GROWTH HORMONE, PROLACTIN, LUTEINIZING HORMONE AND GLUCOCORTICOID RESPONSES TO PROSTAGLANDIN F_{2a} IN CATTLE

Dissertation for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

JOHN NORMAN STELLFLUG

1976

This is to certify that the

thesis entitled

GROWTH HORMONE, PROLACTIN, LUTEINIZING HORMONE AND GLUCOCORTICOID RESPONSES TO PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

presented by

John Norman Stellflug

has been accepted towards fulfillment
of the requirements for

PhD. degree in Dairy Science

Major professor

Date November 12, 1976

ABSTRACT

GROWTH HORMONE, PROLACTIN, LUTEINIZING HORMONE AND GLUCOCORTICOID RESPONSES TO PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

By

John Norman Stellflug

The purpose of this thesis was to determine if prolactin (PRL), growth hormone (GH), luteinizing hormone (LH) and glucocorticoids were released after luteolytic doses of $PGF_{2\alpha}$ in cattle. After administration of 15, 30 or 60 mg $PGF_{2\alpha}$ to diestrous heifers (n=4, 6, 6, respectively), (1) PRL increased ($P<.01$) more than 3-fold, within 10 minutes, to a peak 6-fold above basal values, and returned to pre-injection values within 4 hours, (2) growth hormone increased ($P<0.05$) in a dose-related manner, peaking at 30 minutes and remaining above pre-injection values for over 1 hour, (3) LH increased ($P<0.05$) 2-fold or greater above pre-injection values, within 1.5 to 6 hours, and (4) glucocorticoid (indicator of ACTH release) increased ($P<0.01$) more than 6-fold at 30 minutes, and returned to pre-injection values by 4 hours.

A second experiment was conducted to determine the site of action of $PGF_{2\alpha}$ on glucocorticoid release. $PGF_{2\alpha}$ (25 mg) and saline were given im 7 days after a pretreatment with triamcinolone acetonide (TA) to suppress serum glucocorticoid to less than 0.5 ng/ml within 24 hours. In saline-treated heifers not given TA, glucocorticoid fluctuated between 10 and 20 ng/ml without relation to the saline

injection whereas it increased ($P<0.01$) 5-fold by 30 minutes after $PGF_{2\alpha}$ and returned to pre-injection values by 3 to 4 hours. In TA-pretreated heifers, peak glucocorticoid response was depressed 50 percent after ACTH and 88 percent after $PGF_{2\alpha}$ in comparison with heifers not given TA. Because glucocorticoid response after ACTH in TA-pretreated heifers was partially inhibited, another experiment was conducted to minimize possible adrenal regression after TA, and to maximize the effectiveness of TA. Submaximal doses of porcine ACTH (200 IU) and $PGF_{2\alpha}$ (5mg) were administered to heifers 6 hours after TA pretreatment when glucocorticoid was maximally inhibited by TA. In animals not pretreated with TA, the first 30 minutes of glucocorticoid response to $PGF_{2\alpha}$ resembled that after ACTH, but the peak response to ACTH was much greater ($P<0.01$), and the duration of response to ACTH was much more prolonged ($P<0.01$) than that after $PGF_{2\alpha}$. TA-pretreatment reduced the glucocorticoid response to ACTH by 50 percent, but it essentially abolished the response to $PGF_{2\alpha}$.

Three added treatments consisting of simultaneous or sequential administration of $PGF_{2\alpha}$ and ACTH were included in this third experiment. Glucocorticoid response was more prolonged ($P<0.01$) when $PGF_{2\alpha}$ and ACTH were injected simultaneously, or when $PGF_{2\alpha}$ followed ACTH-treatment by comparison to that after ACTH alone. In contrast, when $PGF_{2\alpha}$ was administered before ACTH, peak glucocorticoid response to ACTH was suppressed by ($P<0.05$) by comparison to that after ACTH alone.

In conclusion, the secretion of PRL, GH, LH, and glucocorticoid after administration of $PGF_{2\alpha}$ may represent relatively specific action

at the hypothalamus or pituitary. No evidence was derived from these experiments for the site of action of $PGF_{2\alpha}$ on GH, PRL, or LH secretion, but on the basis of the last two experiments, I suggest $PGF_{2\alpha}$ acts on the hypothalamo-pituitary axis to cause glucocorticoid secretion. Further research is required to distinguish between these sites of $PGF_{2\alpha}$ action and to determine the physiological importance of $PGF_{2\alpha}$ in pituitary hormone secretion in cattle. Regardless of whether or not the diversified hormone release after $PGF_{2\alpha}$ affects the decision of prostaglandin use for control of ovulation, the results from this research raise the possibility of using prostaglandins to regulate intermediary metabolic hormones in food producing animals.

GROWTH HORMONE, PROLACTIN, LUTEINIZING HORMONE AND GLUCOCORTICOID RESPONSES TO PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

By

John Norman Stellflug

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy Science

1976

TO SHERYL

ACKNOWLEDGMENTS

The Department of Dairy Science at Michigan State University provided facilities, faculty and financial support for this graduate program.

I express my sincerest thanks and appreciation to my major advisor and friend, Dr. H. D. Hafs, for his enthusiastic leadership and encouragement. The participation and consent of the other members of my guidance committee, Drs. J. L. Gill, J. Meites and W. L. Smith, also are gratefully acknowledged. I also wish to thank Dr. R. Neitzel for his assistance with statistical and hormone assay programs.

I wish to thank my fellow colleagues, N. Beck, Dr. T. Beck, D. Bolenbaugh, Dr. R. Gorewit, R. Kittok, T. Kiser, Dr. T. Louis, Dr. K. Mongkonpunya, J. Plog and D. Vines, for their assistance at the barn and with laboratory tasks. For their assistance with radioimmunassay procedures, I thank E. Bostwich, P. Kaneshiro, J. Mapes, J. Martin, C. Rankin and J. Yonker.

I express a very special note of gratitude to Dr. E. L. Moody whose interest and enthusiasm for reproductive biology was fundamental in leading to my decision to pursue a career in reproductive endocrinology.

To my wife, Sheryl, to whom this dissertation is dedicated, I express my gratitude for her patience, understanding and support throughout my doctoral program.

BIOGRAPHICAL SKETCH

John Norman Stellflug was born on May 24, 1947, in Glasgow, Montana. He attended Newton School for 6 years prior to entering Glasgow High School and graduated in June, 1965. Pursuing his interest in agriculture, he completed requirements for a Bachelor of Science degree at Montana State University in December, 1969. He accepted a graduate assistantship in the Animal Science and Range Management Department at Montana State University in January, 1970. From February to June, 1970, he completed his active training in the United States Army Reserve before reinitiating his graduate studies and received the Master of Science degree in December, 1972. His thesis was entitled "Periparturient Estrogen Levels in the Plasma of Beef Cows."

He then enrolled at Michigan State University studying under the directorship of Dr. Harold D. Hafs. He completed the requirements for the Ph.D. in December, 1976, and accepted a position as a Research Physiologist with the Agricultural Research Service at the United States Sheep Experiment Station near Dubois, Idaho.

TABLE OF CONTENTS

LIST OF FIGURES

Page

INTRODUCTION

Artificial insemination to obtain genetic improvement has been hindered by mandatory detection of estrus. This has resulted in development of techniques to sychronize ovulation. One method of ovulation control in cattle is to administer prostaglandin $F_{2\alpha}$ twice at 12-day intervals and inseminate at 80 hours after the second injection of $PGF_{2\alpha}$ without estrus detection. During the developmental stages of this research other investigators reported that prostaglandins of the E series stimulate release of growth hormone, prolactin, and adrenocorticotropin in several species. In addition, the F series of prostaglandins release luteinizing hormone in sheep, and growth hormone and cortisol but not LH, FSH or TSH in humans.

Because $PGF_{2\alpha}$ shows potential for reproductive control in farm animals, and because of the known diversified actions of PGE's and of PGF's, the first study in this thesis was to evaluate the responses of anterior pituitary hormones in cattle to luteolytic doses of $PGF_{2\alpha}$. Two additional experiments were executed to determine the site of action of $PGF_{2\alpha}$ on glucocorticoid release.

This research has two kinds of practical significance. First, $PGF_{2\alpha}$ already has been approved for commercial ovulation control in cattle and horses in several countries, and it may be approved in the United States as early as 1977. Whether or not $PGF_{2\alpha}$ causes release of

pituitary hormones, at the time of its use to control ovulation, may affect decisions on the extent of its use. Secondly, if $PGF_{2\alpha}$ causes release of intermediary metabolic hormones, such as growth hormone, prolactin and glucocorticoid, perhaps out of such a discovery new methods may arise to regulate these hormones to improve efficiency of food producing animals.

LITERATURE REVIEW

This literature review initially is a brief review of reproductive endocrine events during a normal estrous cycle. The second and larger part is a description of endocrine patterns after luteolytic doses of prostaglandin $F_{2\alpha}$ to provide a basis for comparison with the normal changes.

Endocrine Events During the Normal Bovine Estrous Cycle

Days of the estrous cycle in this discussion are numbered from the day of estrus (day 0), which is a 12- to 18-hour period of sexual receptivity. In sequence, the remaining three categories of a normal estrous cycle are: (1) metestrus, the interval from day 1 to day 4 during luteal development; (2) diestrus, the interim of luteal function which persists from day 4 to day 17 or 18; and (3) proestrus, the period from about day 18 to day 21 or 22 during luteal regression and follicular growth.

The most recent comprehensive description of the progesterone, estradiol and LH interrelationships from late diestrus until ovulation in the cow was published by Chenault et al. (1975). They integrated and referenced numerous endocrine studies on the bovine estrous cycle.

Blood serum progestins averaged 5.6 ng/ml on day 15 and declined to less than 1 ng/ml by day 19 (Chenault et al., 1975). Progesterone normally may begin to decline in individual cows at any

time from day 16 to day 19 (Hansel et al., 1973), and progesterone synthesis fails rapidly once luteolysis begins. Progestins monitored at 6-hour intervals during the late luteal phase and early proestrus revealed a precipitous decline to less than 50 percent of diestrus concentration within 12 to 18 hours after onset of luteolysis (Chenault et al., 1975). Progestins remained near 0.5 ng/ml thoughout proestrous, estrus, and early metestrus (Kazama and Hansel, 1970; Chenault et al., 1975). Most reports showed no evidence of a proestrus progesterone surge in the cow as was suggested by Ayalon and Shemesh (1974).

Luteolysis or death of the corpus luteum, indicated by declining blood progesterone, is initiated by production of the luteolytic factor from the uterus (reviewed by Anderson et al., 1969). There are several kinds of evidence for uterine control of luteolysis. For example, ligation of the uterine vein prevents normal luteal regression in sheep (McCracken and Baird, 1969). In addition, ovarian transplants to the neck in sheep (McCracken et al., 1971) prolonged the life span of the corpus luteum, but estrous cycles were normal when the uterus was transplanted with the ovary to the neck (McCracken et al., 1971); this suggested that a proximal anatomical relationship is required between the uterus and the ovary for normal luteal regression. Following the suggestion of Babcock (1966) that prostaglandin may be the uterine luteolytic factor, Hansel et al. (1975) isolated a precursor of prostaglandin $F_{2\alpha}$, arachadonic acid, from uterine extracts and found it to be luteolytic in pseudopregnant hysterectomized hamsters. Although prostaglandin $F_{2\alpha}$ has received extensive attention, and Goding et al. (1972) and McCracken et al. (1972) concluded that $PGF_{2\alpha}$ is the uterine

luteolytic factor in sheep, more evidence is required to confirm this role of $PGF_{2\alpha}$ in sheep and cattle.

Once progesterone declines during proestrus, a wave of follicular growth occurs (Rajakowski 1960; Cole et al. 1969) which is thought to be controlled by follicle stimulating hormone (FSH). In support of this notion, Hackett and Hafs (1969) found the decrease of pituitary content of FSH from day 19 to day 0 indicative of FSH release. However, FSH was not elevated in the blood plasma until near the onset of estrus concident with the ovulatory LH surge (Akbar et al., 1974). Follicle stimulating hormone induces formation of FSH receptors in the rat follicle, while FSH and LH jointly are thought to be necessary for estrogen production (Richards and Midgley, 1976). Estrogen increases estradiol receptors in the follicle and estrogen plus FSH induce LH receptors (Richards and Midgley, 1976). However, the control of follicular growth and its temporal relationship with FSH secretion in cattle must await further clarification.

In the cow, serum estrogen increases gradually during proestrus, then increases abruptly before estrus (Chenault et al., 1975). This high estrogen stimulates the preovulatory surge of LH (Hobson and Hansel, 1972). Once this LH surge occurs, estrogen decreases precipitously to basal values before the end of estrus (Chenault et al., 1975). In addition to the estradiol increase at proestrus, smaller increases of estradiol may occur (Glencross et al., 1973) during metestrus and diestrus. Hansel and Echternkamp (1972) found smaller increases of estrone instead of estradiol during diestrus. The small

estrogen increase during diestrus coincides with midcycle follicular development which lasts about 7 days (Cole et al., 1969).

During the last day of proestrus, LH increases abruptly from 1 ng/ml to between 10 or 20 ng/ml, before it returns to basal concentrations (0.5 to 1 ng/ml) within 6 to 12 hours (Swanson and Hafs, 1971). This surge of LH not only stimulates final maturation of the follicle and oocyte, but it also initiates corpus luteum formation. The corpus luteum starts producing singificant amounts of progesterone at the end of metestrus (day 3-4) and then proliferates dramatically, secreting its highest progesterone concentrations by day 15 (Christensen et al., 1974).

Prolactin is another hormone reported to increase in the blood of cattle near estrus (Sinha and Tucker, 1969; Swanson and Hafs, 1971), but Shams and Karg (1970) found no relationship of serum prolactin to stages of the bovine estrous cycle. Perhaps prolactin increases during proestrus in cattle in response to high estrogen, as reported in rats (Ojeda and McCann, 1974), or it may reflect increased physical activity during behavioral estrus. The functional role of prolactin in relation to estrus, ovulation, and luteal growth in cattle is unclear.

Prostaglandins

As a prelude to the subsequent discussion of the endocrine events after $PGF_{2\alpha}$-induced luteolysis, here follows a brief background on prostaglandins.

Historical Review

Prostaglandin, the generic name for a family of biologically active lipids, was first discovered by two gynecologists (Kurzrok and Lieb, 1930). They reported contraction and relaxation of strips of human uterus after applying human seminal fluid. Goldblatt (1933) and Von Euler (1935) described similar muscle contractile properties of human seminal fluid, and Von Euler (1935) related this property to a lipid he named prostaglandin. It was not until the late 1950's that mass spectrophotometric and chromatographic techniques were developed to allow isolation and chemical identification of prostaglandins. Bergstrom et al. (1962) stimulated new interest in the physiological and pharmacological properties of prostaglandin when he elucidated the chemical structures of three naturally occurring prostaglandins.

Nomenclature

Prostaglandins (PG's) are analogs of prostanoic acid, the hypothetical parent 20-carbon fatty acid with a cyclopentane ring at C-8 to C-12. All naturally occurring prostaglandins have a hydroxyl group at C-15 and a 13, 14 transdouble bond. The four major subdivisions of prostaglandins are designated A, B, E, and F which identify groups on the cyclopentane ring. For example, $PGF_{2\alpha}$ has an α-hydroxyl group which lies below the plane of the ring while beta (β) indicates a group above the plane. The numerical subscripts, which follow the letter, indicate the degree of unsaturation in the alkyl and carboxyl side chains.

Temporal Endocrine Events During the Bovine Estrous Cycle Initiated by Prostaglandin $F_{2\alpha}$

Following the initial discovery by Phariss and Wyngarden (1969) that $PGF_{2\alpha}$ was luteolytic in pseudopregnant rats, Louis et al. (1972) and Rowson et al. (1972) reported that $PGF_{2\alpha}$ induced luteal regression in cattle. These results and an increasing supply of synthetic $PGF_{2\alpha}$ stimulated numerous investigations on its luteolytic action. The most recent review article is by Thatcher and Chenault (1976). They characterized plasma progestin, estradiol and LH changes from injection of $PGF_{2\alpha}$ until the time of ovulation. Progesterone decreased 50 percent within 7 hours after $PGF_{2\alpha}$, similar to previous studies (Hafs et al., 1974; Stellflug et al., 1975). When progesterone was measured at frequent intervals after 5, 10 or 15 mg $PGF_{2\alpha}$ given to six heifers during three consecutive estrous cycles, Stellflug et al. (1976) found progesterone decreased for the first hour and then rebounded slightly at 2 to 3 hours, as Hixon and Hansel (1974) reported. Then progesterone decreased to less than 1 ng/ml by 24 hours and remained below 1 ng/ml until day 3 to 5. Similarly to Chenault et al. (1975) and Thatcher and Chenault (1976, Stellflug et al. (1976) found no evidence for the preovulatory progesterone surge reported by Ayalon and Shemesh (1972).

Estradiol increased linearly from 2.7 pg/ml at time of $PGF_{2\alpha}$ injection to 4.6 pg/ml at 76 hours post-treatment (Thatcher and Chenault, 1976). This estradiol increase induced a preovulatory LH surge like the response during normal proestrus. The LH surge was centered at 65 $\pm$ 5 hours after $PGF_{2\alpha}$, coincident with onset of estrus (Louis et al., 1974). Louis et al. (1974), Chanault et al. (1975), and

Thatcher and Chenault (1976) found little difference in magnitude of the peak and duration of the LH surges which occur during normal or $PGF_{2\alpha}$-induced luteolysis. LH peaked between 10 to 20 ng/ml, persisted for 11.2 $\pm$ 7.5 hours ($\bar{x} \pm$ sd) and follicles ovulated on the average 21 hours after the LH peak (Thatcher and Chenault, 1976). This is similar to the interval from peak LH to ovulation reviewed by Hafs et al. (1974) after $PGF_{2\alpha}$ and described by Chenault et al. (1975) during a normal estrous cycle.

I conclude that endocrine patterns and interrelationships of progesterone, estrogen, and lutenizing hormone after $PGF_{2\alpha}$ in the cow are strikingly similar to these events during a normal cycle. Unfortunately, the studies on normal events have not been as intensive as those on events after $PGF_{2\alpha}$. Some further evidence for the similarity of $PGF_{2\alpha}$-induced estrus to the normal estrus, although indirect at best, is that conception rate from artificial insemination of cattle treated with $PGF_{2\alpha}$ is not reduced when compared to controls (Lauderdale et al., 1974, and Hafs et al. 1975).

Anterior Pituitary Hormone Responses after Prostaglandin

$PGF_{2\alpha}$ has promising potential for control of ovulation in farm animals if it does not cause undesirable side effects. If $PGF_{2\alpha}$ causes release of metabolic hormones, it may provide a means to regulate these important hormones. Previous studies provided intriguing evidence that prostaglandins of the E series stimulated prolactin, growth hormone, gonadotropin and adenocorticotropin (ACTH) release in

several species, but hormone responses to the F series of prostaglandins were equivocal.

Prolactin

$PGF_{2\alpha}$ caused prolactin (PRL) release as well as lactogenesis and abortion on day 18 of pregnancy in the rat (Vermouth and Deis, 1972). Infusion of PGE_1 into the third ventricle of ovariectomized rats also released PRL by 15 minutes, but equal amounts of PGE_2, $PGF_{1\alpha}$ or $PGF_{2\alpha}$ failed to release PRL (Harms et al., 1973). In contrast, no PRL synthesis or release occurred during incubation of rat pituitaries with PGE_1, PGE_2, PGA_1 or $PGF_{2\alpha}$ (MacLoed and Lehmeyer, 1970).

Thus, previous literature supports an in vivo release of PRL in ovariectomized rats induced only by PGE_1 and in pregnant rats during $PGF_{2\alpha}$-induced lactogenesis and abortion, but suggests no in vitro pituitary response to prostaglandins.

Growth Hormone

Prostaglandins E_1 and E_2 induced growth hormone (GH) release in vitro. For example, PGE_1 (5×10^{-6}M) added to the incubation media of heifer pituitary slices stimulated 30 percent more GH production than controls. By monitoring incorporation of 4,5-H^3 leucine into GH and its subsequent release from bisected rat pituitaries into medium 199, PGE_1 increased GH release 165 percent with normal amounts of GH still present in the pituitary tissue (MacLoed and Lehmeyer, 1970). In addition, PGE_2 caused similar GH release while PGA_1 increased GH synthesis but not release, and $PGF_{2\alpha}$ had no effect on synthesis or release. In agreement with this study, Hertelendy (1971) found

increased GH release into media 148 percent after infusion of male rat anterior pituitaries with PGE_1, but PGE_2 stimulated GH to a lesser extent.

In vivo studies with sheep and rats also indicated a prostaglandin induced GH release. When PGE_1 was infused iv into male castrate sheep, GH increased from 2.0 to 14 ng/ml by 20 minutes and decreased to 2.1 ng/ml by 60 minutes (Hertelendy *et al.*, 1972). However, this GH response to PGE_1 was abolished when PGE_1 was administered during infusion of epinephrine. GH peaked at 60 to 90 minutes after a 30-minute iv infusion of 50, 100 or 140 μg of PGE_1/kg/min into two adult men per treatment dose (Ito *et al.*, 1971). Coudert and Faiman (1973) also observed a slight increase in GH after 2.0 μg of $PGF_{2\alpha}$/kg/min iv infusion for 30 minutes to 5 men (23-35 years of age). In addition, blood GH increased over 4-fold to a maximum by 10 to 20 minutes after PGE_1 (5 μg) injection into the carotid of male rats anesthetized with pentobarbital (Hertelendy *et al.*, 1972).

In general, *in vitro* studies indicate PGE_1 and PGE_2 stimulate GH release and PGA_1 appears to stimulate only synthesis, while $PGF_{2\alpha}$ has no effect on GH release. *In vivo* studies also reveal that PGE_1 induces GH release and suggest that $PGF_{2\alpha}$ also causes slight stimulation of GH release.

Gonadotropins

Prostaglandin involvement in gonadotropin release is equivocal. Although as little as 0.1 μg/ml PGE_1 stimulated rat anterior pituitary cyclic AMP (cAMP) concentrations, 20 μg/ml of PGE_1 had little if any effect on LH release during a 20-minute incubation (Zor et al., 1970). Similarily, PGA_1, PGB_1 and $PGF_{1\alpha}$, which augmented cyclic AMP to a lesser extent than PGE_1, failed to release LH. Infusion of $PGF_{2\alpha}$ (iv) at 0.05, 0.20 or 2.0 μg/kg/min caused no changes in serum LH or FSH in adult men (Coudert and Faiman, 1973). And infusion of 5 μg $PGF_{1\alpha}$ or $PGF_{2\alpha}$ into the third ventricle of rats caused no significant changes in blood concentrations of LH or FSH (Harms et al., 1973).

On the other hand, PGE_2 given iv early in the afternoon of proestrus elicited a rise in blood LH in rats in which the ovulatory LH-surge had been blocked by pentobarbital (Tsafriri et al., 1973). For instance, the LH response after PGE_2 was 470 ± 87, versus 106 ± 17 ng/ml (mean ± se) for controls, and PGE_2 induced ovulation in 66 percent of the pentobarbital blocked rats. More convincingly, infusion of 5, 10 and 20 μg of PGE_1 into the third ventricle of cycling rats caused release of LH half as high as the peak and half as long in duration as the ovulatory LH surge in normal proestrus females (Spies and Norman, 1973). Concurrently, 5 μg PGE_2 induced a 4- to 5-fold increase in blood LH at 15 minutes and a slight increase of FSH at 15, 30 and 60 minutes after infusion into the third ventricle (Harms et al., 1973). Also, Carlson et al. (1973) reported increased LH after intracarotid injection of $PGF_{2\alpha}$ into diestrous sheep.

Thus, as of 1973, the role of prostaglandins in gonadotropin release remained uncertain.

Glucocorticoid Response after Prostaglandins

Prostaglandins of the E and F series have been reported to affect adrenal steroidogenesis in rats, cattle and humans. For example, PGE_1, PGE_2, $PGF_{1\alpha}$, ACTH, and cAMP increased corticosterone during superfusion of rat adrenals (Flack et al., 1969), but the most effective prostaglandin (PGE_2) stimulated corticosterone production 1 hour less in duration than the response after ACTH or cAMP. PGE_1 and PGE_2 also released aldosterone, corticosterone and cortisol from slices of beef adrenals, but PGA_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were ineffective (Saruta and Kaplan, 1972). In contrast, infusion of 25 ng $PGF_{2\alpha}$ (iv) at 50 µg/min into women (ages 21-40) increased daily urinary cortisol output 3-fold, and plasma cortisol remained high throughout the 8-hour interval (Wentz et al. (1973). Coudert and Faiman (1973) also observed a slight increase in cortisol concentration after infusion of 2.0 µg $PGF_{2\alpha}$/kg/min into five men (23-35 years old).

In overview, PGE_1 and E_2 induced adrenal steroidogenesis in superfused rat and beef adrenals while $PGF_{1\alpha}$ stimulated steroidogenesis only in rat adrenals, and $PGF_{2\alpha}$ was ineffective in both species. However, $PGF_{2\alpha}$ induced cortisol production in men and women.

Site and Mechanism of Action of Prostaglandins on Hormone Release

Prolactin

Prostaglandin E_1 apparently acts on the hypothalamus and not at the pituitary to cause PRL release, since Harms et al. (1973) found PRL release after intraventricular injections but failed to observe a PRL response after equivalent injections directly into each lobe of the anterior pituitary. In support of PGE_1 not acting at the pituitary, MacLeod and Lehmeyer (1970), reported no prolactin synthesis or release during incubation of rat pituitaries with PGE_1, PGE_2, PGA_1 or $PGF_{2\alpha}$. Because prostaglandins have been implicated in transmission across adrenergic synapses in superior cervical ganglion and in the cerebellum (Hoffer et al. 1970) Harms et al. (1973) suggests prostaglandins may act by mediating or modulating the action of synaptic transmitters such as norepinephrin and dopamine, which already have been shown to possess capabilities of altering PRL release (Hoffer et al., 1970). Harms et al. (1973) also presumed that PGE_1 may stimulate prolactin releasing factor (PRF) or inhibit prolactin inhibiting factor (PIF).

Although further research is required to determine the mechanism of action of prostaglandin-induced PRL release most evidence suggests a hypothalamic site of action.

Growth Hormone

The site of action for PGE_1- and PGE_2 -induced growth hormone release is thought to be the pituitary. The most substantial evidence

for this is that PGE_1 and PGE_2 stimulated GH release during *in vitro* pituitary incubation (Schofield, 1970; Hertelendy, 1971).

The mechanism of action might involve cAMP, since PGE_1 increased cAMP concentration in pituitary cultures (Zor *et al.*, 1969). Moreover, theophylline, a cyclic nucleotide phosphodiesterase inhibitor, potentiated PGE_1 (Schofield, 1970) and PGE, and PGE_2 stimulation of GH (Cooper *et al.*, 1972), suggesting that this increased GH release was a result of increased cyclic AMP content. Adenyl cyclase also increased in the anterior pituitary after PGE_1, PGE_2, PGA, and $PGF_{2\alpha}$ treatment, but GH synthesis and release was observed only with the PGE's and not after $PGF_{2\alpha}$, while PGA_1 increased synthesis but not release of GH (MacLoed and Lehymer, 1970).

When 7-oxa-13-prosynoic acid, a prostaglandin synthesis antagonist, was added to pituitaries *in vitro* it blocked PGE_1 stimulation of GH release and cyclic AMP accumulation but failed to block the stimulatory action of theophylline and dibutryl cyclic AMP (dcAMP), substantiating involvement of PGE_1 with cyclic AMP (Ratner *et al.*, 1973). On the other hand, according to Hertelendy *et al.* (1972), PGE_1 stimulation of GH release was abolished when PGE_1 was given to castrated rams and anesthetized rats during epinephrin infusion and neither alpha- nor beta-adrenergic blocking drugs could overcome this block. They suggested that the site of epinephrin block was beyond the adenyl cyclase-cAMP step and argue against the idea that PGE_1, in this instance may, act as a mediator of releasing factors, or that releasing factors first activate biosynthesis which converts fatty acids to prostaglandins which activate adrenyl cyclase.

Thus, further investigation is required to determine the mechanism of action of PGE_1-induced GH release. PGE_1 probably acts directly on the pituitary, independently of adrenergic receptors.

Gonadotropins

The sites and mechanism of action of prostaglandins on gonadotropin release are less well defined than those for prolactin and growth hormone. The general consensus up to 1974 was that prostaglandins of the E series cause release of LH *in vivo*; but not *in vitro*. The F series of prostaglandin did not cause release of LH *in vitro* nor *in vivo* except in sheep (Carlson *et al*. 1973). The negative results from *in vitro* pituitary incubation (Zor *et al*., 1970) may not contradict the positive *in vivo* results because infusion of prostaglandins directly into the anterior pituitary did not increase blood LH, but infusion of the same doses of PGE_1 and PGE_2 into the third ventricle of the rat increased LH concentrations significantly (Spies and Norman, 1973 and Harms *et al*., 1973). From the demonstration that the PGE's stimulate release of gonadotropins only when put into the third ventricle, Spies and Norman (1973) and Harms *et al*. (1973) suggested that PGE_1 and PGE_2 activate a neurally controlled gonadotropin releasing mechanism. PGE's may be an intermediate step in release of hypothalamic releasing factors, or may act by mediating or modulating the action of synaptic transmitters such as norepinephrin or dopamine which are capable of altering releasing factors (Harms *et al*., 1973).

Tsafriri *et al*. (1973) also suggested that PGE_2 has a central effect and that indomethacin (a prostaglandin biosynthesis inhibitor)

does not block LH release, but rather it interferes with ovulation at the level of the ovary. Carlson et al. (1973) suggested that $PGF_{2\alpha}$ stimulates LH release in ewes during day 5 to 10 of the estrous cycle, probably by action primarily at the hypothalamus, but action at the pituitary could not be ruled out.

In overview, most of the literature supports a PGE- but not a PGF-induced LH release at the hypothalamus, but verification of this stimulatory action, the site of action, and its physiological significance will require further research.

ACTH and Glucocorticoid

Two schools of thought exist on the site and mechanism of action of prostaglandins on adrenal steroidogenesis. Initially, corticosterone synthesis increased with addition of PGE_1, PGE_2 or $PGF_{2\alpha}$ during in vitro adrenal culture; this increase was mimiced by ACTH and cAMP (Flack et al., 1969). Cycloheximide, which inhibits formation of proteins controlling the adrenal steroidogenic pathway, inhibited increase of corticosterone in vitro, indicating that these stimulating substances induce synthesis as well as release of glucocorticoids. In addition, preliminary in vivo studies portrayed the stimulatory action of PGE_2 to be similar to that of ACTH and cAMP, because corticosterone concentrations in plasma and the adrenal increased 70 and 40 percent in response to PGE_2, respectively (Flack et al., 1969). Pursuing this concept of similarity, Flack and Ramwell (1972) demonstrated that the initial rate of synthesis of corticosterone was equivalent during PGE_2 and ACTH incubation, with peak production occurring at 60 minutes,

considerably sooner than the peaks after cAMP (90 min) and dibutryl cyclic AMP (dbcAMP, 150 min). Corticosterone responses to ACTH, cAMP and dbcAMP decayed at comparably slow rates, but the response to PGE_2 decreased more rapidly indicating another marked difference from ACTH. They concluded that PGE_2 stimulates corticosteroidogenesis by direct action on the adrenal cortex.

Saruta and Kaplan (1972) reported results similar to those from Flack and Ramwell (1972) for PGE_1 and PGE_2 in beef adrenals. In their *in vitro* system, PGE_1 and PGE_2 significantly induced synthesis of aldosterone, corticosterone and cortisol, while PGA, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were ineffective. PGE_1 stimulated steroidogenesis in a manner similar to that of ACTH. For example, PGE_1 required calcium, was inhibited by puromycin but not actinomycin D, and increased cAMP concentrations, and its effects on endogenous cAMP were not additive with those from ACTH. However, PGE_1 did not have additive effects with maximal or submaximal concentrations of ACTH. These findings support the hypothesis that PGE_1 shares receptor sites on the plasma membrane with ACTH (Saruta and Kaplan, 1972).

Others hypothesize a specific role of PGE_1 and PGE_2 (in the regulation of adrenal steroidogenesis) on the central nervous system (CNS) instead of a direct action on the adrenals. PGE_1 and PGE_2 were the only substances of low molecular weight found in the brain and capable of stimulating ACTH discharge in a number of assay systems that are commonly used as CRF (Corticotropin releasing factor) assays (rats pretreated with pentobarbital and chlorpromazine or pentobarbital and dexamethasone, deWied *et al.* 1969). In agreement with deWied *et al.*

(1969), PGE_1 failed to alter adrenal ascorbic acid in cortisol-pretreated hypophysectomized rats, indicating PGE_1 has no ACTH-like effect on the adrenal but acts by stimulating ACTH release (Peng et al., 1970). This stimulation could be at the site of the pituitary or hypothalamus because cortisol may inhibit adrenal steroidogenesis at either or both sites (Ganong, 1963). Similarly, the plasma cortisol increase observed throughout infusion of $PGF_{2\alpha}$ into women was eliminated by dexamethasone pretreatment, suggesting that $PGF_{2\alpha}$ does not act directly on the adrenal to stimulate cortisol biosynthesis, but probably operates through induction of ACTH release (Wentz et al., 1973). More convincingly, Peng et al. (1970) inhibited the adrenal ascorbic acid response to PGE_1 in intact rats anesthetized with sodium pentobarbital by pretreating them with morphine, which inhibits secretion of CRF.

Hedge (1972) defined more clearly the effects of prostaglandins on ACTH secretion; rats were anesthetized with sodium pentobarbital and pretreated with dexamethasone. PGE_1, and $PGE_{1\alpha}$ and $PGF_{2\alpha}$ (1.0, 0.5 and 0.5 μg, respectively) increased ACTH comparably when injected into the median eminence, but were ineffective when injected into nearby regions of the basal hypothalamus, the anterior pituitary or the tail vein. In addition, the ACTH responses to PG's were abolished by pretreatment with morphine and were partially inhibited by a higher dose of dexamethasone. The stimulatory effect of $PGF_{1\alpha}$ was abolished by incubation in rat plasma at 37°C for only 1 minute before injection but the effect of PGE_1 was unaltered. Hedge (1972) also concluded that prostaglandins stimulate ACTH secretion indirectly by acting at

the median eminence presumably via CRF, but the mechanism, as well as the physiological significance, remains unanswered.

In overview, the evidence for prostaglandins mediating adrenal steroidogenesis through ACTH release is more convincing to me, but a direct action of prostaglandins on the adrenal cannot be ruled out completely.

MATERIALS AND METHODS

One experiment was conducted to determine if anterior pituitary hormones were released in response to luteolytic doses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Glucocorticoids (indicator of adrenocorticotropin, ACTH, release), luteinizing hormone (LH), prolactin (PRL) and growth hormone (GH) were monitored for 18 hours after intramuscular (im) injections of $PGF_{2\alpha}$. As the results will indicate, this first experiment permitted description of surges of anterior pituitary hormones and glucocorticoid in cattle after $PGF_{2\alpha}$ treatment. Consequently, two additional experiments were designed to determine the site(s) where $PGF_{2\alpha}$ acts to cause the surge of glucocorticoid. Prior to the first of these two experiments, heifers in a perliminary trial were given a long-acting synthetic glucocorticoid (triamcinolone acetonide, TA), and were bled daily via tail vein puncture, for 1 week to verify that TA inhibited glucocorticoid production. Then, TA-pretreated heifers and control heifers were given saline or $PGF_{2\alpha}$ to identify the site of $PGF_{2\alpha}$ action.

Based upon the results from the second experiment, there was concern that the adrenals may have atrophied by 1 week after TA, retarding corticoid response to ACTH and $PGF_{2\alpha}$. Therefore, another preliminary trial was conducted to ascertain precisely how rapidly TA decreased glucocorticoid production to basal concentrations. Then,

a third experiment was conducted to provide more information about the site of action of $PGF_{2\alpha}$ on glucocorticoid release. Detailed descriptions of these three main experiments follow.

Experimental Design

Experiment I: Anterior Pituitary Hormone Response to $PGF_{2\alpha}$

Because im injection of 0.85 percent sodium chloride did not alter ($P>0.05$) glucocorticoid, prolactin (PRL), or growth hormone (GH) concentrations during a 6-hour post-injection interval, controls (given saline vehicle) were not included in Experiment I. Three luteolytic doses of $PGF_{2\alpha}$ tham salt diluted in 2 ml saline were injected im to evaluate their effects on blood plasma PRL, GH, LH and glucocorticoids. Diestrous heifers were given (1) 15 mg $PGF_{2\alpha}$ at time 0 and 6 hours later (n=4), (2) 30 mg PGF_2 (n=6) or (3) 60 mg $PGF_{2\alpha}$ (n=6). Blood was put into heparinized tubes in an ice bath immediately after collection through jugular cannulae. Blood was sampled just before treatment (time 0), at 10-minute intervals for 1 hour and then at 1.5, 2, 4, 6, 12, and 18 hours. After the second 15-mg $PGF_{2\alpha}$ injection, blood was collected at the same frequency as after the injections at time 0. The blood was centrifuged and the plasma was decanted and frozen within 4 to 6 hours after collection. Plasma glucocorticoids were measured by protein-binding assay described by Smith et al. (1973). GH, LH, and PRL were analyzed by radio immunoassays (RIA) described by Purchas et al. (1970), Oxender et al. (1972), and Tucker (1971), respectively.

Experiment II: Site of Action of $PGF_{2\alpha}$ Induced Glucocorticoid Release

The experimental animals consisted of 9 diestrus and 9 ovariectomized heifers. To ensure each diestrus heifer had a functional corpus luteum, each intact heifer was given 25 mg $PGF_{2\alpha}$ (Tham salt, im) 11 days before beginning experimental treatments. This pretreatment was intended to induce luteolysis and to provide a 7- to 9-day corpus luteum when treatments were given. TA (22 mg) was injected (sc) into 3 intact and 3 ovariectomized heifers, and the heifers were bled (tail vein puncture) daily for 1 week before they were given (im) the experimental dose (25 mg) of $PGF_{2\alpha}$ tham salt. The remaining 12 animals were not pretreated with TA; 3 diestrus and 3 ovariectomized heifers were given (im) 2 ml saline and 3 of each were given (im) 25 mg $PGF_{2\alpha}$. The 25-mg im dose of $PGF_{2\alpha}$ was chosen because it was known to cause luteolysis in heifers.

The experimental blood samples were collected through jugular cannulae at half-hour intervals for 4 hours before saline or $PGF_{2\alpha}$ injections, then at 5-minute intervals for 30 minutes after the experimental treatments, followed by 10-minute samples for another 30 minutes, then half-hour samples for another 11 hours. At 12 hours after saline or $PGF_{2\alpha}$, 2 of the 3 heifers within each treatment group were given (sc) 5000 IU ACTH. This ACTH treatment was intended to insure that the heifers could produce glucocorticoids. Blood sample collection continued at half-hour intervals for 4 hours after ACTH treatment. Blood samples clotted at 25°C for 2 to 4 hours before they were stored at

4°C for 8 to 12 hours. Then, samples were centrifuged and serum was decanted and stored at -20°C prior to analysis for glucocorticoids, as in Experiment I.

Experiment III: $PGF_{2\alpha}$ - Versus ACTH-Induced Glucocorticoid Release

Results from Experiment II suggested that the TA pretreatment may have caused some atrophy of the adrenal cortex within 7 days after TA was given. Consequently, a preliminary trial was conducted to determine the minimal period required for TA to maximally suppress glucocorticoid production. Each of two diestrous heifers was given 20 mg of TA (sc) and bled at 3-hour intervals for 12 hours, then at 16 and 24 hours. Inspection of these preliminary data indicated that TA maximally suppressed glucocorticoid within 6 hours. Consequently, treatments were given 6 hours after TA pretreatment in the following experiment.

Eighteen heifers were given 25 mg $PGF_{2\alpha}$, (im) 11 days before administration of experimental treatments, to obtain diestrous heifers at the time of treatment. Nine of the 18 diestrous animals were pretreated with 20 mg TA (sc) 6 hours before treatments then, 2 ml of saline, 5 mg of $PGF_{2\alpha}$ and ACTH were chosen to stimulate a submaximal glucocorticoid response to enable observation of any additional release induced by an added stimulus. Blood samples were collected through jugular cannulae at 6, 3, 1, 0.5, 0.25 and 0 hours before treatment. After treatment, samples were taken at 15-minute intervals for 3 hours, followed by 30 minute intervals to 4 hours post-treatment, and then

hourly for 6 hours. Serum glucocorticoid was monitored as described under Experiment I.

Statistical Analysis

The hormone data from the three principal experiments were analyzed by the split-plot method described by Gill and Hafs (1971) because of repeated measurements within animals. Due to this repeat measurement, the probability of type 1 error may be artificially low because of the high correlation of errors if there is heterogeneity of the variance and covariance of samples taken at several times from one animal. Thus, the type 1 error may exceed its actual value if measurements at close intervals of time are more significantly correlated than those more distant in time. A conservative F-test (Gill and Hafs, 1971) was used to confirm results that were marginally significiant. Thus, analysis of all three experiments was a variation of the split-plot theme. The first experiment was a split-plot design with time as a factor and selected contrasts were compared via Scheffés procedure (Kirk, 1968). Both the second and third experiments were split-plot designs where animals were assigned to two pretreatment and three treatment combinations with time as a factor, and selected contrasts were compared via Bonferroni T-test (Miller, 1968).

RESULTS AND DISCUSSION

Experiment I: Anterior Pituitary Hormone Response to $PGF_{2\alpha}$

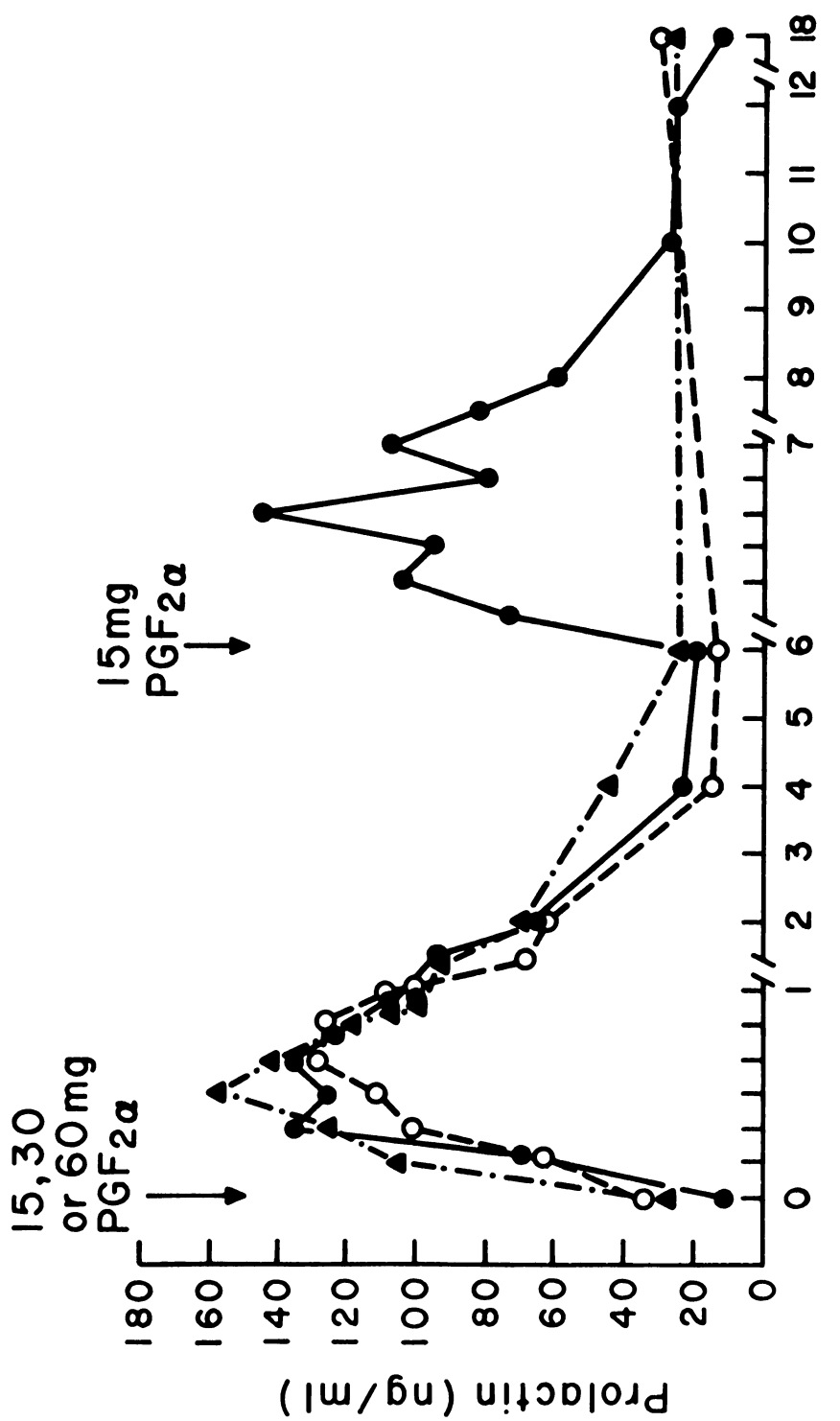
The objectives of the first experiment were to monitor anterior pituitary hormones and glucocorticoid in blood plasma after luteolytic doses (15, 30 or 60 mg) of $PGF_{2\alpha}$ given intramsucularly. The results for each hormone will be discussed separately.

Prolactin

Prolactin (PRL) response did not differ ($P<0.05$) among the three $PGF_{2\alpha}$ doses during the first 6 hours after injection. On the average, plasma PRL increased ($P<0.01$) from 26 to 81 ng/ml within 10 minutes after $PGF_{2\alpha}$ injection and remained above pre-injection concentrations for approximately 4 hours (figure 1). A similar PRL response occurred after the second 15-mg injection of $PGF_{2\alpha}$ given 6 hours after the first injection in hour heifers. These PRL surges were: (1) greater in magnitude than those normally observed in response to milking (Tucker, 1971); (2) similar to the PRL release induced by iv injection of thyrotropin releasing hormone (Convey et al., 1973); and (3) considerably less than those which normally occur at parturition in cows (Ingalls et al., 1973).

Earlier, $PGF_{2\alpha}$ had been reported to induce release of PRL when administered to pregnant rats; it also caused lactogenesis and abortion suggesting that decreasing progesterone (precipitated by $PGF_{2\alpha}$-induced

Figure 1.--Plasma PRL after 15 (2x) (●-●) 30 (▲-.-▲) or 60 (O---O) mg $PGF_{2\alpha}$ (im). Standard errors of means ranged from 1 to 27 ng/ml and were generally proportional to the mean.



Plasma Prolactin after
im PGF_{2a} in heifers

luteolysis) might stimulate the increase in PRL (Vermouth and Deis, 1972). In partial agreement with this concept, Vermouth and Deis (1972) found delayed onset of lactogenesis and parturition with progesterone replacement therapy in $PGF_{2\alpha}$-treated pregnant rats, but this only partially blocked PRL release. In addition, no PRL release occurred after intraventricular injection of $PGF_{2\alpha}$ into ovariectomized rats (Harms et al., 1973) or after iv administration of $PGF_{2\alpha}$ into adult men (Coudert and Faiman, 1973). To my knowledge, at the outset of my research, the only other reported increase of PRL after prostaglandin occurred after PGE_1 infusion into the third ventricle of ovariectomized rats, but not after an identical dose was infused into the pituitary (Harms et al., 1973). This specific PGE_1-induced PRL release suggests a hypothalamic site of action.

During the course of my research, more evidence has accumulated concerning the role of prostaglandins in PRL release. Yue et al. (1974) observed a greater increase of serum PRL concentration after 20 intrauterine injections of 500-1500 µg $PGF_{2\alpha}$ at intervals of 1 to 2 hours or intra-amniotic injection of 30 mg $PGF_{2\alpha}$ during induction of abortions than after normal birth or hypertonic saline-induced abortions in women. In contrast, five post-menopausal women had no increase of serum PRL after intravenous infusion of 18 mg $PGF_{2\alpha}$ over a 6-hour period (Vanderheyden et al., 1974).

Some further information on release of PRL by $PGF_{2\alpha}$ is available from studies with rats. Serum PRL increased 10 to 60 minutes after a single iv injection of $PGF_{2\alpha}$, PGE_1 or PGE_2 (670 µg/rat)

into ovariectomized rats primed with estrogen and progesterone (Ojeda et al., 1974a). In fact, as little as 20 μg of $PGF_{2\alpha}$, 2 μg of PGE_1 or PGE_2 increased blood PRL in the same rats, confirming that the PRL release is stimulated by some prostaglandins of the E and F series. In contrast (unlike the response to PGE_1), $PGF_{2\alpha}$, $PGF_{1\alpha}$ and PGE_2 failed to increase plasma PRL when infused into the third ventricle of ovariectomized estrogen-pretreated rats (Ojeda et al., 1974a).

In the male rat, PGE_2 increased PRL when it was infused into the lateral ventricle but not when infused into the hypophyseal portal vessels (Eskay et al., 1975). Similarly, PRL increased 6- to 7-fold at 30 minutes after infusion of 5 μg PGE_2 or 20 μg $PGF_{2\beta}$ (3- to 4-fold greater than controls) into the lateral ventricle but, 20 μg of $PGF_{2\alpha}$, $PGF_{1\alpha}$ $PGF_{1\beta}$, PGA_1, PGA_2, PGB_1, PBG_2 or 10 μg of PGE_1 did not alter the basal secretion of PRL in male rats anesthetized with sodium pentobarbital (Warbert et al., 1976).

In further support of the PRL release induced by $PGF_{2\alpha}$ in heifers, release of PRL in bulls was related directly to the dose of $PGF_{2\alpha}$ injected im (Hafs, 1975). In addition, plasma PRL increased 25-fold after an intracarotid injection of 50 μg $PGF_{2\alpha}$ into mature bulls (Stellflug, unpublished data).

The PRL release after systemic administration of $PGF_{2\alpha}$ is contradicted by the lack of PRL release after intraventricular infusion of $PGF_{2\alpha}$. But this contradiction might be expected since the rat possesses a reversible enzyme (PGE_2-9-Keto-reductase) which converts PGE_2 to $PGF_{2\alpha}$ (Leslie and Levine, 1973). And, their preliminary experiments have shown this activity to be present in heart homogenates

of chickens, rabbits, cats, cattle and guinea pigs and in rabbit kidney and guinea pig liver homogenates. Enzyme location may explain how $PGF_{2\alpha}$ induces PRL release when administered systemically and not when infused into the third ventricle. However, caution must be taken when comparing species; for instance, an enzyme present in the cellular fraction of sheep blood reduces PGE_2 to $PGF_{2\alpha}$ but is not reversible or coenzyme-dependent like the enzyme in rats (Hensby, 1974). In contrast to $PGF_{2\alpha}$, PGE_1 and PGE_2 stimulated PRL release when infused into the third ventricle but not when PGE_1 was infused into the pituitary and not when PGE_2 was infused into the hypophysial portal vessel--suggesting action on the central nervous system, possibly the hypothalamus. However, some evidence indicates that under certain conditions (ovariectomized rats), PGE_1 may also stimulate a small PRL release by action on the adenohypophysis but this PRL increase was less than when PGE was infused in the third ventricle and it could be abolished by estrogen pretreatment unlike the PRL release after intraventricular infusion (Ojeda et al., 1974).

Regarding the mechanism of action for PRL release induced by E prostaglandins, Ojeda et al. (1974b) suggested a possible role of cAMP mediation and PGE_1 modulation in the dopaminergic control of PRL release.

In summary, the $PGF_{2\alpha}$-induced PRL release in heifers (figure 1) is supported by in vivo studies on pregnant rats and women, on ovariectomized rats primed with estrogen and progesterone, on adult men given $PGF_{2\alpha}$ intravenously, and on bulls after intramuscular or intracarotid injections. In contrast, PRL was not released when $PGF_{2\alpha}$

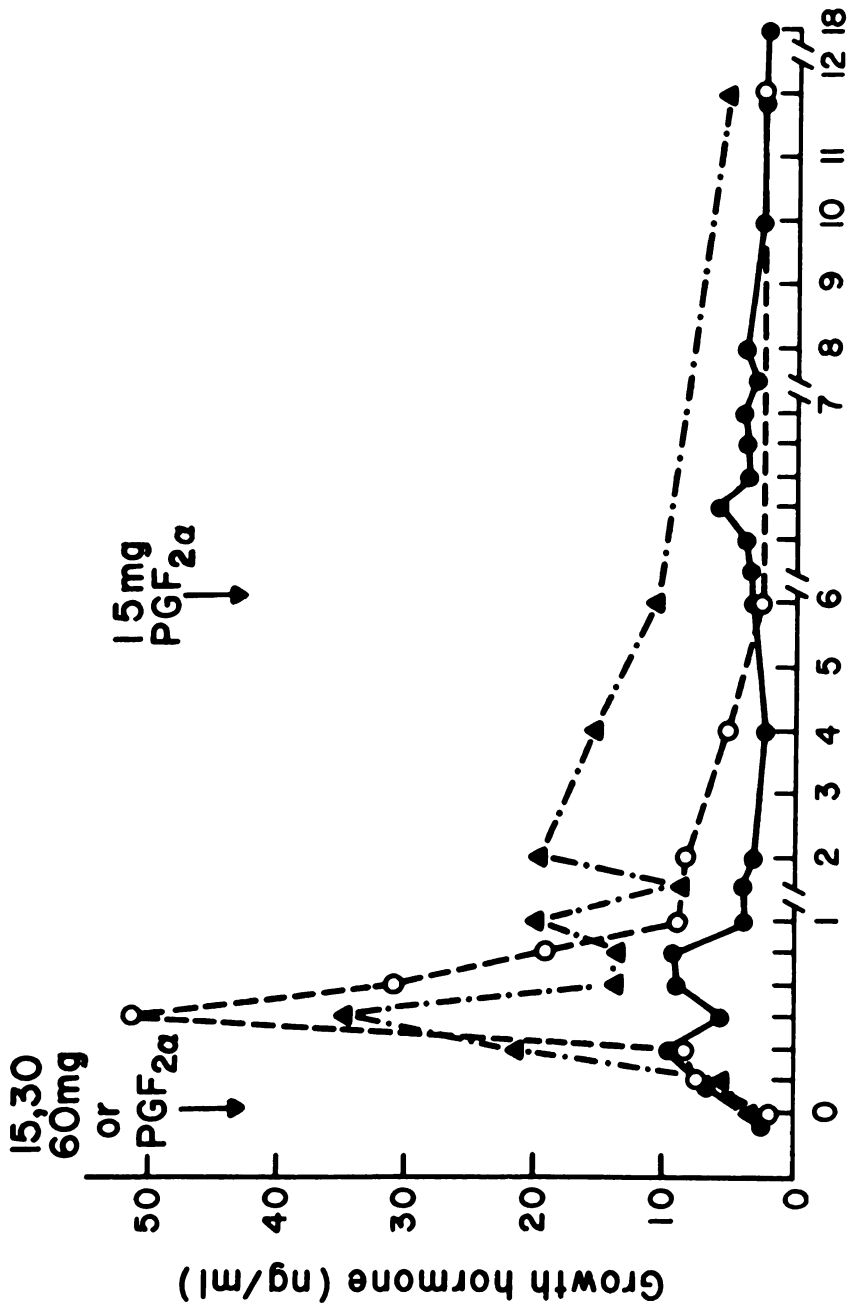
was (1) infused into the third ventricle of ovariectomized estrogen-treated rats and adult male rats, (2) after infusion directly into the anterior pituitary of rats or (3) injected iv into post-menopausal women. These results suggest that the steroid environment modulates release of PRL after $PGF_{2\alpha}$ in the female, but further studies will be required to verify this hypothesis especially since it is based on information from several species.

Growth Hormone

Before $PGF_{2\alpha}$, plasma GH averaged 3 ng/ml (figure 2). Although GH at least doubled after each of the two 15-mg im injections of $PGF_{2\alpha}$, these increases did not differ from pre-injection values ($P>0.05$). GH increased ($P<0.01$) to 35 and 51 ng/ml within 30 minutes after 30- and 60-mg $PGF_{2\alpha}$, respectively, and remained above pre-injection values for at least 1 hour. The 30-minute peak plasma GH concentrations were linearly related to the log of the dose of $PGF_{2\alpha}$ ($P<0.05$). This GH increase in our heifers was greater than that induced by TRH in lactating cows (Convey et al., 1973), but less than those which occur normally at parturition (Ingalls et al., 1973).

Previously, only one study had indicated GH release after $PGF_{2\alpha}$ Coudert and Faiman (1972) reported a slight increase in GH after 2.0 μg of $PGF_{2\alpha}$/kg/min in five men, but no increase occurred after 0.2 μg of $PGF_{2\alpha}$/kg/min or less. To my knowledge, just two other studies monitored GH after $PGF_{2\alpha}$ and both reported no effect on GH release in vitro; one incubated heifer pituitary slices (Cooper et al., 1972) and the other cultured bisected male and female rat pituitaries

Figure 2.--Plasma GH after 15 (2x) (●--●) 30 (▲-.0.▲) or 60 (0---0) mg $PGF_{2\alpha}$ (im). Standard errors of means ranged from 1 to 14 ng/ml and were generally proportional to the mean.



Plasma G.H. after
im PGF_{2a} in heifers

(MacLeod and Lehmeyer, 1970). Both of these *in vitro* systems responded to PGE_1 and PGE_2 with GH synthesis and release.

During this dissertation research, $PGF_{2\alpha}$ infusion (50 µg/min for 6 hours) induced a biphasic increase in plasma GH in post-menopausal women (Vanderheyden *et al.*, 1974). In four diestrous heifers, serum GH increased from 10.1 to 19.4 ng/ml within 8 minutes after a 5-mg iv injection of $PGF_{2\alpha}$ given during infusion of thyrotropin releasing hormone (TRH) and returned to pretreatment concentrations (10.1 ng/ml) by 30 minutes after $PGF_{2\alpha}$ (Tucker *et al.*, 1975). When four metestrous heifers were infused with 30 mg $PGF_{2\alpha}$/hour, GH also increased from 9.6 to 45 ng/ml within 40 min and returned to pre-infusion values within the first 3 hours of PGF_2 infusion (Tucker *et al.*, 1975). Injection of TRH during this $PGF_{2\alpha}$ infusion (5 hours after onset) increased GH from 11 to 78 ng/ml within 10 minutes. Similarly, when metestrous heifers were infused with TRH for 6 hours and injected with 5 mg $PGF_{2\alpha}$ at 5 hours after onset of TRH infusion, GH increased from 9.5 to 30 ng/ml at 20 minutes (Tucker *et al.*, 1975).

A recent *in vitro* study also supports a $PGF_{2\alpha}$ role in GH release. $PGF_{2\alpha}$ increased GH from 34 to 84 µg/ml/4 hour incubation of rat pituitaries (Sundbert *et al.*, 1975). They found $PGF_{1\alpha}$ released GH as effectively as $PGF_{2\alpha}$; PGE_2 was twice as effective as $PGE_{2\alpha}$ for stimulating GH release, and there was a parallel increase in cAMP. But LH, FSH, PRL and TSH release were not altered by PG's in this system.

In overview, the $PGF_{2\alpha}$-induced GH release in heifers (figure 2) confirms the substantial evidence for $PGF_{2\alpha}$-induced GH release as

reviewed above, but little information exists on the site or mechanism of action of $PGF_{2\alpha}$ on GH release. One recent study by Hafs et al. (1976) demonstrated that somatostatin (SIRF) inhibits the GH response to $PGF_{2\alpha}$ in bulls, similar to its effect on GH response to $PGE_{2\alpha}$ or to extracts of porcine stalk median eminence in rats (Szabo and Frohman, 1975). These reports suggest a site of action on the pituitary, but action on the central nervous system cannot be ruled out.

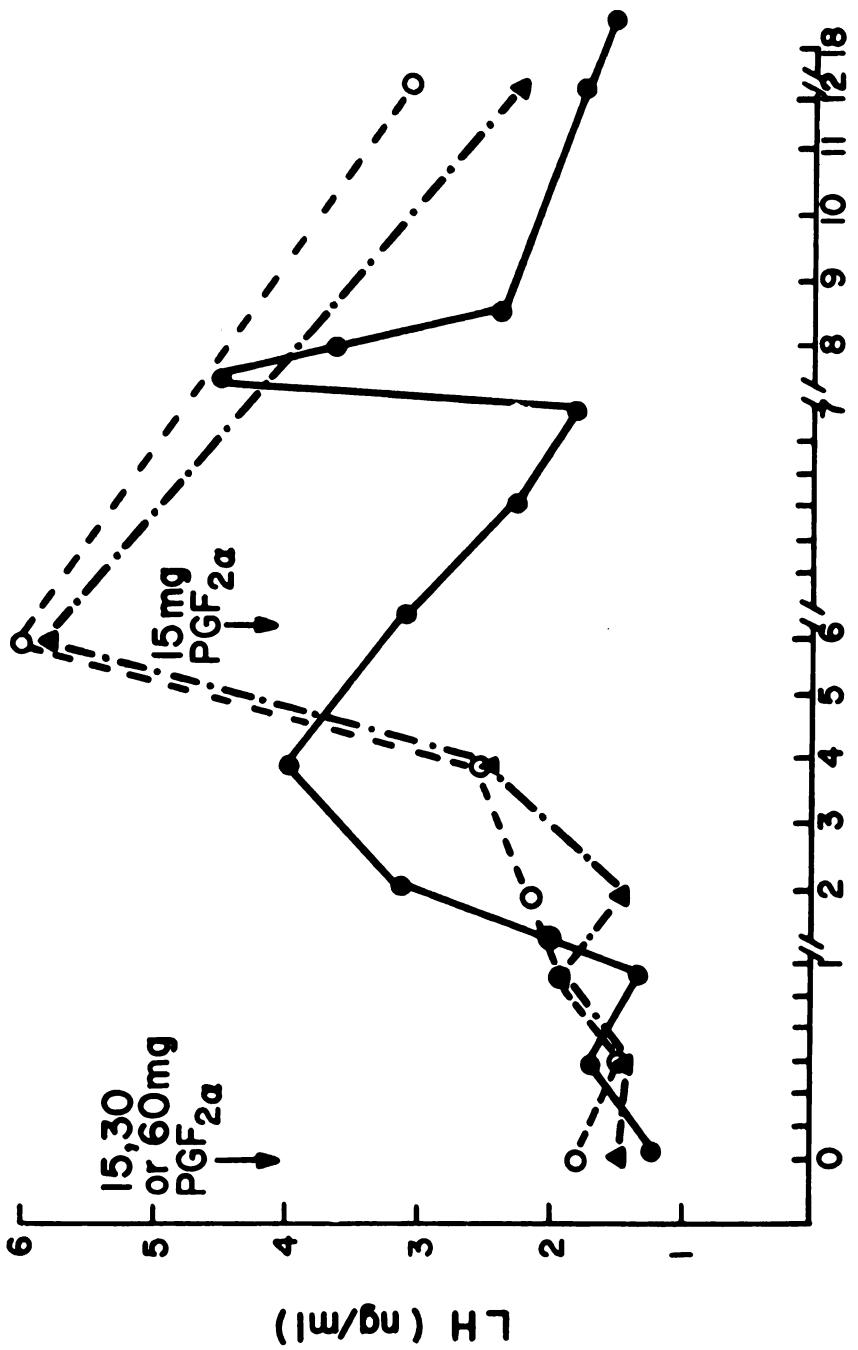
LH

Average plasma LH remained at pre-injection values for at least 1 hour, and then increased ($P<0.05$) 2-fold or greater over pre-injection LH (figure 3) at 1.5 to 6 hours after im administration of 15, 30 or 60 mg PGF_2 . However, peak LH was highly variable among heifers (range from 2.7 to 17 ng/ml), as was the interval from $PGF_{2\alpha}$ injection to LH increase. This LH release was at least 1 hour later than the prolactin and growth hormone surges after prostaglandin treatment; thus, the mechanism for LH release probably differs from those for prolactin and growth hormone.

In view of the previous literature, I expected either no gonadotropin response after $PGF_{2\alpha}$ (Harms et al., 1973; Spies and Norman, 1973) or a response within 10 to 15 minutes after $PGF_{2\alpha}$ injection (Carlson et al., 1973). In contrast, LH release caused by prostaglandins of the E series has been well established while FSH response is equivocal.

During this research, an additional study reported no effect of $PGF_{2\alpha}$ and a stimulatory effect for E prostaglandins on LH release

Figure 3.--Plasma LH after 15 (2x) (●--●) 30 (▲-.-.▲) or 60 (0---0) mg $PGF_{2\alpha}$ (im). Standard errors of means ranged from 0.1 to 2.1 ng/ml and were generally proportional to the mean.



Plasma LH after
im PGF_{2a} in heifers

(Harms et al., 1974). Neither intravenous nor intraventricular (third ventricle) infusion of $PGF_{1\alpha}$ or $PGF_{2\alpha}$ stimulated LH release in rats, but PGE_2 was effective by both routes of administration, and PGE_1 stimulated LH release only after intraventricular infusion (Harms et al., 1974).

In agreement with the LH release after $PGF_{2\alpha}$ in heifers (figure 3), $PGF_{2\alpha}$, as well as PGE_1 and PGE_2 produced a decrease in ovarian content of ascorbic acid in intact and hypophysectomized immature rats pretreated with pregnant mare serum (PMS), indicating an LH-like action (Sato et al., 1974). In addition, a single iv injection of PGE_1, PGE_2, or $PGF_{2\alpha}$ increased serum LH concentration within 10 minutes in rats (Sato et al., 1974). These results were verified by observation of plasma LH increase 10 minutes after iv injection of $PGF_{2\alpha}$, PGE_1 or PGE_2 into ovariectomized mature rats with hypothalamic lesions (Sato et al., 1975). LH release also increased after addition of $PGF_{2\alpha}$ (200 μg/ml), PGE_1 (2 or 20 μg/ml) or PGE_2 (0.002 or 0.02 μg/ml) to incubation medium containing rat anterior pituitaries (Sato et al., 1975). However, plasma LH failed to increase following direct injection of $PGF_{2\alpha}$ into the rat pituitary, unlike the response after PGE_1 or PGE_2 (Sato et al., 1975).

More convincingly, $PGF_{2\alpha}$ $PGF_{2\beta}$ and PGE_2 were found to be potent stimulators of LH release when infused into the lateral ventricle of adult male rats (Warberg et al., 1976). By comparison they found the LH release was considerably less when PGE_1, $PGF_{1\alpha}$, PGA_2 or PGB_2 were infused and suggested that the cis double bond in the

5,6 position and the 11-hydroxyl group are essential for LH releasing activity of prostaglandins. Another study in males reported a blood LH increase after administration of $PGF_{2\alpha}$ either sc or by iv infusion into mature bulls (Kiser et al., 1976). Thus, substantial evidence exists which demonstrates $PGF_{2\alpha}$-induced LH release, but most of the responses occurred within 10 to 30 minutes, unlike the LH response after $PGF_{2\alpha}$ in my diestrous heifers (figure 3). This delayed response suggests an indirect action of prostaglandin, rather than a direct action on the pituitary or hypothalamus.

The steroid environment may modulate the LH release in response to prostaglandins, because sex steroids play important regulatory roles on gonadotropin release. In fact, in my heifers, plasma progesterone began to fall within 10 minutes after $PGF_{2\alpha}$ treatment while estradiol increased 2- to 3-fold by 24 hours (Stellflug et al., 1975).

Hafs et al. (1975) determined that the increase in LH (figure 3) after a luteolytic dose of $PGF_{2\alpha}$ was caused by alteration of progesterone secretion rather than a direct effect of $PGF_{2\alpha}$ on the head. Exogenous progesterone eliminated the serum LH increase precipitated by the 25-mg $PGF_{2\alpha}$ injection in diestrous heifers in one experiment. In another trial, LH remained at pre-$PGF_{2\alpha}$ values while progesterone pessaries were in place, but LH began to increase within 1 hour following pessary removal at 6 hours after $PGF_{2\alpha}$. Similarly blood estradiol remained at pre-$PGF_{2\alpha}$ values until removal of the pessaries, and began to rise 2 hours later. From these results, Hafs

et al. (1975) concluded that the serum LH-increase observed after $PGF_{2\alpha}$ treatment in diestrous cattle was dependent upon withdrawal of progesterone and, probably, was not due to increased serum estradiol or a direct effect of $PGF_{2\alpha}$ on LH release. Thus, similarly to in vivo prolactin release induced by prostaglandin, steroid environment modulates some prostaglandin-induced LH release.

Recent studies with rats suggest that the central nervous system is the site of LH release action for PGE's (Eskay et al., 1975, and Warberg et al., 1976) and some PGF's (Warberg et al., 1976); PGE's apparently do not act at the pituitary (Eskay et al., 1975). However, sufficient doses of PG can also stimulate LH secretion by acting directly on the pituitary (Sato et al., 1975), so both the pituitary and the hypothalamus may be sites of PG action under some specific conditions.

Recent evidence indicated that the mechanism of action for PGE_2-induced gonadotropin release in vivo is to enhance the release of LHRH into the portal vessels (Eskay et al., 1975). By use of receptor blocking agents, Harms et al. (1976) observed that the PGE_2-induced LH release was not mediated by adrenergic, dopaminergic, serotoninergic, or cholinergic receptors. They also suggested PGE_2 does not act trans-synaptically, but probably acts directly on the LHRH neuron to induce LHRH release.

The site and mechanism of action for $PGE_{2\alpha}$-induced LH release in my heifers is less certain than for PGE's especially because the LH release is retarded (beginning at about 2 hours) after $PGF_{2\alpha}$ by comparison with prolactin and growth hormone, which are released within

5 minutes (figures 1 and 2). Thus, withdrawal of progesterone (luteolysis) caused by $PGF_{2\alpha}$ probably participates in LH release in heifers after $PGF_{2\alpha}$, as reported by Hafs et al. (1975).

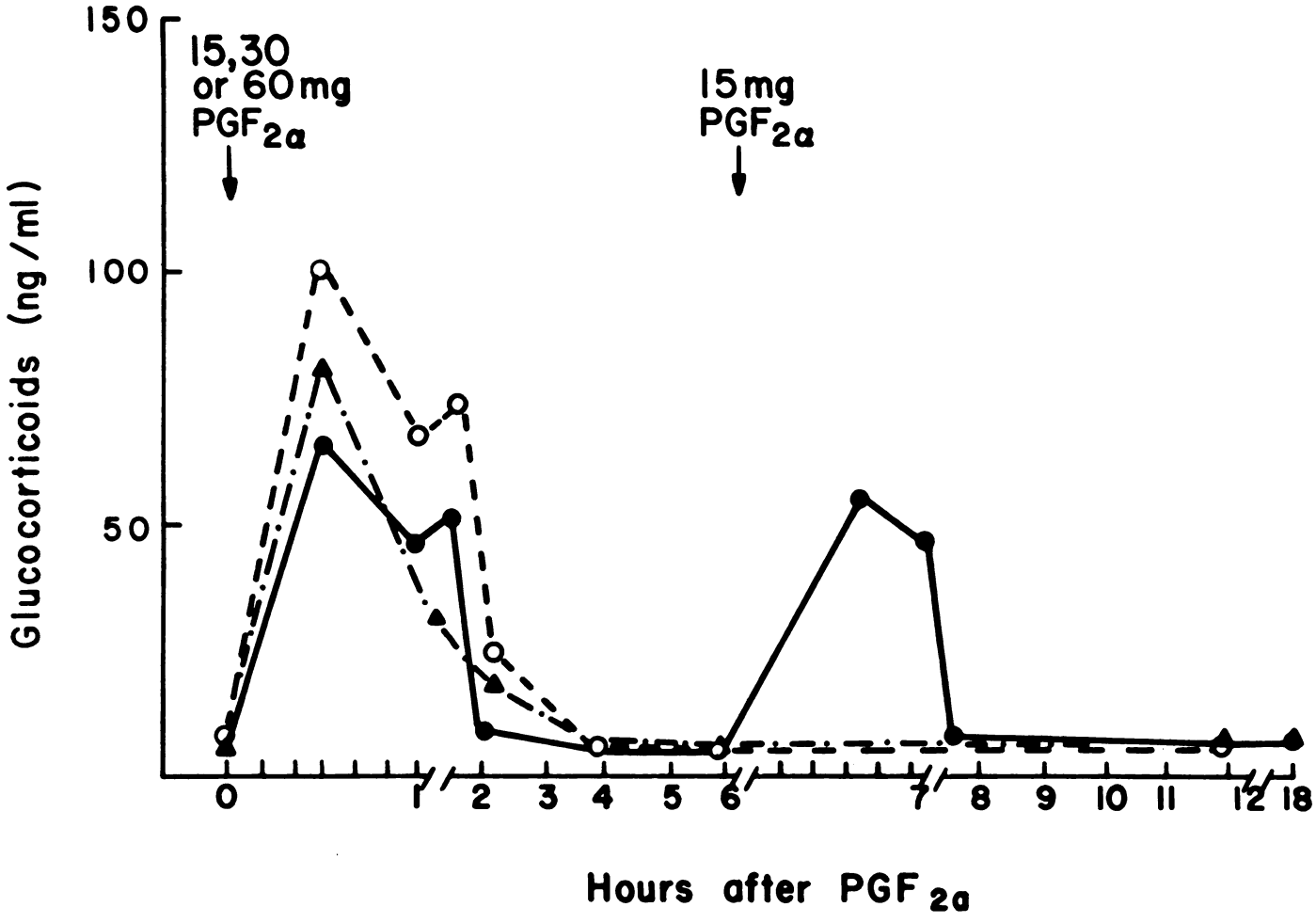
Glucocorticoids

Before $PGF_{2\alpha}$ was injected, plasma glucocorticoids average 11.8 ng/ml (figure 4). Total glucocorticoid increased ($P<0.01$) more than 6-fold at 30 minutes after 15, 30 or 60 mg of PGF_2 and returned to pre-injection values after 4 hours. The 60-mg injection of $PGF_{2\alpha}$ stimulated a larger ($P<0.05$) glucocorticoid release than the 15- or 30-mg doses of $PGF_{2\alpha}$ which resulted in glucocorticoid responses which did not differ signficiantly ($P<0.05$) from each other. In addition, the peak glucocorticoid concentrations were linearly related to the log of the dose of $PGF_{2\alpha}$.

Earlier, $PGF_{2\alpha}$ as well as PGE_1 and PGE_2 induced corticosterone production during superfusion with rat adrenals (Flack et al., 1969). Subsequently, PGE_1 and PGE_2 but not $PGF_{2\alpha}$ stimulated adrenal steroidogenesis when incubated with bovine adrenals (Saruta and Kaplan, 1972). Thus, both groups of researchers advocate a direct action of at least PGE's on the adrenals, and Saruta and Kaplan (1972) found PGE_1 stimulated corticosteroidogenesis similarly to that induced by ACTH, suggesting that PGE_1 may play a part in the mechanism of action by ACTH, or that PGE shares receptor sites with ACTH on plasma membranes.

On the other hand, Coudert and Faiman (1973), Wentz et al. (1973) and Hedge (1972) proposed that prostaglandins act by stimulating release of ACTH. $PGF_{2\alpha}$ infusion stimulated increased plasma cortisol

Figure 4.--Plasma glucocorticoid after 15 (2x) (●-●), 30(▲-.-.▲) or 60 (0---0) mg $PGF_{2\alpha}$ (im). Standard errors of means ranged from 1 to 19 ng/ml and were generally proportional to the mean.



Plasma glucocorticoids after
im $PGF_{2\alpha}$ in heifers

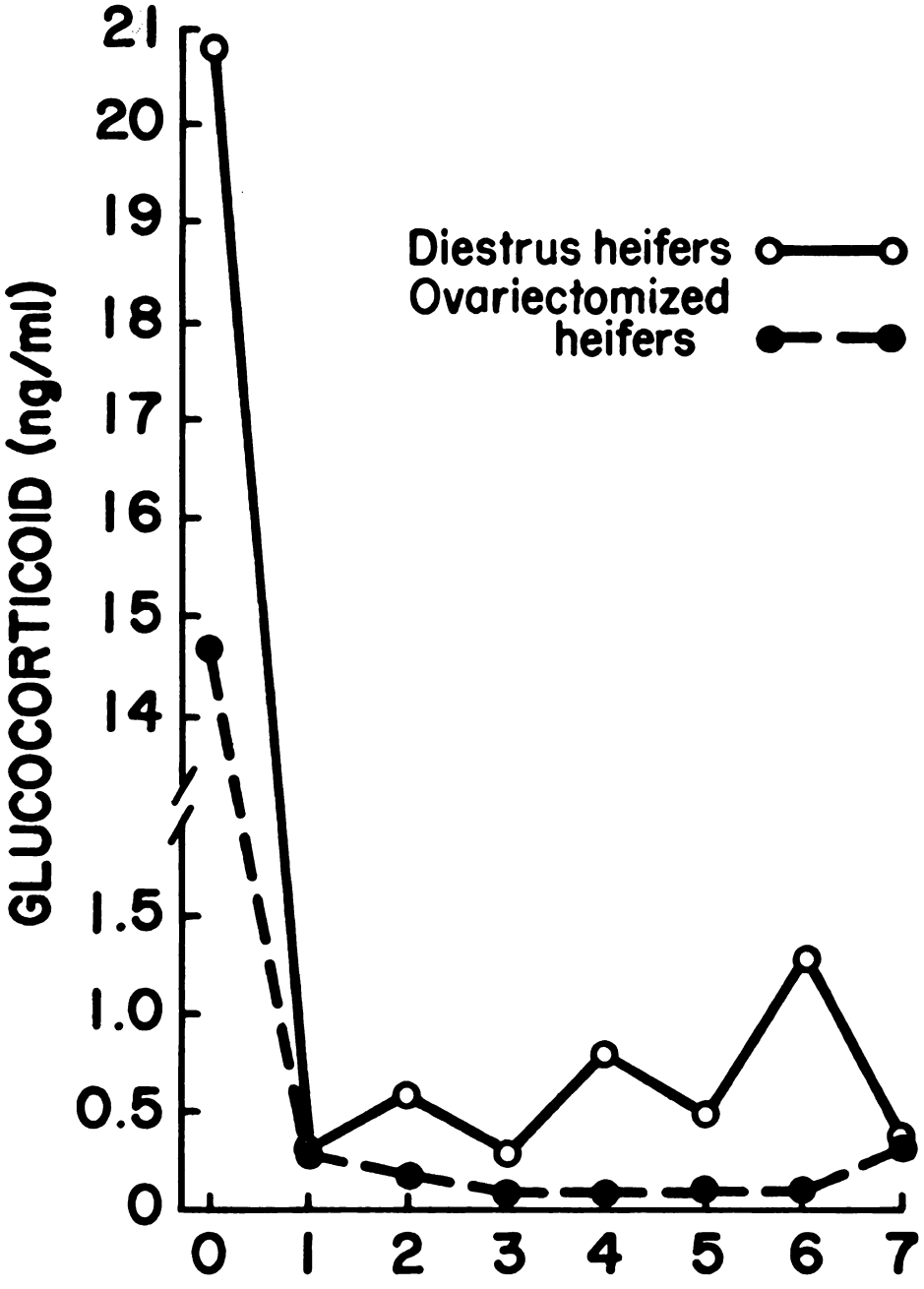
in man (Coudert and Faiman, 1973) and a 3-fold increase in daily cortisol out-put in non-pregnant women. Dexamethasone pretreatment abolished the cortisol response (Wentz et al., 1973), suggesting $PGF_{2\alpha}$ does not directly stimulate adrenal cortisol biosynthesis. Furthermore, injections of PGE_1, PGF_1 and $PGF_{2\alpha}$ into the median eminence (ME) of rats anesthetized with pentobarbital and pretreated with dexamethasone, increased ACTH (Hedge, 1972). But they were ineffective when injected into nearby regions of the basal hypothalamus, the anterior pituitary or the tail vein. In addition, the glucocorticoid response to prostaglandin injection into the ME was eliminated by pretreatment with morphine, suggesting that cortisol response to PG does not reflect action directly on the pituitary, but rather at some central nervous system site, presumably to release corticotropin releasing factor (CRF) (Hedge, 1972).

Thus, three sites of action are possibilities for prostaglandin to stimulate adrenal steroidogenesis: adrenal, pituitary and hypothalamus. More information was required to narrow the selection, especially in cattle, and consequently further experiments were undertaken to determine the site of action of $PGF_{2\alpha}$-induced glucocorticoid release in experiment I (figure 4).

Experiment II: Site of action of $PGF_{2\alpha}$-Induced glucocorticoid release.

Average serum glucocorticoids decreased ($P<0.01$) from 20.9 and 14.8 ng/ml in 3 diestrous and 3 ovariectomized heifers, respectively, to 0.2 ng/ml by 24 hours after sc administration of 22 mg triamcinolone acetonide (TA, figure 5). During the interval from

Figure 5.--Blood Glucocorticoid after sc Injection of Triamcinolone Acetonide (22 mg, sc) in Heifers. Standard errors of the means ranged from 3.09 to 0.04 ng/ml and were generally proportional to the mean.

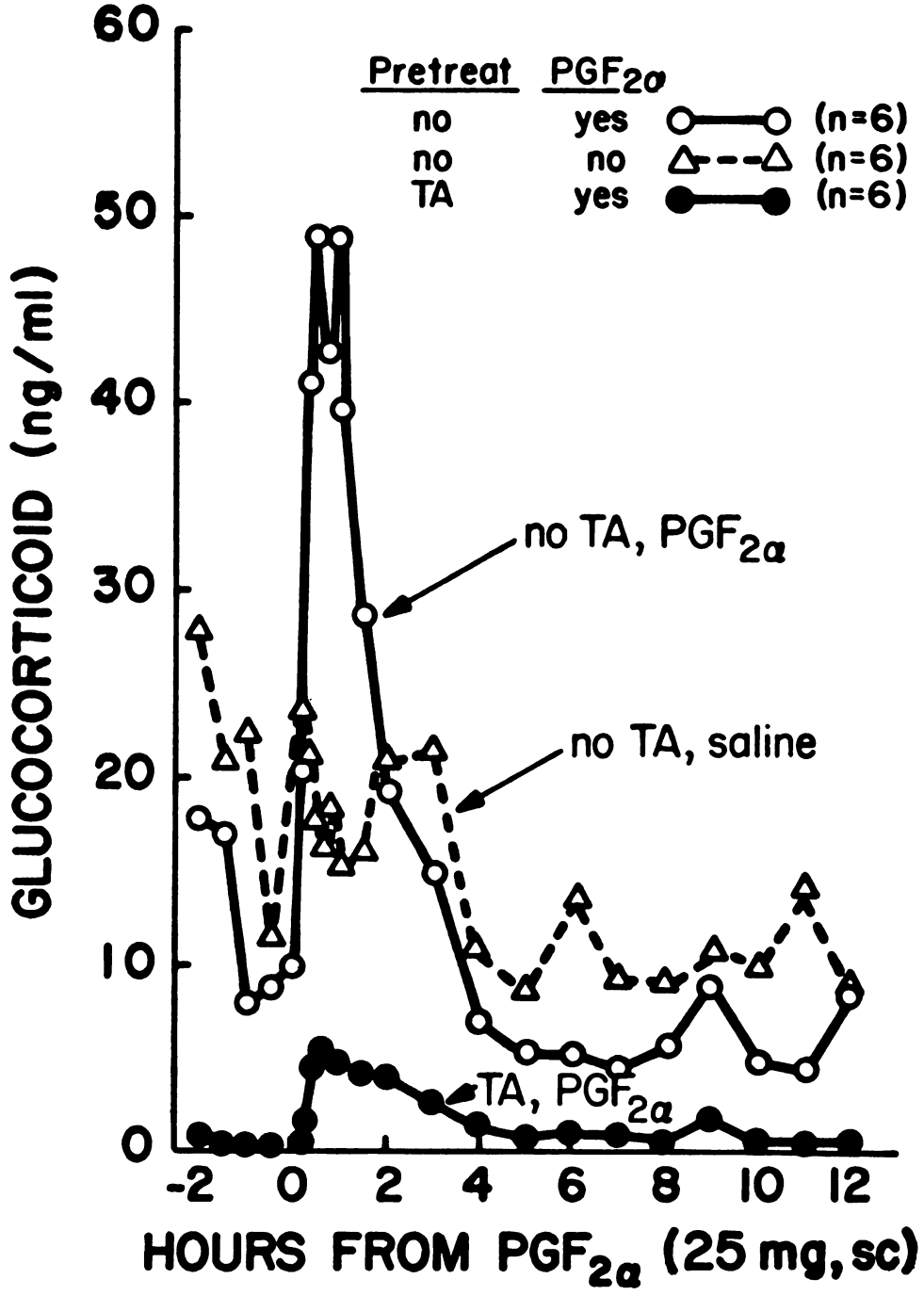


BLOOD GLUCOCORTICOID AFTER sc INJECTION OF TRIAMCINOLONE ACETONIDE (22 mg, sc) IN HEIFERS

days 1 to 7 after TA treatment, daily glucocorticoid values did not differ ($P>.05$) between diestrous and ovariectomized heifers. A similar, prolonged decrease in glucocorticoids also was observed in people given TA. For example, plasma cortisol in women did not increase above 1.2 ng/ml until 11 days after a 25-mg TA injection (Cunningham et al., 1975). That is greatly lower than normal average cortisol value of 72 ng/ml (Czeisler et al., 1976).

Seven days after the TA pretreatment, the experimental doses of 25 mg $PGF_{2\alpha}$ or saline were given im. Since glucocorticoid responses to $PGF_{2\alpha}$ treatments did not differ ($P>0.5$) between diestrous and ovariectomized heifers, in these results, treatment glucocorticoid values are averaged. Glucocorticoid response to the three treatments (figure 6) differed significantly ($P<0.001$) from each other. Blood glucocorticoid increased ($P<0.01$) from 10 to almost 50 ng/ml by 30 minutes after $PGF_{2\alpha}$, and remained above pre-injection values for 3 to 4 hours in heifers without TA pretreatment. In contrast, blood glucocorticoid increased ($P<0.01$) from 0.5 to 7 ng/ml at 30 minutes after $PGF_{2\alpha}$ and remained above pre-injection concentration for 4 to 5 hours in TA pretreated heifers. Thus, peak glucocorticoid response in TA pretreated heifers was much less ($P<0.001$) than that in heifers not given TA. In saline treated controls, blood glucocorticoid fluctuated between 10 and 20 ng/ml during the 12-hour observation period; saline injection induced no glucocorticoid response like those observed after $PGF_{2\alpha}$. In summary, increased glucocorticoid ($P<0.01$) in heifers without TA pretreatment (figure 6) resembled the increase observed after $PGF_{2\alpha}$ in experiment

Figure 6.--Blood Glucocorticoid after $PGF_{2\alpha}$ (25 mg, sc) or Saline Treatment in Heifers with or without Triamcinolone Acetonide (TA) Pretreatment. The standard errors of the means ranged from 9.2 to 1.3 ng/ml (NOTA, Saline), 5.7 to 1.0 ng/ml (NOTA, $PGF_{2\alpha}$), 1.4 to 0.1 ng/ml (TA, $PGF_{2\alpha}$) and were generally proportional to the mean.



BLOOD GLUCOCORTICOID AFTER $PGF_{2\alpha}$ (25 mg, sc) OR SALINE TREATMENT IN HEIFERS WITH OR WITHOUT TRIAMCINOLONE ACETONIDE (TA) PRETREATMENT

I, and TA partially blocked ($P<0.001$) the $PGF_{2\alpha}$-induced peak glucocorticoid response in these heifers.

In addition to the main part of experiment II, 5000 IU ACTH was administered iv to two of the three heifers in each state (diestrous or ovariectomized) of each treatment group at 12 hours after the injection of saline or $PGF_{2\alpha}$. The purpose of the ACTH injection was to test whether the heifers could respond with glucocorticoid secretion 7 days after TA pretreatment. Glucocorticoid increased ($P<0.01$) in all ACTH-treated heifers without TA from 8.6 ± 2.9 ng/ml to a peak of 57.3 ± 4.6 ng/ml ($\bar{x} \pm SE$), but glucocorticoid response to ACTH in the TA pretreated heifers was reduced ($P<0.01$) to 21.7 ± 5.4 ng/ml in comparison with that for heifers not receiving TA.

The inhibition of glucocorticoid by TA illustrated in figure 6, is similar to inhibition of the $PGF_{2\alpha}$-induced cortisol response by dexamethasone in women (Wentz et al., 1973). Dexamethasone pretreatment also blocked PGE_1-, PGE_2- or $PGF_{2\alpha}$- induced glucocorticoid release when these PG's were injected into the basal hypothalamus, the anterior pituitary or a tail vein but not after injection into the median eminence of rats anesthetized with pentobarbital (Hedge, 1972).

Thus, the results of the present study agree with previous research. However, TA did not completely abolish the glucocorticoid response to $PGF_{2\alpha}$ in my heifers. This incomplete inhibition might indicate that TA lost some inhibitory action within 7 days. In support of this notion, glucocorticoid increased slightly although not

significantly between day 6 and 7 in the ovariectomized heifers and between days 1 and 6 in the diestrous animals after TA pretreatment (figure 6), however, the glucocorticoid values in the TA pretreated heifers given saline did not increase ($P<0.01$) throughout the entire 8 day sampling period. Cortisol apparently did not increase within 11 days after TA treatment in humans as mentioned previously (Cunningham et al., 1975), but extrapolation across species may be unjustified and to my knowledge there are no other reports on glucocorticoids after TA in cattle. Similarly, larger doses of TA might be required to completely suppress glucocorticoid in cattle, since inhibition of glucocorticoid was proportional to the amount of dexamethasone in rats (Hedge, 1972). Another explanation could be that TA pretreatment prevented action by some facilitating factors required for full glucocorticoid response to $PGF_{2\alpha}$. For example, an interaction of anterior pituitary hormones is a possibility because both basal secretion and hypoglycemia-induced release of prolactin, ACTH, and growth hormone are suppressable by glucocorticoids (Copinschi et al., 1975). Dexamethasone also blocked stress-induced prolactin release in a dose dependent manner (Harms et al., 1975).

The reduction of glucocorticoid response to ACTH appears anomalous since synthetic corticoids are thought to inhibit glucocorticoid production by inhibiting ACTH release (Kendall et al., 1966 and Arimira et al., 1969). However, dexamethasone, another glucocorticoid, accelerated adrenal protein and RNA degradation and this rate of degradation appeared to be correlated with the degree of dexamethasone-induced atrophy (Ichii et al., 1974). Thus,

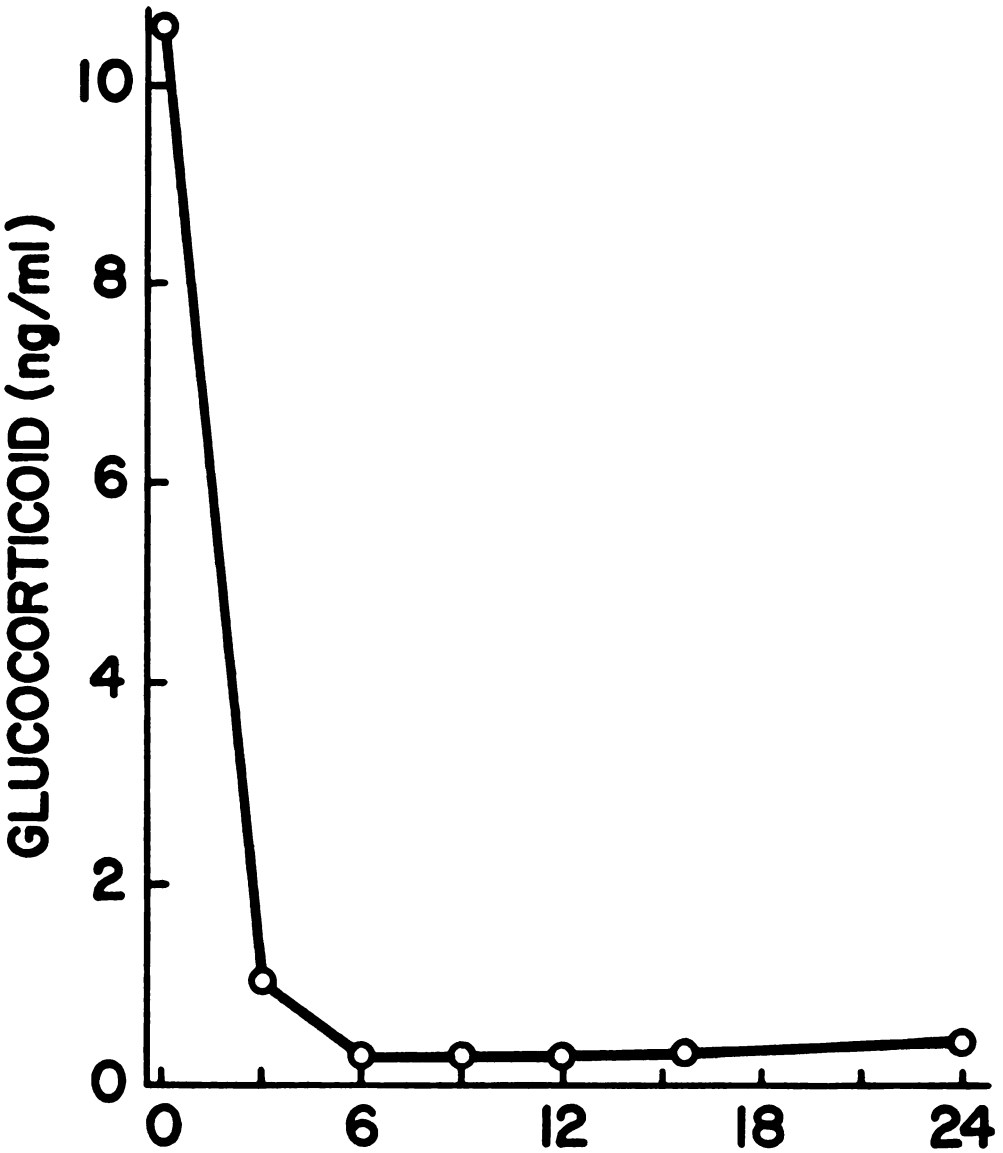
dexamethasone not only acts on the pituitary and CNS to inhibit ACTH secretion, but it also may inhibit steroidogenesis by direct action on the adrenals, especially if one waits for long periods of time after pretreatment with a synthetic glucocorticoid because the rate of degradation appeared to be correlated with the degree of synthetic glucocorticoid-induced atrophy. This factor could prevent one from observing the direct action of a prostaglandin on the adrenal so perhaps $PGF_{2\alpha}$ does have some direct action on the adrenal. Consequently, another experiment was conducted to minimize possible adrenal atrophy and to maximize the effectiveness of TA.

Experiment III: $PGF_{2\alpha}$ - Versus ACTH-Induced Glucocorticoid Release.

In the preliminary experiment with two heifers, serum glucocorticoid declined ($P<0.01$) abruptly from 10.6 to 0.2 ng/ml, within 6 hours after sc injection of 20 mg TA, and remained below 0.5 ng/ml throughout a 24-hour observation period (figure 7). Therefore, 6 hours was selected as the interval between TA pretreatment and the time of injection (iv) of 5 mg $PGF_{2\alpha}$ or 200 iu ACTH in experiment III.

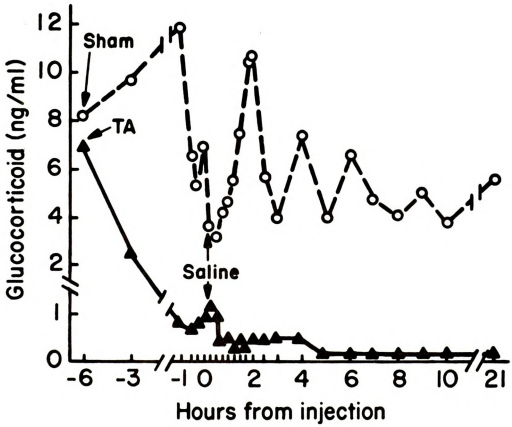
The split plot analysis of this experiment revealed significant treatment ($P<0.01$) and pretreatment effects ($P<0.05$). Serum glucocorticoid for the saline injected controls was significantly higher ($P<0.01$; figure 8) than that for saline-injected heifers pretreated with TA. This response to TA resembled that in the preliminary trial and verifies the inhibition of glucocorticoid secretion in response to TA observed in experiment II.

Figure 7.--Blood Glucocorticoid after sc Injection of Triamcinolone Acetonide (20 mg) in Heifers. The standard errors of the means ranged from 0.7 to 0.1 ng/ml and were generally proportional to the mean.



BLOOD GLUCOCORTICOID AFTER sc INJECTION OF TRIAMCINOLONE ACETONIDE (20 mg) IN HEIFERS

Figure 8.--Blood Glucocorticoid (n=3) after Injection (iv) of Saline with or without Pretreatment with 20 mg Triamcinolone Acetonide (TA, sc) in Heifers. The standard errors of the means ranged from 4.1 to 0.7 ng/ml (Sham) and 2.1 to 0.7 ng/ml (TA, Saline) and were generally proportional to the mean.

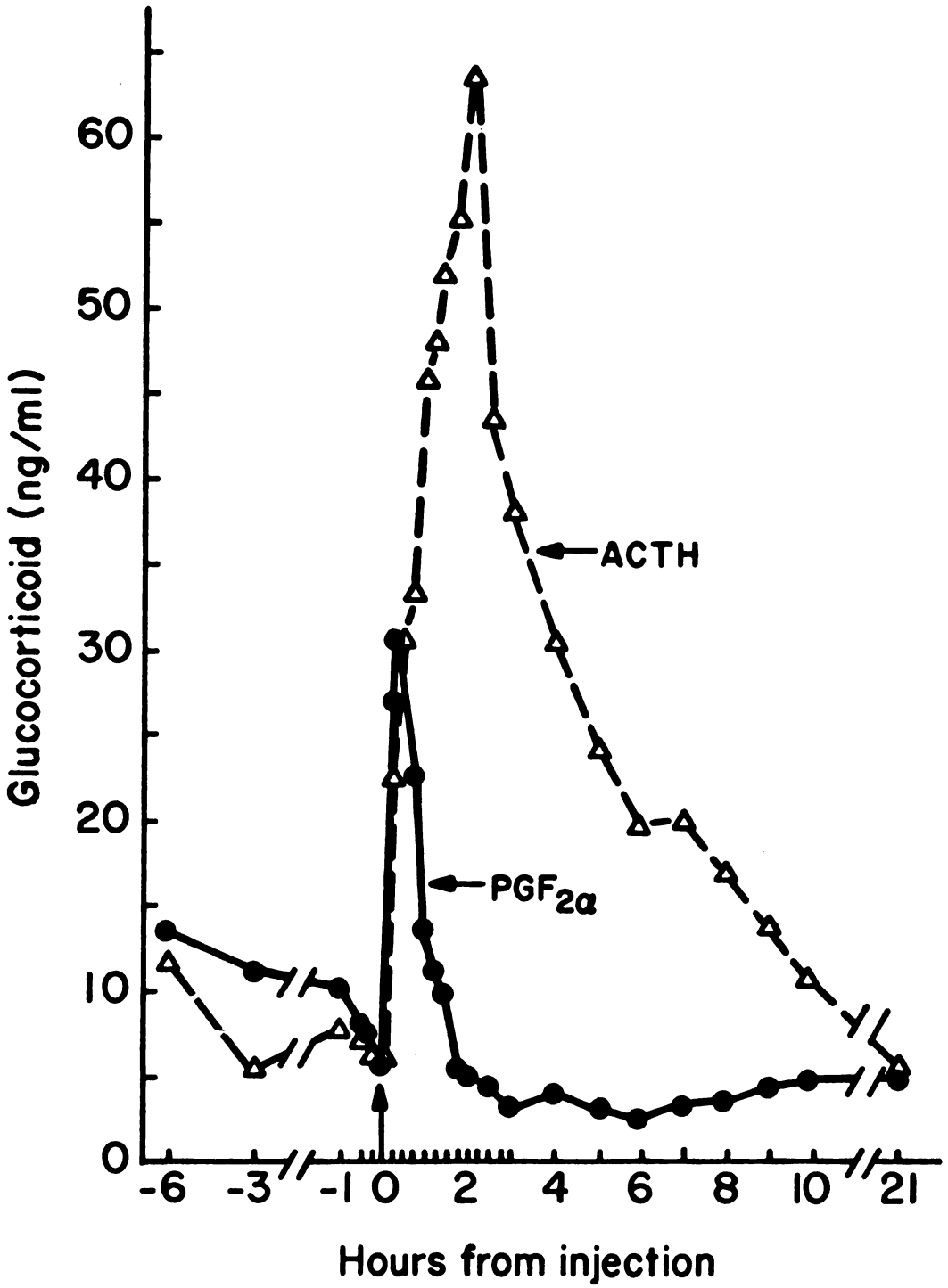


BLOOD GLUCOCORTICOID (n=3) AFTER INJECTION (iv) OF SALINE WITH OR WITHOUT PRETREATMENT WITH 20 mg TRIAMCINOLONE ACETONIDE (TA, sc) IN HEIFERS

In heifers not given TA, the glucocorticoid response after ACTH was greater ($P<0.01$) than that after $PGF_{2\alpha}$. Glucocorticoid increased ($P<0.01$) to 30 ng/ml within 30 minutes after $PGF_{2\alpha}$ and ACTH alike (figure 9), but glucocorticoid continued to increase ($P<0.01$) to over 60 ng/ml at 2 hours after ACTH, whereas it began to fall ($P<0.01$) 30 minutes after $PGF_{2\alpha}$ treatment toward, pre-injection values. Glucocorticoid remained above pre-injection values for about 2 hours after $PGF_{2\alpha}$, much less ($P<0.01$) than the 10-hour period of elevated glucocorticoid after ACTH injection (figure 9). In other words, the initial glucocorticoid response to $PGF_{2\alpha}$ resembled that from ACTH, but the duration of the response to the porcine ACTH was much more prolonged than that after $PGF_{2\alpha}$. The differences in ACTH- and $PGF_{2\alpha}$-induced glucocorticoid release may indicate that the dose of $PGF_{2\alpha}$ was smaller than the dose of ACTH relative to their glucocorticoid stimulatory activity. More likely, perhaps $PGF_{2\alpha}$ was cleared more rapidly than the porcine ACTH; $PGF_{2\alpha}$ is known to have a metabolic clearance rate of 17 liters/minute in cattle (Stellflug et al., 1975). The simultaneous increases of glucocorticoid after $PGF_{2\alpha}$ and ACTH suggest that the iv $PGF_{2\alpha}$ may have caused rapid ACTH release. If this hypothesis is correct, one might expect prolonged glucocorticoid elevation during infusion of $PGF_{2\alpha}$ for several hours to prolong its action.

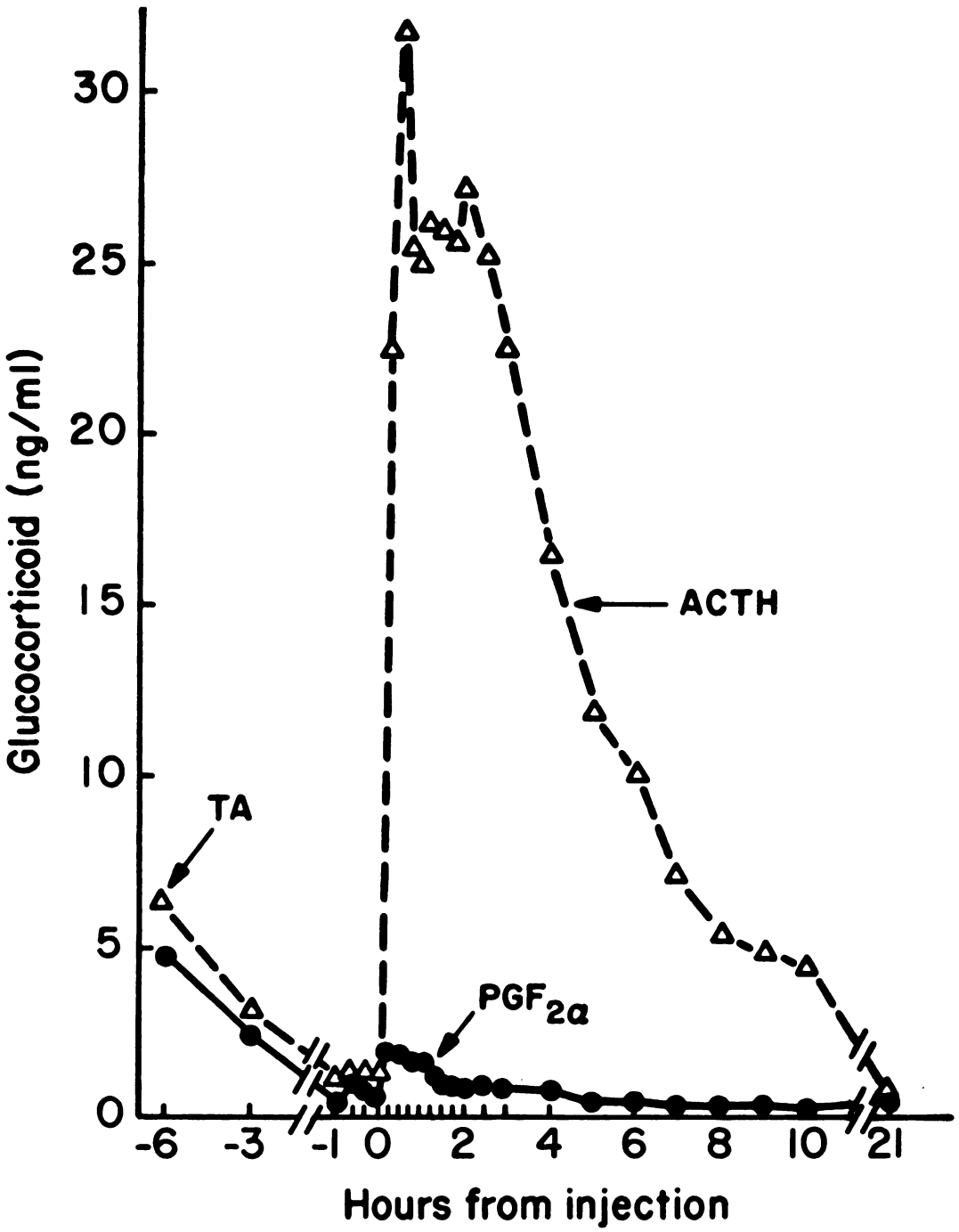
In heifers pretreated with TA (figure 10), peak glucocorticoid response to ACTH (30 ng/ml) was much greater ($P<0.01$) than that after $PGF_{2\alpha}$ (2ng/ml). In fact, the glucocorticoid response to $PGF_{2\alpha}$ was not significant ($P>0.05$) in TA-pretreated animals. In other words,

Figure 9.--Blood Glucocorticoid (n=3) after Injection (iv) of 5 mg $PGF_{2\alpha}$ or 200 IU ACTH in Control Heifers. The standard errors of the means ranged from 0.2 to 6.2 ng/ml ($PGF_{2\alpha}$) and 0.3 to 19.4 ng/ml (ACTH) and were generally proportional to the mean.



BLOOD GLUCOCORTICOID (n=3) AFTER INJECTION (iv) OF 5 mg $PGF_{2\alpha}$ OR 200 IU ACTH IN CONTROL HEIFERS

Figure 10.--Blood Glucocorticoid (n=3) in Response to Injection (iv) of 5 mg $PGF_{2\alpha}$ or 200 IU ACTH 6 Hr after Pretreatment of Heifers with 20 mg Triamcinolone Acetonide (TA, sc). The standard errors of the means ranged from 0.1 to 0.3 ng/ml ($PGF_{2\alpha}$) and 0.3 to 24.3 ng/ml (ACTH) and were generally proportional to the mean.



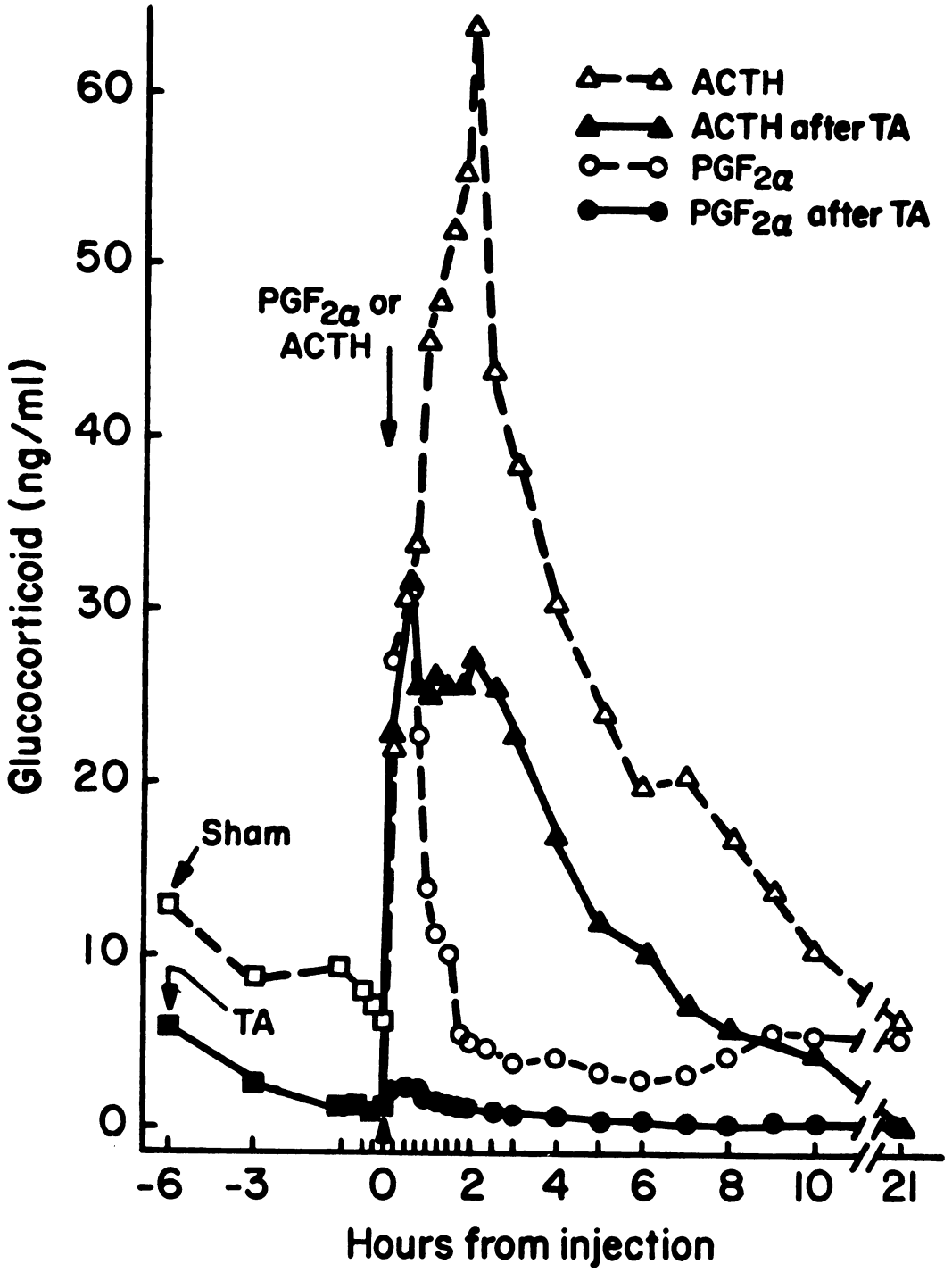
BLOOD GLUCOCORTICOID (n=3) IN RESPONSE TO INJECTION (iv) OF 5 mg $PGF_{2\alpha}$ OR 200 IU ACTH 6 HR AFTER PRETREATMENT OF HEIFERS WITH 20 mg TRIAMCINOLONE ACETONIDE (TA, sc)

TA pretreatment reduced ($P<0.01$) from 64 to 27 ng/ml peak glucocorticoid response to ACTH; by contrast, glucocorticoid did not respond significantly to $PGF_{2\alpha}$ after TA (figure 10).

To facilitate comparisons of the glucocorticoid responses, all ACTH and $PGF_{2\alpha}$ treatments are illustrated in figure 11. Glucocorticoid was averaged for the six TA-pretreated heifers and for the six non-TA-pretreated heifers before $PGF_{2\alpha}$ or ACTH was injected. In overview, the peak glucocorticoid response to ACTH after TA was about 50 percent of that in controls; by comparison peak glucocorticoid response to $PGF_{2\alpha}$ in non-TA-pretreated heifers was over 30 ng/ml, but it was not significant in TA-pretreated heifers. In other words, while TA reduced the glucocorticoid response to ACTH it essentially abolished the response to $PGF_{2\alpha}$ supporting the results from experiment II that the major site of $PGF_{2\alpha}$-induced glucocorticoid release is at the hypothalamopituitary axis, presumably to release ACTH.

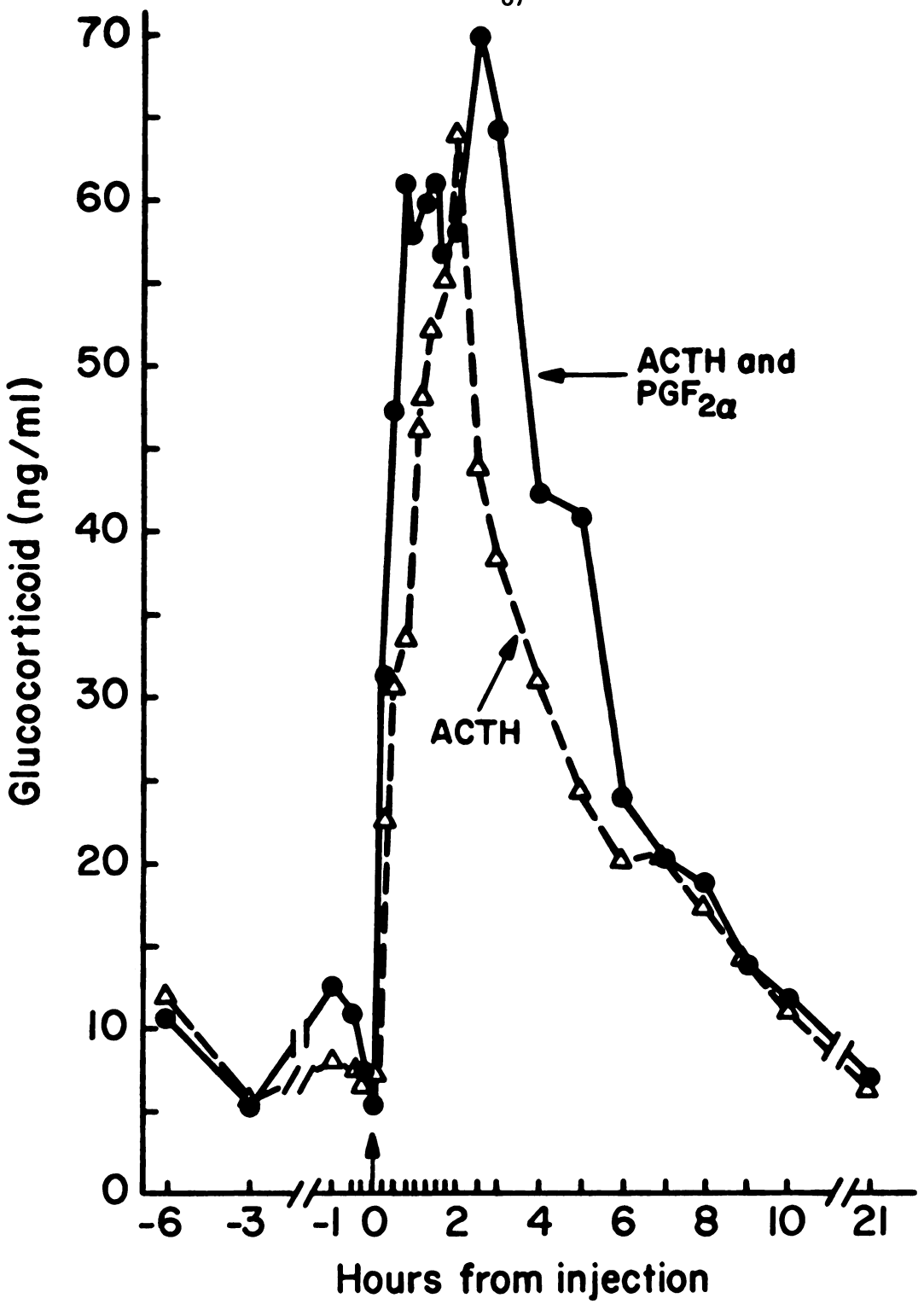
Three additional treatments on three heifers each were included as an adjunct to the principal experiment. The first treatment consisted of a simultaneous (iv) injection of 200 IU ACTH and 5 mg $PGF_{2\alpha}$. The submaximal dose of ACTH was chosen so an additional stimulus released by $PGF_{2\alpha}$ (presumably ACTH) could be monitored by an increase of glucocorticoid. Thus, the more prolonged glucocorticoid response ($P<0.01$) after the simultaneous injection of ACTH and $PGF_{2\alpha}$ then that after ACTH alone (figure 12) also supports an ACTH release after $PGF_{2\alpha}$. The results from this simultaneous injection do not rule out a direct action on the adrenals, however, the almost complete

Figure 11.--Blood Glucocorticoid (n=3) after 5 mg PGF2α or 200 IU ACTH ($\uparrow$, iv) with or without Pretreatment of Heifers with 20 mg Triamcinolone Acetonide (TA, sc). Standard errors of the means are listed on figures 9 and 10.



BLOOD GLUCOCORTICOID (n=3) AFTER 5 mg $PGF_{2\alpha}$ OR 200 IU ACTH (↓,iv) WITH OR WITHOUT PRETREATMENT OF HEIFERS WITH 20 mg TRIAMCINOLONE ACETONIDE (TA, sc)

Figure 12.--Blood Glucocorticoid (n=3) after Injection (iv) of 200 IU ACTH with or without 5 mg $PGF_{2\alpha}$ in Heifers. The standard errors of the means ranged from 0.3 to 19.4 ng/ml (ACTH) and 0.6 to 30.1 ng/ml (ACTH and $PGF_{2\alpha}$) were generally proportional to the mean.

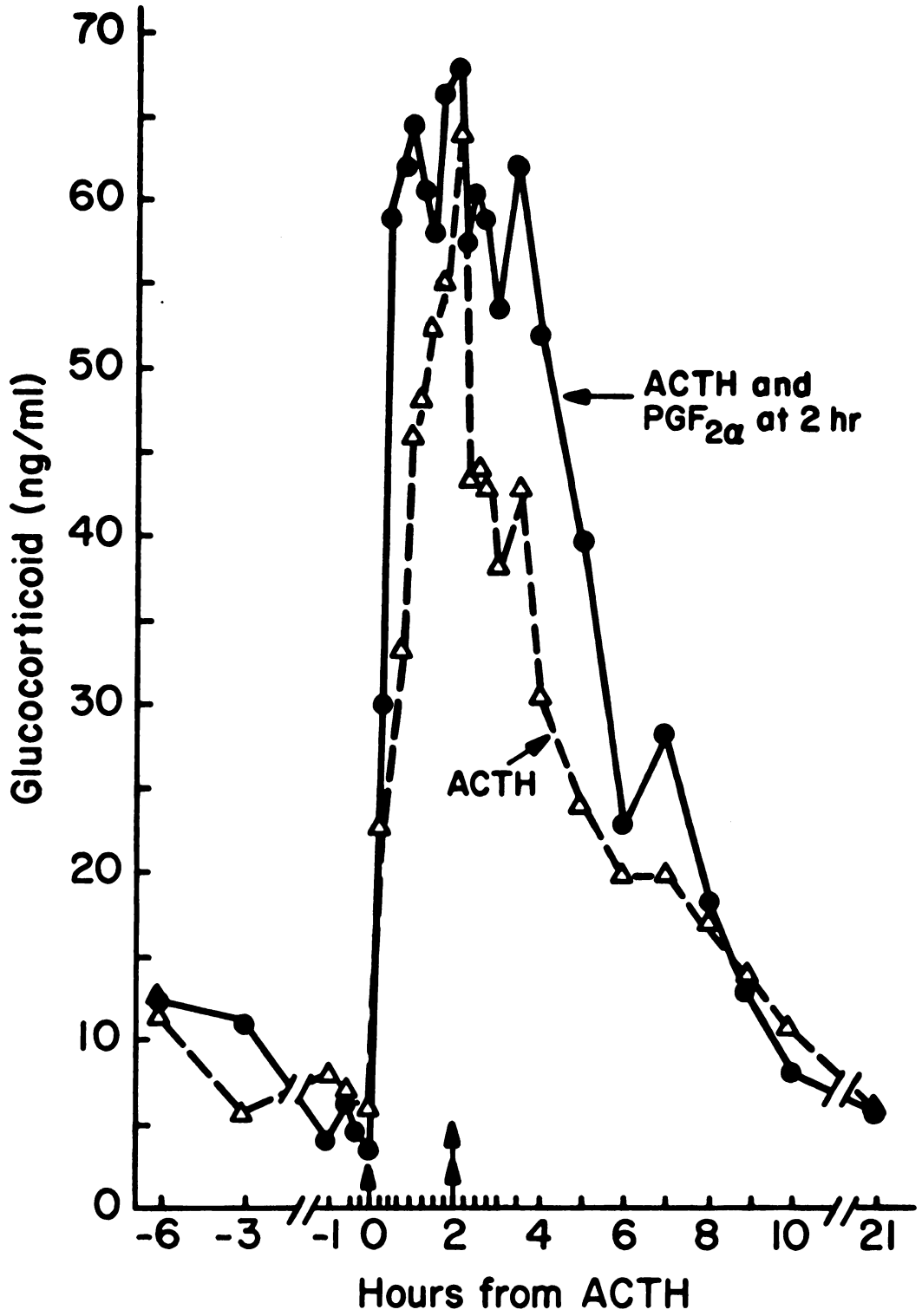


BLOOD GLUCOCORTICOID (n=3) AFTER INJECTION (iv) OF 200 IU ACTH WITH OR WITHOUT 5 mg $PGF_{2\alpha}$ IN HEIFERS

inhibition of $PGF_{2\alpha}$-induced glucocorticoid release by TA provide strong evidence that the major action of $PGF_{2\alpha}$ is not at the adrenal.

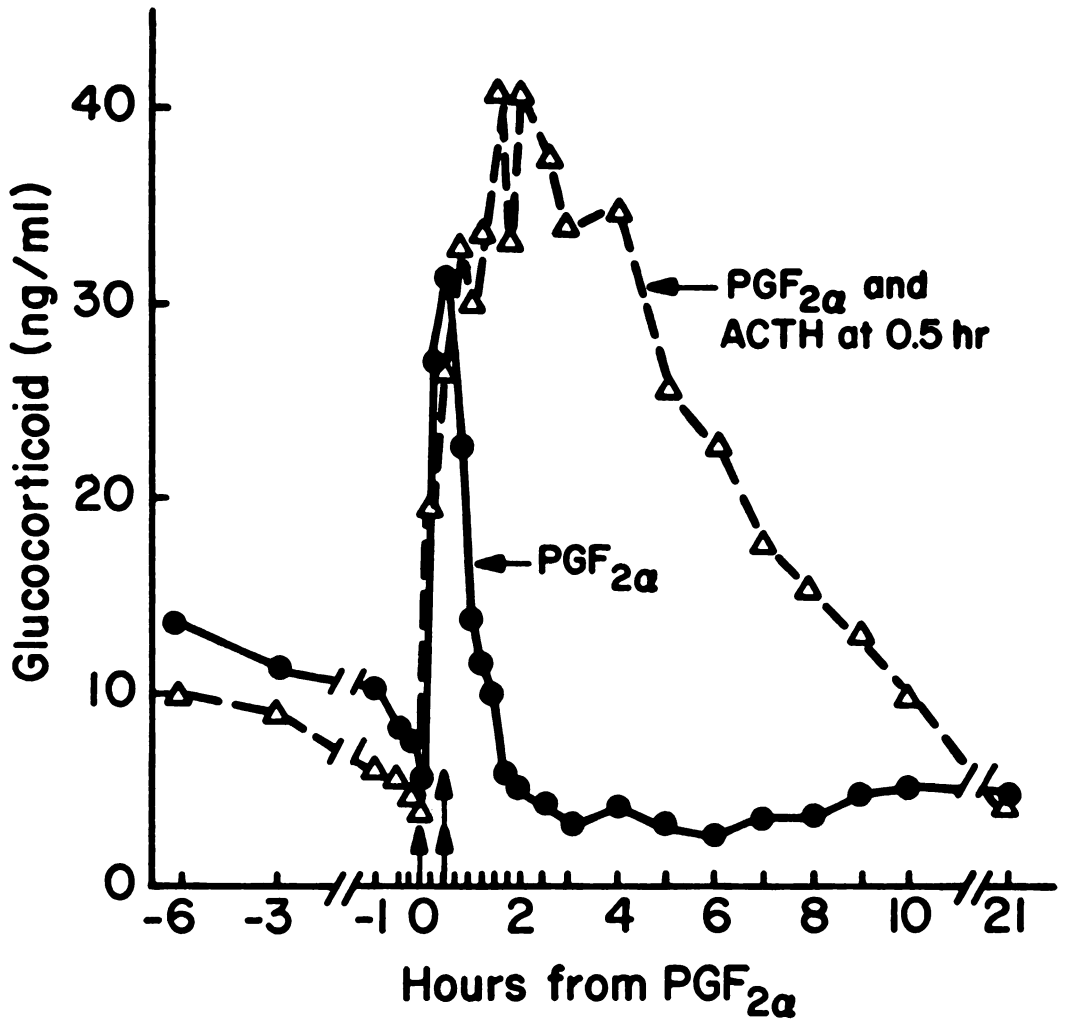
The other two treatments were included to determine if the sequential order of ACTH and $PGF_{2\alpha}$ administration altered the duration of glucocorticoid response in comparison to when the initial stimulus was injected alone. The intervals between sequential treatments were chosen so the second injection would be near the peak glucocorticoid response induced by the first injection. ACTH was injected at time 0 and $PGF_{2\alpha}$ 2 hours later, glucocorticoid response was more prolonged ($P<0.01$) than the response after ACTH alone (figure 13). Again the glucocorticoid response to these submaximal doses of $PGF_{2\alpha}$ appears to be additive and is consistant with the notion that $PGF_{2\alpha}$ acts to increase glucocorticoid secretion by causing ACTH release but does not competely rule out a direct action on the adrenals. In addition when $PGF_{2\alpha}$ was injected at time 0 and ACTH 30 minutes later, glucocorticoid response was prolonged ($P<0.01$) more than that after $PGF_{2\alpha}$ alone (figure 14), but glucocorticoid peaked significantly lower ($P>0.05$) in comparison to that after ACTH alone (figure 9) even though the duration of response was similar. Perhaps the $PGF_{2\alpha}$ injection modified sterol precursor stores in the adrenal, curtailing glucocorticoid production in response to the subsequent ACTH injection. More probable, the $PGF_{2\alpha}$-induced glucocorticoid release might resemble injection of synthetic corticoid to partially inhibit the ACTH-induced glucocorticoid release, as observed in the main body of this experiment and experiment II. In addition, ACTH increases the half-lives of adrenal protein and RNA (Ichii et al., 1974). This effect

Figure 13.--Blood Glucocorticoid (n=3) after Injection (iv) of 200 IU ACTH (↑) with or without 5 mg $PGF_{2\alpha}$ (↑↑) at 2 Hr in Heifers. The standard errors of the means ranged from 0.3 to 19.4 ng/ml (ACTH) and 0.3 to 18.0 ng/ml (ACTH and $PGF_{2\alpha}$ at 2 hr) and were generally proportional to the mean.



BLOOD GLUCOCORTICOID (n=3) AFTER INJECTION (iv) OF 200 IU ACTH (↑) WITH OR WITHOUT 5 mg $PGF_{2\alpha}$ (↕) AT 2 HR IN HEIFERS

Figure 14.--Blood Glucocorticoid (n=3) after Injection (iv) of 5 mg $PGF_{2\alpha}$ (↑) with or without 200 IU ACTH (↕) at 0.5 Hr in Heifers. The standard errors of the mean ranged from 0.2 to 6.2 ng/ml ($PGF_{2\alpha}$) and 1.0 to 7.2 ng/ml ($PGF_{2\alpha}$ and ACTH at 0.5 hr) and were generally proportional to the mean.



BLOOD GLUCOCORTICOID (n=3) AFTER INJECTION (iv) OF 5mg $PGF_{2\alpha}$ (↑) WITH OR WITHOUT 200 IU ACTH (↕) AT 0.5 HR IN HEIFERS

of ACTH might account for the prolonged glucocorticoid release after ACTH in figure 11 in comparison to the reduced response when $PGF_{2\alpha}$ was given before ACTH. That is, perhaps, glucocorticoids released in response to $PGF_{2\alpha}$ suppressed the protein and RNA sustaining action of ACTH given 30 minutes later.

In overview of the results from experiements II and III, I believe the surge of glucocorticoid which follows $PGF_{2\alpha}$ treatment in heifers represents an action of $PGF_{2\alpha}$ on the pituitary of hypothalamus. One cannot determine from my data whether the action is on the pituitary, or the hypothalamus, because dexamethasone (a corticoid similar to TA) suppressed the synthesis and release of ACTH through action on the pituitary *in vivo* (Kendall *et al.*, 1966 and Yasuda *et al.*, 1976) and *in vitro* (Arimura et al., 1969). Glucocorticoid receptor sites in the pituitary presumably facilitate this inhibitory action of dexamethasone, but in addition the dorsal hippocampus contains glucocorticoid receptor sites in rats (Rotsztein *et al.*, 1975). In other words, glucocorticoid also inhibits ACTH secretion by action on the CNS. To determine whether the $PGF_{2\alpha}$ acts on the pituitary or the hypothalamus in cattle, future experiments might include (1) treatments with small doses of $PGF_{2\alpha}$ given into brain ventricles or into the pituitary, (2) *in vitro* incubations of pituitaries with $PGF_{2\alpha}$ with and without CRF extracts from median eminence, and (3) treatments such as morphine to block CRF production before $PGF_{2\alpha}$ treatment.

SUMMARY AND CONCLUSIONS

Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) potentially may be used widely for control of ovulation in farm animals. Some other prostaglandins altered secretion of important metabolic hormones. Thus, the purpose of the three main experiments in this thesis was to determine if anterior pituitary hormones were released after luteolytic doses of $PGF_{2\alpha}$ in cattle.

In the first experiment after administration of 15, 30 or 60 mg PGF_2 to diestrous heifers, prolactin increased over 3-fold within 10 minutes and returned to pre-injection values within 4 hours; growth hormone increased in a dose-related manner, peaking at 30 minutes and remaining above pre-injection values for 1 hour; LH increased at least 2-fold over pre-injection values within 1.5 to 6 hours; glucocorticoids (indicator of ACTH release) increased more than 6-fold at 30 minutes and returned to pre-injection values by 4 hours.

A second experiment was conducted to determine the site of action of $PGF_{2\alpha}$ on glucocorticoid release. $PGF_{2\alpha}$ (25 mg) or saline was given im 7 days after a triamcinolone acetonide (TA) pretreatment which decreased serum glucocorticoid to less than 0.5 ng/ml within 24 hours. In saline-treated heifers not given TA, blood glucocorticoid fluctuated at random whereas glucocorticoid increased from 10 to 50 ng/ml by 30 minutes after $PGF_{2\alpha}$ and returned to pre-injection values 4

hours later in heifers without TA. However, in TA-pretreated heifers peak glucocorticoid response to $PGF_{2\alpha}$ was depressed to 12 percent of that in heifers not given TA. The results suggested that the TA inhibition might have been partially lost and that the adrenals might have been partially regressed by 7 days after TA, thereby reducing the glucocorticoid response to a stimulus.

Consequently, another experiment was conducted to minimize possible adrenal regression and to maximize the effectiveness of TA. Submaximal doses of porcine ACTH (200 IU) and $PGF_{2\alpha}$ (5mg) were administered to heifers 6 hours after TA-pretreatment, when glucocorticoid secretion was fully inhibited by TA. In animals not pretreated with TA, the first 0.5 hours of glucocorticoid response to $PGF_{2\alpha}$ resembled that after ACTH, but the peak response to ACTH was much greater and the duration of response to ACTH was much more prolonged than that after $PGF_{2\alpha}$. The TA-pretreatment reduced the glucocorticoid response to ACTH by 50 percent but it essentially abolished the response to $PGF_{2\alpha}$. Three added treatments which consisted of simultaneous or sequential administration of the same amount of $PGF_{2\alpha}$ and ACTH were included in this third experiment. The glucocorticoid response was more prolonged when $PGF_{2\alpha}$ and ACTH were injected simultaneously or when $PGF_{2\alpha}$ followed ACTH-treatment by 2 hours. In contrast when $PGF_{2\alpha}$ was administered 30 minutes before ACTH, peak glucocorticoid response to ACTH was suppressed by comparison to that after ACTH alone.

In conclusion, prolactin, growth hormone, luteinizing hormone and glucocorticoids are secreted transitorily in relatively large

amounts in response to treatment of heifers with luteolytic doses of $PGF_{2\alpha}$. Review of the literature for other species justifies the hypothesis that prostaglandins may normally mediate pituitary hormone secretion. Consequently, the pituitary hormone releases reported in response to $PGF_{2\alpha}$ in this thesis may represent relatively specific action at the hypothalamus or pituitary. I have no evidence in these three experiments for a pituitary-hypothalamic site of $PGF_{2\alpha}$ action for growth hormone, prolactin or LH secretion. However, on the basis of the last two experiments, I believe $PGF_{2\alpha}$ acts primarily on the hypothalamus or the pituitary to cause glucocorticoid secretion. Further research is required to discriminate between these sites of $PGF_{2\alpha}$ action, and to determine whether $PGF_{2\alpha}$ normally participates in pituitary hormone secretion in cattle. Whether or not it does, the results from this thesis raise the possibility of using prostaglandins to regulate intermediary metabolic hormones in food-producing animals.

BIBLIOGRAPHY

BIBLIOGRAPHY

Akbar, A.M., L.E. Reichert, Jr., T.G. Dunn, C.C. Kaltenbach and G.D. Niswender. 1974. Serum levels of follicle stimulating hormone during the bovine estrous cycle. J. Anim. Sci. 39:360-365.

Anderson, L.L., K.P. Bland, and R.M. Melampy. 1969. Comparative aspects of uterine-luteal relationships. Rec. Prog. Hormone Res. 25:57.

Arimura, A., C.Y. Bowers, A.V. Schally, M. Saito and M.C. Miller III. 1969. Effect of corticotropin-releasing factor, dexamethasone and actinomycin D on the release of ACTH from rat pituitaries *in vivo* and *in vitro*. Endocrinol. 85:300.

Ayalon, N. and M. Shemesh. 1974. Pro-oestrous surge in plasma progesterone in the cow. J. Reprod. Fertil. 36:239.

Babcock, J.C. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. In: "Ovarian regulatory mechanisms." J. Reprod. Fertil. Suppl. 1, p. 47.

Bergstrom, S., R. Ryhage, B. Samuelsson and J. Stoval. 1962. The structure of prostaglandin E_1, F_1 and F_2. Acta Chem. Scand. 16:501.

Carlson, J.C., B. Barcikowski and J.A. McCracken. 1973. Prostaglandin F_{2a} and the release of LH in sheep. J. Reprod. Fertil. 34:357.

Chenault, J.R., W.W. Thatcher, P.S. Kalra, R.M. Abrams and C.J. Wilcox. 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:709.

Christensen, D.S., M.L. Hopwood and J.N. Wiltbank. 1974. Levels of hormones in the serum of cycling beef cows. J. Anim. Sci. 38:577.

Cole, H.H. and R.B. Snook. 1964. Proc. 5th Intern. Congr. Animal Reprod., Trento II, 143.

Convey, E.M., H.A. Tucker, V.G. Smith, and J. Zolman. 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. Endocrinol. 92:471.

Cooper, R.H., M. McPherson and J.G. Schofield. 1972. The effect of prostaglandins on Ox pituitary content of adenosine 3':5'-cyclic monophosphate and the release of growth hormone. Biochem. J. 127:143.

Copinschi, G., M. L'Hermite, R. Leclercq, J. Golstein, L. Vanhaelst, E. Virasoro, and C. Robyn. 1975. Effects of glucocorticoids on pituitary hormonal responses to hypoglycemia. Inhibition of prolactin release. J. Clin. Endocrinol. Metab. 40:442.

Coudert, S.P., and C. Faiman. 1973. Effect of prostaglandin F_{2a} on anterior pituitary function in man. Prostaglandins 3:89.

Cunningham, G.R., E.M. Caperton, Jr., and J.W. Goldzieher. 1975. Antiovulatory activity of synthetic corticoids. J. Clin. Endocrinol. Metab. 40:265.

Czeisler, C.A., M.C.M. Ede, Q.R. Regestein, E.S. Kisch, V.S. Fang and E.N. Ehrlich. 1976. Episodic 24-hour cortisol secretory patterns in patients awaiting elective cardiac surgery. J. Clin. Endocrinol. Metab. 42:273-283.

de Wied, D., A. Witter, D.H.G. Versteeg, and A.H. Mulder. 1969. Release of ACTH by substances of central nervous system origin. Endocrinol. 85:561.

Eskay, R.L., J. Warberg, R.S. Mical, and J.C. Porter. 1975. Prostaglandin E_2-Induced Release of LHRH into hypophysial portal blood. Endocrinol. 97:816.

Flack, J.D. and P.W. Ramwell. 1972. A comparison of the effects of ACTH, cyclic AMP, dibutyryl cyclic AMP, and PGE_2 on corticosteroidogenesis in vitro. Endocrinol. 90:371.

Flack, J.D., R. Jessup, and P. W. Ramwell. 1969. Prostaglandin stimulation of rat corticosteroidogenesis. Science 163:691.

Ganong, W.F., In Nalbandov, A.V. (ed.). 1963. Advances in Neuroendocrinology, Univ. Ill. Press, Urbana, p. 92.

Gill, J.L., H.D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Glencross, R.G., I.B. Munro, B.E. Senior, and G.S. Pope. 1973. Concentrations of oestradiol-17_β, oestrone, and progesterone, in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta Endocrinol. 73:374.

Goding, J.R., M.D. Cain, J. Cerini, M. Cerini, W.A. Chamley, I.A. Cumming. 1972. Prostaglandin F_{2a} "the" luteolytic hormone in the ewe. J. Reprod. Fertil. 28:146.

Goldblatt, M.W. 1933. A depressor substance in seminal fluid. J. Soc. Chem. Ind., Lond. 52:1056.

Hackett, A.J., H.D. Hafs. 1969. Pituitary and hypothalmic endocrine changes during the bovine estrous cycle. J. Anim. Sci. 28:531.

Hafs, H.D., T.E. Kiser, N.B. Haynes, J.S. Kesner, J.N. Stellflug. 1976. Release of pituitary hormones, cortisol, testesterone and insulin in response to prostaglandin $F_{2\alpha}$ given during intracarotid infusion of somatostatin in bulls. J. Anim. Sci. In press.

Hafs, H.D., T.M. Louis, P.A. Noden, and W.D. Oxender. 1974. Control of the estrous cycle with prostaglandin $F_{2\alpha}$ in cattle and horses. J. Anim. Sci. Suppl. 1, p. 10.

Hafs, H.D. 1975. Prostaglandins and the control of anterior pituitary hormone secretion. In Hypothalamic Hormones. Eds., M. Motta, P.G. Crosignani and L. Martini. Academic Press, New York, p. 183.

Hafs, H.D., J.G. Manns and B. Drew. 1975. Onset of oestrus and fertility of dairy heifers and suckled beef cows treated with prostaglandin $F_{2\alpha}$. Anim. Prod. 21:13.

Hafs, H.D., T.M. Louis, J.N. Stellflug, E.M. Convey, and J.H. Britt. 1975. Blood LH after $PGF_{2\alpha}$ in diestrous and ovariectomized cattle. Prostaglandins 10:1001.

Hansel, W., P.W. Concannon, and J.H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222.

Hansel, W., S.E. Echternkamp. 1972. Control of ovarian function in domestic animals. Am. Zool. 12:225.

Hansel, W., M. Shemesh, J. Hixon, and J. Lukaszewska. 1975. Extraction, isolation and identification of a luteolytic substance from bovine endometrium. Biol. Reprod. 13:30.

Harms, P.G., P. Langlier, and S.M. McCann. 1975. Modification of stress-induced prolactin release by dexamethasone or adrenalectomy. Endocrinol. 96:475.

Harms, P.G., S.R. Ojeda, and S.M. McCann. 1973. Prostaglandin involvement in hypothalamic control of gonadotropin and prolactin release. Science 181:760.

Harms, P.G., S.R. Ojeda, and S.M. McCann. 1974. Prostaglandin-induced release of pituitary gonadotropins: central nervous system and pituitary sites of action. Endocrinol. 94:1459.

Harms, P.G., S.R. Ojeda, S.M. McCann. 1976. Failure of monoaminergic and cholinergic receptor blockers to prevent prostaglandin E_2-induced luteinizing hormone release. Endocrinol. 98:318.

Hedge, G.A. 1972. The effects of prostaglandins on ACTH secretion. Endocrinol. 91:925.

Hertelendy, F. 1971. Studies on growth hormone secretion II. Stimulation by prostaglandin in vitro. Acta Endocrinol. 68:355.

Hertelendy, F., G. Peake, and H. Todd. 1971 Studies on growth hormone secretion: Inhibition of prostaglandin, theophylline and cyclic AMP stimulated growth hormone release by valinomycin in vitro. Biochem. Biophys. Res. Commun. 44:253.

Hertelendy, F., H. Todd, K. Ehrhart and R. Blute. 1972. Studies on growth hormone secretion: IV. In vivo effects of prostaglandin E_1. Prostaglandins 2:79.

Hensby, C.N. 1974. Reduction of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by an enzyme in sheep blood. Biochimica et Biophysica Acta 348:145.

Hixon, J.E. and W. Hansel. 1974. Evidence for preferential transfer of prostaglandin $F_{2\alpha}$ to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11:543-552.

Hobson, W.C. and W. Hansel. 1972. Plasma LH levels after ovariectomy, corpus luteum removal and estradiol administration in cattle. Endocrinol. 91:185.

Hoffer, B.S., G.R. Siggins, and F.E. Bloom. 1970. Possible cyclic AMP-mediated adrenergic synapses to rat cerebellar purkinje cells: Combined structural, physiological, and pharmacological analyses. In Advan. Biochem. Psychopharmacol. Eds. P. Greengard and E. Costa. Raven Press, N.Y., p. 349.

Ichii, S., N. Murakami, and M. Izawa. 1974. Effect of ACTH and dexamethasone on the rate of protein and RNA degradation in rat adrenal glands. Endocrinol. Japan 21:465.

Ingalls, W.G., E.M. Convey, and H.D. Hafs. 1973. Bovine serum LH, GH, and prolactin during late pregnancy, parturition and early lactation. Proc. Soc. Exp. Biol. and Med. 143:161.

Ito, H., G. Momose, T. Katayama, H. Takagishi, L. Ito, H. Nakajima, and Y. Takei. 1971. Effect of prostaglandin on the secretion of human growth hormone. J. Clin. Endocrinol. and Metab. 32:857.

Kato, Y., J. Dupre, and J.C. Beck. 1973. Plasma growth hormone in the anesthetized rat: Effects of dibutyryl cyclic AMP, prostaglandin E_1, adrenergic agents, vasopressin, chlorpromazine, amphetamine L-dopa. Endocrinology 93:135.

Kazama, N. and W. Hansel. 1970. Preovulatory changes in progesterone level of bovine peripheral blood plasma. Endocrinol. 86:1252.

Kendall, J.W., A.K. Stott, C. Allen, and M.A. Greer. 1966. Evidence for ACTH secretion and ACTH suppressibility in hypophysectomized rats with multiple heterotropic pituitaries. Endocrinol. 78:533.

Kirk, R.E. 1968. Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole Pub. Co., Belmont, Calif., p. 90.

Kiser, T.E., H.D. Hafs, and W.D. Oxender. 1976. Increased blood LH and testosterone after administration of prostaglandin $F_{2\alpha}$ in bulls. Prostaglandins 11:545.

Kurzrok, R., and C.C. Lieb. 1930. Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med. 26:268.

Lauderdale, J.W., B.E. Seguin, J.N. Stellflug, J.R. Chenault, W.W. Thatcher, C.K. Vincent, and A.F. Loyancano. 1974. Fertility of cattle following $PGF_{2\alpha}$ injection. J. Anim. Sci. 38:964.

Leslie, C.A., and L. Levine. 1973. Evidence for the presence of a prostaglandin $E_{2\alpha}$-9-Keto reductase in rat organs. Biochem. and Biophys. Res. Comm. 52:717.

Louis, T.M., H.D. Hafs, and D.A. Morrow. 1972. Estrus and ovulation after $PGF_{2}\alpha$ in cows. J. Anim. Sci. 35:1121 (Abstr.).

Louis, T.M., H.D. Hafs, and D.A. Morrow. 1974. Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347.

MacLeod, R.M. and J.E. Lehmeyer. 1970. Release of pituitary growth hormone in prostaglandins and dibutyryl adenosine cyclic 3':5'-monophosphate in the absence of protein synthesis. Proc. Natn. Acad. Sci. U.S.A. 67:1172.

McCracken, J.A. and D.T. Baird. 1969. The study of ovarian function by means of transplantation of the ovary in the ewe. The Gonads, K. McKerns (ed.), Appleton-Century-Crofts. New York. Chap. 7, p. 175.

McCracken, J.A., D. T. Baird, and J. R. Goding. 1971. Factors affecting the secretion of steroids by the transplanted ovary in the sheep. Rec. Progr. Hormone Res. 27.

McCracken, J.A., J.C. Carlson, M.E. Glew, J.R. Goding, D.T. Baird, K. Green, and B. Samuelsson. 1972. Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. Nature New Biol. 238:129.

Miller, R.G., Jr. 1966. Simultaneous Statistical Inference. McGraw Hill Book Co. New York, p. 67.

Ojeda, S.R., P.G. Harms, and S.M. McCann. 1974a. Central effect of prostaglandin E_1 (PGE_1) on prolactin release. Endocrinol. 95:613.

Ojeda, S.R., P.G. Harms, and S.M. McCann. 1974b. Possible role of cyclic AMP and prostaglandin E_1 in the dopaminergic control of prolactin release. Endocrinol. 95:1694.

Ojeda, S.R., and S.M. McCann. 1974. Development of Dopaminergic and estrogenic control of prolactin release in the female rat. Endocrinol. 95:1499.

Oxender, W.D., H.D. Hafs, and L.A. Edgerton. 1972. Serum growth hormone, LH and prolactin in the pregnant cow. J. Anim. Sci. 35:51.

Peng, T.C., K.M. Six, and P.L. Munson. 1970. Effects of prostaglandin E_1 on the hypothalamo-hypophyseal-adrenocortical axis in rats. Endocrinol. 86:202.

Pharriss, B.B., and L.J. Wyngarden. 1969. The effect of prostaglandin $F_{2\alpha}$ on the progestin content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92.

Purchas, R.W., K.L. Macmillan, and H.D. Hafs. 1970. Pituitary and plasma growth hormone levels in bulls from birth to one year of age. J. Anim. Sci. 31:358.

Rajakowski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinol. (Kbh.), 34 (Suppl. 52):1-68.

Ratner, A., M.C. Wilson, and G.T. Peake. 1973. Antagonism of prostaglandin-promoted pituitary cyclic AMP accumulation and growth hormone secretion in vitro by 7-oxa-13-prostynoic acid. Prostaglandins 3:413.

Richards, J.S., and A.R. Midgley, Jr. 1975. Protein hormone action: A key to understanding ovarian follicular and luteal cell development. Biol. Reprod. 14:82.

Rotsztejn, W.H., M. Normand, J. LaLonde, and C. Fortier. 1975. Relationship between ACTH release and corticosterone binding by the receptor sites of the adenohypophysis and dorsal hippocampus following infusion of corticosterone at a constant rate in the adrenalectomized rat. Endocrinol. 97:223.

Rowson, L.E.A., R. Tervit, and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J. Reprod. Fertil. 29:145.

Saruta, T., and N.M. Kaplan. 1972. Adrenocortical steroidogenesis: The effects of prostaglandins. J. Clin. Invest. 51:2246.

Sato, T., M. Hirono, T. Jyujo, T. Iesaka, K. Taya, and M. Igarashi. 1975. Direct action of prostaglandins on the rat pituitary. Endocrinol. 96:45.

Sato, T., T. Iesaka, T. Jyujo, K. Taya, J. Ishikawa, and M. Igarashi. 1974. Prostaglandin-induced ovarian ascorbic acid depletion. Endocrinol. 95:417.

Schams, D., and H. Karg. 1970. Untersuchungen uber prolactin im rinderblut mit einer radio-immunologischen bestimmungsmethode. 2 bl. Vet. Med. 17:193.

Schofield, J.G. 1970. Prostaglandin E_1 and the release of growth hormone in vitro. Nature 228:179.

Sinha, Y.N., and H.A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507.

Smith, V.G., L.A. Edgerton, H.D. Hafs, and E.M. Convey. 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. J. Anim. Sci. 36:391.

Spies, H.G., and R.L. Norman. 1973. Luteinizing hormone release and ovulation induced by the intraventricular infusion of prostaglandin E_1 into pentobarbital blocked rats. Prostaglandins 4:131.

Stellflug, J.N., T.M. Louis, R.C. Gorewit, W.D. Oxender, and H.D. Hafs. 1976. Luteolysis induced by prostaglandin $F_{2\alpha}$ before and after hysterectomy in heifers. Biol. Reprod. In Press.

Stellflug, J.N., T.M. Louis, H.D. Hafs, and B.E. Seguin. 1975. Luteolysis, estrus and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglandin $F_{2\alpha}$. Prostaglandins 9:609.

Sundberg, D.K., C.P. Fawcett, P. Illner, and S.M. McCann. 1975. The effect of various prostaglandins and a prostaglandin synthetase inhibitor on rat anterior pituitary cyclic AMP levels and hormone release in vitro. Proc. Soc. Exp. Biol. and Med. 148:54-59.

Swanson, L.V., and H.D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038.

Szabo, M., and L.A. Frohman. 1975. Effects of porcine stalk median eminence and prostaglandin E_2 on rat growth hormone secretion in vivo and their inhibition by somatostatin. Endocrinol. 96:955.

Tucker, H.A. 1971. Hormonal response to milking. J. Anim. Sci. 32, Suppl. 1:137.

Tucker, H.A., D. T. Vines, J. N. Stellflug, and E.M. Convey. 1975. Milking, thyrotropin-releasing hormone and prostaglandin induced release of prolactin and growth hormone in cows. Soc. Exp. Biol. Med. Proc. 149:462.

Thatcher, W.W., and J.R. Chenault. 1976. Reproductive physiological responses of cattle to exogenous prostaglandin $F_{2\alpha}$. J. Dairy Sci. 59:1366.

Tsafriri, A., Y. Koch, and H.R. Lindner. 1973. Ovulation rate and serum LH levels in rats treated with indomethacin or prostaglandin E_2. Prostaglandins 3:461.

Vanderheyden, K., M. Dhont, D. Vandekerckhove, A. Vermuelen, and M. Thiery. 1974. Prostaglandin $F_{2\alpha}$: Effect on cortisol, growth hormone, luteinizing hormone, follicle-stimulating hormone, prolactin and thyroid-stimulating hormone. Obstet. and Gynecol. 2:1164.

Vermouth, N.T., and R.P. Deis. 1972. Prolactin release induced by prostaglandin $F_{2\alpha}$ in pregnant rats. Nature New Biol. 238:248.

Von Euler, V.S. 1935. A depressor substance in the vesicular gland. J. Physiol., Lond. 84:21.

Warberg, J., R.L. Eskay, and J.C. Porter. 1976. Prostaglandin-induced release of anterior pituitary hormones: Structure-activity relationships. Endocrinol. 98:1135.

Wentz, A.C., G.S. Jones, and T. Bledsoe. 1973. Effects of $PGF_{2\alpha}$ infusion on human cortisol biosynthesis. Prostaglandins 3:155.

Yasuda, N., K. Takeba, and M.A. Greer. 1976. Studies on ACTH dynamics in cultured adenohypophyseal cells: Effect of adrenalectomy or dexamethasone *in vivo*. Endocrinol. 98: 717.

Yue, D.K., I.D. Smith, J.R. Turtle, and R.P. Shearman. 1974. Effect of $PGF_{2\alpha}$ on the secretion of human prolactin. Prostaglandins 8:387.

Zor, U., T. Kaneko, H.P.G. Schneider, S.M. McCann, and J.B. Field. 1970. Further studies of stimulation of anterior pituitary cyclic adenosine 3':5'-monophosphate formation by hypothalamic extract and prostaglandins. J. Biol. Chem. 245:2883.