Serum progesterone concentration (ng/ml) increased ($p < 0.05$) from an average of 1.5 before, to 3-4 following placement of progesterone pessaries and remained elevated for at least 5 days following ovariectomy. Serum estradiol concentration (pg/ml) increased ($p < 0.05$) from an average of 8 before, to 24 following placement of estradiol implants and also remained elevated following ovariectomy. Serum concentrations of these hormones decreased ($p < 0.05$) in untreated heifers within 24 hr after ovariectomy. When depot steroids were removed the respective hormone concentrations in serum decreased ($p < 0.05$) to levels comparable to concentrations in untreated ovariectomized heifers. There were no changes ($p > 0.05$) in serum concentrations of prolactin and GH attributable to steroid treatment. Neither

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did ovariectomy or removal of depot steroids affect ($p > 0.05$) serum concentration of these hormones.

In a subsequent study, placement of estradiol implants into ovariectomized heifers appeared to increase serum concentration of GH but not prolactin. Neither was there any difference ($p > 0.05$) in TRH-induced prolactin release between controls and estradiol-treated heifers. However, serum GH concentration was 53% greater ($p > 0.05$) in heifers bearing 4 estradiol implants than in controls and 132% greater ($p < 0.05$) in heifers bearing 8 estradiol implants than in controls. Thus estradiol at concentrations which approximate those found at estrus in cattle do not influence prolactin concentration in serum. Hence, increase prolactin at or near estrus in cattle is probably due to factors other than increased serum estrogens. Similarly changes in serum progesterone of a magnitude expected during the estrous cycle of cows probably do not play a major role in control of prolactin and GH.

FACTORS INFLUENCING BOVINE PROLACTIN AND GROWTH HORMONE

By

Valdin G. Smith

A DISSERTATION

Submitted to
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To his co-graduate students he also expresses gratitude for their help in different laboratory chores involved in this research.

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BIOGRAPHICAL SKETCH

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Valdin G. Smith

I was born in Jamaica, West Indies on December 12, 1942. After completion of elementary and secondary education I entered Jamaica School of Agriculture in 1960 and received a diploma in agriculture in 1963. Thereafter I worked for 3 years as an artificial insemination technician with the Ministry of Agriculture and Lands.

In 1967 I entered Tuskegee Institute and received a B.S. in animal science in 1969. I was accepted by the Department of Dairy Science, Michigan State University where I completed a M.S. in dairy physiology in 1971 under the guidance of Dr. E. M. Convey. Immediately thereafter I started working towards the Doctor of Philosophy degree which will be completed in Fall term 1974.

TABLE OF CONTENTS

MATERIAL

A.

B.

C.

D.

E.

Table

LIST OF TABLES

Figure

1. S
c
c

2. P
i
m

3. P
i

4. P
i
m

5. P
i
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6. E
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a
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12. S
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LIST OF FIGURES

Figure

13. S
n
i

14. S
n
i

15. S
t
i

16. T
t
p

17. T
h
i

18. S
m
i

19. T
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20. T
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Appendix

LIST OF APPENDICES

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INTRODUCTION

Although endocrinology did not attain importance as a scientific discipline until the twentieth century, much has been accomplished during this relatively short period toward understanding hormone-regulated mechanisms. Today we know that growth, reproduction and lactation of laboratory and economically important animals appear to be endocrine regulated but the mechanism by which these events are controlled is not fully understood.

In view of the importance of milk and dairy products, research has been directed toward finding a means of obtaining more milk from dairy cows. It appears that not only proper breeding, feeding and management influence milk production in dairy cows but hormones are also involved. Prolactin and growth hormone have been implicated as part of the lactogenic complex in certain laboratory species but their role in bovine lactation is not fully elucidated.

Therefore the purpose of these studies was to investigate the physiological role and mechanisms by which prolactin and growth hormone are controlled in the bovine. An understanding of the mechanism by which these hormones are controlled in the bovine may provide basic information required to manipulate hormones and achieve increase milk production.

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REVIEW OF LITERATURE

A. Prolactin and Growth Hormone (GH) requirement for lactation

Based on their observations that milk secretion was induced in psuedopregnant rabbits injected with anterior pituitary extracts, Stricker and Grueter (1928) suggested that anterior pituitary secretions were involved in lactation. Grueter and Stricker (1929) also obtained increase milk production in cows injected with ox pituitary extracts.

With identification and isolation of prolactin (Riddle et al. 1933) and GH (Li and Evans 1944) many experiments were executed to demonstrate the requirements of these two hormones for lactation. Fredrikson (1939) induced milk secretion in hypophysectomized rabbits by daily injections of sheep prolactin. Subsequently, this result was confirmed by Lyons (1942) who injected sheep prolactin into a single galactophore of the mammary gland of rabbits and observed initiation of milk secretion in the segment of the gland served by that galactophore. In an excellent review, Cowie (1969) summarized his work and that of others that showed either prolactin or GH in combination with an adrenal corticoid could initiate lactation in hypophysectomized, ovariectomized and adrenalectomized rats and mice with developed mammary glands.

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cows and ewes (Folley and Young 1940, Sulman and Twersky 1948, Cotes et al. 1949, Wrenn and Sykes 1953 and Morag et al. 1971). However, Gotsulenko (1968) observed increase lactational performance in goats following injection of prolactin into the arterial system of the mammary gland. In addition, Schams et al. (1972) suggested that prolactin was required for lactogenesis in cattle since inhibition of the prepartum rise of serum prolactin resulted in decreased milk yield in the subsequent lactation. Koprowski and Tucker (1973) reported a significant correlation between the magnitude of prolactin release to milking stimuli and milk production in the bovine which may suggest that prolactin released at milking may influence subsequent milk yield as reported for rats (Grosvenor and Mena 1973).

Although, Cowie (1969) reported that sheep prolactin alone could induce traces of mammary secretion in the hypophysectomized goat, administration of prolactin, GH, corticoid and thyroid hormone was required to restore lactation to prehypophysectomy levels. However, following restoration of lactation, withdrawal of prolactin had no effect on milk yield for several weeks but cessation of GH treatment caused an immediate suppression of lactation. Administration of bovine GH to lactating cows has also been reported to enhance lactational performance (Cotes et al. 1949, Donker and Petersen 1951, 1952, Chung et al. 1953, Wrenn and Sykes 1953, Brumby and Hancock 1955, Hutton 1957 and Machlin 1973).

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B. Effect of ergot alkaloids on Prolactin and GH secretion

1. In vivo

Shelesnyak (1954, 1955, 1958) presented the first indirect evidence that ergot drugs inhibited prolaction secretion when he observed that administration of ergotoxine to rats suppressed deciduoma formation and terminated psuedopregnancy and early pregnancy. Many of these effects could be reversed with progesterone or prolactin administered concurrently or within 24 hr after ergotoxine treatment. Similarly, ergocornine blocked nidation in hypophysectomized rats bearing pituitary transplants (Varvuhidi et al. 1966) and when given to rats on the morning after coitus it inhibited pregnancy (Carpent and Desclin 1969). These effects could be reversed with progesterone given concurrently with ergocornine.

Yanai and Nagasawa (1970) Meites et al. (1972), Shaar and Clemens (1972) and Wuttke and Meites (1972) demonstrated that ergot drugs suppressed serum and pituitary prolactin concentrations of rats. In addition, ergocornine and ergocryptine inhibited growth of either dimethylbenzanthrene-induced or spontaneous mammary tumors of rats, presumably by inhibiting prolactin secretion (Nagasawa and Meites 1970, Heuson et al. 1970, Quadri and Meites 1971 and Cassell et al. 1971). Following termination of treatment, growth of spontaneous mammary tumors was resumed (Quadri and Meites 1971). Pearson et al. (1969) also reported that exogenous bovine prolactin but not GH reactivated regressed mammary tumors of

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Injection of ergocornine into rats on the morning of proestrus inhibited the characteristic increase in serum prolactin concentration observed on the afternoon of proestrus in this species (Yokoyama et al. 1971, Wuttke et al. 1971; 1972 and Yanai and Nagasawa 1974). Ergocornine also inhibited estrogen-induced prolactin release in ovariectomized rats in vivo and from pituitary explants in vitro (Lu et al. 1971). In addition ergot drugs suppressed lactation in rats, (Zielmaker and Carlson 1962, Shaar and Clemens 1972) mice (Nagasawa and Yanai 1972) and humans (del Pozo et al. 1972, Varga et al. 1972 and Lutterbeck et al. 1971). In rats, administration of prolactin but not oxytocin could reverse the suppression of lactation induced by ergocornine (cited in Nagasawa and Yanai 1972). Although ergocryptine decreased basal serum prolactin concentration of goats (Hart 1973, McMurty and Malven 1974) and cows (Karg et al. 1972) and inhibited prolactin release in response to milking stimuli in cows (Schams et al. 1972a) this compound did not affect milk yield.

Recently ergocryptine has been reported to suppress serum prolactin concentration in ewes (Niswender 1974) and in rats bearing pituitary homografts (Malven and Hoge 1971). Furthermore, Schams et al. (1972) demonstrated that when ergocryptine was administered to pregnant cows 3-4 days before parturition it inhibited the prepartum rise in serum prolactin concentration characteristic of this species (Ingalls et al. 1973). In

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addition ergocryptine greatly diminished the magnitude of serum prolactin release in response to thyrotropin releasing hormone in cattle (Schams 1972).

2. In vitro

Nassar et al. (1950) first suggested that ergot drugs acted directly on the anterior pituitary to cause necrosis of the gland. At autopsy, when pituitaries of rats treated with ergotoxine were examined microscopically a number of pyknotic nuclei were present in the anterior portion of the gland. However Pasteels et al. (1971) reported that ergocornine and ergocryptine inhibited prolactin release from rat and human hypophyses in vitro without causing overt cellular destruction of the gland as prolactin secretion could be restored by washing the ergots from the explants. Similarly, Ectors et al. (1972) reported that ergocryptine caused no cellular destruction of the pituitary gland but rather caused an inhibition of exocytocis from prolactin cells. Moreover, explants treated with ergocryptine contained greater prolactin concentration than control explants as assayed by proliferation of the pigeon crop sac.

Yanai and Nagasawa (1970a) used polyacrylamide gel electrophoresis to determine pituitary prolactin and GH concentrations and reported that chronic administration of ergocryptine to mice suppressed pituitary prolactin content and concentration with no apparent effect on GH concentration. Similarly, when pituitaries from rats treated with ergocryptine were incubated in vitro with ^{14}C-leucine, prolactin release, but not its

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Although it is apparent that ergot drugs can act directly on the adenohypophysis to inhibit prolactin release, the possibility of an additional effect on the hypothalamus cannot be excluded. When ergocornine was implanted into the median eminence of rats or when hypothalami from ergocornine-treated rats were coincubated *in vitro* with normal pituitary explants, serum and pituitary prolactin concentrations were decreased (Wuttke *et al.* 1971). Hokfelt and Fuxe (1972) by use of fluorescence staining, reported that ergocryptine and ergocornine decreased the rate of disappearance of dopamine from the median eminence of lactating and pregnant rats. In general, these investigators suggest that ergocornine and ergocryptine may act at the hypothalamus to increase prolactin inhibiting factor, thus decreasing pituitary and serum prolactin levels.

C. Thyrotropin-Releasing Hormone (TRH)

1. General

Isolation of porcine TRH (Schally *et al.* 1969) was followed quickly with elucidation of its structure (Nair *et al.* 1970) and synthesis of the tripeptide (Boler *et al.* 1969). With synthesis and availability of TRH, many experiments have been conducted in different species to understand the physiology of TRH.

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(Fleischer et al. 1970, Labella and Vivian 1971, Porter et al. 1971, Vale et al. 1972 and Haigler et al. 1972). In addition to TSH the response of growth hormone and glucocorticoids was also investigated but only TSH concentration was consistently changed by TRH (Ormston et al. 1971, 1971a and Gaul et al. 1972).

2. Effect of TRH on Prolactin and GH concentrations

Bowers et al. (1971) and Jacobs et al. (1971) first reported that administration of TRH to humans increased serum prolactin concentration. These initial reports were confirmed in humans (Friesen et al. 1972, L'Hermite et al. 1972, Bowers et al. 1972, 1973, Jacobs et al. 1973, Wilber 1973 and Noel et al. 1974).

Administration of TRH to lactating women not only increased serum prolactin concentration but caused breast engorgement, milk let down and increased milk fat and protein content of milk (Tyson et al. 1972, 1972a). Similarly, Convey et al. (1973a) reported that administration of TRH to 20 lactating dairy cows increased milk yield by 0.66 kg/cow/day, but neither milk fat nor protein content of milk was affected. In contrast, Kelly et al. (1973) observed no change in milk yield or its composition following administration of TRH to four lactating cows and Adams et al. (1973) reported that administration of TRH to lactating rats (day 5-21) had no effect on milk production as determined by litter weight gain at weaning.

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prolactin concentration in sheep (Davis and Borger 1972, Debeljuk et al. 1973, Fell et al. 1973, Moseley et al. 1973) and in cattle (Schams 1972, Convey 1973, Convey et al. 1973, 1974, Kelly et al. 1973 and Vines et al. 1973, 1974). But rats appeared to be the least responsive to TRH-induced prolactin release since doses of TRH (5-10 ug) which effectively released prolactin in man failed to stimulate prolactin release from rat pituitary explants in vitro (Friesen and Hwang 1973). Lu et al. (1972) also reported that a single injection of 5 or 7.5 ug TRH into male rats failed to increase serum prolactin concentration, and 50 ug TRH injected daily for 6 days greatly increased pituitary prolactin concentration but caused only a slight increase in serum prolactin concentration. In contrast Mueller et al. (1973) reported that TRH increased serum prolactin concentration in normal and estrogen-primed male rats and in normal female rats treated on the morning of proestrus. The tripeptide also increased serum prolactin concentration in proestrus and lactating rats (Deis and Alonso 1973, Blake 1974) and prolactin and GH in male rats (Takahara et al. 1974).

Although TRH consistently increased serum GH concentration in dairy heifers, (Vines et al. 1974) lactating cows (Convey 1973, Convey et al. 1973) and in acromegalic humans (Saito et al. 1971, Irie and Tsushima 1972, Schalch et al. 1972, Cryder et al. 1973 and Fagalia et al. 1973) the effect of the tripeptide on GH release in normal humans is not clear. In normal humans, Fleischer et al. (1970), Bowers et al.

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(1971) and Torjesen et al. (1973) reported that TRH increased serum GH concentration but Anderson et al. (1971) Ormston et al. (1971a), Saito et al. (1971) and L'Hermite et al. (1972) failed to confirm these results.

3. Mechanism of TRH action

Several investigators have attempted to elucidate the mechanism by which TRH can affect prolactin and GH concentrations. Tashjian et al. (1971) suggested the pituitary as one site of action when they observed that TRH increased prolactin secretion and decreased GH secretion from cloned rat pituitary tumor cells in vitro. Vale et al. (1973) also demonstrated that TRH released prolactin from rat pituitary cell cultures and hemi-pituitaries in vitro. Similarly, Dibbet et al. (1973) demonstrated that TRH increased prolactin release from rat pituitary explants in vitro and Dannies et al. (1973) reported that TRH increased both synthesis and release of prolactin from rat pituitary explants in vitro.

Although Labella and Vivian (1971) reported that TRH stimulated prolactin and GH release from bovine pituitary explants in one of three experiments, Convey et al. (1973) did not observe any TRH stimulation of prolactin release from steer pituitary explants in vitro. Recently, Machlin and Jacobs (1973) and Smith and Convey (1974) reported that TRH increased media prolactin and GH concentration from primary cell cultures of bovine pituitaries. Bourne et al. (1974) also demonstrated TRH stimulation of prolactin

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release from bovine pituitary cell cultures. Thus TRH repeatedly caused prolactin release from bovine pituitary cells in culture but prolactin release from bovine pituitary explants is not demonstratable or at best variable.

The reports of Labrie et al. (1972) and Wilber (1973) that TRH selectively binds to plasma membrane of anterior pituitaries of rats and cattle also support the hypothesis that TRH can stimulate prolactin release by a direct action on the pituitary. Furthermore, Labrie et al. (1973) demonstrated that within 2-6 min after addition of TRH to rat pituitary explants in vitro, cyclic AMP increased (100-150%). These authors suggested that TRH might activate adenyl cyclase to increase cyclic AMP concentration which then served as the messenger for the action of TRH.

Based on their observations that TRH was an antidepressant agent, Kastin et al. (1972) and Prange et al. (1972) suggested that TRH can act at sites other than the anterior pituitary. Bowers et al. (1972), Noel et al. (1973) and Jaffe et al. (1973) reported that TRH-induced prolactin release was suppressed in humans treated with L-dopa 1-2 hr before TRH. These results were interpreted to mean that TRH acted on the hypothalamus, since L-dopa or its metabolite (dopamine) had been previously demonstrated to reduce serum prolactin concentration in rats, presumably by increasing hypothalamic prolactin inhibiting factor (Kamberi et al. 1971, Lu and Meites 1972). But addition of dopamine to rat pituitary explant in vitro also inhibited prolactin secretion (Koch et al. 1970) suggesting a dual site of action for

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4. Interaction of TRH with thyroid hormone

Hollander et al. (1972) reported that administration of TRH to humans increased serum thyroxine (T_4) and triiodothyronine (T_3). Since T_4 and T_3 increased prolactin release from rat pituitary explants in vitro (Nicoll and Meites 1963) and increased rat pituitary prolactin content in vivo (Chen and Meites 1969) the possibility existed that prolactin released in response to TRH was mediated via thyroid hormones. But in cattle this was unlikely since serum prolactin concentration increased within minutes after TRH injection and serum T_4 increased only after several hours (Convey et al. 1973). Results reported by Shaw et al. (1972) lend credence to this hypothesis since it was demonstrated that feeding of thyroprotein to dairy cows increased serum T_4 from 6 to 13 ug/100 ml without affecting serum prolactin concentration. Recently Vanjonack et al. (1974) reported that administration of TRH to cows resulted in a biphasic response in serum T_4. Within the first 30 min after injection, serum T_4 increased, then decreased to a nadir by 2 hr followed by another increase within 6 hr. Unfortunately these authors did not quantify serum prolactin concentration.

In general, although the evidence is not conclusive, it appears that hypothyroidism stimulates while hyperthyroidism

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suppresses the effect of TRH (Wilber 1973). Vale et al. (1972 and 1973) demonstrated that thyroid hormones diminished the magnitude of TRH-induced prolactin and TSH release from rat pituitary explants and primary pituitary cell cultures. These observations were confirmed in vivo in sheep (Debeljuk et al. 1973) and humans (Snyder et al. 1973 and Yamaji 1974). However, Bowers et al. (1972) and Rapoport et al. (1973) reported that administration of T_3 to humans suppressed TRH-induced TSH release but not prolactin release.

D. Effect of gonadal steroids on prolactin and GH concentrations

1. Estrogen

As early as 1937 Reece and Turner reported that exogenous estrogen increased pituitary prolactin content of rats. These results were confirmed by Nicoll and Meites (1962) and Ben-David et al. (1964) who reported that estrogen increased prolactin release from rat pituitary explants in vitro. Similarly, intrahypophyseal implants of estrogen promoted prolactin release from rats (Ramirez and McCann 1964) and rabbits (Kanematsu and Sawyer 1963).

Prolactin secretion was increased when rat pituitary explants were coincubated in vitro with hypothalamic extract from estrogen-primed rats (Ratner and Meites 1964), and enovid-treated rats (Minaguchi and Meites 1967), compared to hypothalamic extract from normal cycling rats. These results were interpreted to mean that hypothalami taken from rats exposed to estrogens, contained less PIF, therefore more prolactin was released from pituitaries coincubated with these

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hypothalami. Sar and Meites (1967) also reported that hypothalami from rats killed during proestrus and estrus contained less PIF than hypothalami from rats killed during diestrus.

In many laboratory species pituitary and serum prolactin concentrations are greater at proestrus and estrus compared to diestrus (Reece 1939, Sar and Meites 1967, Kwa and Verhofstad 1967, Amenomori et al. 1970 and Voogt et al. 1970). In contrast no change in serum prolactin concentration was apparent during the menstrual cycle of women (Hwang et al. 1971, Jaffe et al. 1973 and Tyson and Friesen 1973). Apparently, the prolactin surge at proestrus in rats is estrogen-dependent since it could be eliminated with an antiestrogen administered on the day preceding proestrus (Neil et al. 1971, Freeman et al. 1972 and Yokoyama and Tomogane 1973).

Although estrogens stimulate prolactin secretion there are reports that large doses of estrogens and estrogenic oral contraceptives can inhibit lactation in several species (Meites 1961, Cowie 1961, Morris 1967 and Koetsawang et al. 1972). The mechanism whereby large doses of estrogen are inhibitory to an established lactation is not clearly understood, but one possibility is that large doses of estrogen suppress prolactin secretion. However, large doses of estrogen administered to ovariectomized rats did not inhibit serum prolactin concentration relative to non-treated ovariectomized rats (Chen and Meites 1970). Therefore Meites et al. (1972) suggested that inhibition of lactation may be due to estrogen interference with the peripheral action of prolactin at the mammary gland. Bruce and Ramirez (1970) supported this

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hypothesis when they observed that estrogen implanted into the mammary gland of rats inhibited lactation, but it enhanced lactation when it was placed in the anterior pituitary gland. Griffith and Turner (1962) suggested that inhibition of lactation was due to estrogen interference with the milk ejection reflex since rats treated with estrogen had their mammary glands engorged with milk. Estrogen may also inhibit lactation by stimulating mammary gland growth, thereby changing the ability of the gland to respond to the lactogenic complex.

Evidence of the effect of gonadal steroids on prolactin concentration in farm animals is not conclusive. In heifers Sinha and Tucker (1969) reported that pituitary prolactin content increased from two days before to the day of estrus, then significantly decreased until two days after estrus. They suggested that the decrease in pituitary prolactin content may reflect release of prolactin into the serum. However during the estrous cycle there was no change in serum prolactin concentration of lactating and non-lactating cows (Schams and Karg 1970) and heifers (Wetteman and Hafs 1973). Similarly, serum prolactin concentration before, immediately after and 1 hr after milking dairy cows did not change significantly during the estrous cycle (Koprowski and Tucker 1973). Raud et al. (1971) also failed to establish any relationship between the stage of the estrous cycle and serum prolactin concentration when blood was collected from cycling cows via jugular puncture. In contrast, when animals were bled via jugular cannulae serum prolactin concentration

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increased at proestrus and estrus relative to diestrus. These authors suggested that stress associated with venipuncture can cause erratic alterations in serum prolactin concentration to negate the response to other stimuli. But Swanson and Hafs (1971) and Swanson et al. (1972) collected blood from heifers via venipuncture or jugular cannulae and reported increase serum prolactin concentrations 3-4 days preceding estrus, it peaked at estrus then subsequently declined during diestrus.

In ewes, Reeves et al. (1970), Bryant et al. (1971) and Davis et al. (1971) demonstrated increase serum prolactin concentrations at proestrus and estrus relative to concentrations during metestrus and diestrus. Injection of estradiol benzoate into anestrus ewes also increased plasma prolactin concentration (Fell et al. 1972). Day et al. (1959) also observed a linear increase in pituitary prolactin content of pigs between days 2-19 of the estrous cycle.

Changes in estradiol concentration may also influence GH levels. In rats (Dickerman 1971) and mice (Sinha et al. 1972) serum and pituitary GH concentrations were greater at proestrus and estrus than during diestrus. Spellacy et al. (1969) reported increase GH concentration during the ovualatory and pre-menstrual phases of women, a time when estrogen levels were elevated in urine (Brown 1960). Similarly, Unger (1965) demonstrated that fasting levels of serum GH were higher in women than men presumably due to higher levels of estrogen in women. Koprowski and Tucker (1973) also observed increase serum GH concentration during the estrogenic phase of

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the estrous cycle of lactating cows, and during late pregnancy. Increased serum GH concentration at parturition in cattle (Ingalls et al. 1973) may be due to estrogens which are reported to be elevated in serum of heifers (Smith et al. 1973).

Administration of diethylstilbestrol to steers increased serum GH concentration (Trenkle 1970). Similarly, Lloyd et al. (1971 and 1973) demonstrated that injection of diethylstilbestrol into rats increased pituitary weight and serum GH concentration. In humans, administration of diethylstilbestrol or estrogenic oral contraceptives also increased basal serum GH concentration (Frantz and Rabkin 1965) and increased the magnitude of GH release in response to arginine (Merimee et al. 1966 and Vela and Yen 1969).

In contrast to these reports Ieiri et al. (1971) observed no change in GH synthesis or release in rats at estrus despite marked increases in prolactin concentration. Similarly, Vines et al. (1974) found no effect of the estrous cycle on basal serum GH concentration of dairy heifers. Neither did the stage of the estrous cycle affect the magnitude of GH release in response to thyrotropin releasing hormone.

2. Progesterone

Reece and Bivins (1942) demonstrated that administration of progesterone (15 mg) concurrently with estradiol benzoate (33 ug) to ovariectomized rats inhibited the increase in pituitary prolactin content normally associated with estrogen therapy. However, pituitary prolactin content was increased

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in rats receiving 15 mg of progesterone but no estrogen. Chen and Meites (1970) confirmed these results when they reported that progesterone (0.5 - 4 mg) administered concurrently with estradiol benzoate (1.0 ug) inhibited estrogen-induced prolactin release in rats but progesterone (10 mg) when given alone resulted in nearly a doubling of serum prolactin concentration. Sar and Meites (1968) also reported that 10 mg of progesterone administered daily for 21 days to ovariectomized rats increased pituitary prolactin content relative to untreated ovariectomized rats. Furthermore, when hemi-pituitaries from normal rats were coincubated in vitro with hypothalami from progesterone-treated-rats more prolactin was released into the media than when hemi-pituitaries were incubated with hypothalami from control rats. These results were interpreted to mean that progesterone either directly, or indirectly via conversion to estrogens, reduced prolactin inhibiting factor. Apparently, only high doses of progesterone can stimulate prolactin release since addition of low doses to rat pituitary explant in vitro (Nicoll and Meites 1964) and to intact or hypothalamic-lesioned rats (Bishop et al. 1972) had no effect on media or serum prolactin concentration.

The ratio of estrogen:progesterone also appears to influence prolactin secretion in vivo since at proestrus and estrus when serum estrogens are elevated and serum progesterone concentration is at a nadir, pituitary and serum prolactin concentrations are elevated in some species. Similarly, during pregnancy in cattle, serum prolactin

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concentration was highest at approximately 24 hr before parturition (Schams and Karg 1970, Ingalls et al. 1973) and this coincided with high levels of serum estrogens and low concentration of serum progesterone (Smith et al. 1973). Meites (1959) also reported that in rats and guinea pigs, the increase in pituitary prolactin concentration associated with estrogen therapy was inhibited with estrogen:progesterone ratios of 1:1000 to 1:2000.

Additional evidence also suggests that progesterone can inhibit lactogenesis in certain species, although the mechanisms by which this is accomplished are poorly understood. Yoshinaga et al. (1969) suggested that failure to observe increase prolactin concentration during most of pregnancy in some species may be attributed to the ratio of estrogen:progesterone. Towards the end of pregnancy the fall in progesterone concentration and the increase in serum estrogens change the ratio of estrogen:progesterone which may stimulate prolactin secretion and precipitate lactation. In support of this view, Kuhn (1969) and Herrenkohl (1971) reported that progesterone effectively blocked lactogenesis in rats and suggested that the fall in serum progesterone near to parturition may be the trigger for lactogenesis. Furthermore, injection of prolactin into pregnant rabbits prevented the inhibitory effect of progesterone on lactogenesis (Denamur and Delouis 1972).

There is a paucity of information regarding the effect of progesterone on GH concentration. Administration of progesterone (Bhatia et al. 1972) or medroxyprogesterone acetate

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(Simon et al. 1967, Lawrence and Kirsteins 1970) to humans, suppressed GH release in response to arginine and hypoglycemia. Malarkey and Daughaday (1971) also reported that administration of medroxyprogesterone acetate to acromegalic humans decreased serum GH concentration. But during pregnancy, GH concentration was unchanged in serum of heifers (Oxender and Hafs 1971) and rats (Dickerman 1971).

3. Relationship of gonadal steroids and TRH on prolactin and GH concentrations

Bowers et al. (1971), Friesen et al. (1972), Torjesen et al. (1973), Jacobs et al. (1973) and Noel et al. (1974) reported that TRH-induced prolactin release was greater in human females than males, presumably due to higher estrogen levels in the females. This view was supported by Jaffe et al. (1973) who reported that TRH-induced prolactin release was augmented in early postpartum women, treated with estrogen and testosterone to suppress lactation. Carlson et al. (1973) demonstrated that diethylstilbestrol enhanced TRH-induced prolactin release but not GH release in humans. But Takahara et al. (1974) observed no difference in TRH-induced prolactin release between estradiol-treated rats and controls. Tyson et al. (1972) also found no difference in the magnitude of prolactin released from women treated with TRH during the luteal or menstrual phase of the cycle. Neither did Vines et al. (1974) observe any difference in the magnitude of prolactin or GH released from cows treated with TRH on different days of the estrous cycle.

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MATERIALS AND METHOD - GENERAL

A. Animals

Lactating Holstein cows maintained in the Michigan State University herd and primiparous Holstein heifers, purchased at a local auction, were used in this study. All purchased heifers were palpated per rectum, and four which were diagnosed pregnant were aborted by cesarean section approximately two months before they were involved in any experiment. At Michigan State University, these heifers were maintained under loose housing conditions with free access to pasture.

On the day preceding each *in vivo* experiment, an indwelling jugular cannula (Vinyl IV Tubing, Clay Adams Inc., New York) was inserted into each animal. Approximately 45 cm of the 240 cm cannula were inserted into one jugular vein and affixed to the neck and withers with tag cement (Nasco, Fort Atkinson, Wis.) on 7.6 x 12.7 cm adhesive tape. Each cannula was flushed with 10 ml of 3.5% sodium citrate and sealed until used for blood collection.

At different time intervals, depending on the experimental design, 10 ml of blood were collected according to the following procedure:

(1) Approximately 5 ml of blood which contained residual citrate used to keep the cannulae patent were collected and discarded.

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(2) Ten ml of blood were withdrawn and dispensed into polypropylene centrifuge tubes (Sorval, Inc., Newton, Conn.).

(3) After each blood sample was withdrawn, cannulae were filled with 3.5% sodium citrate which would be removed and discarded at the next collection period.

Blood was allowed to clot at 4°C for 24 hr after which serum was obtained by centrifugation (2500 xg at 4°C) for 30 min. Serum samples were stored frozen until assayed for hormones.

B. In vitro procedures

Preparation of bovine anterior pituitary cell cultures and design of experiments are shown in figure 1. Bovine pituitaries were collected within 30 min of death of the animals at a local abbattoir and transported at 37°C to the laboratory. Within 1 hr of death of animals, posterior pituitaries were discarded and anterior pituitaries were minced with scissors and washed four times with the medium that was used for culture (Appendix 1).

Approximately 1.5 - 2.5 g of minced pituitary tissue were placed in 25 ml Erlenmeyer flasks. Ten ml of 0.2 - 0.3% collagenase (Type 1 - 135 u/mg Lot 13C2430, Sigma Chemical Co.) in culture medium were added to each flask, and the contents of each flask were incubated (37°C) with constant shaking in an Eberbach metabolic shaker at 180 ocillations/min for 45 - 60 min. In some of the later experiments cell suspensions were obtained more quickly by stirring the erlenmeyer flasks containing tissue and collagenase on a Corning

Figure

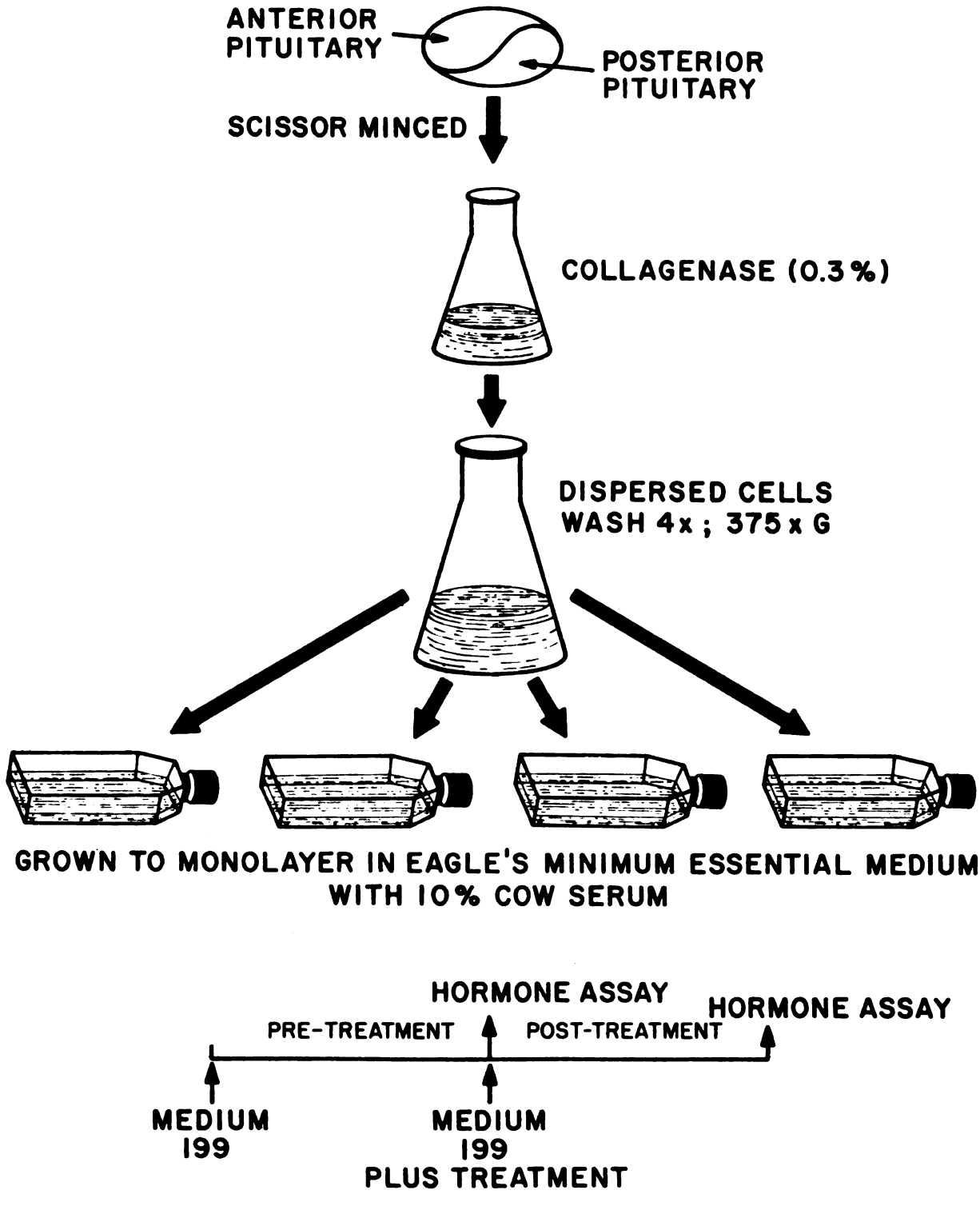

Figure 1. Schematic representation of the preparation of anterior pituitary cell cultures and design of <u>in vitro</u> experiments.

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magnetic mixer (Fisher Scientific Co., Pittsburg, Penn.). The resulting cell suspension obtained by either method was filtered through cheesecloth (2 layers) into 50 ml conical plastic tubes (Falcon Plastics, Oxnard, Cal.) and centrifuged at 375 xg for 3 min at 25°C. The supernatant was discarded and the cells were washed with medium (Appendix 1) and centrifuged at least four times to remove residual collagenase.

Following the final centrifugation cells from four pituitaries were suspended in 120 ml medium containing 10% cow serum (growth medium). Four ml of this cell suspension were transferred with serological pipettes to culture flasks (25 cm^2, 30 ml tissue culture flasks, Falcon Plastics) and incubated at 37°C for 3-4 days by which time confluent monolayers were established. Medium was first replaced after 48 hr and thereafter at 24 hr intervals. The cells were used for experiments on day 3 or 4 depending on the time taken to establish confluent monolayers. For all *in vitro* experiments, treatments were administered in 4 ml of Tissue Culture (TC) medium 199 (Appendix 2).

C. Hormone Assays

1. Protein Hormones

Prolactin, growth hormone and luteinizing hormone in sera and/or tissue culture media were quantified by double antibody radioimmunoassay (RIA) procedures previously described by Tucker (1971) Purchas *et al.* (1970) and Oxender *et al.* (1972), respectively.

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2. Steroid Hormones

Serum progesterone and estradiol were quantified by RIA procedures previously reported (Louis et al. 1973 and Wetteman et al. 1972), respectively.

Total corticoids were extracted from serum as previously reported by Smith et al. (1972). Cortisol was isolated from the total corticoid-fraction by column chromatography (Lin, Oxender and Hafs, unpublished). Briefly, the fractionation procedure was as follows. Approximately 0.2 ml of chromatography solvent (chloroform:methanol; 99:1) was added to each tube containing the isolated total corticoid-fraction. The content of each tube was agitated with a disposable pippette then transferred to a LH-20 sephadex column (0.5 x 12 cm pippette; fitted with a 12-ml reservoir on top). Tubes containing the corticoid-fraction were rinsed a second time with an additional 0.2 ml of chromatography solvent which was also transferred to the columns. Approximately 8 ml of chromatography solvent were used to elute corticoids from the column which were collected in 1 ml-fractions. The elution profile was determined by quantifying radioactivity in each fraction in a liquid scintillation spectrophotometer (Nuclear Chicago Model, Mark 1). Cortisol was eluted in fractions 6, 7 and 8 and the fraction with the greatest amount of radioactivity or in some cases a pooled fraction was assayed for cortisol by protein binding procedures previously reported by Smith et al. (1972). Extraction efficiency and procedural losses were estimated from the difference in radioactivity between ^{3}H-cortisol added to the serum

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D. Specific Objectives and Experimental Procedures

Experiment 1.-- Effect of Ergocryptine on Bovine Prolactin, GH, Cortisol and Milk Yield

Objective 1: Effect of Ergocryptine in vivo

Experimental design: Ten non-pregnant Holstein cows lactating an average of 42.8 days (range 10-97) were used in this experiment. Cows selected for this experiment were in the early stages of lactation since those in late lactation may not respond to milking stimuli with an increase in serum prolactin concentration (Johke 1970, Koprowski and Tucker 1973). During the experimental period all cows were milked twice daily at 0500-0600 hr and 1700-1800 hr. Cows were assigned randomly to one of two groups to receive subcutaneously either 5 ml of 50% ethanol (controls) or 80 mg of ergocryptine in 5 ml of 50% ethanol on two consecutive days. Treatments were administered at 0700 hr and blood was collected via indwelling jugular cannulae according to the following schedule; every 2 hr following treatment until 1500 hr then at 30 min intervals to 1700 (initiation of milking). In addition, blood was collected via jugular cannulae at 1700-1800 hr on the day preceding treatment, each day of treatment and the day following treatment. Blood was also collected once daily from the coccygeal artery or vein by venipuncture on days 2, 3 and 4 after treatment. Prolactin, GH and cortisol were quantified in selected serum samples. Milk yield was recorded at each milking.

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Objective 2: Effect of Ergocryptine in vitro

Experimental design: Bovine anterior pituitary cell cultures which had grown to confluent monolayers by 96 hr were used for this study. On the day of the experiment growth medium was replaced with TC medium 199, (Appendix 2) cells were incubated for 4 hr and the medium was decanted and stored frozen. Then each of four flasks received 4 ml of TC medium 199 containing either 0, 0.01, 1.0, or 10.0 ug ergocryptine/ml and was incubated for 4 hr after which the medium was again decanted and stored frozen. Ergocryptine was dissolved in ethanol prior to addition to TC medium 199 and the final concentration of ethanol in each flask was 0.1%. Prolactin and GH were quantified in the media.

Experiment 2.--Thyrotropin Releasing Hormone (TRH): Effect on Prolactin and GH Release from Bovine Pituitary Cell Cultures

Objective 1: Effect of TRH on Prolactin and GH release in vitro

Experimental design: Cell cultures prepared from pituitaries of four cows were in culture for 72 hr when they were incubated for 2 hr with TC medium 199. Thereafter, cell cultures (four flasks/treatment) were incubated for 2 hr with TC medium 199 containing either 0.0, 0.01, 0.1, 1.0 or 10.0 ng TRH/ml.

In a second experiment, cell cultures prepared from pituitaries of four cows and three steers were in culture for 96 hr when they were incubated for 2 hr with TC medium 199. Then, cell cultures (four flasks/treatment) were incubated for 2 hr with either 0.0, 0.01, 0.1, 1.0 or 100.0 ng TRH/ml medium.

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Following each incubation period the media were decanted and stored frozen until assayed for prolactin and GH.

Objective 2: Prolactin Release in vitro: TRH vs GnRH

Rationale: The rationale for using gonadotropin releasing hormone (GnRH) was to test if bovine pituitary cell cultures retained their ability to distinguish different secretagogues. If there was no difference in the type of hormone released in response to different secretagogues one could argue that TRH-induced prolactin release was a non-specific response.

Experimental design: Cell cultures prepared from pituitaries of four cows were in culture for 96 hr when they were incubated for 2 hr with TC medium 199. Then each of four flasks was incubated for 2 hr with TC medium 199 containing either 0, 1, 10 or 100 ng GnRH/ml or 10 ng TRH/ml. Prolactin and luteinizing hormone (LH) were quantified in the media.

Objective 3: Effect of triiodothyronine (T_3) and thyroxine (T_4) on TRH-induced Prolactin Release in vitro

Experimental design: Bovine pituitary cells were in culture for 96 hr when they were incubated for 2 hr with TC medium 199. Thereafter cells, (four flasks/treatment) were incubated for 2 hr with TC medium 199, containing thyroid hormone and/or TRH. The design of the experiment was a 2x3x2 factorial employing 2 thyroid hormones (T_3 and T_4) at 3 levels (0, 0.1 and 1.0 ug/ml) and 2 doses of TRH (0 and 10 ng/ml).

In a second experiment, bovine pituitary cells that were in culture for 96 hr were preincubated for 6 hr with TC medium 199 containing either 0 or 0.1 ug T_3/ml. Then cells, (four flasks/treatment) were incubated for 2 hr with TC medium 199

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In the final study of this series, 96-hr pituitary cell cultures (5 flasks/treatment) were incubated for two 2-hr periods with TC medium 199 containing either: 1) 0 ug T_4/ml then 10 ng TRH/ml; 2) 5 ug T_4/ml then 10 ng TRH/ml; 3) 5 ug T_4/ml then 10 ng TRH + 5 ug T_4/ml; 4) 50 ug T_4/ml then 10 ng TRH/ml or 5) 50 ug T_4/ml then 10 ng TRH + 50 ug T_4/ml. Media collected at the end of each incubation period were assayed for prolactin.

Experiment 3.-- Prolactin and Growth Hormone Release after Gonadal Steroids and TRH in Vivo and in Vitro

Objective 1: Serum Prolactin and GH after Gonadal Steroids in vivo

Experimental design: Fifteen Holstein heifers (Section A of Materials and Method) were randomly assigned to receive at three days before ovariectomy either no steroids (n = 3); a progesterone pessary (n = 4); estradiol -17β (n = 4) or both estradiol and progesterone (n = 4). Estradiol -17β was contained in polydimethylsiloxane implants (I.D. 3.35, O.D. 4.65 x 50 mm). Four implants were placed subcutaneously, two in each ear. Heifers were ovariectomized on the third day after steroid treatment. Blood collection was accomplished via jugular vein puncture before steroid treatment; thereafter via jugular vein cannulae. A blood sample was collected from each heifer immediately before steroid treatment and just prior to ovariectomy. After ovariectomy blood samples were collected at 2 hr intervals for 48 hr then twice daily for 4 days. Thereafter depot steroids were removed and blood was collected every

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2 hr for 48 hr then once daily for 4 days. Prolactin was quantified in all serum samples and GH, progesterone and estradiol were quantified only in selected serum samples.

Objective 2: Effect of Estradiol -17β on TRH-induced Prolactin and GH release in vivo

Experimental design: Approximately 60 days after ovariectomy, ten heifers were randomly assigned to receive either no steroid (control) or four implants containing estradiol -17β. Following treatment with estradiol -17β blood was collected at 2 hr intervals for 36 hr. Beginning at 72 hr after treatment, blood was collected at 30 min intervals for 90 min, then every 5 min for 30 min at which time all heifers received intravenously 33 ug TRH/100 kg body wt. Following TRH, blood was collected at 4, 6, 8 and 10 min, at 5 min intervals until 30 min, at 15 min intervals until 60 min then at 90 and 120 min.

Eight of these 10 heifers plus two additional ovariectomized heifers were used in a subsequent experiment. All heifers had just completed an experiment wherein they were treated with GnRH and all were bearing four implants containing estradiol -17β. Implants were removed from five heifers and the remaining five received an additional four implants i.e. five heifers had no steroid (controls) and five had eight implants. Blood was collected at 2 hr intervals for 36 hr at which time all heifers received intravenously 33 ug TRH/100 kg body wt. Following TRH, the schedule for blood collection was similar to that used for heifers with four estradiol implants.

Serum prolactin, GH and estradiol were quantified in samples selected from among those collected before TRH administration, and after TRH treatment prolactin and GH were quantified in all serum samples.

Objective 3: Effect of Estradiol -17β on baseline prolactin concentration and TRH-induced prolactin release in vitro

Experimental design: Cell cultures prepared from pituitaries of cows were 96 hr in culture when they were incubated for 2 hr with TC medium 199. Thereafter, cell cultures (four flasks/treatment) were incubated for 2 hr with TC medium 199 containing either 0, 1, 10 or 100 pg estradiol/ml or 10 ng TRH/ml.

In a second experiment, pituitary cell cultures at 96 hr of culture were incubated for 2 hr with TC medium 199. Then cultures (four flasks/treatment) were incubated for 6 hr with TC medium 199 containing either 0, 1, 10 or 100 pg estradiol/ml or 10 ng TRH/ml. Following this incubation period, two flasks from each treatment group were incubated for 2 hr with TC medium 199 and the remaining two with 10 ng TRH/ml TC medium 199.

In the final study of this series, pituitary cell cultures were 96 hr in culture when they were incubated for 12 hr with TC medium 199 or TC medium 199 containing 10 ng estradiol -17β/ml. Thereafter, each of four flasks was incubated for 2 hr with either: (1) TC medium 199 (2) 10 ng estradiol/ml TC medium 199 (3) 10 ng TRH/ml TC medium 199 or (4) 10 ng estradiol + 10 ng TRH/ml TC medium 199. Prolactin was quantified in the media.

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E. Statistical Procedure

The data were analysed by analysis of variance (Sokal and Rohlf 1969) and the procedure of Dunnett (1955) was used to test differences among means.

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RESULTS AND DISCUSSION

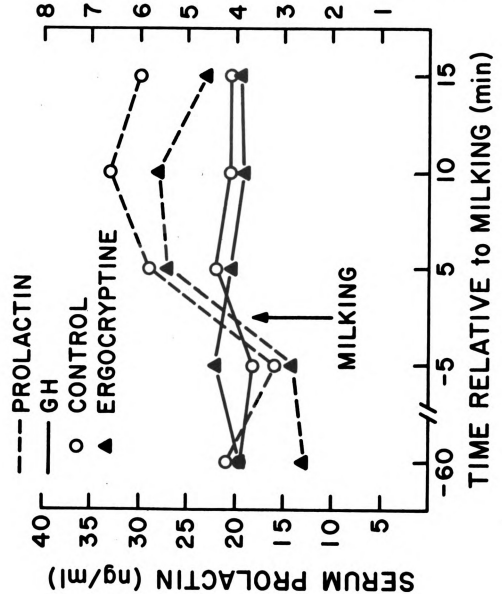
Experiment 1. Effect of Ergocryptine on Bovine Prolactin, GH, Cortisol and Milk Yield

1. Effect of Ergocryptine in vivo

On the day preceding treatment, serum prolactin concentration in cows assigned to receive ethanol (control) averaged 16 and 33 ng/ml (figure 2) at 5 min before and 10 min after the start of milking respectively, and the difference between the means was significant ($p < 0.05$). Comparable averages for cows assigned to receive ergocryptine were 14 and 28 ng/ml and the difference between means was also significant ($p < 0.05$). But differences between treatment groups were not significant ($p > 0.05$). When GH was quantified in these same samples its concentration in serum remained at approximately 4 ng/ml throughout the milking period (figure 2).

Serum prolactin concentration (ng/ml) of control cows on days 1 and 2 of treatment averaged 20 and 17 at 5 min before and 35 and 27 at 5 min after the start of milking, respectively, (figures 3 and 4) and the difference between means within day was significant ($p < 0.05$). Comparable averages for cows treated with 80 mg of ergocryptine were 1.3 and 1.1 at 5 min before and 1.4 and 1.1 at 5 min after milking, respectively, and differences between means were not significant ($p > 0.05$). On both days of treatment serum pro-

Figure 2. Prolactin and growth hormone response to milking on the day preceding ergocryptine treatment.


SERUM PROLACTIN (ng/ml)
SERUM GROWTH HORMONE (ng/ml)
TIME RELATIVE to MILKING (min)
PROLACTIN
GH
CONTROL
ERGOCRYPTINE
MILKING
40
35
30
25
20
15
10
5
8
7
6
5
4
3
2
1
-60
-5
5
10
15

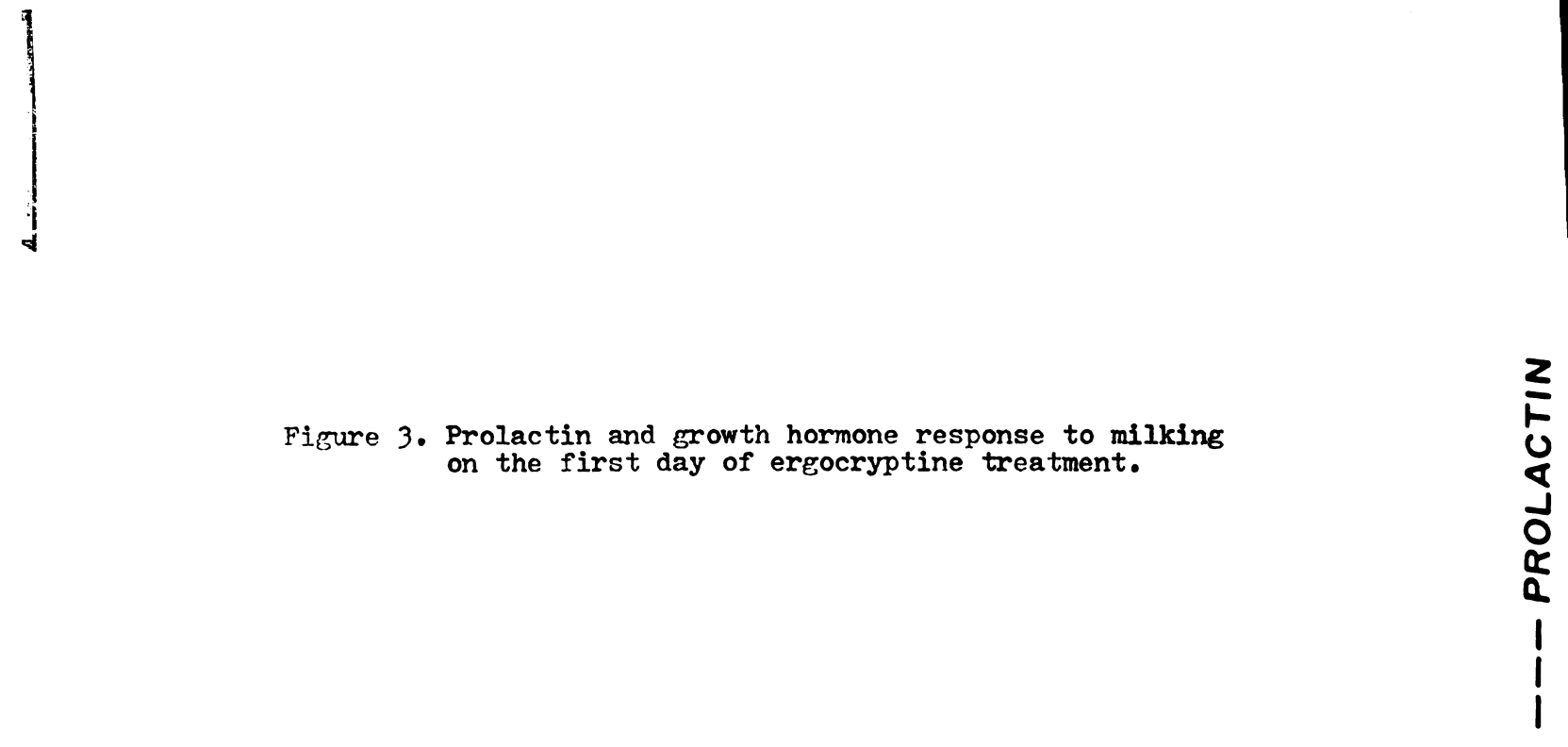


Figure 3. Prolactin and growth hormone response to milking on the first day of ergocryptine treatment.

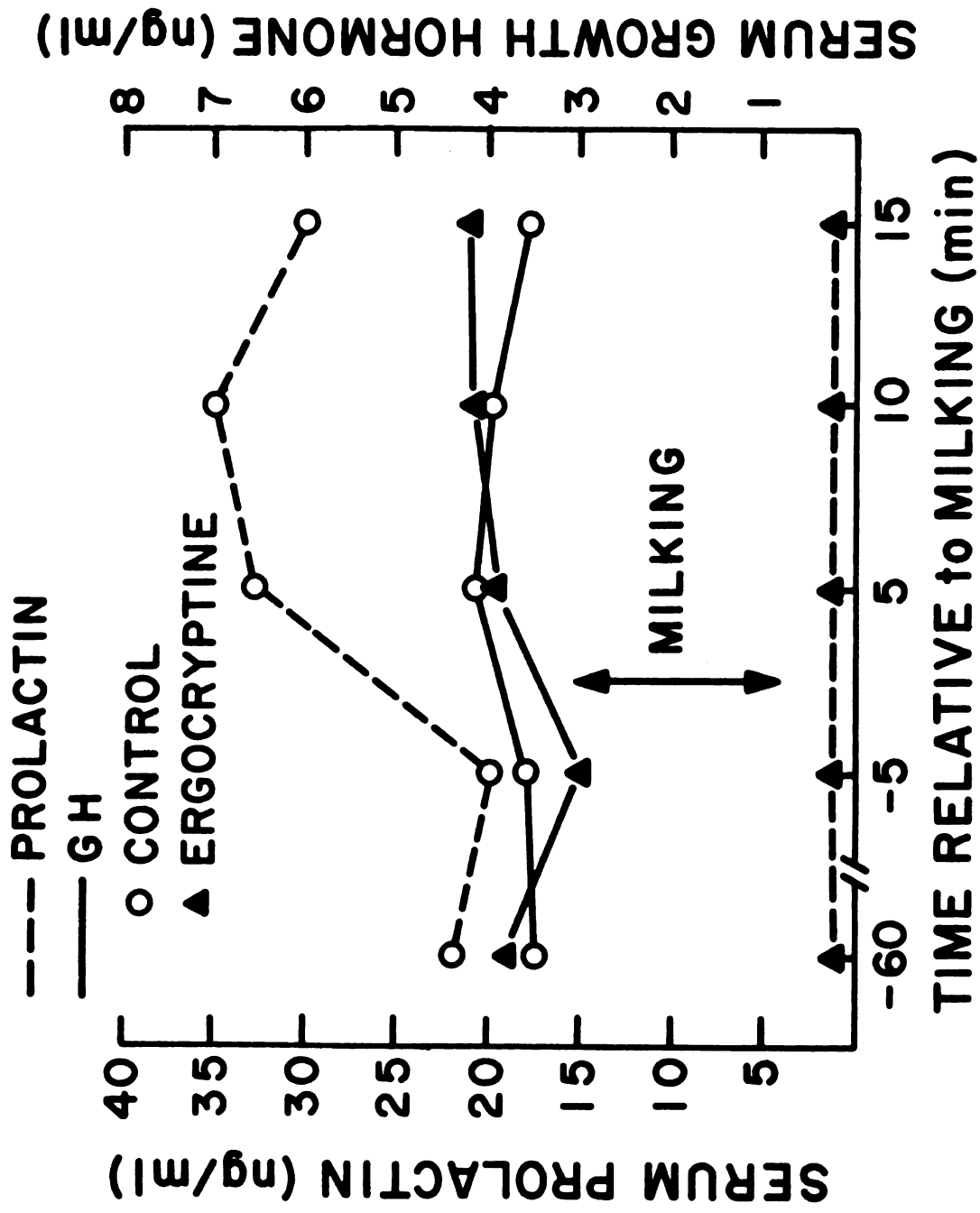
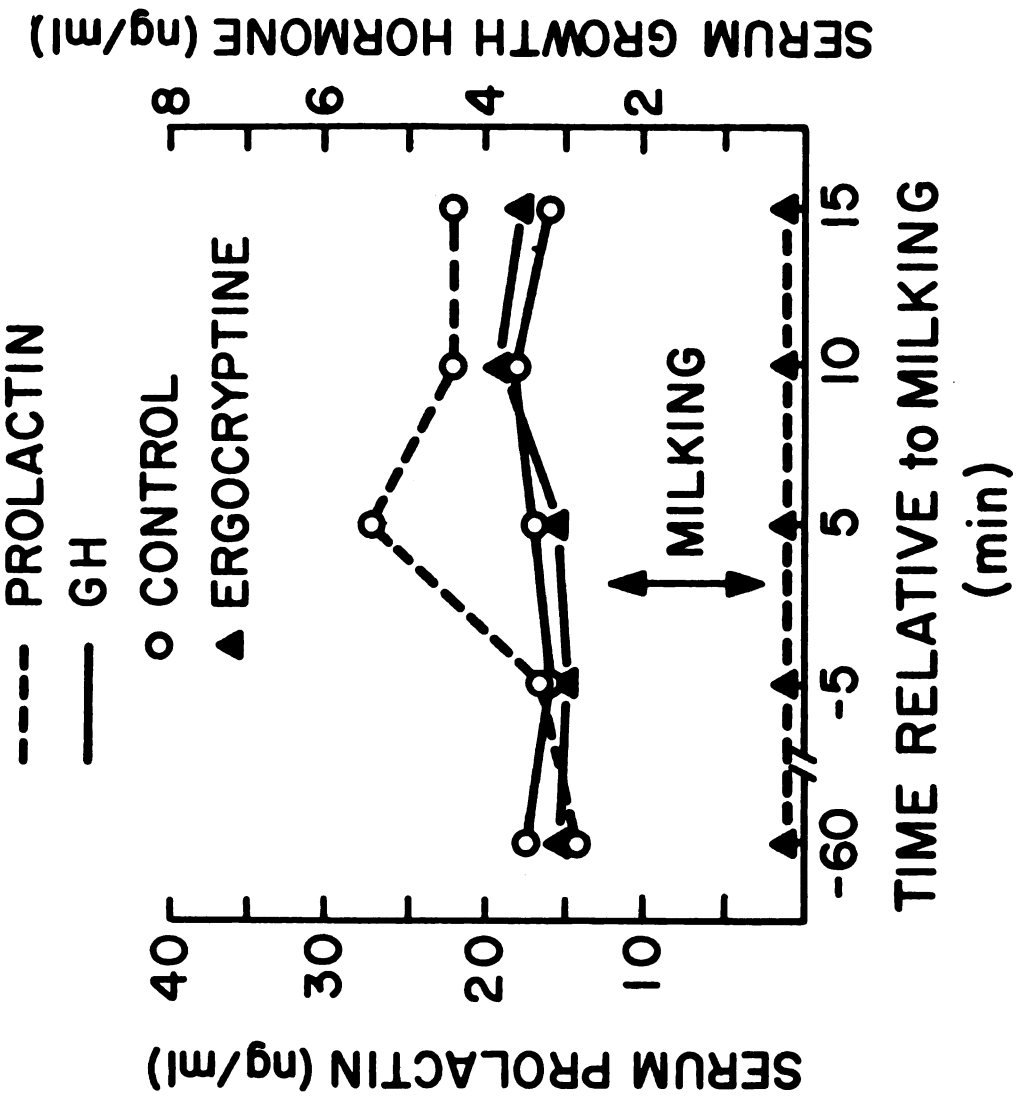

SERUM GROWTH HORMONE (ng/ml)
8
7
6
5
4
3
2
1
PROLACTIN
GH
CONTROL
ERGOCRYPTINE
MILKING
-60
-5
5
10
15
TIME RELATIVE to MILKING (min)
SERUM PROLACTIN (ng/ml)
40
35
30
25
20
15
10
5

Figure 4. Prolactin and growth hormone response to milking on the second day of ergocryptine treatment.


SERUM GROWTH HORMONE (ng/ml)
8
6
4
2
PROLACTIN
GH
o CONTROL
▲ ERGOCRYPTINE
MILKING
40
30
20
10
SERUM PROLACTIN (ng/ml)
-60
-5
5
10
15
TIME RELATIVE to MILKING
(min)

lactin
relativ
cryptin
treatme
GH conc
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Th
prolact
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ing was
with er
lactin
and 5 m
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and 1.1
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cryptine
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4.1 ng/m
cryptine
and 13.7
milking.
Ser

lactin concentration was greater ($p < 0.01$) in control cows relative to comparable averages for cows treated with ergocryptine. In contrast to prolactin, neither ergocryptine treatment nor stimuli associated with milking affected serum GH concentration (figures 3 and 4). On both days of treatment GH concentration in serum of both groups of cows remained at 3-4 ng/ml throughout the milking period.

The effect of ergocryptine on suppression of serum prolactin was long lasting. Thus, on the day following treatment, prolactin in serum of blood collected around milking was greater ($p < 0.01$) in control cows than cows treated with ergocryptine (figure 5). In control cows, serum prolactin concentration averaged 11 and 24 ng/ml at 5 min before and 5 min after the start of milking respectively, and the difference between means was significant ($p < 0.05$). Comparable averages for cows treated with ergocryptine were 1.1 and 1.1 ng/ml. Serum GH concentration was unchanged throughout the milking period (figure 5).

Serum cortisol concentration (figure 6) was determined only in samples collected around milking on the first day of treatment. There was no apparent effect ($p > 0.05$) of ergocryptine treatment on serum cortisol concentration. At 5 min before milking, serum cortisol concentration averaged 7.3 and 4.1 ng/ml in cows treated with ethanol (controls), and ergocryptine respectively, and was increased ($p < 0.05$) to 12.2 and 13.7 ng/ml respectively, at 10 min after the start of milking.

Serum prolactin concentration decreased rather quickly

Figure 5. Prolactin and growth hormone response to milking on the day following ergocryptine treatment.

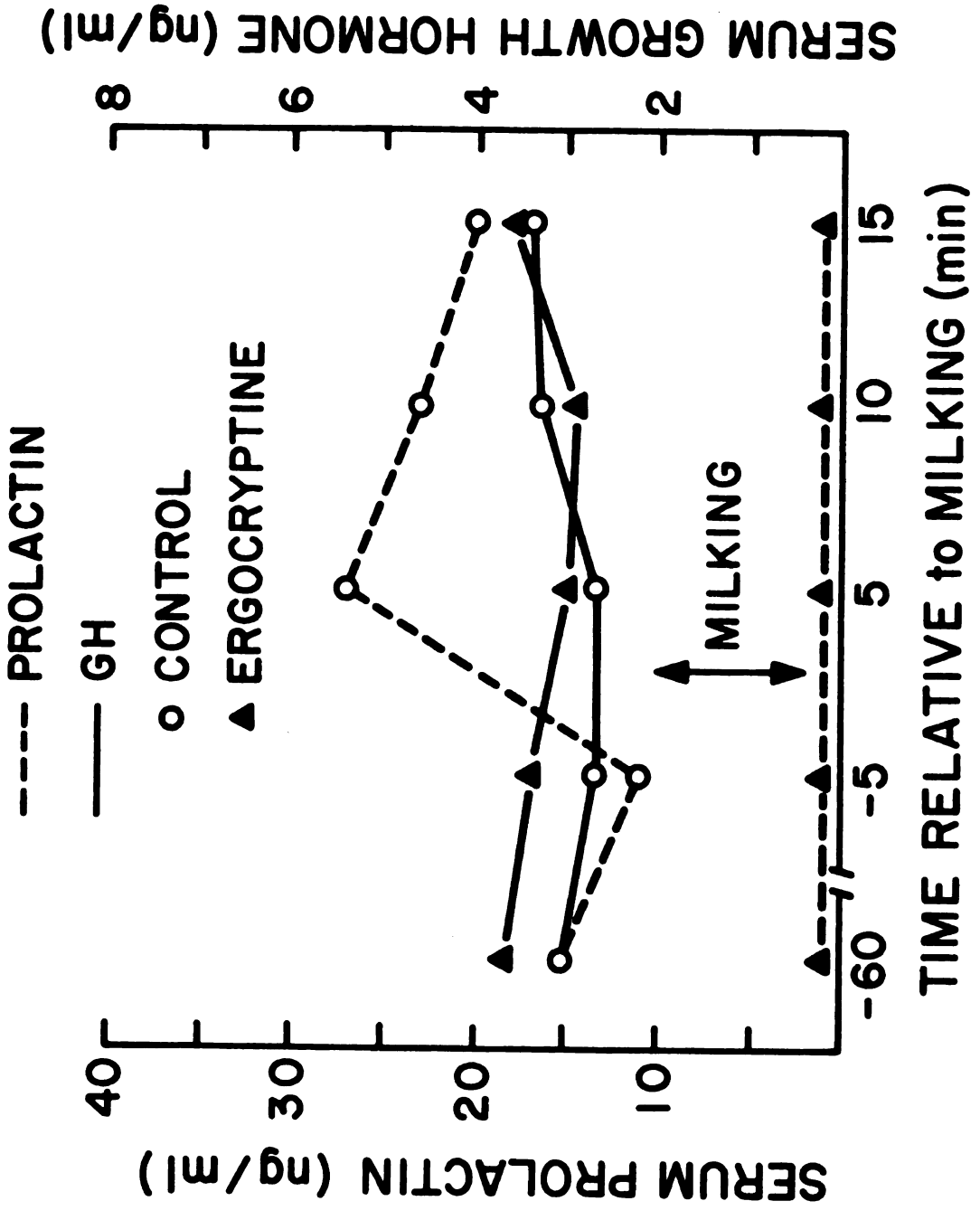
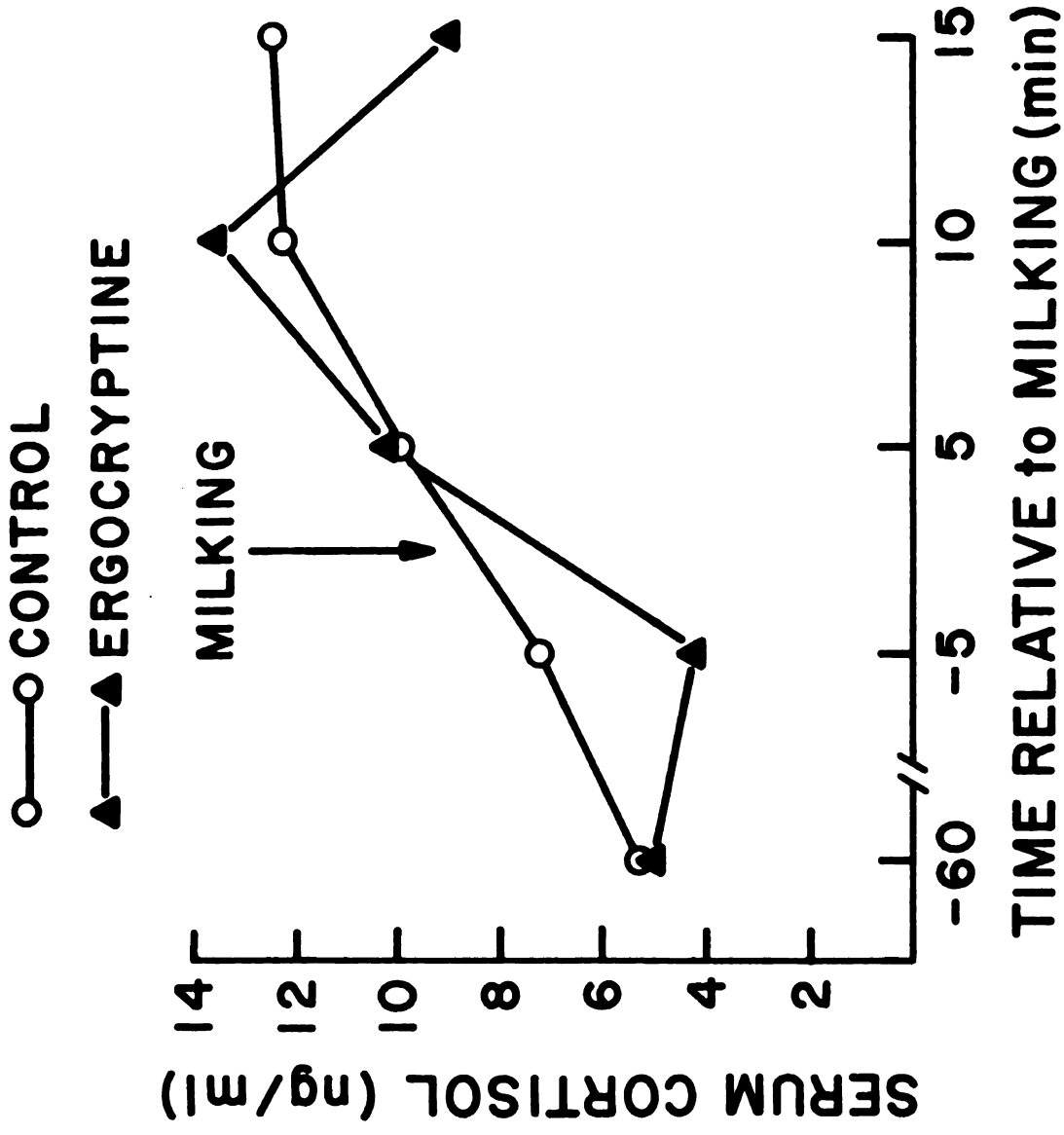

SERUM GROWTH HORMONE (ng/ml)
8
6
4
2
SERUM PROLACTIN (ng/ml)
40
30
20
10
PROLACTIN
GH
CONTROL
ERGOCRYPTINE
MILKING
-60
-5
5
10
15
TIME RELATIVE to MILKING (min)

Figure 6. Effect of ergocryptine and milking on serum cortisol concentration on the first day of ergocryptine treatment.


CONTROL
ERGOCRYPTINE
MILKING
SERUM CORTISOL (ng/ml)
14
12
10
8
6
4
2
-60
-5
5
10
15
TIME RELATIVE to MILKING (min)

after ergocryptine treatment. On the first day of treatment, prolactin concentration at 2, 4 and 6 hr after treatment averaged 30, 22 and 53 ng/ml respectively, for cows treated with ethanol (control) and 5, 2 and 2 ng/ml respectively, for cows treated with ergocryptine. Comparable averages (ng/ml) on the second day of treatment were 12, 24 and 54 (control) and 1.3, 1.4 and 1.1 for cows treated with ergocryptine and differences between group means were significant.

Following ergocryptine treatment, the decrease in serum prolactin concentration persisted for at least five days (figure 7). In cows treated with ergocryptine, serum prolactin concentration averaged 13.2 ng/ml on the day preceding treatment and 1.4 ng/ml thereafter. Serum prolactin concentration in cows treated with ethanol averaged 18.2 ng/ml on the day preceding treatment, the two days of treatment and the day following treatment when blood was collected via jugular cannulae but increased ($p < 0.05$) to 31.5 ng/ml for the next three days when blood was collected via venipuncture.

Although serum prolactin concentration decreased to approximately 1 ng/ml for at least five days after 160 mg of ergocryptine had been administered to lactating cows, there was no effect ($p > 0.05$) on milk yield (figure 8). During the two days of treatment, average daily milk yields were 23.7 and 24.0 kg for control- and ergocryptine-treated cows, respectively. By 10 days after treatment, control cows were producing an average of 1 kg more milk daily than cows treated with ergocryptine but differences between means were not significant ($p > 0.05$). Considering the entire experimental period

Figure 7. Chronic effect of two consecutive doses of ergocryptine on serum prolactin and growth hormone concentrations.

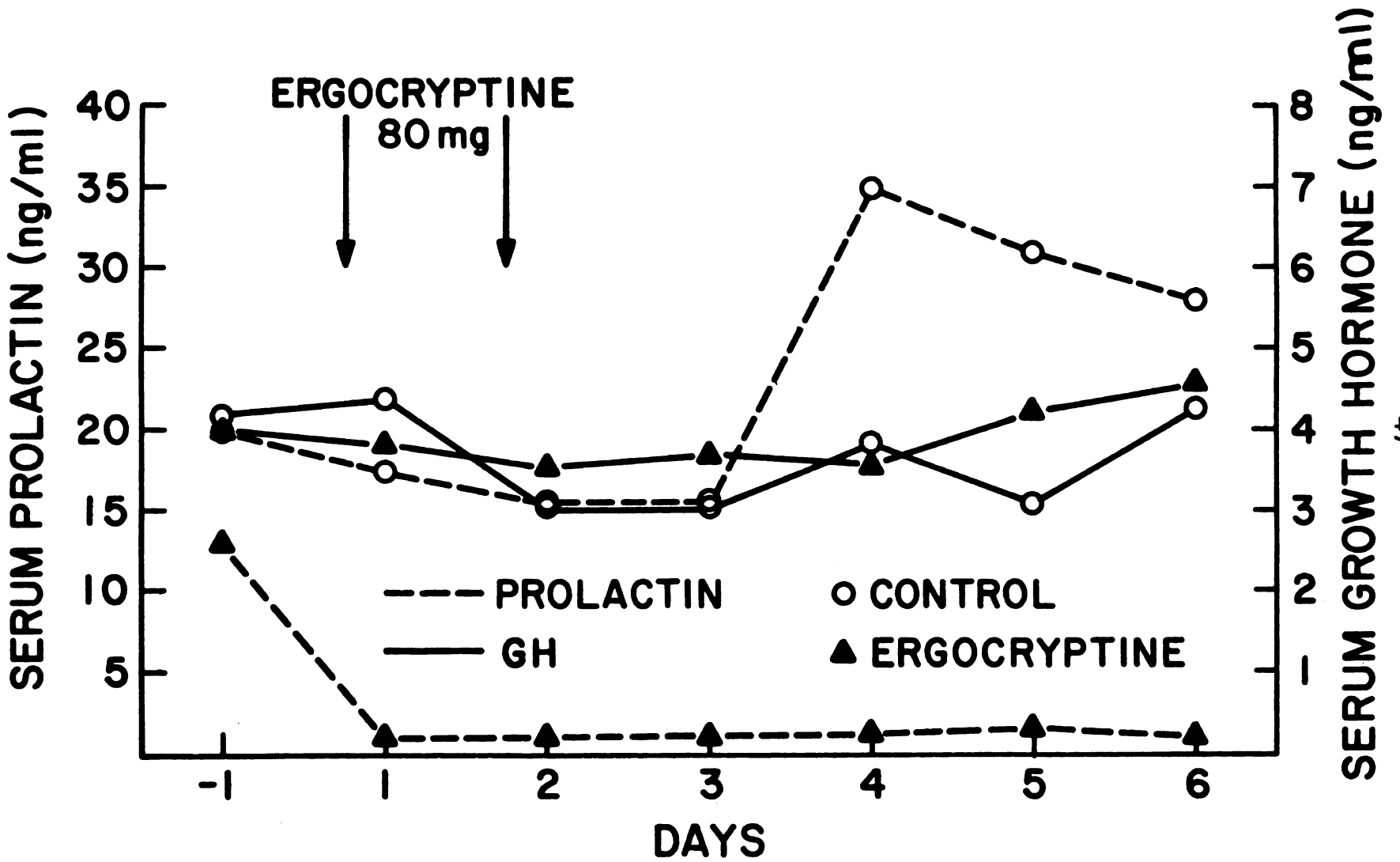
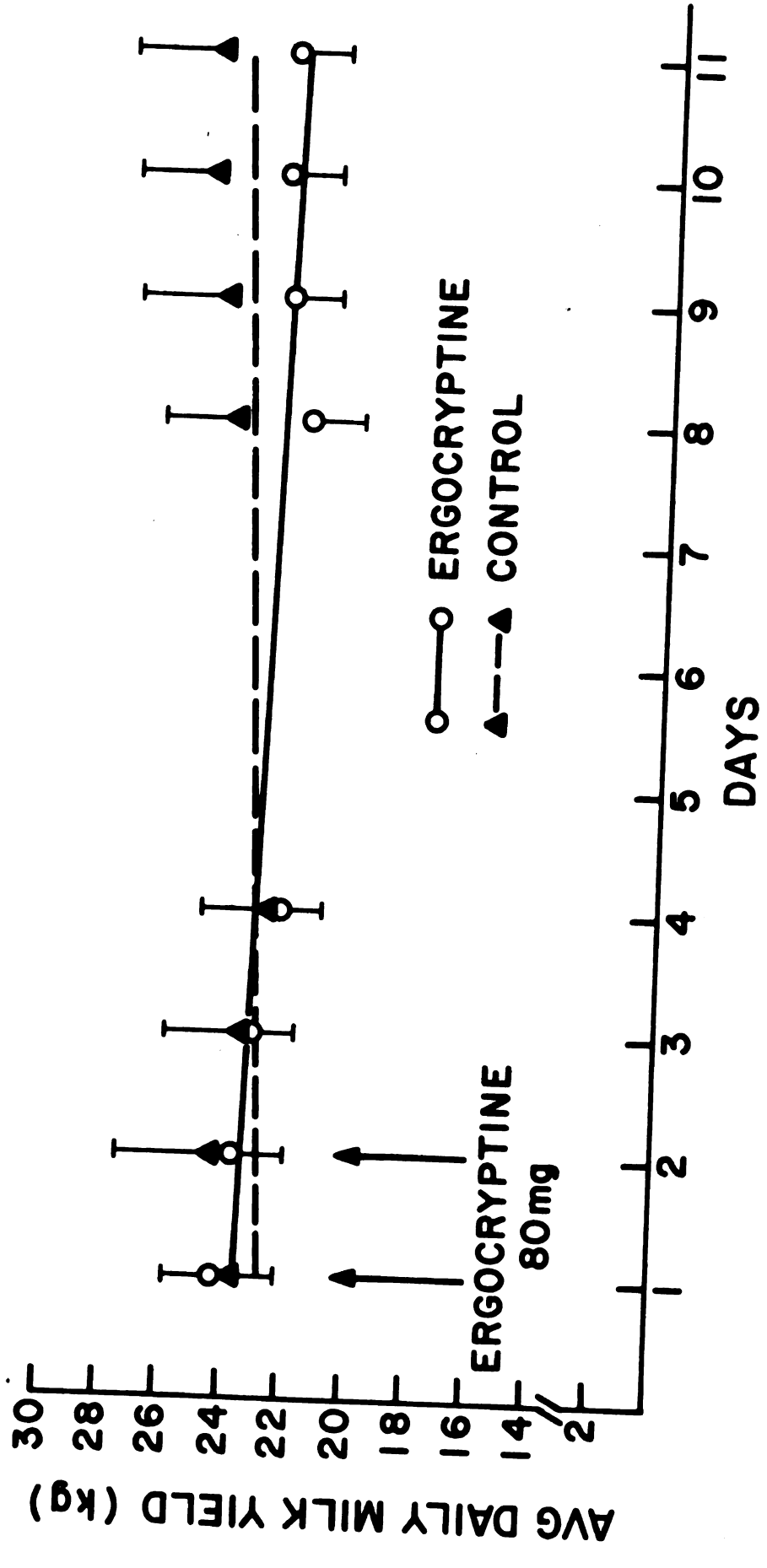

ERGOCRYPTINE
80 mg
SERUM PROLACTIN (ng/ml)
SERUM GROWTH HORMONE (ng/ml)
40
35
30
25
20
15
10
5
8
7
6
5
4
3
2
1
PROLACTIN
GH
CONTROL
ERGOCRYPTINE
-1
1
2
3
4
5
6
DAYS

Figure 8. Milk yield in cows treated with ergocryptine on days 1 and 2.


AVG DAILY MILK YIELD (kg)
30
28
26
24
22
20
18
16
14
12
ERGOCRYPTINE 80mg
ERGOCRYPTINE
CONTROL
1
2
3
4
5
6
7
8
9
10
11
DAYS

following treatment, regression of milk yield on time after treatment revealed no difference ($p > 0.05$) in slope between the two treatments.

2. Effect of Ergocryptine in vitro

Incubation of bovine pituitary cell cultures for 4 hr with ergocryptine in doses ranging from 0.01 to 10 ug/ml TC medium 199, resulted in approximately a 60% reduction in media prolactin concentration compared to prolactin concentration in media from pituitary cell cultures, not exposed to ergocryptine (table 1). Following 4 hr of incubation, media prolactin concentration averaged 57.8 and 32.9 ng/ml from control- and ergocryptine-treated cultures respectively, and the difference between means was significant ($p < 0.001$). However, differences due to dose of ergocryptine were not significant ($p > 0.05$).

Although ergocryptine had no effect ($p > 0.05$) on media GH concentration the amount of GH released during the treatment incubation period appeared to be dependent upon pretreatment media GH concentration. Hence GH concentrations of the treatment incubation period were adjusted by covariance based on pretreatment GH levels. These adjusted means are presented (table 1). Following 4 hr of incubation, media GH concentration averaged 29.7 ng/ml in cultures not exposed to ergocryptine and 25.1 ng/ml in cultures treated with ergocryptine but the difference between means was not significant ($p > 0.05$).

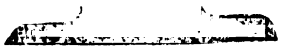


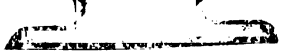
Table 1. Prolactin and growth hormone release from bovine pituitary cell cultures treated with ergocryptine.

Ergocryptine, ug/ml	Hormone in media	
	Prolactin ng/ml	Growth hormone[a] ng/ml
0	57.8	29.7
0.01	34.0[c]	23.1
0.10	31.2[c]	26.2
1.0	35.2[c]	25.8
10.0	31.2[c]	25.3
SEM[b]	2.7	2.5

[a]Means adjusted for variation in growth hormone release during pre-treatment incubation.

[b]Standard error of mean calculated from error mean square (prolactin) and deviations mean square (growth hormone); n=4.

[c]Less than average of control flasks (0 ug/ml); $p < 0.001$.



Results of this experiment clearly demonstrate that ergocryptine injected subcutaneously, significantly decreased resting concentrations of serum prolactin and prevented the increase in serum prolactin concentration that normally follows milking in cows (Johke 1969, Tucker 1971, Koprowski and Tucker 1973). The action of this drug appears to be rapid, since 2 hr after administration serum prolactin concentration was significantly decreased and it remained suppressed for at least five days indicating a prolonged effect of ergocryptine on prolactin inhibition. The decline in serum prolactin concentration caused by ergocryptine agrees with results for rats, (Nagasawa and Meites 1970, Brooks and Welsch 1974, Dohler and Wuttke 1974) humans, (del Pozo et al. 1972, Varga et al. 1972) cows (Schams et al. 1972, Karg et al. 1972, Fell et al. 1974) and sheep (Niswender 1974).

These data also confirm reports in cows (Karg et al. 1972) and goats (Hart 1973) that ergocryptine significantly decreased serum prolactin concentration without affecting milk yield. However, Fell et al. (1974) observed a reduction in milk yield and protein content of milk following administration of ergocryptine to cows just prior to and after parturition. Schams et al. (1972, 1973) and Karg and Schams (1974) also reported that ergocryptine given to cows in late pregnancy inhibited the rise in serum prolactin concentration that occurs prior to parturition and suppressed milk yield in the subsequent lactation. Karg et al. (1972) had previously reported that ergocryptine suppressed bovine serum prolactin

concentration to near 1 ng/ml, but failed to affect established lactations. Therefore these authors suggested that prolactin may be required for lactogenesis but not galactopoiesis in the bovine. Ergocryptine however, will inhibit established lactation in rats (Shaar and Clemens 1972) humans (del Pozo et al. 1973, Varga et al. 1972) and mice (Nagasawa and Yanai 1972) presumably due to inhibition of prolactin.

Serum concentrations of GH and cortisol were not affected by ergocryptine treatment, indicating a relative specificity of this drug with regard to prolactin suppression. Failure of ergocryptine to suppress serum GH concentration confirms a previous report (Hart 1973) showing that ergocryptine suppressed serum prolactin but not GH concentration in lactating goats. Failure to detect an increase in serum GH concentration due to stimuli associated with milking corroborates previous reports for cows (Tucker 1971, Reynaert and Peeters 1972 and Koprowski and Tucker 1973a). But stimuli associated with milking or suckling increase serum GH concentration in lactating goats (Hart and Flux 1973) and decreased pituitary GH concentration in lactating rats presumably by causing release of GH into the circulation (Grosvenor et al. 1968 and Sar and Meites 1969).

To my knowledge this is the first report which demonstrates that ergocryptine does not affect serum glucocorticoid concentration. The increase in serum cortisol concentration in response to milking reported herein confirms previous results from our laboratory (Smith et al. 1972 and Koprowski and Tucker

1973a) and those of Wagner (1969) that showed increase total serum glucocorticoids following milking in cows.

The increase in serum prolactin concentration of control cows, which began three days after treatment, might have resulted from stress associated with venipuncture which has been reported to increase serum prolactin concentration (Johke 1970, Raud et al. 1971 and Tucker 1971). If this is true, then failure of cows treated with ergocryptine to show increase serum prolactin concentration in response to venipuncture similar to cows treated with ethanol, indicates the effectiveness of ergocryptine to suppress prolactin release evoked by a stimulus other than milking.

Although ergocryptine reduced serum prolactin concentration to approximately 1 ng/ml without affecting milk yield for 10 days after treatment, these results should not be interpreted as evidence that prolactin is not required for lactation in the bovine. Assuming a blood to milk ratio of 400:1, a cow with serum prolactin concentration of 1 ng/ml and producing 25 kg of milk/day, the mammary gland would be exposed to approximately 20 mg of prolactin daily which may be adequate to sustain milk secretion. Conceivably, under normal conditions, far more prolactin may be present in bovine serum than is required to maintain milk secretion. Hence serum prolactin levels may not reflect only lactational events and other physiological roles for this hormone should be considered.

Results presented here also demonstrate that ergocryptine can act directly on bovine pituitary cell cultures in vitro

to inhibit prolactin release. These data confirm previous results that ergocryptine or ergocornine suppressed prolactin release *in vitro* from anterior pituitary explants of different species (Pasteels *et al.* 1971, Lu *et al.* 1971, Ectors *et al.* 1972 and Yanai and Nagasawa 1974). Failure of this drug to affect GH release *in vitro* is in agreement with results of Yanai and Nagasawa (1970a and 1974) which showed that ergocryptine suppressed prolactin release *in vitro* from pituitary explants of rats and mice but had no effect on GH concentration.

Furthermore, failure of ergocryptine to affect GH release despite significant suppression of prolactin release, suggests to us that its effect on prolactin release is not simply a noxious action. Ectors *et al.* (1972) also provided evidence to refute any idea that the effect of ergocryptine on prolactin release was simply that of a noxious drug. They observed that ergocryptine caused no overt cellular destruction of anterior pituitary explants *in vitro*, but rather, suppressed prolactin release by inhibiting exocytosis from prolactin cells. In addition, when ergocryptine was rinsed from cultures previously treated, prolactin release was restored (Pasteels *et al.* 1971).

Although the results reported here would suggest that the *in vivo* influence of ergocryptine on serum prolactin concentration is at least in part via a direct action on the anterior pituitary, one cannot ignore the possibility that this drug inhibited prolactin release by acting at higher centers of the brain, or simply by affecting the metabolic clearance rate of

prolactin.

Experiment 2. Thyrotropin Releasing Hormone (TRH): Effect on Prolactin and GH Release from Bovine Pituitary Cell Cultures

1. Effect of TRH on Prolactin and GH release in vitro

Addition of TRH to pituitary cell cultures from cows, at 72-hr of culture increased ($p < 0.01$) prolactin release into the media approximately 2-5 times relative to prolactin concentration for the 2-hr pretreatment period (table 2). Media prolactin concentration ranged from 508-587 ng/ml for all treatment groups during the 2-hr pretreatment period. Following 2 hr of incubation with TRH, average media prolactin concentration in control cultures (0 ng TRH/ml) showed a 4% decreased ($p > 0.05$) relative to comparable averages for the 2-hr pretreatment period. In contrast, media prolactin concentration was increased ($p < 0.01$) to approximately 1.4 ug/ml in cultures to which 0.01 ng TRH/ml was added and to 2.5 ug/ml in cultures that received the higher doses of TRH. The difference between pre- and post-treatment prolactin concentration after 0, 0.01, 0.1, 1.0 and 10.0 ng TRH/ml was -23, 799, 1966, 1926 and 1976 ng/ml, respectively. Apparently maximum prolactin release was achieved with 0.1 ng TRH/ml, as there were no differences ($p > 0.05$) in the magnitude of prolactin release among cell cultures, that received TRH at doses greater than 0.1 ng/ml medium.

Growth hormone concentration in these same media is shown in table 3. Although TRH increased ($p < 0.05$) GH release, the magnitude of response was small compared to prolactin release. Media GH concentration from control cultures

Table 2. Prolactin release from bovine pituitary cell cultures treated with thyrotropin releasing hormone (TRH).[a]

TRH (ng/ml)	Prolactin in media		
	Pre-treatment[b]	Post-treatment	[c]
	(ng/ml)		
0	576±39	553±79	-23±78
0.01	587±38	1386±204	799±174
0.1	560±52	2526±59	1966±42
1.0	548±6	2474±116	1926±120
10.0	508±51	2484±157	1976±117

Table 3. Growth hormone release from bovine pituitary cell cultures treated with thyrotropin releasing hormone (TRH).[a]

TRH (ng/ml)	Growth hormone in media		
	Pre-treatment[b]	Post-treatment	[c]
	ng/ml)		
0	90±3	85±4	-5±3
0.01	111±12	120±7	9±6
0.1	102±11	114±9	12±4
1.0	109±7	130±5	21±4
10.0	101±4	122±8	21±5

[a]Values are means ± standard error.

[b]Mean prolactin or growth hormone concentration of four flasks for the 2-hr period preceding treatment.

[c]Difference between pre- and post-treatment.

(0 ng TRH/ml) averaged 90 ng/ml for the 2-hr period preceding treatment and 85 ng/ml for the second 2-hr incubation period. But addition of TRH at levels as low as 0.01 ng/ml medium, increase media GH concentration as evidenced from the mean differences in GH concentration between pre and post-treatment incubation periods. These differences in GH concentration were -5, 9, 12, 21 and 21 ng/ml at 0.0, 0.01, 0.1, 1.0 and 10.0 ng TRH/ml medium, respectively. Maximum GH release was apparently achieved with 1.0 ng TRH/ml which is in contrast to prolactin where maximum release was apparent with 0.1 ng TRH/ml medium.

Addition of TRH to pituitary cell cultures from cows, steers and a bull at 96-hr of culture increased ($p < 0.05$) prolactin release into the media (table 4). Similar to 72-hr pituitary cell cultures there was a decrease in media prolactin concentration from all control cultures during the treatment incubation period relative to prolactin averages for the pretreatment period. In addition, media prolactin concentration from pituitary cell cultures of cows was 37% less than averages from the 72-hr cultures. But addition of TRH to cultures from cow pituitaries evoked prolactin release as evidenced from the mean difference in prolactin concentration before and after TRH. These differences in prolactin concentration were -27, 63, 109, 207, 226 and 137 ng/ml at 0, 0.01, 0.1, 1.0, 10.0 and 100 ng TRH/ml respectively. Apparently, maximum prolactin release from these cultures was achieved with 1.0 ng TRH/ml which is in contrast to 72-hr cell cultures when maximum prolactin release was

Table 4. Prolactin release from bovine pituitary cell cultures treated with thyrotropin releasing hormone (TRH).

TRH (ng/ml)	Sex[b]	Prolactin in Media[a] Pre-treatment[c] (ng/ml)	Post-treatment (ng/ml)	d (ng/ml)
0	Cow (3)	173±7	146±9	-27±14
0.01		167±21	230±23	63±3
0.1		193±23	302±42	109±20
1.0		201±9	408±21	207±29
10.0		258±15	484±42	226±27
100.0		230±21	367±37	137±20
0	Steer (3)	123±21	109±12	-14±10
0.01		110±9	123±12	13±13
0.1		140±4	176±11	36±8
1.0		140±14	293±26	153±30
10.0		109±9	275±7	166±6
100.0		112±8	192±11	80±10
0	Bull (1)	57±4	51±5	-6±6
10.0		64±8	78±10	14±6
100.0		72±6	103±18	31±12

[a] 96-hr pituitary cell cultures.

[b] Number in parentheses equals n.

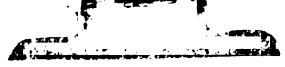
[c] Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

[d] Difference between pre- and post-treatment.

obtained with 0.1 ng TRH/ml medium. Extending the dose of TRH to 100 ng/ml, apparently cause a reduction in prolactin release compared to that observed after 10 ng TRH/ml ($p < 0.05$).

Thyrotropin releasing hormone also augmented ($p < 0.05$) prolactin release from pituitary cell cultures of steers (table 4). The mean difference in prolactin concentration between the pre- and post-treatment incubation periods were -14, 13, 36, 153, 166 and 80 ng/ml for 0.0, 0.01, 0.1, 1.0, 10.0 and 100 ng TRH/ml medium respectively. In 50% of the cultures that received 0.01 ng TRH/ml, prolactin concentration was reduced during the treatment incubation period relative to the pretreatment period, and this accounted for the large standard error. Similar to results of 96-hr pituitary cell cultures from cows, maximum prolactin release was achieved with 1.0 ng TRH/ml and extending the dose of TRH to 100 ng/ml resulted in a diminution ($p < 0.05$) in prolactin release compared to the response obtained with 10 ng TRH/ml.

Addition of TRH to pituitary cell cultures of a bull also increased ($p < 0.05$) prolactin release into the media (table 4). The mean difference in prolactin concentration between the pre- and post-treatment incubation periods was -6, 14 and 31 ng/ml at 0, 10 and 100 ng TRH/ml respectively. Relative to prolactin concentration after 10 ng TRH was added per ml of medium, there was a doubling in prolactin concentration (14 vs 31 ng/ml) after 100 ng TRH/ml. Media prolactin concentration was greater in pituitary cell cultures of cows



at 96-hr of culture, relative to prolactin concentration in cultures from pituitaries of steers and a bull.

When GH concentration was determined in these media from 96-hr pituitary cell cultures, there was a decrease ($p > 0.05$) in GH concentration during the treatment incubation period, relative to GH averages for the pretreatment period (table 5). The decrease was observed among pituitary cell cultures of cows, steers and a bull and was independent of the dose of TRH (0.0 to 100 ng/ml medium). This reduction in GH concentration could not be attributed to TRH inhibition since the magnitude of decrease among TRH-treated cultures was not different ($p > 0.05$) from control cultures.

2. Prolactin Release in vitro: TRH vs GnRH

Addition of GnRH to 96-hr pituitary cell cultures of cows slightly stimulated prolactin release (table 6). Media prolactin concentration ranged from 326-360 ng/ml for all treatment groups during a 2-hr incubation period with TC medium 199. Following 2 hr of incubation with GnRH prolactin concentration decreased 13-38% relative to concentrations during the pretreatment period. The change in media prolactin concentration expressed as the difference in quantity of prolactin release before and after GnRH treatment averaged -133, -80, -47 and -54 ng/ml after 0, 1, 10 and 100 ng GnRH/ml respectively. Prolactin concentration was greater ($p < 0.05$) in media from cultures treated with 10 and 100 ng GnRH/ml compare to prolactin concentration in control cultures (0 ng GnRH/ml). This increase however, was minimal relative to the comparable average 386 ng/ml from cultures treated with

Table 5. Growth hormone release from bovine pituitary cell cultures treated with thyrotropin releasing hormone (TRH).

TRH	Sex[b]	Growth hormone in media[a]		
		Pre-treatment[c]	Post-treatment	d
(ng/ml)		ng/ml		
0	Cow (3)	104±6	58±11	-46±16
0.01		108±12	71±12	-37±2
0.1		103±15	53±11	-50±5
1.0		112±3	60±5	-52±7
10.0		146±21	89±10	-57±16
100.0		115±12	51±8	-64±5
0	Steer (3)	96±10	58±5	-38±8
0.01		100±12	69±10	-31±11
0.1		123±7	60±7	-63±3
1.0		130±12	79±8	-51±16
10.0		100±12	80±7	-20±12
100.0		106±2	60±7	-46±6
0	Bull (1)	55±7	41±5	-14±5
10.0		68±7	47±8	-21±6
100.0		77±7	40±8	-37±2

[a]96-hr pituitary cell cultures.

[b]Number in parentheses equals n.

[c]Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

[d]Difference between pre- and post-treatment.

Table 6. Prolactin release from bovine pituitary cell cultures treated with gonadotropin releasing hormone (GnRH) or thyrotropin releasing hormone (TRH).[a]

Treatment		Prolactin in media		
		Pre-treatment[b]	Post treatment	[c]
(ng/ml)		ng/ml		
GnRH	0	347±8	214±23	-133±20
	1	326±21	246±27	-80±18
	10	360±48	313±24	-47±25[d]
	100	342±10	288±12	-54±11[d]
TRH	10	349±34	735±43	386±9[e]

[a]Values are means ± standard error.

[b]Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

[c]Difference between pre- and post-treatment means.

[d]Greater ($p < 0.05$) than average of control flasks (0 ng/ml).

[e]Greater than comparable means for flasks not treated with TRH ($p < 0.01$).

10 ng TRH/ml.

When luteinizing hormone (LH) was quantified in these media, GnRH but not TRH increased ($p < 0.05$) media LH concentration. The difference between pre- and post-treatment LH concentration after 0, 1, 10 and 100 ng GnRH/ml and 10 ng TRH/ml was -6, 15, 18, 17 and -2 ng/ml respectively.

3. Effect of Triiodothyronine (T_3) and Thyroxine (T_4) on TRH-induced Prolactin Release in vitro

Prolactin release from cell cultures was not affected by inclusion of either triiodothyronine (T_3) or thyroxine (T_4) at 0.0, 0.1 or 1.0 ug/ml in the incubation medium (table 7). Media prolactin concentration ranged from 23-29 ng/ml in cultures incubated for 2 hr with either TC medium 199 or thyroid hormones and was increased ($p < 0.01$) to 40-45 ng/ml in cultures incubated for 2 hr with either TRH or TRH and thyroid hormones. There was no difference ($p > 0.05$) in mean prolactin concentration between cultures exposed to TRH alone and those exposed to TRH and thyroid hormones.

Pretreatment of cell cultures for 6 hr with 0.1 ug T_3/ml medium affected neither baseline prolactin concentration nor the amount of prolactin released in response to TRH during a subsequent 2-hr incubation period (table 8). After 2 hr of incubation with TC medium 199, prolactin released into the media averaged 21 and 26 ng/ml from cell cultures pretreated for 6 hr with 0 and 0.1 ug T_3/ml medium, respectively. Comparable averages after 2 hr of incubation with either T_3, TRH, or T_3 + TRH were 25 and 23; 90 and 78 and 67 and 66 ng/ml, respectively. Media prolactin concentration after

Table 7. Prolactin release (ng/ml) from bovine pituitary cell cultures treated with triiodothyronine (T_3), thyroxine (T_4) and thyrotropin releasing hormone (TRH).[a,b]

Thyroid hormone (ug/ml)		Thyrotropin releasing hormone (ng/ml)	
		0	10
T_3	0.0	25 ± 3	42 ± 2[c]
	0.1	23 ± 2	43 ± 3[c]
	1.0	29 ± 1	43 ± 5[c]
T_4	0.0	24 ± 1	40 ± 1[c]
	0.1	24 ± 4	45 ± 4[c]
	1.0	24 ± 3	42 ± 4[c]

[a]Values are means $\pm$ standard error.

[b]Mean prolactin concentration of four flasks for the 2-hr treatment period.

[c]Greater than comparable means for flasks not treated with TRH ($p < 0.01$).

Table 8. Prolactin release from bovine pituitary cell cultures pretreated for 6 hr with triiodothyronine (T_3) and then incubated for 2 hr with T_3 and thyrotropin releasing hormone (TRH).[ab]

Treatment	Pretreatment media[c]	
	TC medium 199	TC medium 199 + T_3[d]
Non-treated control	21±4	26±5
T_3[d]	25±2	23±1
TRH[e]	90±18	78±7
TRH[e] + T_3[d]	67±13	66±6

[a]Values are means ± standard error.

[b]Mean prolactin concentration of four flasks for the 2-hr treatment period.

[c]Cell cultures pretreated for 6 hr with TC medium 199 or TC medium 199 + T_3.

[d]Concentration = 0.1 ug/ml medium.

[e]Concentration = 10 ng/ml medium.

2 hr of treatment with TRH was greater ($p < 0.05$) than that of cell cultures receiving no treatment or T_3 alone. Although concurrent addition of T_3 and TRH appeared to suppress TRH-induced prolactin release (90 vs 67) and 78 vs 66) ng/ml, differences between means were not significant ($p > 0.05$). In addition the presence of T_3 for 2 hr or 8 hr (6 hr pre-treatment + 2 hr post-treatment) did not affect ($p > 0.05$) TRH-induced prolactin release (67 vs 66) ng/ml.

Incubation of cell cultures with higher doses of T_4 resulted in a suppression of spontaneous prolactin release and the quantity of prolactin released by TRH (table 9). Following a 2-hr incubation period with 0, 5 and 50 ug T_4/ml TC medium 199, media prolactin concentration averaged 161, 119.5 and 82.5 ng/ml respectively, and difference between means was significant ($p < 0.05$). When these cultures were further incubated for 2 hr with 10 ng TRH/ml medium, prolactin concentration averaged 261, 224 and 167 ng/ml in cultures previously exposed to 0, 5 and 50 ug T_4/ml medium respectively. Comparable averages resulting from concurrent addition of TRH and T_4 during the second incubation period were 220 and 176 ng/ml in cultures previously exposed to 5 and 50 ug T_4/ml respectively. The increase in media prolactin concentration after TRH was significant ($p < 0.05$) relative to averages before TRH treatment. Since the presence of T_4 did not appear to affect the magnitude of TRH-induced prolactin release (224 vs 220) and (167 vs 176) ng/ml, these averages were pooled to examine the main effects of T_4 on the quantity of prolactin released by TRH. Overall treatment averages

Table 9. Prolactin release from bovine pituitary cell cultures treated with thyroxine (T_4) and thyrotropin releasing hormone (TRH).[a]

Period 1		Period 2		
T_4	Media prolactin[b]	T_4	TRH	Media prolactin
ug/ml	ng/ml	ug/ml	ng/ml	ng/ml
0	161±14	0	10	261±10
5	127±16	0	10	224±13
5	112±2	5	10	220±3
Avg	119.5±8*			222±2*
50	84±3	0	10	167±11
50	81±6	50	10	176±11
Avg	82.5±2**			171.5±4**

[a]Values are means ± standard error.

[b]Mean prolactin concentration of five flasks for the 2-hr treatment periods.

Less than the comparable average for period control.
* = $p < 0.05$
** = $p < 0.01$

for media prolactin concentration were 261, 222 and 171.5 ng/ml after TRH challenge of cultures previously exposed to 0, 5, or 50 ug T_4/ml respectively, and difference between means was significant ($p < 0.05$). Thus thyroxine did not appear to affect the action of TRH, but rather the releasable quantity of prolactin.

Results reported here clearly demonstrate that TRH stimulates prolactin release from bovine pituitary cell cultures *in vitro*. These results agree with a previous report by Tashjian *et al.* (1971) showing that TRH stimulated prolactin release from cloned rat pituitary tumor cells *in vitro*. More recently Vale *et al.* (1973) also reported that TRH at concentrations of 10^{-9} to 10^{-6}M enhanced prolactin release from rat pituitary cell cultures, and in a preliminary report Machlin and Jacobs (1973) observed increase prolactin release from calf pituitary cell cultures treated with TRH. In the present investigation prolactin release from bovine pituitary cell cultures was augmented with TRH at concentrations of approximately $3x10^{-11}$ to $3x10^{-7}$M. The decrease in magnitude of prolactin release in response to 100 ng TRH/ml, has not to my knowledge been previously reported and may be due to toxicity of the tripeptide when used at high concentrations.

Previously, Convey *et al.* (1973) reported no stimulation of prolactin release from steer pituitary explants incubated *in vitro* with TRH. Labella and Vivian (1971) using bovine pituitary explants reported that TRH caused only a

marginal stimulation of prolactin release in one of three experiments. Lu et al. (1972) also failed to demonstrate prolactin release from rat hemipituitaries incubated with TRH. Vale et al. (1973) reported that TRH caused only a minimal stimulation of prolactin release from normal rat hemipituitaries in vitro but significantly increased prolactin release from hemipituitaries of hypothyroid rats. In the present investigation and those of Machlin and Jacobs (1973), TRH consistently stimulated prolactin release from bovine pituitary cell cultures, but release from bovine pituitary explants was inconsistent.

Although the reasons for the different results are not apparent, several possibilities could be put forth. Perhaps enzymatic dispersion of bovine pituitary cells causes some cellular transformation that allows TRH to release prolactin from these cells but not from pituitary explants. Alternately excessive cutting of bovine pituitary explants may cause a great non-specific release of hormones which could mask smaller amounts released by a secretagogue. Holding pituitary cells in culture for 3-4 days would allow damaged cells that would release hormone non-specifically, to be eliminated during media changes. But these views were not supported by Vale et al. (1973) who established that TRH stimulated prolactin release in vitro from both primary pituitary cell cultures and hemipituitaries of hypothyroid rats. Dibbet et al. (1973) also demonstrated TRH-induced prolactin release from rat pituitary explants in vitro and recently

Porteus and Malven (1974) reported that TRH increased serum prolactin concentration in rats bearing pituitary homografts following hypophysectomy and lesion of the median eminence.

Although TRH unequivocally increased serum GH concentration in cows (Convey 1973, Convey et al. 1973) and acromegalic humans (Irie and Tsushima 1972) the effect of the tripeptide on GH release in vitro is not consistent. Tashjian et al. (1971) reported that TRH inhibited GH release in vitro from the same tumor cells that secreted increase quantities of prolactin in response to TRH. But Lu et al. (1972) observed no change in media GH concentration following incubation of rat hemipituitaries with TRH. Labella and Vivian (1971) demonstrated that TRH enhanced GH release in vitro from bovine pituitary explants in only one of three experiments. But recently Machlin and Jacobs (1973) and Carlson et al. (1974) reported that TRH significantly increased GH release in vitro from primary cell cultures and hemipituitaries of calves and rats respectively.

Results of the present experiment do not clarify the effect of TRH on GH release in vitro. The reason why TRH stimulated GH release from 72-hr but not 96-hr pituitary cell cultures is not clear. That prolactin release from 72-hr pituitary cell cultures was greater than from 96-hr cell cultures raises the possibility that the GH response may be attributed to cross reaction of the GH assay with high concentrations of prolactin in the media. The possibility is improbable however, since the GH assay used cannot measure

NIH-bovine prolactin at levels less than 50 ng/tube (Koprowski and Tucker 1971) and the dilutions that were used in the GH assay would allow a maximum prolactin concentration of 25 ng/tube which should not interfere with the GH assay.

The difference in baseline prolactin concentration and the magnitude of prolactin release in response to TRH by pituitary cell cultures of cows, steers and a bull cannot be attributed only to the physiological status of the donor animals. Differences could be due to age of the cell cultures, the number of viable cells present at the onset of the experiment and the sex of the pituitary donor. Likewise, differences in TRH-induced prolactin release between 72- and 96-hr pituitary cell cultures of cows, could be due to age of the cultures, the number of viable cells present or the physiological status of the pituitary donor.

The specificity of the response of pituitary cell cultures to TRH and GnRH was demonstrated by failure of TRH to increase media LH concentration, and the observation that GnRH only slightly stimulated prolactin release. Vale et al. (1972) reported that addition of TRH to rat pituitary cell cultures in vitro stimulated release of prolactin and thyroid stimulating hormone (TSH) but not LH or follicle stimulating hormone (FSH). Luteinizing hormone releasing hormone (LH-RH) stimulated release of LH and FSH but not TSH or prolactin. In addition, Bowers et al. (1971) observed an increase in serum concentration of prolactin but not LH following administration of TRH to humans and Kastin et al. (1973) reported that LH-RH

increased serum LH concentration but not prolactin in men. Thus similar to the pituitary *in situ*, cells in culture are also capable of discriminating between specific releasing hormones.

Failure of T_3 or T_4 to increase baseline prolactin concentration from bovine pituitary cell cultures, supports previous results from our laboratory (Shaw *et al.* 1972) which showed that feeding of thyroprotein to lactating cows had no effect on baseline serum prolactin concentration despite marked increases in serum thyroxine. In view of these reports, it is unlikely that the galactopoietic effect of thyroxine and thyroactive substances in cattle (Blaxter *et al.* 1949) is attributable to stimulation of prolactin secretion but presumably results from a general increase in body metabolism. In contrast to results obtained with the bovine cell cultures Meites (1963) demonstrated that both T_3 and T_4 significantly increased prolactin release from rat pituitary explants *in vitro*. In addition, Chen and Meites (1969) showed that T_4 increased pituitary prolactin content of rats, presumably by a direct action on the anterior pituitary since there was no change in the activity of prolactin inhibiting factor.

The reduction in magnitude of prolactin release from cell cultures treated with 5 or 50 ug T_4/ml medium, confirms a previous report by Vale *et al.* (1973) which showed that T_3 or T_4, in doses similar to those used in the present study, decreased spontaneous release of prolactin from hemipituitaries of hypothyroid rats. The mechanism by which these doses

of T_4 inhibit prolactin release *in vitro* is equivocal but failure to affect LH release (Vale *et al.* 1973) suggests that the effect on prolactin release is not simply a noxious action. Results reported herein, suggest that T_4 had no effect on the action of TRH with regard to prolactin release, but might have affected the quantity of releasable prolactin.

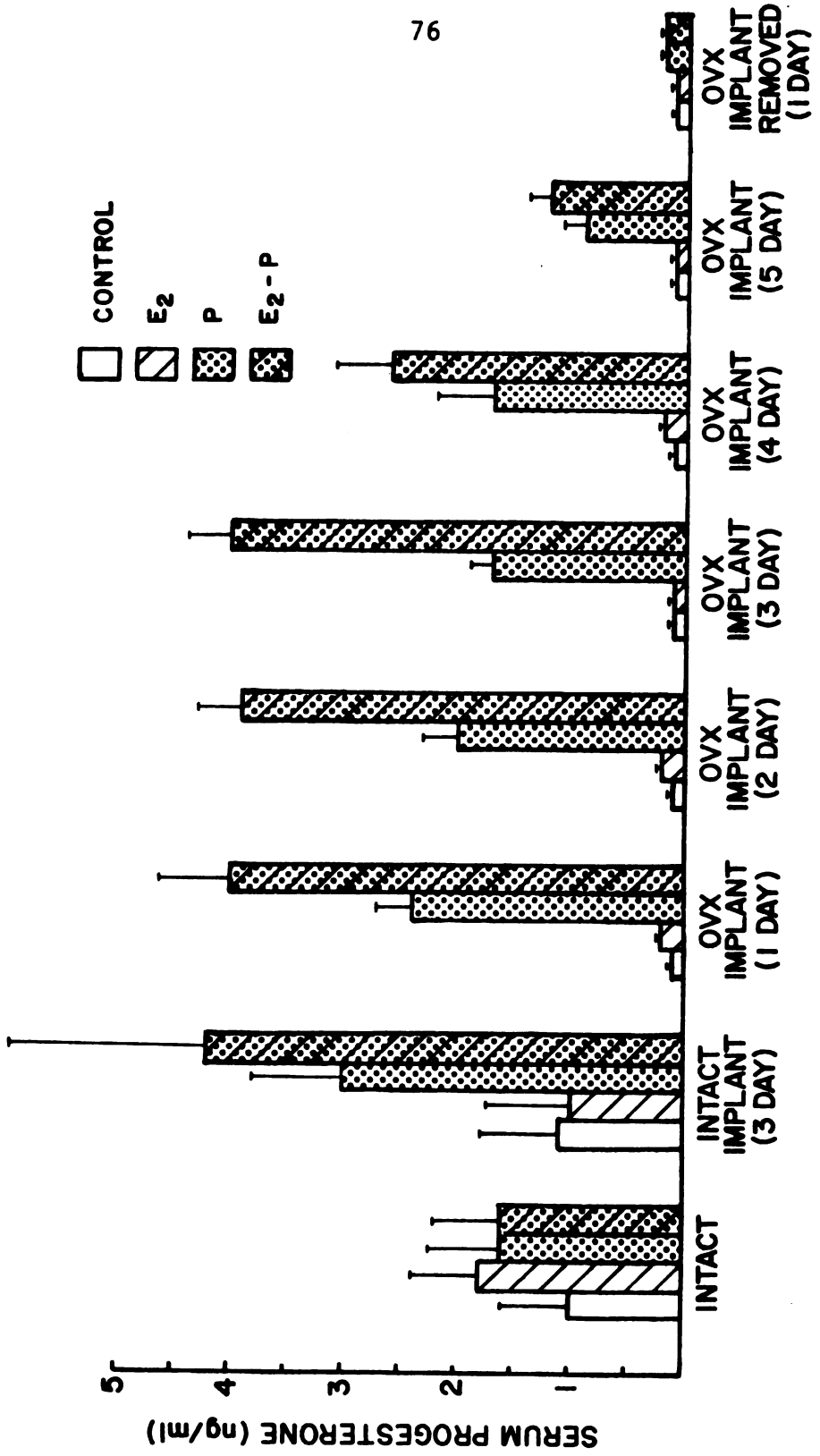
In contrast to results obtained *in vitro*, Bowers *et al.* (1971) and Snyder *et al.* (1973) demonstrated that administration of thyroid hormones to humans decreased the magnitude of prolactin release in response to TRH. These results were confirmed in sheep by Debeljuk *et al.* (1973), but the mechanism by which this decrease was effected is equivocal.

Experiment 3: Prolactin and Growth Hormone Release after Gonadal Steroids and TRH in vivo and in vitro

1. Serum Prolactin and GH after Gonadal Steroids in vivo

Serum progesterone concentration averaged 1.0, 1.8, 1.6, and 1.6 ng/ml (figure 9) in intact heifers immediately before they received no steroid treatment, estradiol (E_2), progesterone (P) or E_2+P, respectively, and differences among group means were not significant ($p > 0.05$). Progesterone concentration in serum of heifers was increased ($p < 0.05$) to 3–4 ng/ml at 72 hr following insertion of progesterone pessaries but remained unchanged in heifers not receiving pessaries. By 24 hr after ovariectomy progesterone concentration decreased ($p < 0.05$) to approximately 0.2 ng/ml in heifers without pessaries, but remained greater than 1.0 ng/ml for at least five days after ovariectomy in heifers bearing pessaries.

Figure 9. Serum progesterone concentration of heifers after placement of depot steroids and subsequent ovariectomy. Heifers were ovariectomized 3 days after placement of steroid implants. Implants were removed 6 days after ovariectomy.

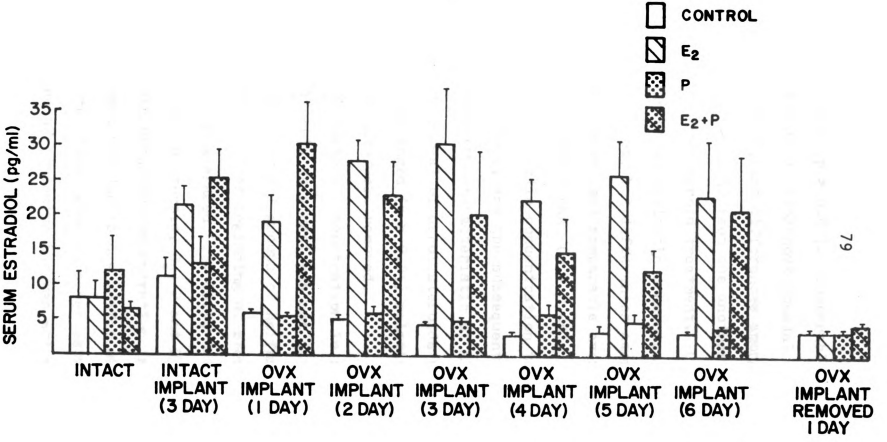

SERUM PROGESTERONE (ng/ml)
5
4
3
2
1
CONTROL
E_2
P
E_2 - P
INTACT
INTACT IMPLANT (3 DAY)
OVX IMPLANT (1 DAY)
OVX IMPLANT (2 DAY)
OVX IMPLANT (3 DAY)
OVX IMPLANT (4 DAY)
OVX IMPLANT (5 DAY)
OVX IMPLANT REMOVED (1 DAY)

Progesterone concentration was greater ($p < 0.05$) the first three days after ovariectomy, in serum of heifers receiving both estradiol and progesterone than in serum of those receiving only progesterone. Within 24 hr after depot steroids were removed, progesterone concentration averaged 0.2 ng/ml in serum of all heifers regardless of treatment.

Serum estradiol concentration averaged 8.2, 7.9, 12 and 6.4 pg/ml (figure 10) in intact heifers immediately before they received no steroid treatment, E_2, P or E_2+P respectively ($p > 0.05$). Serum estradiol concentration then increased ($p < 0.05$) to 24 pg/ml at 72 hr, in heifers bearing four estradiol-filled implants but was unchanged (average 12 pg/ml) in heifers without estradiol implants. Estradiol concentration was decreased ($p < 0.05$) to an average of 5 pg/ml at 24 hr after ovariectomy, in serum of heifers not receiving estradiol implants but remained greater than 20 pg/ml in heifers with estradiol implants during the six days after ovariectomy when the implants remained in place. Average serum estradiol concentration was 5 pg/ml at 24 hr after depot steroids were removed, and there was no difference ($p > 0.05$) between means for each treatment group.

Despite marked increases in serum estradiol and progesterone concentrations there were no significant changes in serum prolactin and GH concentrations attributable to steroid treatment. Serum prolactin concentration prior to placement of depot steroids averaged 24, 20, 19 and 34 ng/ml (figure 11) in heifers assigned to receive no steroid treatment, E_2,

Figure 10. Serum estradiol concentration of heifers after placement of depot steroids and subsequent ovariectomy. Heifers were ovariectomized 3 days after placement of steroid implants. Implants were removed 6 days after ovariectomy.


CONTROL
E2
P
E2+P
SERUM ESTRADIOL (pg/ml)
35
30
25
20
15
10
5
INTACT
INTACT IMPLANT (3 DAY)
OVX IMPLANT (1 DAY)
OVX IMPLANT (2 DAY)
OVX IMPLANT (3 DAY)
OVX IMPLANT (4 DAY)
OVX IMPLANT (5 DAY)
OVX IMPLANT (6 DAY)
OVX IMPLANT REMOVED 1 DAY

P or E_2+P respectively ($p > 0.05$). Comparable averages after 72 hr of exposure to exogenous gonadal steroids were 34, 29, 23 and 24 ng/ml, and differences among means were neither different ($p > 0.05$) from one another nor from comparable averages before steroid treatment. Considerable fluctuation in mean serum prolactin concentration was observed following ovariectomy but differences among treatment means or among these means and comparable averages before ovariectomy were not significant ($p > 0.05$). The decrease ($p < 0.05$) in serum prolactin concentration to a nadir at 4-10 hr after ovariectomy, and the subsequent return to pre-ovariectomized levels were characteristic of all treatment groups. Following removal of depot steroids serum prolactin concentration was unchanged ($p > 0.05$) relative to comparable averages before implants were removed.

Average growth hormone concentration in serum of intact heifers prior to steroid treatment was not different ($p > 0.05$) among groups; average for all groups of heifers was 5 ng/ml (figure 12). At 72 hr after beginning of steroid treatment serum GH concentration averaged 6.5, 10.9, 5.8 and 7.4 ng/ml in heifers that received no steroid, E_2, P and E_2+P respectively. The increase in mean serum GH concentration in heifers treated with estradiol alone, was due to one heifer whose serum GH concentration was ten times greater after estradiol treatment than before. Serum GH concentration was not affected ($p > 0.05$) by either ovariectomy or removal of depot steroids when compared with appropriate averages before ovariectomy.

Figure 11. Serum prolactin concentration of heifers after placement of depot steroids and subsequent ovariectomy. Heifers were ovariectomized 3 days after placement of steroid implants. Implants were removed 6 days after ovariectomy.

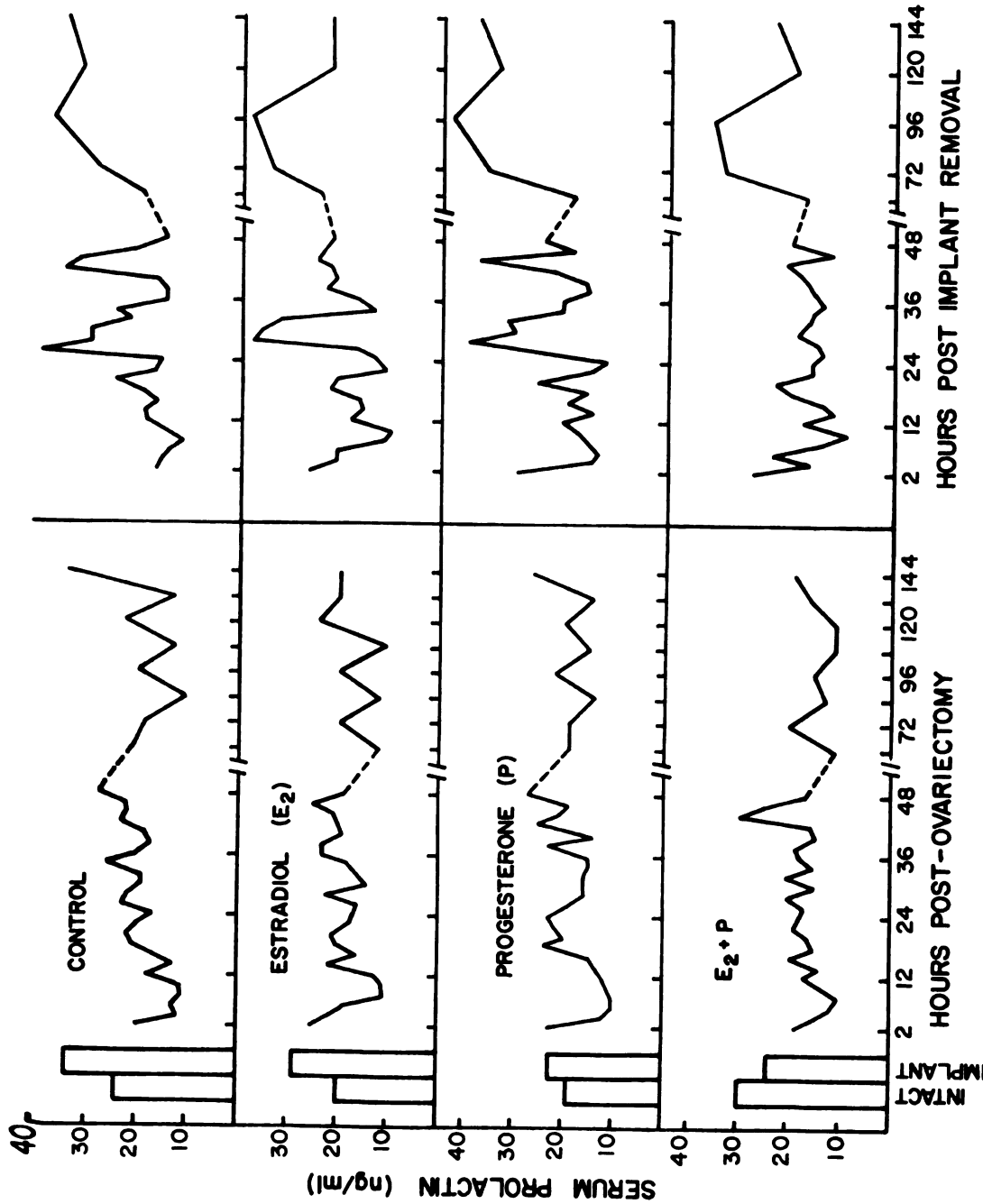
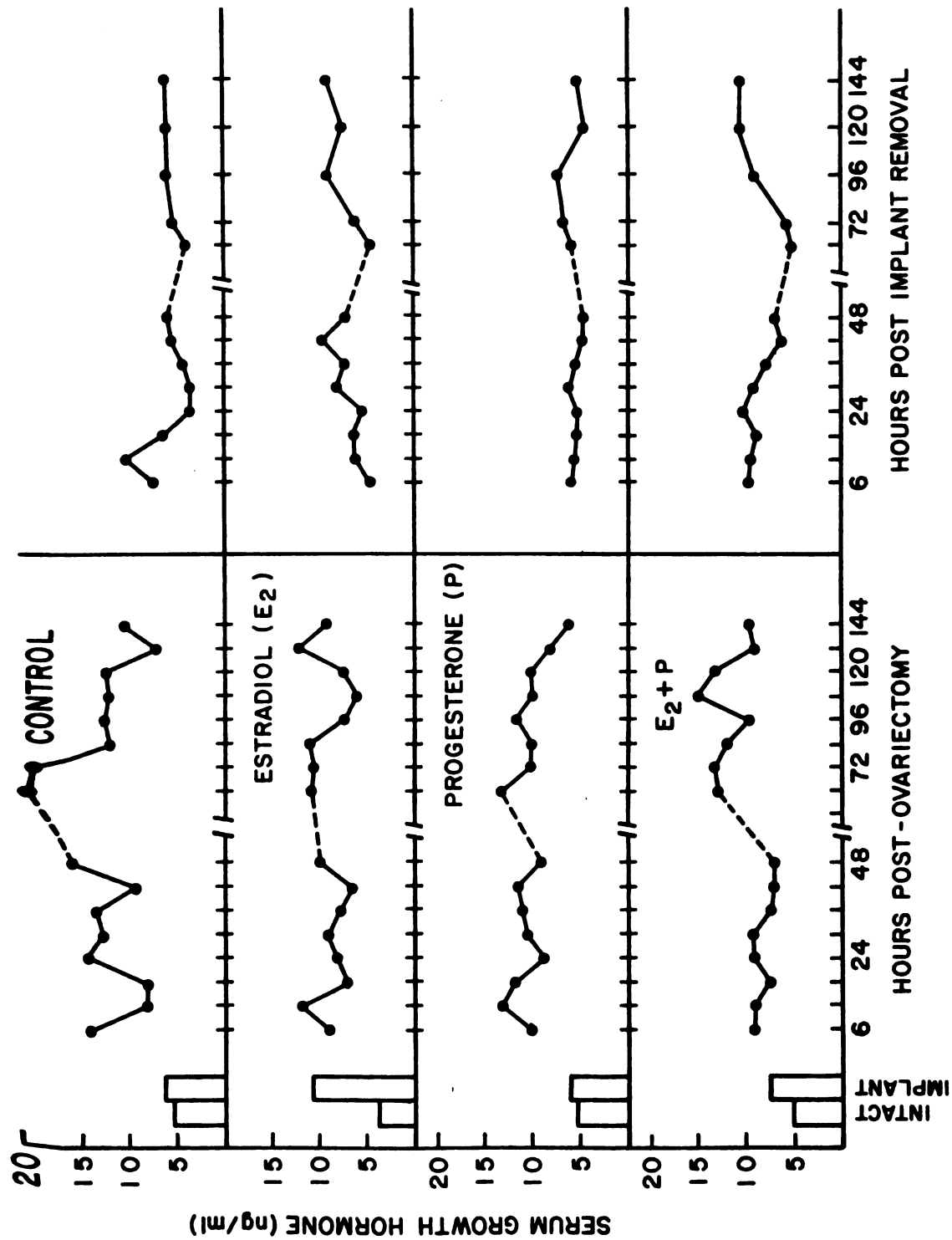

CONTROL
ESTRADIOL (E_2)
PROGESTERONE (P)
E_2 + P
SERUM PROLACTIN (ng/ml)
40
30
20
10
INTACT
IMPLANT
2 12 24 36 48 72 96 120 144
HOURS POST-OVARIECTOMY
2 12 24 36 48 72 96 120 144
HOURS POST IMPLANT REMOVAL

Figure 12. Serum growth hormone concentration of heifers after placement of depot steroids and subsequent ovariectomy. Heifers were ovariectomized 3 days after placement of steroid implants. Implants were removed 6 days after ovariectomy.


CONTROL
ESTRADIOL (E_2)
PROGESTERONE (P)
E_2+P
SERUM GROWTH HORMONE (ng/ml)
HOURS POST-OVARIECTOMY
HOURS POST IMPLANT REMOVAL
INTACT
IMPLANT
6 24 48 72 96 120 144

2. Bovine Serum Prolactin and Growth Hormone Response to Estradiol -17β and TRH

Estradiol concentration averaged 6 and 8 pg/ml in serum of ovariectomized heifers immediately before they received no steroid treatment (control) or four estradiol-filled implants respectively, (figure 13). Estradiol concentration was increased ($p < 0.01$) to 55 pg/ml at 6 hr in serum of heifers receiving estradiol implants but decreased ($p < 0.05$) to 35 pg/ml by 18 hr after treatment. Serum estradiol concentration was unchanged ($p > 0.05$) in serum of control heifers. By 72 hr after treatment, serum estradiol concentration averaged 27 pg/ml in estradiol-treated heifers which was greater ($p < 0.05$) than comparable average in controls.

Serum prolactin concentration determined in blood collected at intervals during the first 36 hr after estradiol treatment is shown in figure 14. Analysis of treatment differences revealed that serum prolactin concentration was not affected ($p > 0.05$) by increased serum estradiol concentration. However, time by treatment interaction was significant ($p < 0.05$) and was attributable to a difference in prolactin concentration between control and estradiol-treated heifers at 5 hr after treatment; 31 vs 10 ng/ml for control and estradiol-treated heifers respectively. Growth hormone (figure 15) measured in these same samples, averaged 11 ng/ml in serum of heifers immediately before estradiol treatment. After treatment, average GH concentration was greater in serum of estradiol-treated heifers relative to controls, but the difference

Figure 13. Serum estradiol concentration in ovariectomized heifers implanted with four estradiol-filled-implants at time zero.

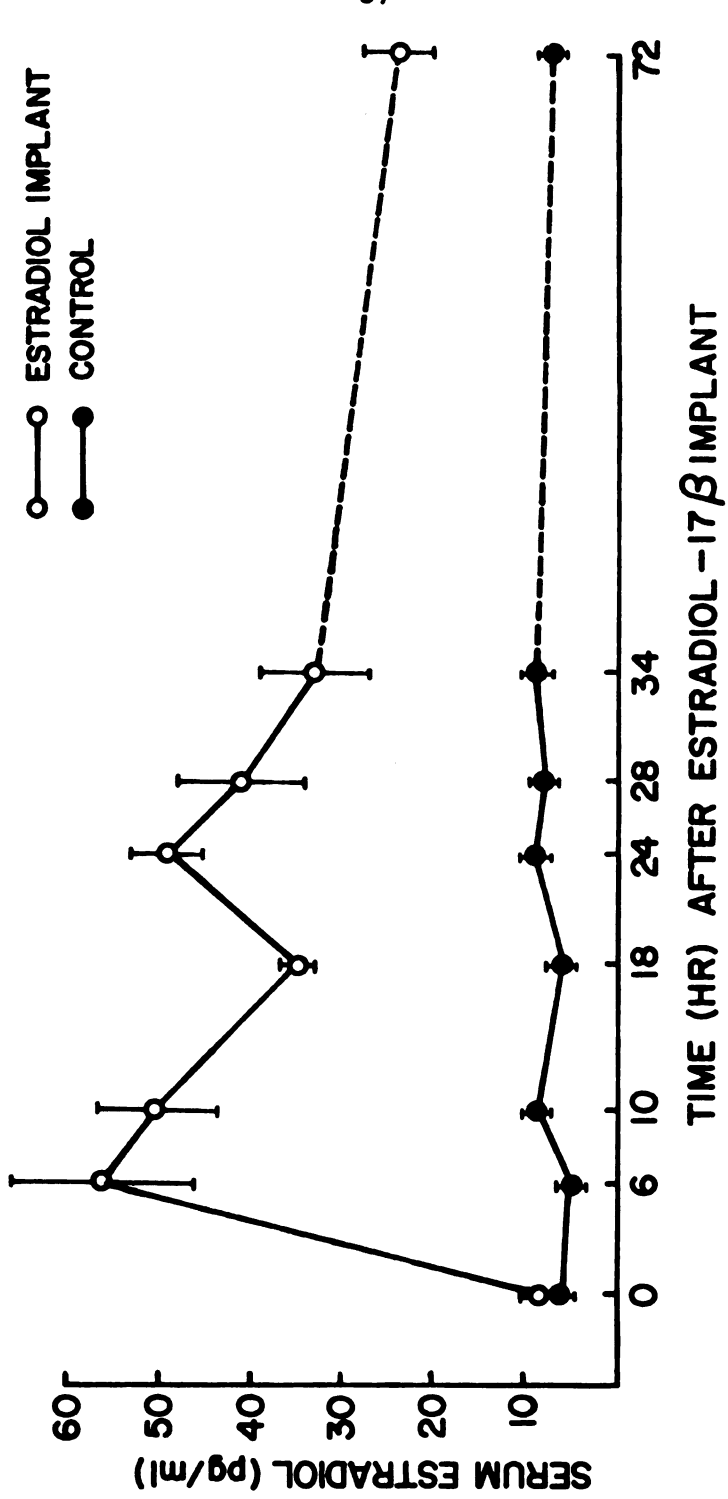

ESTRADIOL IMPLANT
CONTROL
SERUM ESTRADIOL (pg/ml)
60
50
40
30
20
10
0
6
10
18
24
28
34
72
TIME (HR) AFTER ESTRADIOL-17β IMPLANT

Figure 14. Serum prolactin concentration in ovariectomized heifers implanted with four estradiol-filled-implants at time zero.

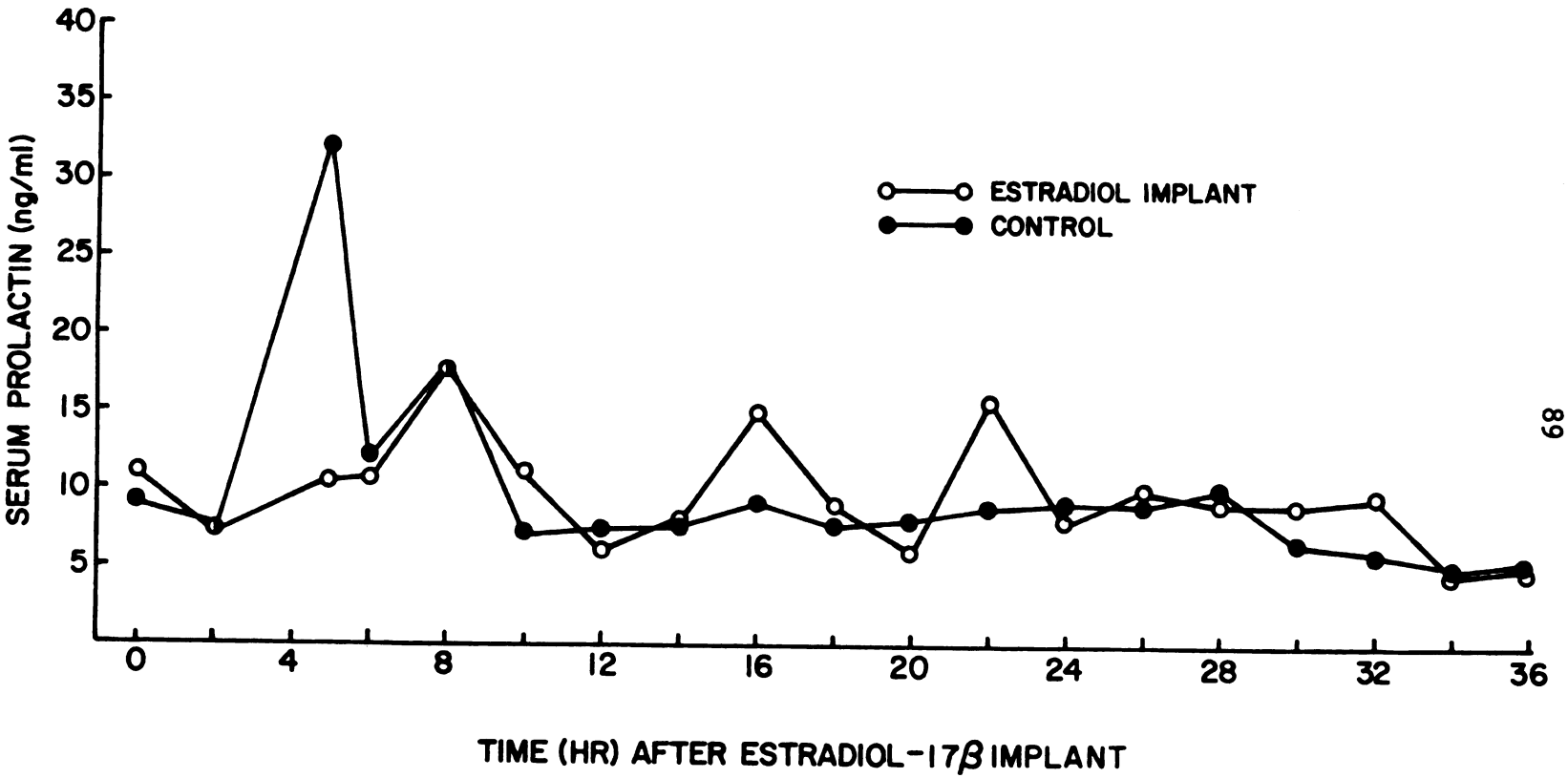
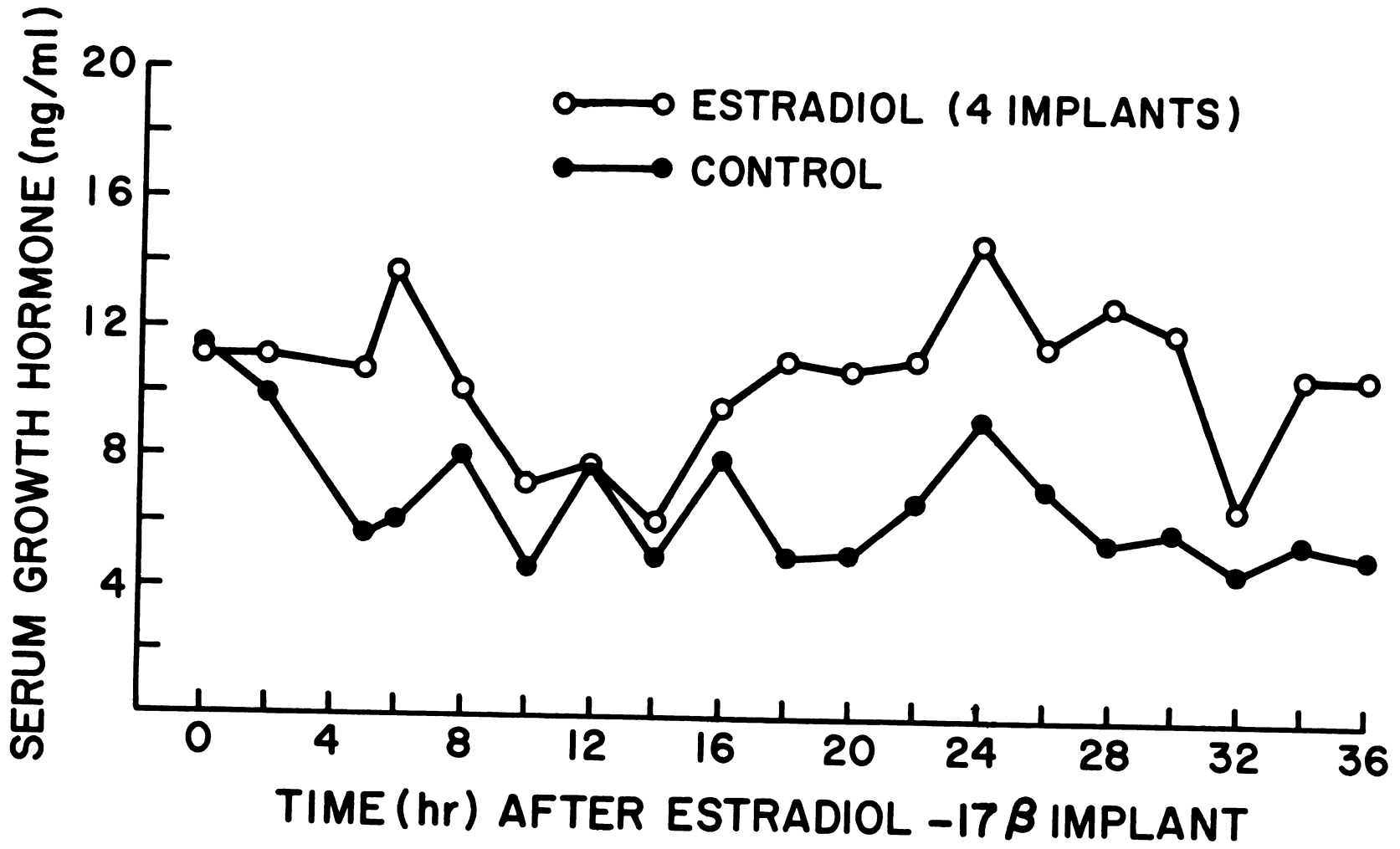

SERUM PROLACTIN (ng/ml)
40
35
30
25
20
15
10
5
0
4
8
12
16
20
24
28
32
36
ESTRADIOL IMPLANT
CONTROL
TIME (HR) AFTER ESTRADIOL-17β IMPLANT

Figure 15. Serum growth hormone concentration in ovariectomized heifers implanted with four estradiol-filled implants at time zero.


SERUM GROWTH HORMONE (ng/ml)
20
16
12
8
4
ESTRADIOL (4 IMPLANTS)
CONTROL
0
4
8
12
16
20
24
28
32
36
TIME (hr) AFTER ESTRADIOL -17β IMPLANT

between group means was not significant ($p > 0.05$). However, analysis of variance revealed a significant ($p < 0.05$) effect of time.

Serum prolactin concentration increased in heifers following injection of TRH at 72 hr after estradiol treatment (figure 16). Immediately before TRH, serum prolactin concentration averaged 4.7 and 3.5 ng/ml in control and estradiol-treated heifers respectively. Serum prolactin concentration then increased ($p < 0.01$) to 75, 82, 69, 60 and 52 ng/ml at 4, 6, 8, 10 and 15 min respectively, following TRH administration to control heifers. Comparable averages for estradiol-treated heifers were 62, 68, 61, 64 and 57 ng/ml. Serum prolactin concentration decreased ($p < 0.05$) towards pretreatment averages by 2 hr after TRH was injected. Although TRH increased ($p < 0.01$) serum prolactin concentration relative to pretreatment averages, neither prolactin concentration at the peak after TRH, nor the mean area under the prolactin response curve was different ($p > 0.05$) between controls and estradiol-treated heifers.

Growth hormone concentration also increased in serum of ovariectomized heifers in response to TRH (figure 17). Immediately before TRH injection serum GH concentration averaged 7 and 15 ng/ml in control and estradiol-treated heifers respectively, but the difference between means was not significant ($p > 0.05$). Serum GH concentration then increased ($p < 0.05$) to 25, 26, 24, 24 and 21 ng/ml at 4, 6, 8, 10 and 15 min respectively, following TRH injection into control

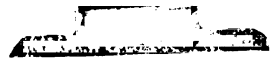


Figure 16. Thyrotropin releasing hormone-induced prolactin release from ovariectomized heifers implanted with four estradiol-filled-implants. TRH (33 ug/100 kg body wt) given at time zero, 72 hr after estradiol treatment.

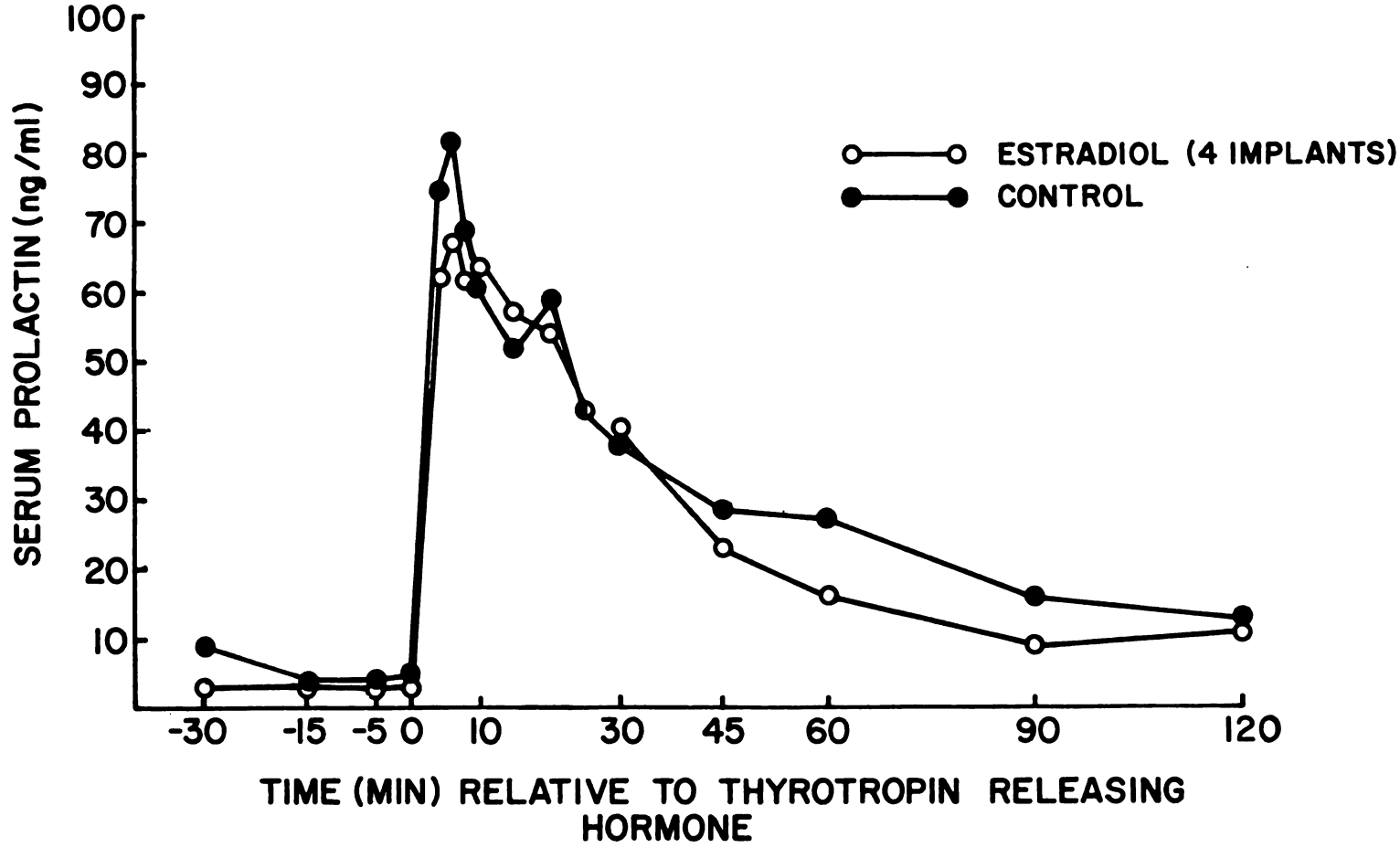
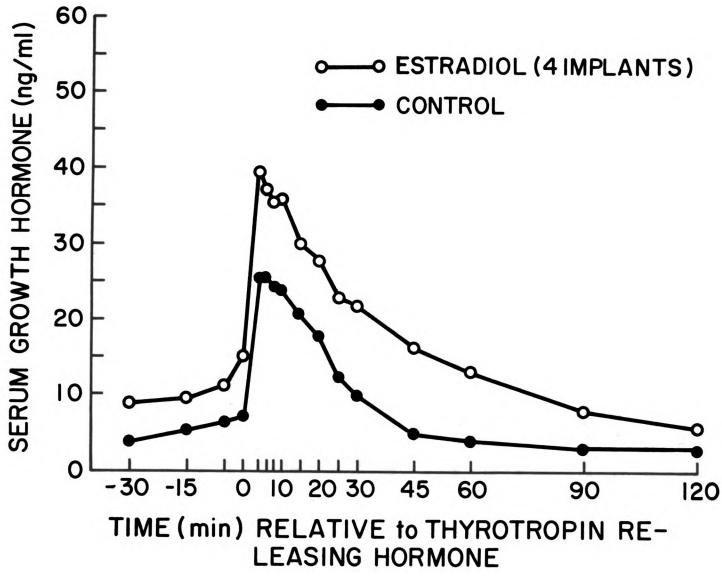

SERUM PROLACTIN (ng/ml)
100
90
80
70
60
50
40
30
20
10
-30
-15
-5
0
10
30
45
60
90
120
TIME (MIN) RELATIVE TO THYROTROPIN RELEASING HORMONE
ESTRADIOL (4 IMPLANTS)
CONTROL

Figure 17. Thyrotropin releasing hormone-induced growth hormone release from ovariectomized heifers implanted with four estradiol-filled-implants. TRH (33 ug/100 kg body wt) given at time zero, 72 hr after estradiol treatment.


SERUM GROWTH HORMONE (ng/ml)
60
50
40
30
20
10
0
ESTRADIOL (4 IMPLANTS)
CONTROL
-30 -15 0 10 20 30 45 60 90 120
TIME (min) RELATIVE to THYROTROPIN RE-
LEASING HORMONE

heifers. Comparable averages for estradiol-treated heifers were 40, 37, 36, 36 and 30 ng/ml. Serum GH then decreased ($p < 0.05$) towards pretreatment averages within 2 hr of TRH injection. Similar to prolactin serum GH concentration increased ($p < 0.05$) in response to TRH, but the difference between group means was not significant, ($p > 0.05$) although after TRH injection serum GH concentration was 53% greater in estradiol-treated heifers than controls.

Average serum estradiol concentration in ovariectomized heifers bearing eight estradiol-filled implants is shown in figure 18. At the beginning of this experiment all heifers had four estradiol implants and estradiol concentration averaged 42 and 33 pg/ml in serum of heifers immediately before estradiol implants were removed (controls) or heifers which received four additional implants, respectively. Estradiol concentration decreased ($p < 0.05$) to 17 pg/ml in serum of heifers at 6 hr after implants were removed (control) and averaged 10-15 pg/ml thereafter. In contrast, serum estradiol concentration increased ($p < 0.05$) to 71 pg/ml at 6 hr in those heifers receiving an additional four implants (total of 8) and remained elevated throughout the duration of the sampling period (36 hr).

Immediately before TRH injection serum prolactin concentration averaged 7.3 and 8 ng/ml in control and estradiol-treated heifers respectively (figure 19). Serum prolactin then increased ($p < 0.05$) to 57, 52, 50, 47 and 46 ng/ml at 4, 6, 8, 10 and 15 min following TRH administration to control

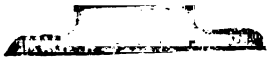


Figure 18. Serum estradiol concentration in ovariectomized heifers implanted with estradiol implants. At time zero all heifers were bearing four implants. Immediately thereafter, implants were removed from heifers in the control group and four additional implants were placed in heifers in the estradiol-treated group.

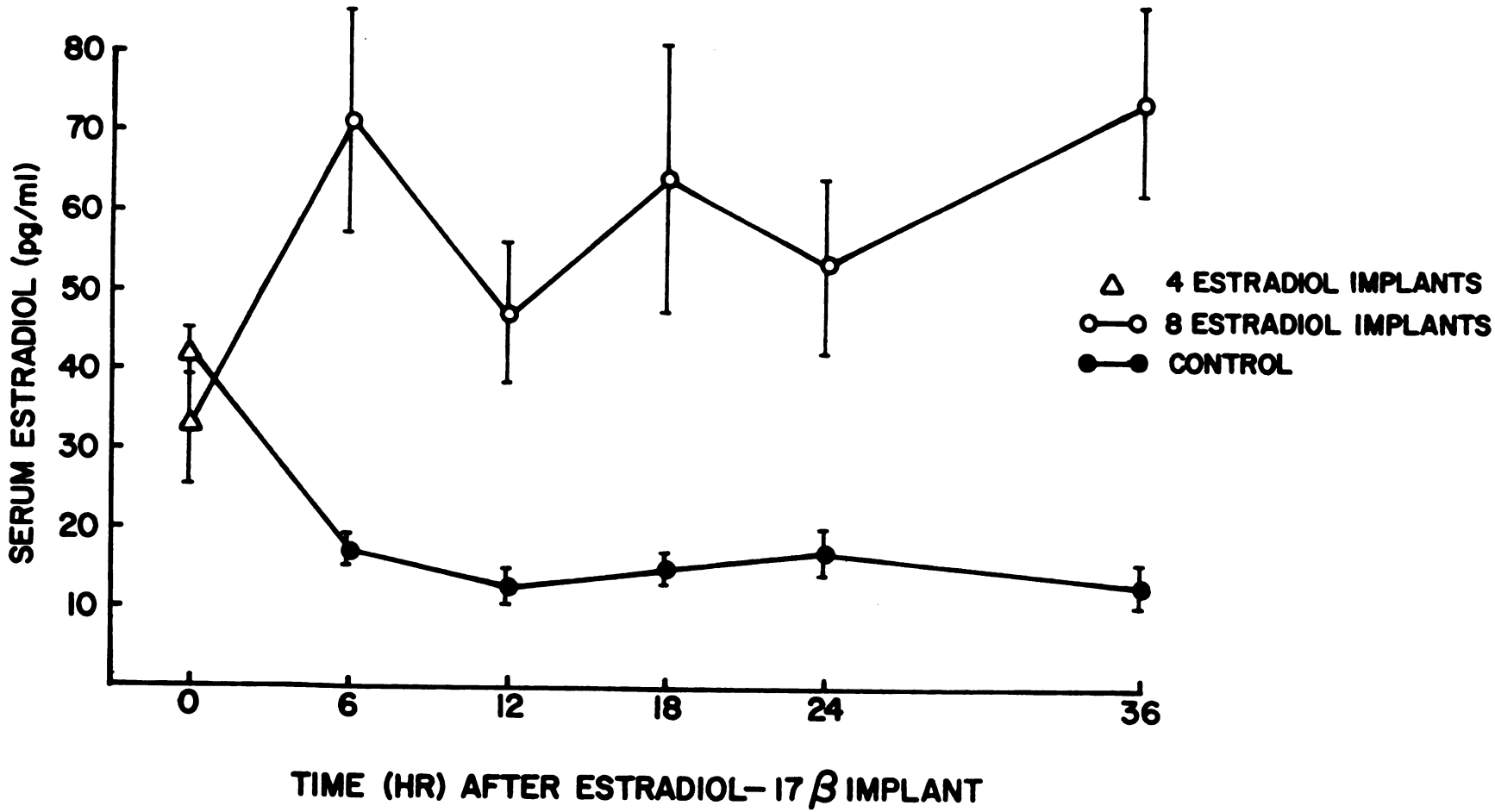
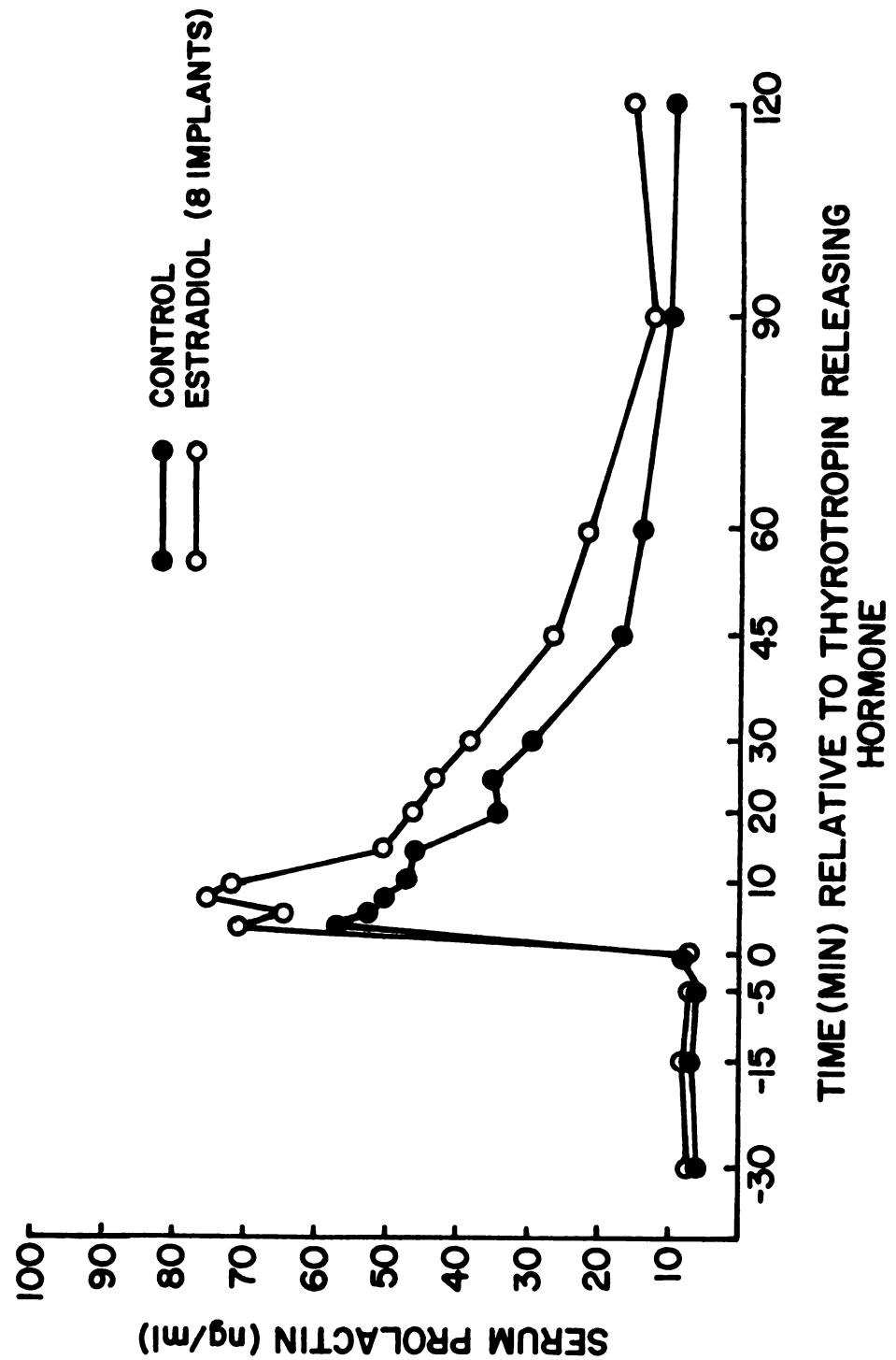

SERUM ESTRADIOL (pg/ml)
80
70
60
50
40
30
20
10
0
6
12
18
24
36
TIME (HR) AFTER ESTRADIOL-17β IMPLANT
4 ESTRADIOL IMPLANTS
8 ESTRADIOL IMPLANTS
CONTROL

Figure 19. Thyrotropin releasing hormone-induced prolactin release from ovariectomized heifers implanted with eight estradiol-filled-implants. TRH (33 ug/100 kg body wt) given at time zero, 36 hr after estradiol treatment.


CONTROL
ESTRADIOL (8 IMPLANTS)
SERUM PROLACTIN (ng/ml)
100
90
80
70
60
50
40
30
20
10
-30
-15
-5
0
10
20
30
45
60
90
120
TIME (MIN) RELATIVE TO THYROTROPIN RELEASING HORMONE

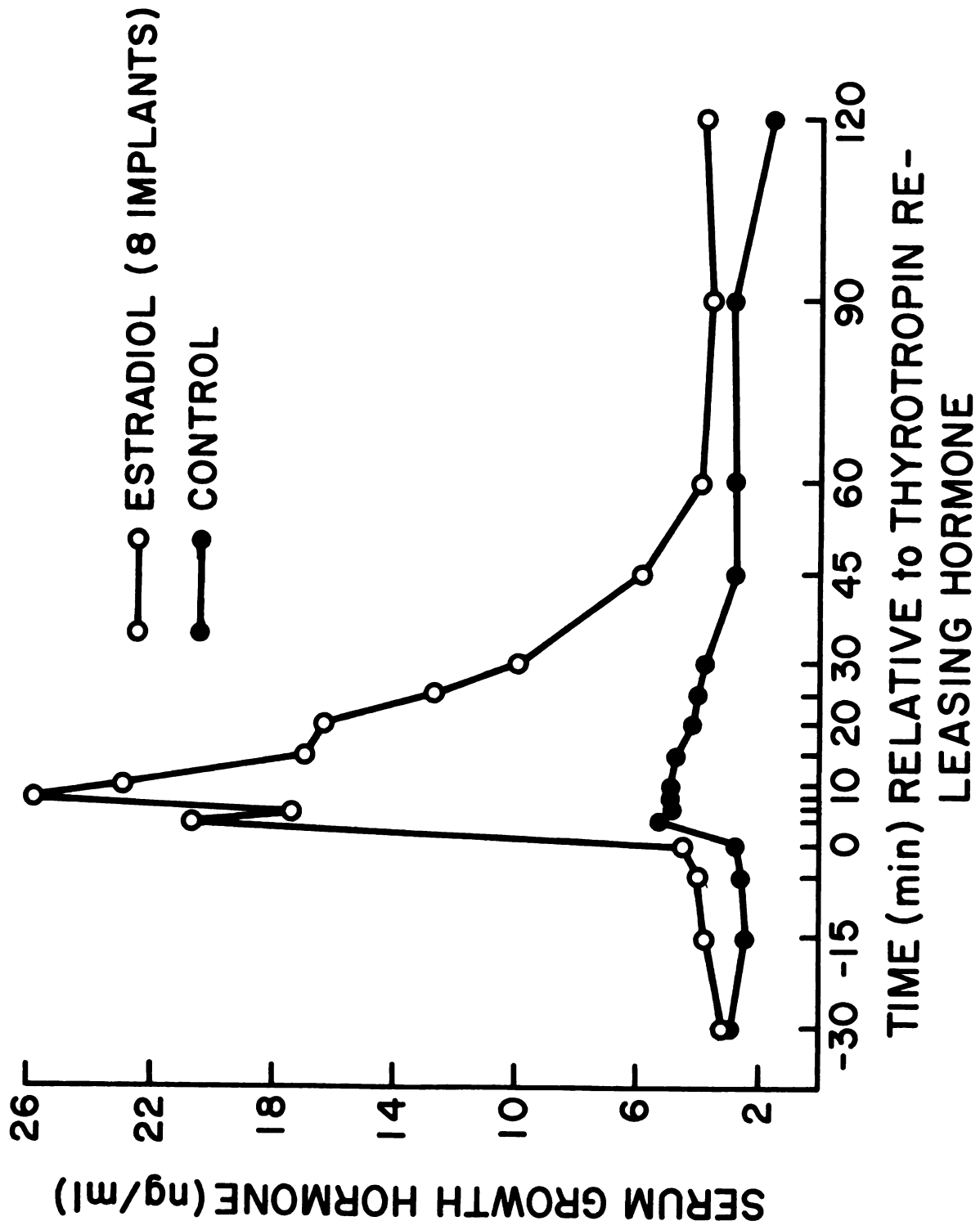
heifers. Comparable averages for estradiol-treated heifers were 71, 64, 75, 72 and 50 ng/ml. Similar to results obtained with heifers bearing four estradiol implants, there was no difference in mean serum prolactin concentration after TRH injection between control heifers and those bearing eight estradiol implants. Serum GH also increased after TRH injection in these heifers (figure 20). Immediately before TRH injection serum GH concentration averaged 2.7 and 4.6 ng/ml in control and estradiol-treated heifers, respectively, ($p > 0.05$). Serum GH then averaged 6, 5, 6, 6 and 9 ng/ml at 4, 6, 8, 10 and 15 min respectively following TRH injection into control heifers. Comparable averages for estradiol-treated heifers were 21, 17, 26, 23 and 17 ng/ml. The increase in serum GH concentration after TRH was 132% greater in estradiol-treated heifers than controls and this increase was significant ($p < 0.01$).

3. Prolactin Release in Vitro in Response to Estradiol -17β and Thyrotropin Releasing Hormone (TRH)

Media prolactin concentration averaged 138, 128, 147 and 157 ng/ml following incubation of bovine pituitary cell cultures for 2 hr with 0, 1, 10 and 100 pg estradiol -17β/ml TC medium 199, respectively, and difference between means was not significant ($p > 0.05$). In contrast, addition of 10 ng TRH/ml medium stimulated prolactin release such that prolactin concentration in the media was 384 ng/ml which was greater ($p < 0.01$) than prolactin concentration for other treatment groups.

Neither baseline prolactin concentration nor TRH-induced

Figure 20. Thyrotropin releasing hormone-induced growth hormone release from ovariectomized heifers implanted with eight estradiol-filled implants. TRH (33 ug/100 kg body wt) given at time zero, 36 hr after estradiol treatment.


ESTRADIOL (8 IMPLANTS)
CONTROL
SERUM GROWTH HORMONE (ng/ml)
26
22
18
14
10
6
2
-30 -15 0 10 20 30 45 60 90 120
TIME (min) RELATIVE to THYROTROPIN RE-
LEASING HORMONE

prolactin release was affected by pretreatment of cell cultures with estradiol -17β. Media prolactin concentration ranged from 33-45 ng/ml during a 2 hr incubation period with TC medium 199 (table 10). Following 6 hr incubation with 0, 1, 10 and 100 pg estradiol -17β/ml, prolactin concentration in the media averaged 145, 163, 134 and 146 ng/ml, respectively, ($p > 0.05$). But media prolactin concentration averaged 402 ng/ml after 10 ng TRH/ml, and this was greater ($p < 0.05$) than all other group means. Thyrotropin-releasing hormone stimulated prolactin release from cell cultures treated with estradiol -17β for 6 hr. However the mean difference in prolactin release from these cultures and comparable cultures treated with TC medium 199 for 6 hr was not different ($p > 0.05$). The tripeptide however, did not stimulate prolactin release from cultures previously treated with TRH for 6 hr. Pretreatment of cell cultures for 12 hr with 10 ng estradiol -17β/ml TC medium 199, also failed to affect prolactin release (table 11). Following incubation for 2 hr with TC medium 199 prolactin concentration averaged 49 and 49 ng/ml in media from cultures pretreated for 12 hr with 0 and 10 ng estradiol -17β/ml, respectively. Comparable averages after incubation for 2 hr with 10 ng TRH/ml medium, were 88 and 94 ng/ml and the difference between means was not significant ($p > 0.05$).

The rise in serum concentrations of estradiol and progesterone following placement of depot steroids into heifers, is in agreement with previous reports (Karsch et al. 1973)

Table 10. Effect of estradiol -17β on basal and thyrotropin releasing hormone-induced prolactin release from bovine pituitary cell cultures.

Treatment		Prolactin in media: 2 hr pretreatment	Prolactin in media: 6 hr treatment	Prolactin in media: 2 hr TRH
		ng/ml		
Estradiol (pg/ml)	0	33±2	145±17	39±4[c]
				106±11[d]
	1	45±3	163±19	48±6[c]
				133±1[d]
	10	36±2	134±8	48±2[c]
				107±8[d]
	100	35±2	146±7	44±2[c]
				125±15[d]
TRH (ng/ml)	10	35±1	402±40	36±7[c]
				41±1[d]

[a]Mean prolactin concentration of four flasks for the 2-hr preceding treatment.

[b]Mean prolactin concentration of four flasks for the 6-hr treatment period.

[c]Mean prolactin concentration of two flasks treated with 0 ng TRH/ml medium after the 6-hr treatment period.

[d]Mean prolactin concentration of two flasks treated with 10 ng TRH/ml medium after the 6-hr treatment period.

Table 11. Prolactin release (ng/ml) from bovine pituitary cell cultures treated with estradiol -17β (E_2) and thyrotropin releasing hormone (TRH).[a,b]

Treatment	Pretreatment media[c]	
	TC medium 199	TC medium + E_2[d]
Non-treated control	49±2	49±2
E_2[d]	45±2	50±3
TRH[d]	88±8	94±12
E_2[d] + TRH[d]	80±9	95±6

[a]Values are means ± standard error.

[b]Mean prolactin concentration of four flasks for the 2-hr treatment period.

[c]Cell cultures pretreated for 12 hr with TC medium 199 or TC medium 199 + estradiol -17β.

[d]Concentration = 10 ng/ml medium.

which showed an increase of these hormones in serum of ovariectomized monkeys bearing silastic capsules containing estradiol -17β or progesterone. A positive correlation between serum estradiol concentration and the number of estradiol-filled implants being administered was suggested by results of this experiment, as serum estradiol concentration in heifers bearing eight estradiol implants was twice that of heifers with four implants. Karsch et al. (1973) also reported that serum estradiol concentration could be increased progressively in ovariectomized monkeys by increasing the number of estradiol -17β-filled capsules being administered. The concentration of serum estradiol following placement of four estradiol implants in these heifers, was approximately twice that normally found in heifers at estrus (Beal et al. unpublished) when serum was assayed by methods used in this study. However, after a single progesterone pessary was inserted, serum progesterone concentration approximated concentration found in cows during the luteal phase of the estrous cycle (Kittok et al. 1973).

The observation that estradiol and progesterone concentrations in serum of these heifers were not different at 72 hr after beginning steroid treatment but before ovariectomy, and at four days after ovariectomy may indicate that exogenous steroids either partially or completely inhibited endogenous production of estradiol and progesterone. Therefore the concentration of these hormones in heifers bearing implants may represent the quantity of hormone released from the implants.

Alternately, in the ovariectomized heifers there could be an increase in adrenal synthesis of estradiol and/or progesterone or a change in the metabolic clearance rate of these hormones thus preventing any change in their concentration in serum.

Serum estradiol concentrations reported herein for untreated ovariectomized heifers, as well as concentrations reported by Short et al. (1973) for ovariectomized cows, are high relative to estradiol concentrations in cattle during the luteal phase of the estrous cycle or during the early postpartum period. Thus Wetteman et al. (1972) reported average estradiol concentration of 3.6 pg/ml serum, in heifers during the luteal phase of the estrous cycle and Echternkamp and Hansel (1973) reported 1-2 pg estradiol/ml serum, in cows during the early postpartum period and during the early luteal phase of the estrous cycle. Perhaps the high serum estradiol concentrations observed here were due to adrenal synthesis of the hormone. Similarly, the high serum concentration of estradiol (10-17 pg/ml) found in serum of ovariectomized heifers 36 hr following removal of depot estradiol could be due to residual estradiol or adrenal synthesis. The possibility that these high concentrations were due to assay contamination cannot be excluded, but this is improbable since serum from steers used as internal standards in all assays performed, averaged 2.8 pg estradiol/ml (n=8).

Greater serum progesterone concentration in heifers treated with both estradiol and progesterone relative to those treated with only progesterone may have resulted from vaginal

hyperemia. Estrogens are known to increase blood flow to the vagina and in these experiments the vulvas of estradiol-treated heifers were noticeably hyperemic. Increased blood flow could facilitate absorption of progesterone. Alternately the presence of estradiol might have caused a change in the metabolic clearance rate of progesterone.

Failure of estradiol to alter baseline prolactin concentration does not support previous results in cattle (Schams and Karg 1972, Schams and Reinardt 1973, Karg and Schams 1974 and Schams et al. 1974) that showed increase plasma prolactin concentration following infusion of 2-12 mg of estradiol-17β for 1-3 hr. These authors note a suppression of plasma prolactin concentration during the infusion period but an increase in plasma prolactin concentration when estradiol infusion was completed. However, quantities of estradiol -17β infused would probably increase its concentration in serum to levels in excess of those reported in this study following placement of estradiol implants. Davis and Borger (1974) reported increase plasma prolactin concentration in ovariectomized ewes following a single injection of estradiol benzoate (0.5 ug/kg body wt). The increase in plasma prolactin was observed only at 8-10 pm (8-10 hr after estradiol treatment) and there were no appropriate controls. Thus, increase plasma prolactin concentration may be attributable to stimuli other than estradiol. In the present experiments, failure of estradiol at concentrations near those found during the normal estrous cycle to affect serum prolactin concentration in heifers suggest that changes in estradiol

concentration during the bovine estrous cycle do not influence prolactin secretion. Thus reports of increased prolactin concentration near to or at estrus in the bovine (Raud et al. 1971 and Swanson et al. 1972) may have resulted from stimuli associated with the physical aspects of estrus (riding, butting, nervousness) rather than direct effects of estrogen on components of the prolactin control system. Increased serum prolactin concentration resulting from estrus activities could be expected since a variety of stimuli will increase serum prolactin in cows (Raud et al. 1971, Tucker 1971 and Johke 1970).

In contrast to results reported herein for heifers, Schams et al. (1974) observed increase plasma prolactin concentration following infusion of 5, 10, 40 or 80 mg of progesterone for 1 hr in bulls. Suppression of plasma prolactin concentration was observed during the infusion period but prolactin concentration was increased when infusion was completed. However quantities of progesterone infused would probably increase its concentration in serum above quantities normally found during the luteal phase of the bovine estrous cycle or pregnancy. Unfortunately progesterone concentrations were not determined. Results reported herein however, agree with those of Nicoll and Meites (1964) that showed no change in media prolactin concentration following incubation of rat pituitary explants in vitro with 1 or 2 ug of progesterone/ml medium. In addition, Bishop et al. (1972) demonstrated no change in serum prolactin concentration following administration

of 1.5 mg of progesterone to rats with lesion in the hypothalamus. But higher doses of progesterone, (10 or 15 mg) will stimulate prolactin secretion in rats (Reece and Bivins 1942; Chen and Meites 1970). Whether progesterone per se or a metabolite, was the effective agent in stimulating prolactin secretion is not known.

Failure of estradiol to affect baseline serum GH concentration in these heifers might have been due to the low concentration of estradiol obtained in serum following placement of implants. Serum GH concentration was increased in steers following daily administration of 10 mg diethylstilbestrol for 142 days (Trenkle 1970). Similar results were obtained in men (Carlson et al. 1973) with 2.5 mg diethylstilbestrol given twice daily for 5 days and in rats (Lloyd et al. 1971 and 1973) given a single dose of 1 or 12 mg diethylstilbestrol. In cycling dairy heifers Vines et al. (unpublished) also failed to establish any relationship between serum GH concentration and days of the estrous cycle. But Koprowski and Tucker (1974a) observed a greater concentration of serum GH during the estrogenic phase of the estrous cycle of cows. Perhaps the GH control system of cows responds differently to estrogens than does that of heifers.

The increase in serum prolactin concentration, the time to peak and the subsequent decline following TRH agree with previous results published from our laboratory (Convey 1973 and Convey et al. 1973). Failure to observe any difference in TRH-induced prolactin release between control and estradiol-

treated heifers confirms a report by Vines et al. (1974) which showed no difference in magnitude of prolactin release in response to TRH administered to cycling heifers on the day of estrus or on days 2, 4, 7, 15 and 18 of the estrous cycle. Similar to results reported herein Tyson et al. (1972) found no difference in TRH-induced prolactin release from women when the tripeptide was administered during the luteal or menstrual phase of the cycle. In contrast, Carlson et al. (1973) and Jaffe et al. (1973) demonstrated that estrogenic compounds would augment TRH-induced prolactin release in humans. The increase in TRH-induced GH release of heifers treated with estradiol is comparable to results in women showing that oral contraceptives with estrogenic activity would augment arginine-induced GH release (Vela and Yen 1969). The fact that TRH-induced GH release was significantly greater in heifers with eight estradiol implants relative to their appropriate controls but not in heifers with four estradiol implants relative to their controls, suggests that the quantity of GH releasable by TRH was dependent on serum estradiol concentration. Vines et al. (1974) also failed to show any difference in magnitude of GH release in response to TRH, administered to dairy heifers on different days of the estrous cycle.

Evidence that estradiol can act directly to stimulate prolactin release from rat pituitary explants has been reported by others (Nicoll and Meites 1962, Ben-David et al. 1964). Results presented here and those of Zolman (1973)

suggest that prolactin release by bovine pituitary cell cultures or pituitary explants *in vitro* was not influenced by estradiol at concentrations used in these experiments. The response of cell cultures to TRH attests to the viability of these cells and that prolactin release could be stimulated by proper secretagogues. If one assumes that estradiol increased pituitary prolactin content but not release of the hormone, then after 6-12 hr of exposure to estradiol, intracellular prolactin content should increase in cell cultures and more prolactin should be available for release by TRH. Therefore, failure to observe any difference in TRH-induced prolactin release between estradiol-treated and control cultures, suggests that estradiol at these concentrations and for the time of exposure employed in this design did not affect the releasable source of pituitary prolactin content. These results lend credence to our earlier hypothesis that changes in estradiol concentration of a magnitude expected during the estrous cycle of the bovine do not appear to influence prolactin concentration.

Failure of bovine pituitary cell cultures to respond to a second challenge with TRH may have resulted from either a depletion of releaseable pituitary prolactin stores or the TRH-prolactin-releasing mechanism became refractory to TRH. In humans, Bowers *et al.* (1971) observed a diminution in serum prolactin concentration after each of four consecutive injections (3 hr intervals) with 400-800 ug TRH and Fell *et al.* (1973) also demonstrated similar results in ewes with 20 ug TRH given at 1 hr intervals for 3 hr.

SUMMARY AND CONCULSIONS

Regulation of prolactin and growth hormone (GH) release in the bovine was studied by both *in vivo* and *in vitro* methods.

Subcutaneous administration of 80 mg ergocryptine on two consecutive days to lactating Holstein cows decreased serum prolactin concentration to approximately 1 ng/ml for at least five days after beginning treatment, but neither milk yield nor serum concentrations of GH and cortisol was affected. In addition ergocryptine in doses of 0.01 to 10 ug/ml TC medium 199, decreased release of prolactin but not GH from bovine anterior pituitary cells in culture.

It was concluded from these results that ergocryptine decreased serum prolactin concentration in cattle, at least in part by a direct action on the anterior pituitary. Failure to observe any change in milk yield despite marked reduction of serum prolactin concentration suggests that prolactin may not be required to maintain established lactations in cattle, or that far more prolactin is present in serum than is required for milk secretion.

Prolactin release from bovine pituitary cell cultures *in vitro* was consistently stimulated with thyrotropin releasing hormone (TRH) at doses of 0.01 to 100 ng/ml TC medium 199. The

effect on growth hormone release however, was equivocal, in that TRH stimulated GH release from cell cultures at 72 hr of culture but not at 96 hr. Gonadotropin releasing hormone (GnRH) at 1, 10 and 100 ng/ml TC medium 199, slightly stimulated the release of both prolactin and luteinizing hormone (LH) from 96-hr pituitary cell cultures, but TRH had no effect on LH release from these cultures.

Addition of triiodothyronine (T_3) or thyroxine (T_4) at 0.1 and 1.0 ug/ml TC medium 199 to cell cultures, affected neither baseline prolactin concentration nor the magnitude of prolactin release in response to TRH. However, 5 or 50 ug T_4/ml medium decreased spontaneous release of prolactin but not TRH-induced prolactin release.

It was concluded from these results that: (1) TRH stimulates prolactin release in cattle at least in part by a direct action on the anterior pituitary; (2) The mechanism by which TRH causes prolactin release in cattle appears to be insensitive to inhibition by thyroid hormones and (3) Pituitary cells in culture are capable of discriminating between different releasing hormones similar to their observed effects *in vivo*.

In order to investigate the effect of physiological concentrations of estradiol -17β and progesterone on serum prolactin and GH concentrations in cattle, progesterone pessaries and estradiol -17β-filled polydimethylsiloxane implants were placed into intact Holstein heifers that were subsequently ovariectomized. In addition the effect of estradiol -17β on the magnitude of TRH-induced prolactin and GH release *in*

vivo and prolactin release in vitro was investigated.

Despite increase progesterone and/or estradiol concentration in serum of heifers bearing depot steroids there were no significant changes in baseline concentrations of serum prolactin and GH attributable to treatment.

Thyrotropin releasing hormone administered to heifers bearing four or eight estradiol-filled implants increased serum prolactin concentration relative to pretreatment averages, but neither peak serum prolactin concentration nor area under the prolactin curves was affected by estradiol treatment. Serum GH concentration after TRH injection was 53% greater in heifers bearing four estradiol implants relative to their controls, but this increase was not signifcant. In contrast, serum GH concentration after TRH was 132% greater in heifers bearing eight estradiol implants relative to their controls and this increase was significant.

Neither spontaneous release of prolactin nor TRH-induced prolactin release from pituitary cell cultures was affected by incubating cultures for 2-12 hr with TC medium 199, containing 1, 10 and 100 pg or 10 ng/ml estradiol -17β. Pituitary cell cultures chronically treated with TRH for 6 hr, failed to respond to a subsequent TRH challenge with increase prolactin release.

It was concluded from these results that concentrations of serum estradiol and progesterone which approximate concentrations found during the estrous cycle of cattle do not influence significantly prolactin secretion. Hence, increased

serum prolactin concentration at or near estrus in cattle may be due to physical stimuli associated with estrus activity, rather than a direct effect of estrogen on prolactin control mechanism. In contrast, the control of GH secretion may be associated with serum estradiol concentration. Failure of pituitary cell cultures to respond to consecutive challenges of TRH may be due to refractoriness of the TRH-prolactin releasing mechanism or depletion of releasable prolactin.

APPENDICES

APPENDIX 1

PREPARATION OF MEDIA FOR CELL CULTURE

TC medium 199 10x concentration; 10.4 g/liter

TC Minimal Medium Eagle, Hanks BSS[a]; 10.4 g/liter.

Mix both solutions 50:50 v/v.

Adjust ph to 7.2-7.4 with 10% $NaHCO_3$.

Add antibiotics
Fungizone[b] (Amphotericin B); 5 ug/ml medium.
Penicillin G; 100 units/ml medium
Streptomycin sulfate; 100 ug/ml medium.

Filter sterilize medium. Medium can be used up to 3 weeks after preparation.

For growing cells add 10% cow serum (sterile) to the above medium = growth medium.

[a]DIFCO Laboratories, Detroit, Michigan.

[b]E.R. Squibb and Sons Inc., New York, N.Y.

APPENDIX 2

PREPARATION OF TC MEDIUM 199

TC medium 199 (10x) concentration	100 ml
Sodium bicarbonate solution (2.8% $NaHCo_3$)	80 ml
Antibiotic solution (170 mg penicillin g/100 ml)	40 ml
Sterile glass double distilled H_2O	780 ml
Total	1000 ml

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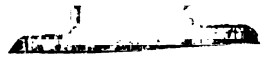

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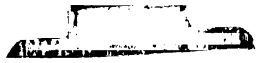

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