ABSTRACT

MAMMARY ARTERIAL AND VENOUS CONCENTRATIONS
OF PROLACTIN, GROWTH HORMONE AND INSULIN
IN LACTATING COWS

By

Neil F. G. Beck

Serum prolactin concentrations were determined in mammary arterial and venous blood samples from six early and six late lactating cows. In early lactators, serum prolactin concentrations increased from an average of 28 ng/ml 30 min before milking to 48 ng/ml 0 to 20 min after milking, then declined to 32 ng/ml 30 to 70 min post milking. During these same periods in late lactators, serum prolactin concentrations were lower ($P < .05$), averaging 9, 17 and 14 ng/ml. Arteriovenous differences in serum prolactin concentrations averaged .20 ng/ml before milking and .70 ng/ml after milking. Serum insulin concentrations in these samples remained constant, averaging 2.6 ng/ml in early and 3.5 ng/ml in late lactating cows ($P < .1$). Arteriovenous differences in serum insulin concentrations were different from zero ($P < .05$) in early (.11 ng/ml) and late (.16 ng/ml) lactating cows between 0 and 20 min after milking.

Serum prolactin concentrations were measured in mammary arterial and venous blood samples before and after intravenous administration of 200 μg thyrotropin releasing hormone (TRH) followed by milking 30 min later. In early lactators, serum prolactin concentrations averaged 29 for 30 min before and 175 ng/ml for 30 min after TRH. Between 0 and 20 min after milking serum prolactin concentrations averaged 243 ng/ml. In six late lactators, serum prolactin concentrations tended to be lower, averaging 13, 79, and 77 ng/ml during the same periods. Arteriovenous differences in serum prolactin concentrations increased from .4 to 7.7 ng/ml after TRH in early and from -.01 to 2.6 ng/ml in late lactating cows. Arteriovenous differences after TRH were different from zero ($P < .05$). Growth hormone concentrations, determined in the same samples, averaged 3.9 ng/ml for 30 min before TRH in early and late lactating cows. During the 30 min interval after TRH serum growth hormone concentrations increased to 14.9 in early and 11.4 ng/ml in late lactating cows. Between 0 and 20 min after milking, serum growth hormone averaged 7.3 in early and 5.1 ng/ml in late lactating cows. Arteriovenous differences in serum growth hormone increased from -.03 to .58 ng/ml after TRH in early and from -.05 to .73 ng/ml in late lactating cows ($P < .05$).

Five early lactating cows were treated with CB-154 (2-bromo-ergocryptine-methanosulphate) and five late

lactating cows were treated with ergocryptine. Serum prolactin concentrations decreased within 24 hr from 22.9 and 11.9 to 7.2 in early and 1.7 in late lactating cows. When the ergot treated cows were injected during early lactation with 500 μg TRH serum prolactin concentrations increased from 9.3 ng/ml 30 min before, to 113 ng/ml for 0 to 20 min after TRH; thereafter, declining to 90 ng/ml 30 to 70 min post injection. Serum prolactin concentrations in late lactation during the same periods were lower ($P < .05$), averaging 2.0, 20.7 and 12.9 ng/ml. Arteriovenous differences in serum prolactin concentrations increased from -.14 to 6.3 ng/ml after TRH in early lactators and from -.18 to -.24 ng/ml in late lactators. Serum growth hormone concentrations averaged 5 ng/ml before, 20.3 ng/ml 0 to 20 min after TRH, and 7.4 ng/ml 30 to 70 min after TRH in early lactating cows. In late lactating cows, serum growth hormone concentrations were reduced to ($P < .05$) 3.5, 12.8 and 5.2 ng/ml during the same periods. Arteriovenous differences in serum growth hormone increased from .08 ng/ml before to .6 ng/ml after TRH in early and late lactating cows.

Prolactin concentrations in skim milk samples collected from early and late lactating cows before administration of TRH or ergot drugs averaged 24.6 and 11.4 ng/ml, respectively. Administration of either 200 or 500 μg TRH 30 to 70 min before milking did not affect prolactin

concentrations in skim milk. However, treatment of early lactating cows with CB-154, and late lactating cows with ergocryptine reduced skim milk prolactin to 11.9 and 7.0 ng/ml, respectively. Intramammary injection of 50 mg prolactin or saline did not increase mammary venous prolactin concentrations in cows treated with ergot drugs.

Within a stage of lactation, high ambient temperatures (> 18 C) compared with low ambient temperatures (< 18 C) were associated with greater serum prolactin concentrations, before and after milking or injecting TRH.

I concluded that greater intensity of lactation in cows is associated with greater serum prolactin concentrations. Also, greater mammary uptake of prolactin and growth hormone occurred at intervals when these hormones were acutely increased in serum.

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INTRODUCTION

Hormonal control of lactation has been investigated for a number of years. The question of how changes in availability of certain hormones control lactation has been approached in a number of ways.

The most direct approach has been simple to remove the source of origin of a particular hormone from the lactating animal and observe the affect on milk production. Then if removal of the hormone decreased milk production, studies are usually made to determine whether replacement of the hormone will restore lactation. This approach will answer the question of whether or not the hormone is needed by the mammary gland. However, it will not answer the question, whether in the normal physiological situation, if variation in availability of a hormone to the lactating mammary gland will result in a corresponding change in milk production.

Initially, the only way to monitor changes in hormone concentrations in vivo was bioassays. However, these assays were usually only sufficiently sensitive to measure hormone concentrations at the endocrine gland where the hormone was produced. Therefore, one could only

relate changes in concentration of a hormone, within endocrine glands with changes in lactational performance. However, concentration of a hormone within an endocrine gland is dependent upon at least two variables, i.e., synthesis and secretion rates, and drawing conclusions from such studies was difficult.

With the advent of radioimmunoassays, it became possible to determine very precisely hormone concentrations in blood. Changes in serum hormone concentration in blood could be related to changes in lactational performance. Therefore, if serum concentrations of a hormone was statistically related to milk production, one might infer that this hormone is importantly associated with lactation.

In the past few years, it has been demonstrated that protein hormones physically bind to their target organ in vitro. The mammary gland of the cow is a large target organ for several hormones. We wished to determine whether variation in serum concentration of prolactin, growth hormone, and insulin, three polypeptide hormones implicated in the control of lactation, was associated with parallel changes in mammary uptake of these hormones in vivo. Furthermore, we wished to evaluate whether the uptake of these hormones was effected by stage of lactation, a variable that importantly affects quantity of milk produced.

REVIEW OF LITERATURE

A. Hormonal Requirements of the Lactating Mammary Gland in Established Lactation

It is now well established that the mammary gland requires hormonal stimulation in order to function (Cowie and Tindal, 1971; Convey, 1974; Meites, 1974; Tucker, 1974). The question of which hormones are required has been approached in three major ways: (1) whether absence of a certain hormone affects lactation; (2) following its absence will subsequent replacement of the hormone restore previous lactational performance; and (3) will increased availability of the hormone alter lactational performance. In spite of species variation, it has been generally established that prolactin, growth hormone, thyroid stimulating hormone (TSH) or thyroid hormones, adenocorticotrophic hormone (ACTH) or glucocorticoids, oxytocin, and to a lesser extent insulin and ovarian steroids all influence lactation. Since this research investigates the change in and mammary uptake of prolactin, growth hormone and insulin during established lactation, these hormones will be discussed in detail, initially by describing their normal variation in their endocrine organ source, in blood, and then how lactational performance is

affected when they are administered or removed from a lactating animal.

1. The Role of Prolactin in Established Lactation

In 1937, Reece and Turner observed that pituitaries of lactating cows contained more prolactin than those from nonlactating animals, and pituitaries from lactating dairy cattle contained more prolactin than those from lactating beef cows. More recently the use of radioimmunoassays has established that the "milking" or "sucking" stimulus temporarily increases blood serum prolactin concentrations in rats (Amenomori et al., 1968), goats (Bryant et al., 1968; Hart, 1975a), sheep (Lamming et al., 1974), and cows (Johke, 1969 and 1970; Tucker, 1971; Schams, 1972a; Koprowski and Tucker, 1973a). In cattle, increased serum prolactin concentrations are observed within 5 minutes of milking (Johke, 1969 and 1970; Schams and Karg, 1970; Schams, 1972a; Koprowski and Tucker, 1973a). Koprowski and Tucker (1973a) determined that serum prolactin concentrations post milking do not return to their pre-milking values for approximately 35 minutes. The half life of serum prolactin in lactating cows is approximately 25 to 29 minutes (Johke, 1969; Schams and Karg, 1970; Tucker et al., 1972). Johke (1970) and Koprowski and Tucker (1973a) observed that the amount of prolactin released from the pituitary at milking declined with

advancing stage of lactation, but Schams (1972a) maintained that the effects of stage of lactation on prolactin release from the pituitary were minor compared with those of season.

Schams and Karg (1970) previously reported that blood serum prolactin concentrations were related more to month of year than to stage of lactation or milk yield. The effect of season on serum prolactin concentrations was confirmed by Koprowski and Tucker (1973a) who observed that basal concentrations of prolactin were higher in summer than in winter. However, greater quantities of prolactin were released in response to milking in winter than in summer. Effect of season on prolactin concentrations are primarily associated with changing day length (Bourne and Tucker, 1975) and ambient temperature (Wettemann and Tucker, 1974).

Koprowski and Tucker (1973a), observed that within-stage-of-lactation correlations between serum prolactin and milk yield were very low and sometimes negative during the first 3 to 6 months of lactation. However, during the remainder of lactation, correlations were consistently positive and ranged from .08 to .48. Average monthly milk production and average serum prolactin concentrations measured immediately after milking were highly correlated (.96). These results suggest prolactin plays a role in milk production in cattle (but

it is not necessarily a direct, cause-effect type relationship).

In addition to lactation, other physiological states influence pituitary and blood serum prolactin concentrations in the bovine. After birth, pituitary prolactin content of heifers increases, reaching a maximum at nine months of age (Sinha and Tucker, 1969). These changes were associated with changes in mammary development. Sinha and Tucker (1969) observed increased pituitary prolactin concentrations in cattle around estrus. However, reports on changes in blood serum concentrations during the estrous cycle in cattle differ. Swanson et al. (1972) observed increased blood serum prolactin concentration around estrus in nonlactating heifers, whereas Koprowski and Tucker (1973a) and Wettemann and Hafs (1971) were unable to detect any differences in serum prolactin concentrations attributable to changes in the estrous cycle in lactating cows and heifers. Serum prolactin concentrations, in blood samples collected one hour before and one hour after milking, were significantly greater in pregnant than in nonpregnant lactating cows; however, serum prolactin concentrations measured immediately after milking were no different in pregnant compared with nonpregnant lactating cows (Koprowski and Tucker, 1973a). Wettemann and Hafs (1971) were unable to detect any significant changes in serum prolactin concentration

during early pregnancy in cattle, but increased serum prolactin (Oxender et al., 1972) and pituitary prolactin concentrations (Reece and Turner, 1937) have been observed in cattle in late pregnancy. Marked increases in serum prolactin concentrations, possibly associated with the initiation of lactation, occur just prior to parturition in cattle (Schams and Karg, 1970; Johke et al., 1971; Ingalls et al., 1973).

Serum prolactin concentrations in lactating cattle are affected by various other stimuli: washing the udder and venipuncture (Tucker, 1971), washing the brisket (Koprowski et al., 1971) and feeding (Johke, 1970; McAtee and Trenkle, 1971). Stress increases blood serum prolactin concentrations in rats (Krulich et al., 1974; Riegle and Meites, 1976). Advancing stage of lactation and season of the year affect prolactin release in goats as in cows (Hart, 1975a). Serum prolactin concentrations were greater post partum than prepartum period in ewes (Arai and Lee, 1967; MacNeilly, 1971). Lactating ewes had greater pituitary secretion rates, metabolic clearance rates, and blood serum concentrations of prolactin than pregnant, anestrus, or ovariectomized animals (Davis and Borger, 1973). Threfall et al. (1974) observed that in suckled lactating sows pituitary and blood serum prolactin concentrations were approximately four times greater

than those of suckled agalactic animals at the same stage post partum.

The author is unaware of any studies in which hypophysectomy has been used as a means of removing prolactin from lactating cattle. In goats surgically hypophysectomized during lactation, milk yield falls to essentially zero (Cowie, 1964). Ovine prolactin alone, or in combination with adrenal corticoids, or bovine growth hormone in combination with glucocorticoids only partially restored milk yield. However, a combination of ovine prolactin, bovine growth hormone and adrenal glucocorticoids caused a substantial restoration of milk yield. When ovine prolactin, bovine growth hormone and adrenal glucocorticoids were given in combination with triiodothyronine, milk yield was fully restored to pre-hypophysectomy levels. In hypophysectomized rabbits, milk yield may be restored by prolactin alone (Cowie et al., 1969). A combination of prolactin, growth hormone, and glucocorticoids will partially restore milk yield in hypophysectomized rats (Lyons, 1958).

Ergot alkaloids and their derivatives markedly decrease serum prolactin concentrations in rats (Nagasawa and Meites, 1970), sheep (Niswender, 1972), goats (Hart, 1973; McMurtry and Malven, 1974a), cows (Karg et al., 1972; Fell et al., 1974; Smith et al., 1974), and women (Brun del Re et al., 1973). Cows treated with CB-154

(2-bromo-α-ergocryptine-methanosulphate) in late pregnancy and early lactation had depressed milk yields (Fell et al., 1974; Karg and Schams, 1974). Although Schams and Karg (1974) claimed to have observed similar effects in cows treated with CB-154 in established lactation, Smith et al. (1974) using a much larger number of experimental animals were unable to detect any significant change in milk yield, even though serum prolactin concentrations were markedly decreased. Similarly, the administration of CB-154 to goats had no effect on milk yield (Hart, 1973). However, in this species, CB-154 increases the quantity of growth hormone released at milking, which may compensate for the comparative absence of prolactin. In lactating rats injected with ergot alkaloids, Shaar and Clemens (1972) observed decreases in mammary weight and depressed litter weight gains. Since CB-154 may also depress food intake, Tomogane et al. (1975) restricted food intake of the control animals to that of the treated animals. Nevertheless litter weight gains of treated animals were still less than those of controls. Administration of CB-154 to rabbits (Taylor and Peaker, 1975) and women (Brun del Re et al., 1973) caused an abrupt cessation of lactation. In summary, CB-154 is a potent inhibitor of prolactin and, except in ruminants, of lactation.

Since the availability of endogenous hormone may be a limiting factor during established lactation,

exogenous hormone has been administered in an attempt to enhance lactation. The effect of administration of ovine prolactin to early lactating rats is dose dependent (Tucker, 1974). Daily injections of one milligram of ovine prolactin had no effect on litter weight gains but 3 mg per day did stimulate milk secretion, possibly through increasing metabolism. Neither injections of sheep prolactin nor prolactin from isotransplanted pituitaries will maintain litter weight gains in rats during extended lactation (Tucker, 1974). Injections of prolactin to lactating cows (Folley and Young, 1940; Cotes et al., 1949), ewes (Denamur and Martinet, 1970; Morag et al., 1971) and guinea pigs (Nagasawa and Naito, 1963) were without effect on milk yields. But injections of ovine prolactin markedly enhance lactation in rabbits (Cowie, 1969; Taylor and Peaker, 1975). Hart (1975b) prevented the seasonal decline in the amount of prolactin released at milking in lactating goats by artificially extending daily photoperiod for three months. But milk yields declined in a manner similar to those of control animals which were exposed to natural changes in daylength.

Synthetic thyrotropin-releasing hormone, pyroglutamyl-histidyl-proline amide (TRH), increases serum concentrations of prolactin, growth hormone, and thyroxine in lactating cows (Convey et al., 1973a), and growth hormone and prolactin in dairy bulls (Convey et al., 1973a;

Tucker et al., 1975) and prepubertal heifers (Vines et al., 1976). Convey et al. (1973a) observed that in lactating cows increases in serum prolactin and thyroxine concentrations, following intravenous administration of TRH, were not proportional to dose. In contrast growth hormone increased proportionally to the dose of TRH. But later Vines et al. (1976) found that in prepubertal heifers, the amount of prolactin and growth hormone released following TRH administration was significantly related to dose of TRH and baseline concentrations of serum hormones. It was suggested by Vines et al. (1976), as a possible explanation for this discrepancy, that the greater variation in serum prolactin concentrations in lactating cows probably masked any dose-response effect.

Since TRH increases serum concentrations of prolactin, growth hormone and thyroxine, three hormones implicated as being important in lactation, Convey et al. (1973b) investigated the galactopoietic potential of this releasing hormone. Forty lactating cows receiving twice daily injections of 50 μg TRH for 10 days had significantly greater milk yields (on average 0.66 kg/cow/day) during the last 5 days of treatment than when they were injected with saline alone. Neither Kelly et al. (1973), nor Schams et al. (1974) who gave greater doses of TRH to fewer animals over a shorter period of time were able to detect any increase in milk yields. Since

TRH caused only a small increment in milk yield (Convey et al., 1973b), it is not surprising that Kelly et al. (1973) and Schams et al. (1974), using only four late lactating cows, were unable to detect any change in milk yield. However, Schams et al. (1974) claimed that TRH given during the peripartum period would increase subsequent milk production 10 to 50%. Adams et al. (1973) failed to stimulate milk production in rats treated with TRH. In lactating women, Tyson et al. (1972) were able to cause breast engorement by injecting TRH intravenously, but Zarate et al. (1976) were unable to enhance lactation in women given 20 mg of TRH orally, three times a day, despite increased serum prolactin concentrations. Zarate et al. (1976) suggested that the difference between their results and those of Tyson et al. (1972) may have been associated with route of administration. In concluding this section, once TRH increases serum concentrations of prolactin, growth hormone and thyroxine, its effect on lactation is probably the result of the galactopoietic action of all three of these hormones and not just the single effect of one of them.

2. The Role of Growth Hormone in Established Lactation

Stimuli associated with suckling or milking increase serum concentrations of growth hormone in rats (Grosvenor et al., 1968; Tucker and Thatcher, 1968) and

goats (Hart and Flux, 1973), but not cows (Reynaert and Peters, 1972; Koprowski and Tucker, 1973b). Serum concentrations of growth hormone decrease significantly during the first 6 weeks of lactation in cattle (Smith et al., 1976) and are negatively correlated with milk yield (Koprowski and Tucker, 1973b). Koprowski and Tucker (1973b) observed that in pregnant, lactating cows, serum growth hormone concentrations remained constant with time. However, the use of covariate analysis showed that when effects of advancing stage of concurrent pregnancy are held constant, serum growth hormone concentrations decrease with advancing lactation. Conversely when effects of advancing stage of lactation are held constant, serum growth hormone concentrations increase with advancing pregnancy. Vines (1976) observed that in cattle TRH released significantly greater quantities of growth hormone during the second month of lactation than at any other stage. Hart et al. (1975) observed that serum growth hormone concentrations of lactating cows were significantly greater in dairy cattle than in lower milk yielding beef animals. Furthermore, Fitko et al. (1969) found that ^{131}I growth hormone disappeared at a slower rate from blood of high producing cows than from blood of low producing or dry cows. Overall these studies suggest that greater serum concentrations of growth hormone are associated with higher milk production in cows.

Other physiological states, apart from lactation, influence pituitary and blood concentrations of growth hormone in cattle. Pituitary growth hormone content is greater in prepubertal cattle than in post pubertal cattle (Armstrong and Hansel, 1956; Purchas et al., 1970). Plasma growth hormone concentrations in cattle were greater at birth than between 2 and 12 months of age, after which time they remained stable (Purchas et al., 1970). Therefore, growth hormone may be more actively synthesized and secreted from the pituitaries of prepubertal cattle during the first two months after birth.

Koprowski and Tucker (1973b) observed that serum growth hormone concentrations were greater during the estrogenic phase than during the luteal phase of an estrous cycle in lactating cows. When effects of advancing stage of lactation are held constant in a covariate analysis, serum growth hormone concentrations increase linearly with advancing pregnancy in cows (Koprowski and Tucker, 1973b). However, in unadjusted data, serum growth hormone concentrations remain constant with advancing pregnancy in lactating cows (Oxender et al., 1972; Koprowski and Tucker, 1973b). Serum growth hormone levels remain constant throughout pregnancy in heifers (Koprowski and Tucker, 1973b). Ingalls et al. (1973) observed constant serum growth hormone concentrations from 26 to 9 days prepartum. Growth hormone concentrations began to

increase at 9 days prepartum and were maximum at parturition. In contrast, Reynaert et al. (1976) were only able to detect increased serum growth hormone concentrations, in cows and heifers, during the act of parturition. The cause of this abrupt increase in serum growth hormone concentrations is debatable. In cattle, serum growth hormone concentrations are inversely related to free fatty acid concentrations (Reynaert et al., 1975a). Stress in cattle causes a decline in blood free fatty acid concentrations and finally an increase in growth hormone concentration (Shirley et al., 1973; Reynaert et al., 1975b). However, in cows and heifers, serum free fatty acid concentrations began to rise 1 day prepartum and remained elevated for at least 5 days post partum (Reynaert et al., 1976). Therefore, it is unlikely that a decrease in serum free fatty acid concentrations was responsible for the abrupt increase in serum growth hormone concentrations at parturition. Reynaert et al. (1976) suggested that increased concentrations of prostaglandin in blood at parturition may cause an increase in growth hormone concentrations.

In rats (Lyons, 1958) and goats (Cowie, 1964), hypophysectomized during lactation, exogenous growth hormone, in combination with other hormones, helped restore lactation. Growth hormone is galactopoietic in ruminants (Cowie, 1961; Meites, 1961). A number of workers

have observed increased milk production in response to growth hormone injections in lactating cows. Hutton (1957) gave single injections of growth hormone and found a significant linear relationship between log weight of hormone injected and increase in milk volume. Shaw et al. (1955) observed appreciable increases in milk production in cows at different stages of lactation given daily injections of 100 mg growth hormone. A marked increase in milk production occurred in twin cattle given subcutaneous injections of 50 mg of growth hormone for a 12-week period beginning at the peak of lactation and for a 4-week period in late lactation (Brumby and Hancock, 1955). It is unlikely that results of these earlier workers reflect independent effects of growth hormone since preparations injected were contaminated with prolactin and thyrotropin. Therefore, Bullis et al. (1965) compared in cows the galactopoietic potencies of commercial grade and highly purified growth hormone preparations. Changes in milk production during a 10-day injection period were 7.6, 3.4, and -4.4%, respectively, for commercial growth hormone, highly purified growth hormone, and saline. The increase in production caused by highly purified growth hormone was not significantly different from that of the saline control group. However, milk production in the cows given highly purified preparation was greater than that during the pre-injection period. In contrast,

Machlin (1973) gave only half the dose of growth hormone used by Bullis et al. (1965) of a highly purified growth hormone preparation for the same period of time and obtained an 18% increase in milk production. In these experiments, Machlin (1973) noted that milk production continued to increase during the injection period and that the increased production persisted for two days after the final injection. Furthermore, the galactopoietic action of growth hormone did not diminish in long-term experiments where lactating cows were injected only three times a week for 10 weeks. Growth hormone is not galactopoietic in goats (Tomov, 1963), ewes (Denamur and Martinet, 1970) and women (Lyons et al., 1968). However, conflicting results have been obtained in rats (Tucker, 1974).

The effect of CB-154 or TRH, alone or in combination, on serum growth hormone concentration varies with species. In cattle, CB-154 has no effect on serum growth hormone concentrations (Smith et al., 1974), however, in goats (Hart, 1974) it increased the amount of growth hormone released to the milking stimulus. In normal women, CB-154 decreases growth hormone concentrations but in acromegalic women it increases them (Camanni et al., 1975). In contrast, Benker et al. (1976) observed that CB-154 depressed serum growth hormone concentration but did not prevent TRH-stimulated release of growth hormone in humans.

Investigations into the effect of TRH on serum growth hormone concentrations in humans have produced conflicting results. Anderson et al. (1971) found no change, whereas, Torjesen et al. (1973) obtained increased serum growth hormone concentrations in response to TRH in normal humans. In acromegalic humans, Cryder et al. (1973) obtained increased serum concentrations of growth hormone in response to TRH. TRH increases serum growth hormone concentrations in normal rats (Takahara et al., 1974) and in hypophysectomized rats bearing ectopic pituitaries (Udeschinia et al., 1976). In lactating cows, the amount of pituitary growth hormone released was in proportion to the dose of TRH used (Convey et al., 1973a). In conclusion, even though growth hormone has a potent galactopoietic action in cattle, its serum concentrations remain remarkably constant throughout lactation.

3. The Role of Insulin in Established Lactation

In cattle, serum insulin concentrations increase during early lactation and are inversely related to serum growth hormone concentrations (Koprowski and Tucker, 1973b; Hart et al., 1975; Hove, 1975; Smith et al., 1976). Koprowski and Tucker (1973b) showed that serum insulin concentrations increased 2 to 3 fold between weeks 4 to 12 of lactation and that blood samples collected during milking contained greater serum concentrations of insulin

than those collected 2 to 4 hours before or one hour after milking. That the milking stimulus may cause the pancreas to release insulin is supported by recent evidence that stimulation of the rat hypothalamus (Curry and Joy, 1974) or stimulation of the vagus nerve of the dog pancreas (Bergman and Miller, 1973) cause insulin release. Hart et al. (1975), using early lactating beef and dairy cows, obtained an inverse relationship between serum concentrations of insulin and growth hormones, and noted that insulin and glucose concentrations were significantly lower in beef as compared with dairy cows. Hart et al. (1975) suggested that higher serum concentrations of insulin and glucose, in the lower producing beef cows, were associated with lower mammary uptake of glucose. This hypothesis is consistent with the negative correlation obtained between serum insulin concentrations and milk yield obtained by Tucker and Koprowski (1973b). Glucose, infused intravenously, increases serum insulin concentrations in cattle (McAtee and Trenkle, 1971). A significant increase in plasma insulin and glucose concentrations occurs during the first 3 to 5 weeks of lactation in cows (Smith et al., 1976) and animals which had very low concentrations of plasma glucose and insulin in early lactation tended to develop ketosis later (Schwalm and Schultz, 1976).

Hove and Blom (1973) observed increased insulin and decreased plasma glucose concentrations at feeding in cows. Bassett (1974) observed, during feeding in sheep, an increase in plasma insulin and a decrease in plasma growth hormone concentrations which were unrelated to changes in blood metabolite concentrations. He suggested that the changes in insulin and growth hormone concentrations were caused by gastrointestinal hormones, reflexly released at feeding. Hove (1975) and Halse et al. (1976) determined growth hormone, insulin and glucose concentrations in blood plasma from lactating cows during the day when blood concentrations of insulin, growth hormone and glucose are effected by feeding and during the night when they are affected to a lesser degree. In plasma samples collected during the daytime, a negative correlation existed between plasma insulin and glucose concentrations but in samples collected during night time the correlation was positive.

The udder of the lactating cow utilizes 60 to 85% of glucose entering the blood system (Annison and Linzell, 1964; Kronfeld and Emery, 1970), and it is therefore of little surprise that hypoglycemia, resulting from administration of insulin causes a depression in milk yields in lactating cows (Cowie, 1961; Kronfeld et al., 1963; Schmidt, 1966). However, if blood glucose concentrations

are maintained constant, infused exogenous insulin stimulates lactation.

The role of insulin in lactation in rats is also important because surgical removal of the pancreas or administration of the diabetogenic agent alloxan reduces litter weight gain in lactating rats (Tucker, 1974).

B. Mammary Binding of Prolactin, Insulin, and Growth Hormone in Vitro and Its Subsequent Effects

It is pertinent to consider whether hormonal maintenance of lactation is the result of the direct action of hormones on the mammary gland; the result of indirect extramammary effects or a combination of both. That hormones have a direct effect on the mammary gland has been established in studies where the mammary gland is isolated in an *in vitro* culture system (Juergens et al., 1957; Wood et al., 1975). However, this does not dismiss the possibility that extramammary effects, e.g., stimulation of other target organs or increased blood metabolite concentrations, are involved. Evidence that prolactin, growth hormone and insulin bind specifically to target organs other than the mammary gland will be presented at the beginning of this section. Later, mammary binding of prolactin, growth hormone and insulin *in vitro*, will be considered, and finally metabolic stimulation of mammary cells by protein hormones and excretion of prolactin in milk will be discussed.

Recently prolactin, growth hormone, and insulin have been found to bind specifically to tissues other than the mammary gland. For example, ^{125}I labelled prolactin, growth hormone, and insulin, bind specifically to liver, adrenal and heart tissue from many species (Kelly et al., 1974; Posner et al., 1974; Aragona and Friesen, 1975; Donatsh and Richardson, 1975; Marshall et al., 1975; Saito and Saxena, 1975; Rolland and Hammond, 1975; Posner, 1976). Liver membranes from a number of species specifically bind ^{125}I labelled prolactin, growth hormone and insulin (Kelly et al., 1974; Posner, 1976; Gelato et al., 1975). The quantity of those hormones specifically bound to liver varied with physiological state. Membranes from fetal and immature female rat livers bound much less human growth hormone and ovine prolactin than those of adult females (Kelly et al., 1974). Furthermore, specific binding of human growth hormone and ovine prolactin increased markedly at puberty and pregnancy. In male rats, Kelly et al. (1974) observed that the liver membranes of adults bound more human growth hormone and ovine prolactin than liver membranes from fetal or immature animals. Quantitatively, the liver membranes of male rats specifically bound less hormone than liver membranes from female rats, irrespective of stage of development. Specific binding of ^{125}I labelled insulin to liver membranes was quantitatively identical in both sexes and at all stages

of development (Kelly et al., 1974). Gelato et al. (1975) observed that prolactin binding capacity is depressed in liver membranes from thyroidectomized, ovariectomized or thyroidectomized-ovariectomized female rats compared with those of intact controls, and hormone therapy with thyroxine or estrogen alone or in combination will restore the specific binding capacity of prolactin to control values.

^{125}I-labelled prolactin, growth hormone and insulin binding to liver membranes has also been investigated in rabbits, guinea pigs and mice. Kelly et al. (1974) observed that liver membranes from fetal or immature rabbits bound significantly less human growth hormone and ovine prolactin than liver membranes from mature female adults. In contrast, liver membranes from pregnant rabbits bound significantly more human growth hormone and ovine prolactin than liver membranes from mature females. Unlike rats, rabbit liver membranes from both sexes bound equal amounts of ovine prolactin and human growth hormone. The amount of ^{125}I insulin which binds to rabbit liver membranes is constant for all stages of development. Liver membranes from guinea pigs did not bind significant amounts of human growth hormone or ovine prolactin, but did bind a significant quantity of insulin. Liver membranes from pregnant animals bound significantly more ^{125}I insulin than liver membranes from animals in other

physiological states. Posner (1976) observed that mouse liver membranes bound significant quantities of human growth hormone, ovine prolactin and insulin. Compared with liver membranes from adult female mice, liver membranes from fetal and male mice bound less, and liver membranes from pregnant mice bound more ovine prolactin and human growth hormone. Furthermore the specific binding of bovine growth hormone to mouse liver membranes was negligible, except during pregnancy when liver membranes bound a significant amount of this hormone. The specific binding of ^{125}I labelled insulin to mouse liver membranes was constant at all stages of development.

Marshall et al. (1975) investigated the effect of different physiological conditions on the specific binding of prolactin to rat kidney membranes. Compared with control animals, specific binding of prolactin to kidney membranes was unaffected by water deprivation, salt loading or unilateralnephrectomy. In the same study, both salt loading and nephrectomy significantly increased the specific binding of prolactin to adrenal tissue.

Collectively, the above studies suggest not only that binding of prolactin, growth hormone and insulin to their various target organs is of physiological significance (binding changes with physiological state), but also that galactopoietic actions of these three hormones may involve target organs in addition to the mammary gland.

A number of workers have investigated protein hormone binding to receptors in the membrane fraction isolated from the mammary glands of laboratory species. However, the author is unaware of similar work in ruminants. Frantz et al. (1974) isolated prolactin receptors from mammary glands of pregnant or lactating mice. These receptors, specifically bound biologically active ^{125}I-ovine prolactin. Unlabelled ovine prolactin and to a lesser extent human growth hormone and placental lactogen competitively displaced labelled prolactin from its receptors. Binding of the ^{125}I-ovine prolactin to the receptor occurred over a range of physiological concentrations and took 3 minutes to complete. Compared with normal receptors, receptors pretreated with trypsin, bound 60% less ^{125}I-prolactin, suggesting a component of the receptor is protein. The number of specific, saturable binding sites of labelled prolactin was greater in lactating mammary tissue than in mammary tissue from mid-pregnant mice, indicating the specific binding of prolactin changes with the physiological state of the gland.

Shiu and Friesen (1974) investigated the physical properties of prolactin receptors from rabbit mammary gland. These receptors bound human, simian, ovine, bovine, and murine prolactin, human growth hormone and human placental lactogen. The binding was dependent on pH, temperature and the ionic composition of the incubation

media. Unlike observations in mice that specific mammary binding of prolactin was greater in lactation than in pregnancy (Frantz et al., 1974), binding capacity of rabbit mammary tissue for prolactin did not change from pregnancy to lactation. Furthermore, under the same physical conditions, the time it took for the binding process to be half completed, was considerably longer in the rabbit (5 hr) than in the mouse (3 min). Enzymic pretreatment with either trypsin or phospholipase C reduced the binding capacity of rabbit mammary receptors for prolactin. This suggested that the receptor contained both a protein and a phospholipid component. Holcomb et al. (1976) studied specific prolactin binding to rat mammary tissue. Under optimal physical conditions the binding process took 3 hr to reach equilibrium. Rat mammary receptors were specific for prolactin and their binding capacity was completely abolished by pretreatment with trypsin and reduced 51% by phospholipase C. Specific binding of ^{125}I-labelled ovine prolactin to rat mammary slices was consistently low during pregnancy but increased 3 to 6 fold after parturition. Holcomb et al. (1976) maintained that low mammary binding of ovine prolactin during pregnancy was the result of high serum concentrations of placental lactogen "masking" the prolactin binding sites, since removal of the gravid uterus and ovaries during pregnancy resulted in a large increase in

binding. However, I question this hypothesis since if rat prolactin and placental lactogen are competing for the same binding sites, then in vitro the placental lactogen should be competitively displaced by ^{125}I-labelled prolactin. The only possible way placental lactogen could "mask" prolactin binding sites is by irreversibly binding to the prolactin receptor.

Qualitative studies using autoradiography on mice, rats, and rabbits indicated that biologically active ^{125}I-prolactin binds to the apical membrane of the mammary cell (Birkinshaw and Falconer, 1972; Rajaniemi et al., 1974). However, those workers were unable to detect any hormone inside the cell. Turkington (1970) demonstrated that prolactin bound to an inert carrier (Sepharose beads) was as biologically active as free hormone in stimulating mammary cell metabolism. Since prolactin bound to Sepharose is unable to enter the cell, this work is evidence for a prolactin receptor located in the surface membrane of the mammary cell. Collectively, the above studies support the concept that mammary cells have specific membrane receptors for prolactin and therefore the hormone does not need to enter the cell to be active. However, the mechanism of action for prolactin may not be exclusively through receptors at the cell surface since Chomczynski and Topper (1974) demonstrated that placental lactogen and prolactin stimulated ribonucleic acid

synthesis in isolated nuclei from lactating rat and mouse mammary cells, but were without effect on nuclei from kidney or liver cells. Insulin failed to stimulate ribonucleic acid synthesis in any of the isolated nuclei, which is consistent with evidence that insulin acts via a cell membrane receptor (Cuatrecassas, 1969). More recently, Nolin and Witorsch (1976) using a horse radish peroxidase immunoprecipitation technique discovered the presence of endogenous immunoreactive prolactin inside rat mammary epithelial cells during lactation. It would therefore seem possible that prolactin may not only act through a cell membrane receptor but also through an intracellular receptor.

Studies using insulin bound to an inert carrier (Sepharose) indicate that the mechanism of action of insulin is through a brief interaction with a membrane receptor on the surface of the mammary cell (Cuatrecassas, 1969; Oka and Topper, 1972). However, more recently Kalb et al. (1975) claimed that insulin dissociated from its carrier and therefore its mechanism of action may not be exclusively a surface phenomena. O'Keefe and Cuatrecassas (1974) investigated ^{125}I-insulin binding to mammary epithelial cells from pregnant and lactating mice. Compared with fat cells, mammary cells exhibited very similar physical binding characteristics and constants for ^{125}I-insulin. Cells from fat tissue and cells from

mammary tissue of pregnant and virgin animals each possessed approximately 1000 binding sites for insulin. The number of binding sites per cell increased four fold for lactating mammary tissue, compared with tissue from pregnant or virgin mice. ^{125}I-labelled insulin stimulated ^{3}H-uridine incorporation into nucleic acid and physiological concentrations of unlabelled native hormone competitively displaced receptor bound ^{125}I-labelled insulin.

The above evidence indicates that protein hormones bind specifically to receptors located on the surface membrane of mammary cells. The hormone receptor interaction probably results in a change in the concentration of some intracellular messenger. For example, Rillema (1975, 1976) observed a stimulatory effect of prolactin on ribonucleic acid and subsequent casein synthesis in mouse and rat mammary explants which could be mimmicked by increasing tissue concentrations of cyclic guanosine monophosphate or prostaglandins of the F series. Conversely, the stimulatory effect of prolactin on cell metabolism could be blocked by increasing intracellular concentrations of cyclic adenosine monophosphate or prostaglandins of the A or E series. Since the effects of prolactin, cyclic mononucleotides and prostaglandin were nonadditive, Rillema (1975, 1976) suggested that cyclic mononucleotides

and prostaglandins probably act as intracellular messengers in the mechanism of action of prolactin.

Speake et al. (1975, 1976) showed that insulin, glucocorticoids and prolactin were essential for synthesis, activity and degradation of the enzyme fatty acid synthetase in mammary explants from mid-pregnant rabbits. Prolactin caused a rapid decrease in the rate of degradation of this enzyme. The enzyme lactose synthetase is also responsive to prolactin. Following hypophysectomy, there is a four fold decline in lactose synthetase activity in the mammary gland of the lactating rabbit (Jones and Cowie, 1972). However, this may be reversed by administration of prolactin, but not growth hormone. Prolactin will normally induce milk secretion in pseudopregnant rabbits but when administered simultaneously with progesterone, secretion is blocked and the expected increase in lactose synthetase activity prevented (Assairi et al., 1974). However, prolactin-stimulated-RNA-synthesis was not affected, suggesting that prolactin may stimulate mammary metabolism by a number of intracellular routes, one of which is unaffected by progesterone. Zinder et al. (1974) observed that in lactating rats, lipoprotein lipase activity was much greater in mammary than in adipose tissue and that hypophysectomy of lactating rats caused a marked decrease in mammary lipoprotein lipase activity and increased adipose lipoprotein lipase activity. However,

treatment of hypophysectomized animals with prolactin restored mammary lipoprotein lipase activity and decreased adipose lipoprotein lipase activity. Field and Coore (1976) showed that insulin and prolactin withdrawal decreased mammary gland pyruvate dehydrogenase activity and activity was restored with insulin and prolactin replacement therapy.

In addition to its effects on nucleic acid synthesis and enzyme systems, prolactin affects milk composition by changing mammary epithelium permeability. Linzell et al. (1975) observed that exogenous prolactin restored ion and lactose concentrations in milk, and permeability of mammary epithelium to ^{14}C-sucrose of late lactating rabbits to that normally found in early lactation. Prolactin stimulates sodium transport, and consequently water movement across the cells of the amniotic membrane (Holt and Perks, 1975), intestine and gall bladder (Mainoya et al., 1974; Mainoya, 1975).

The fate of the prolactin molecule after it has reacted with its receptor is as yet unknown. Since its binding to mammary tissue is competitive, it may be displaced by another prolactin molecule or possibly metabolized after binding to liver and kidney cells. In the lactating animal, one route of excretion of prolactin is into milk. Concentrations of prolactin, similar to those in blood, have been determined in milk of rats, goats,

cows (McMurtry and Malven, 1974a; McMurtry and Malven, 1974b; McMurtry et al., 1975), and humans (Gala et al., 1975). Unless there exists a very rapid transcellular transport system for prolactin in the mammary gland, evidence to date indicates that prolactin enters milk, from the blood stream, via a paracellular route. Since administration of exogenous ovine prolactin to rats (McMurtry and Malven, 1974a) or increased endogenous prolactin concentrations in goats (McMurtry and Malven, 1974b) results in a prompt increase in milk prolactin concentration. The rapid increase in milk prolactin concentrations in response to increased blood prolactin concentrations suggests that prolactin enters the milk by simple diffusion between the mammary cells. It is noteworthy, however, that milk prolactin concentrations remained high after serum prolactin concentrations had returned to their original values (McMurtry and Malven, 1974b; Grosvenor and Whitworth, 1976). Why the return passage of prolactin from the milk into the blood is restricted is unknown. It is possible that the prolactin in milk becomes physically bound to a milk component or that passage of prolactin between blood and milk is more than a process of simple diffusion. Prolactin in milk may be chemically different from prolactin elsewhere, because Grosvenor and Whitworth (1976) observed that time of incubation of rat milk had no effect on endogenous concentrations of

immunoreactive prolactin in milk. However, when they incubated pituitary prolactin or prolactin purified from pituitary cell culture media with milk, the measurable prolactin concentrations decreased.

C. Application of the Arteriovenous Difference Technique to Hormone Utilization

Arteriovenous difference technique has been used extensively to measure mammary utilization of blood metabolites, for example, glucose, amino acids and volatile fatty acids (see chapters by Annison, Linzell and Mepham in Falconer, 1970). The same technique has been employed to determine mammary uptake of steroid hormones in ruminants (Heap and Linzell, 1966; Paterson and Linzell, 1971; Heap et al., 1975a; Heap et al., 1975b). Essentials of the technique were that H^3 labelled hormone was infused intravenously at a rate which maintained its blood concentration constant. When a steady state was obtained, blood samples were obtained simultaneously from the exteriorized carotid artery and the subcutaneous abdominal vein (mammary or milk vein). Mammary uptake of hormone (μCi/min) was calculated as the product of the arteriovenous difference in hormone concentration (μCi/litre) and the mammary blood flow (liters/min). Paterson and Linzell (1971) observed that mammary tissue extracted 50% of the ^{3}H-cortisol entering the gland in pregnant and in nonpregnant lactating goats. A similar study in pregnant and nonpregnant

lactating cows (Heap et al., 1975b) gave a range of mammary extraction of ^{3}H progesterone of 0 to 24% of its arterial concentration.

Cicmanec and Niswender (1973) attempted to find if arteriovenous differences existed in gonadotrophin concentrations across the ovary of sheep during different reproductive states. Prolactin, luteinizing hormone and follicle stimulating hormone concentrations were determined in blood samples obtained simultaneously through indwelling cannulae in the dorsal aorta and ovarian vein. No consistent differences were observed between arterial or venous blood concentrations of gonadotrophins. Burd et al. (1976) were unable to detect any uterine uptake of prolactin, estradiol-17β or cortisol in ewes undergoing induced or spontaneous labor. However, progesterone concentrations in blood samples obtained from the uterine vein were consistently greater than those obtained from the jugular vein and mammary artery.

Summary

In general, increased or decreased concentrations of prolactin, growth hormone and insulin in blood should result in increased or decreased mammary utilization of those hormones accompanied by appropriate changes in rate of synthesis and secretion of milk and ultimately milk yield. However, the literature indicates that increased or decreased availability of protein hormones in the

lactating animal, does not necessarily elicit a mammary response. This is surprising since following total removal of many of those hormones, lactation is generally inhibited and replacement therapy is required to re-establish lactational performance. The specific binding of prolactin, growth hormone and insulin to mammary tissue suggests a role for those hormones in galactopoeisis.

MATERIALS AND METHODS

A. Animals

A total of 12 lactating, nonpregnant Holstein-Fresian cows, six of which were in early lactation (5 to 12 weeks) and six of which were in late lactation (37 to 57 weeks), were used in these studies. During the experimental period, the cows were kept in stanchions, with free access to water and were fed daily 40 lb of corn silage, 10 lb of hay and 1 lb of concentrate per 2.5 lb of milk produced.

B. Surgical Implantation of Pudic Artery and Subcutaneous Abdominal Vein Cannulae

Prior to surgery each cow was deprived of food and water for 24 hr. On the day of surgery the cow was restrained on a tilting surgical table, and anesthesia was induced with 0.3% sodium thyiamylal and 5.0% glycerol guaiacolate solution given intravenously at a dose rate of 0.25 ml/kg body weight. The animal was then intubated and further anesthesized with halothane (4 to 5%) and oxygen (8 to 10 l/min) until the required surgical plane was reached. Anesthesia was then maintained at 2 to 3% halothane and 6 l/min of oxygen throughout surgery.

1. Cannulation of the Pudic Artery

Following surgical preparation of the area, a 10 to 12 cm incision was made over the inguinal region between the medial thigh and lateral surface of the udder. The pudic artery was then located by blunt dissection and the vessel was freed of connective tissue. A purse string suture was placed in the wall of the pudic artery in the region where the cannula was to be inserted.

The cannula was constructed from a 110 cm length of polyvinyl tubing (V-8, Bolab, Inc., Derry, NH) encased in a outer sheath of polyvinyl tubing (.156" x .25", Portex Limited, Hythe, Kent, England) 100 cm long; thus, 10 cm of the inner tubing was exposed. At the point where the 10 cm length of inner tubing left the outer sheath, a silicone sponge (1 cm x 1 cm x 0.5 cm, Bellco Glass Inc., Vineland, NJ) was attached with plastic glue (Woodhill Chemical Sales, Cleveland, OH). The cannula was filled with heparinized saline (10 units/ml, Sigma Chemical Co., St. Louis, MO). The vessel was then occluded by hand with umbilical tape and an incision made in the center of the region surrounded by the purse string suture. The exposed 10 cm of inner polyvinyl tubing was pushed through the incision in the pudic artery wall. The incision was closed by tightening the purse string suture and the cannula sutured to the vessel wall through the silicone sponge. The cannula outside of the vessel was

then brought through the peritoneal cavity to the surface through an incision in the skin of the animal's flank. The end of the cannula was then stored in a pouch which was sutured to the animal's flank.

2. Cannulation of the Isolateral Subcutaneous Abdominal Vein

Following surgical preparation, a 5 cm incision was made in the skin on the ventral surface of the subcutaneous abdominal vein at a point approximately 30 cm from where the vein leaves the front quarter of the udder. The vessel was exposed by blunt dissection and cannulated in a manner identical to that described for the pudic artery. The cannula was implanted subcutaneously between the vein and the surface of the skin of the flank. The venous cannula was brought through the same incision as that for the arterial cannula and stored in the same pouch. In the event of the subcutaneous abdominal vein cannula failing to function during the experimental period, it was replaced with a single wall polyvinyl cannula (V-10, Bolab, Derry, NH) installed by venipuncture.

Following closure of incisions, each animal was maintained on oxygen until the pharyngeal reflexes returned. Immediately following surgery the cannulas were flushed every 2 hr, for 12 hr, with heparin (10 units/ml). Thereafter, the cannulas were flushed every 12 hr for 3 days. Antibiotics (Procaine-Penicillin, Pfizer, NY) were

injected (20 ml every 12 hr) intramuscularly for 5 days. Experimentation was delayed until 3 to 5 days post surgery. Experimentation was begun when milk production approximated that prior to surgery.

C. Common Experimental Protocol

Certain procedures were common to all experiments. All experiments were performed during the afternoon. This enabled the experimental animals to be milked at their normal afternoon milking time of 1500 hr. The complexity of the sampling schedule and the number of experiments meant that we could perform experiments on only one animal at any given time. Consequently, experiments were performed on different animals during different months of the year. Therefore, the day, month and the ambient temperature of each experiment were recorded to enable the data to be adjusted for variation caused by changing season of the year.

On any given experimental day the cannulae were opened 2 hr prior to each experiment and blood samples collected and discarded every 15 min until the beginning of each experiment. This procedure accustomed the experimental animal to periodic disturbance and minimized changes in blood hormone concentrations caused by stress (Tucker, 1971). Between each blood sample the cannulae were flushed with a sterile anticoagulant solution of 0.85% sodium chloride containing 3.5% sodium citrate. Between

each experiment cannulae were filled with an anticoagulant solution of 0.85% sodium chloride containing 3.5% sodium citrate and 50% dextrose. After each experiment, blood samples were left to coagulate at room temperature (25 C) for 2 hr, stored in a cold room (5 C) for 24 to 36 hr and then centrifuged at 2,500 x g for 15 min. Blood serum was then stored at -20 C prior to hormone assay.

Each animal was milked twice daily throughout the series of experiments. The weight of milk obtained at each milking was recorded and a sample of milk representative of each AM and PM yield collected. Milk samples were stored at 5 C for approximately 12 hr and then skimmed by centrifugation at 2,500 x g for 15 min. Skim milk samples were then stored at -20 C prior to assay for prolactin.

D. Hormone Assays

Radioimmunoassays were used to determine serum hormone concentrations. The assay procedures used for prolactin (Tucker, 1971; Koprowski and Tucker, 1971), growth hormone (Purchas et al., 1970), and insulin (Koprowski and Tucker, 1973b) have been described elsewhere. Following validation, prolactin concentrations in skim milk were determined by the same radioimmunoassay used for blood serum. Hormone concentrations were determined in duplicate, and accepted when accuracy was ±5%.

1. Validation of Prolactin Assay for Skim Milk

Prolactin concentrations in skim milk were determined by radioimmunoassay (Tucker, 1971; Koprowski and Tucker, 1971). As greater volumes of skim milk were added to the "total precipitate tubes" of the prolactin assay, radioactivity bound to the antibody decreased progressively, parallel to the standard curve. Addition of 5, 10 or 15 ng of NIH-B3 prolactin to tubes containing 20 µl of a skim milk sample gave quantitative recoveries of 90, 100 and 100%. Measurement of prolactin in 165 milk samples prior to and after skimming indicated that 30.9 ± 1.2% of the prolactin was associated with milk fat. The percentage of prolactin lost from the milk after skimming did not change with advancing stages of lactation. Prolactin concentrations in skim milk samples were significantly correlated with whole milk ($r = .73$; $P < .001$). Prolactin concentrations were determined in eight samples of fresh milk after freezing (-20 C) and thawing (25 C) three times (conducted over a 3 day period). Prolactin averaged 13.8 ± 1.8 and 13.3 ± 2.5 ng/ml ($P > .05$) for fresh and frozen-thawed skim milk samples.

E. Experimental Objectives and Design

Experiment 1. Arterial and Venous Concentrations of Prolactin and Insulin During Milking

The objectives of this experiment were to determine whether increased blood serum concentrations of prolactin (Johke, 1969, 1970; Tucker, 1971; Schams, 1972a) and insulin (Koprowski and Tucker, 1973b) during milking were associated with a greater mammary uptake compared with the period immediately before milking when blood serum concentrations of those hormones would be relatively constant. This was accomplished by measurement of arteriovenous differences in concentrations of prolactin and insulin across the mammary gland before, during and after milking. The second objective was to determine whether stage of lactation affected mammary uptake of prolactin and insulin.

This experiment was repeated on three consecutive afternoons, beginning on day 1 of experimentation. The time of the PM milking was designated as time 0. Simultaneous arterial and venous blood samples were collected at 30, 25, 20, 15, 10, 8, 6, 4, 2 and 0 min before milking. Approximately 15 to 20 sec prior to milking the cows udder was washed and at time 0 the milking machine was attached to the animal. Milking required approximately 3 to 5 min. Blood samples were then collected at 2, 4, 6,

8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, and 70 min after milking.

Experiment 2. Arterial and Venous Concentrations of Prolactin and Growth Hormone After the Administration of TRH Prior to Milking

The primary objectives of this experiment were, firstly, would elevated prolactin concentrations prior to milking saturate mammary prolactin receptors and thereby prevent uptake of prolactin normally associated with increased prolactin concentrations at milking. Secondly, since the milking stimulus does not release growth hormone in cows (Reynaert and Peters, 1972; Koprowski and Tucker, 1973b) but growth hormone is galactopoietic in cows. Whether TRH stimulated release of growth hormone (Convey et al., 1973a) would be associated with a greater mammary uptake of this hormone compared with that associated with basal concentrations of this hormone. Thirdly, I wished to determine if stage of lactation affected the data.

This experiment was repeated three times on 12 cows following the completion of Experiment 1. To minimize carryover effects the replicates of Experiment 3 may have had on each other, a 48-hr interval was allowed between replicates.

Blood sampling was begun 60 min prior to milking. Arterial and venous blood samples were drawn simultaneously 60, 55, 50, 45, 40, 35, and 30 min before milking

to determine basal hormone concentrations. Immediately after the samples at 30 min had been drawn, 200 µg of TRH (Abbott Laboratories, Chicago, IL) in 2 ml of .85% NaCl was injected intravenously to stimulate release of prolactin and growth hormone. Arterial and venous samples were then drawn at 28, 26, 24, 22, 20, 18, 16, 14, 12, 10, 5 and 0 min before milking. Each cow was milked immediately after the 0 min samples were collected for approximately 3 to 5 min. Blood samples were collected at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 min after the start of milking.

Experiment 3. Arterial and Venous Concentrations of Prolactin after Milking in Cows Treated with Ergocryptine

Ergocryptine markedly reduces bovine serum prolactin concentrations and prevents the milking-induced release of pituitary prolactin (Schams and Karg, 1974). Therefore, following administration of ergocryptine, the quantity of serum prolactin available to the mammary gland should be severely reduced. The objective of Experiment 3 was to determine if there is a mammary uptake of prolactin, before, during and after milking when serum prolactin concentrations are chronically depressed, and whether this uptake is affected by stage of lactation.

This experiment was performed in triplicate following the completion of Experiment 2 on five early and five

late lactating cows. The reduction in the number of animals from 12 to 10 was the result of the arterial cannulae in two animals becoming nonfunctional. The replicates were performed on three consecutive afternoons.

Ergocryptine was administered to the five early lactating cows as the salt 2-bromo-α-ergocryptine-methanosulphate (CB-154, Sandoz, Basle, Switzerland) and to the five late lactating as pure ergocryptine (Sigma Chemical Co., St. Louis, MO). Each cow was injected subcutaneously with 80 mg of either CB-154 or ergocryptine dissolved in 5 ml 95% ethanol, 24 hr prior to Experiment 3.

Simultaneous arterial and venous samples were collected 30, 25, 20, 15, 10, 8, 6, 4, 2, and 0 min before milking. After washing the udder and attaching the milking machine at 0 min, blood samples were collected 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60 and 70 min later.

Experiment 4. Arterial and Venous Concentrations of Prolactin and Growth Hormone After Administration of TRH in Cows Treated with Ergocryptine

The quantity of prolactin released in response to TRH is severely diminished in cows treated with ergocryptine, and greater doses of TRH are required to release quantities of prolactin comparable to those released in untreated cows (Schams, 1972b). The objective of this experiment was to determine if prolonged exposure to low

serum concentrations of prolactin as a result of ergocryptine would potentiate the mammary uptake of serum prolactin following administration of 500 μg of TRH. I also wished to know whether mammary uptake of prolactin under these conditions was affected by stage of lactation. Furthermore, since the effects of ergocryptine on TRH-induced growth hormone release were unknown in cattle, serum growth hormone concentrations were also determined.

Experiment 4 was conducted in triplicate after Experiment 3 on five early and five late lactating cows. Serum prolactin concentrations in the 10 cows were still depressed during Experiment 4 by the CB-154 or ergocryptine given prior to Experiment 3. To minimize effects the TRH treatment might have on succeeding replicates, the three experimental replicates of Experiment 4 were conducted on alternate afternoons, thus allowing a 48-hr period between them.

Simultaneous arterial and venous blood samples were collected 30, 25, 20, 15, 10, 8, 6, 4, 2, and 0 min before injection of TRH. Immediately after the collection of the 0 min samples, 500 μg of TRH in .85% NaCl was injected intravenously. Samples were then collected 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60 and 70 min after injection of TRH. The animals were milked following the collection of the 70 min samples.

Experiment 5. Milk Concentrations of Prolactin

Malven and McMurtry (1974a and b) observed that alterations of blood serum concentrations of prolactin in goats and rats resulted in parallel changes in the prolactin concentrations of milk. This evidence would suggest that passage of prolactin from blood into milk is unrestricted. The treatments in Experiments 1, 2, 3 and 4 should have resulted in both acute and chronic changes in blood serum prolactin concentrations. Therefore, I obtained skim milk samples from each cow for AM and PM milkings throughout Experiments 1, 2, 3 and 4. The changes in serum prolactin during the different experiments were compared with prolactin concentrations in skim milk.

Experiment 6. Serum Concentrations of Prolactin in the Mammary Vein Following Intramammary Infusion of Prolactin

To investigate further the relationship between prolactin in blood and milk, the experimental animals were given intramammary injections of prolactin. The objective of this study was to determine if a large quantity of prolactin infused via a streak canal into the milk contained in one quarter of the udder would increase venous blood concentrations of prolactin.

This experiment was performed after Experiment 4 was concluded. To facilitate detection of any increase in

prolactin concentration in mammary venous blood resulting from the intramammary injection of prolactin, 40 mg of CB-154 in 95% ethanol was given subcutaneously to each of the five early lactating cows and 40 mg of ergocryptine in 95% ethanol was given subcutaneously to each of the five late lactating cows, 24 hr prior to the experiment. Use of CB-154 and ergocryptine would reduce concentrations of endogenous prolactin and minimize release of endogenous prolactin as a result of manipulation of the teat during injection of prolactin.

The experiment was performed on three consecutive afternoons. On the first two afternoons 50 mg of prolactin (National Institutes of Health, B3), dissolved in approximately 20 ml of alkaline 0.85% NaCl was injected into the gland cistern of one quarter via the streak canal of the teat. On the third afternoon, 20 ml of alkaline 0.85% saline was injected into the same quarter as a control experiment. Each experiment was performed 90 min before milking when the udder was full of milk and the quarter was massaged immediately after injection to facilitate distribution of the prolactin throughout the milk of the injected quarter.

Mammary venous blood samples were collected 30, 25, 20, 15, 10, 5 and 0 min before the injection of either prolactin or saline and 5, 10, 15, 20, 30, 40, 50, 60 and 90 min after injection.

Experiment 7. The Effect of Ambient Temperature on Serum Prolactin Concentrations Before and After Milking or TRH

Increasing ambient temperature increases basal serum prolactin concentrations in rats (Mueller et al., 1974) and heifers (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976). Furthermore, higher ambient temperatures are associated with increased prolactin release in response to TRH (Tucker and Wettemann, 1976). The objective of this experiment was to determine if ambient temperature affected serum prolactin concentrations in arterial and venous samples before and after milking or TRH.

When the blood samples were collected for each experimental replicate of Experiments 1, 2 and 4, the ambient temperature on that particular afternoon was recorded from a mercury-in-glass thermometer. From the temperatures recorded for each experimental replicate, a mean overall ambient temperature was determined for each experiment for each cow. Then, cows were allocated, within stage of lactation, to either a higher or lower temperature group for a particular experiment. The higher temperature group was composed of cows in which experiments were performed at greater than 18 C and the lower temperature group was composed of cows in which experiments were performed at less than 18 C. This particular

temperature was chosen since the cows within a particular stage of lactation naturally fell into two equal groups on either side of 18 C.

F. Statistical Methods

The statistical analyses described below were calculated with the aid of a CDC 6500 computer.

Average hormone concentrations at each sampling time were calculated for each cow from the three replicates of each experiment. From these values, a mean value and a standard error was calculated for each sampling time for each stage of lactation. The average values for arterial, venous, and arteriovenous difference in hormone concentrations, for each sampling time, for each cow, were then used in a split plot analysis of variance. For the analysis each experiment was divided into three time periods, which were for Experiments 1, 3, and 4: the 30-min prior to milking or injection of TRH, the 20-min period immediately after milking or TRH, and the last 50 min of each experiment. For Experiment 2 the three time periods were: the 30-min period prior to injection of TRH, the 30-min period immediately after the injection of TRH, and the 20-min period beginning at milking. During those time periods, overall means and overall standard errors of arterial and venous concentrations and arteriovenous differences in hormone concentration were calculated.

From the split plot analysis of variance the effects of experimental treatment, period within an experiment, and stage of lactation on arterial, venous and arteriovenous differences in hormone concentrations were determined. The F-test was used to determine if two overall means were different, and the t-test was used to determine if overall mean arteriovenous differences for a period were different from zero.

In Experiment 7, the results of Experiments 1, 2, and 4 were re-analyzed at each stage of lactation according to whether the average ambient temperature when each cow was sampled was high (> 18 C) or low (< 18 C). The same split plot analysis was used except that temperature was entered as an additional variable.

RESULTS

Experiment 1. Arterial and Venous Concentrations of Prolactin and Insulin During Milking

A. Prolactin

Overall mean prolactin concentrations, for arterial and venous serum samples collected from the six early lactating cows during the 30-min pre-milking period were 28.3 ± 11.2 and 27.6 ± 11.0 ng/ml (Figure 1). Immediately following application of the milking machine, serum prolactin concentrations increased to maximum arterial concentrations of 55.8 ± 11.8 to 56.2 ± 11.9 ng/ml within 6 to 12 min ($P < .01$). Serum prolactin concentrations in venous samples changed in a similar manner, reaching maximum concentrations of 56.5 ± 12.1 to 54.1 ± 10.2 ng/ml 8 to 10 min after initiation of milking ($P < .01$). Thereafter arterial and venous prolactin concentrations returned to pre-milking concentrations within 50 min averaging 29.4 ± 11.5 and 28.9 ± 10.9 ng/ml, respectively.

For the 30-min pre-milking period, overall mean serum prolactin concentrations in arterial and venous samples were 9.1 ± .7 and 9.3 ± .6 ng/ml, respectively,

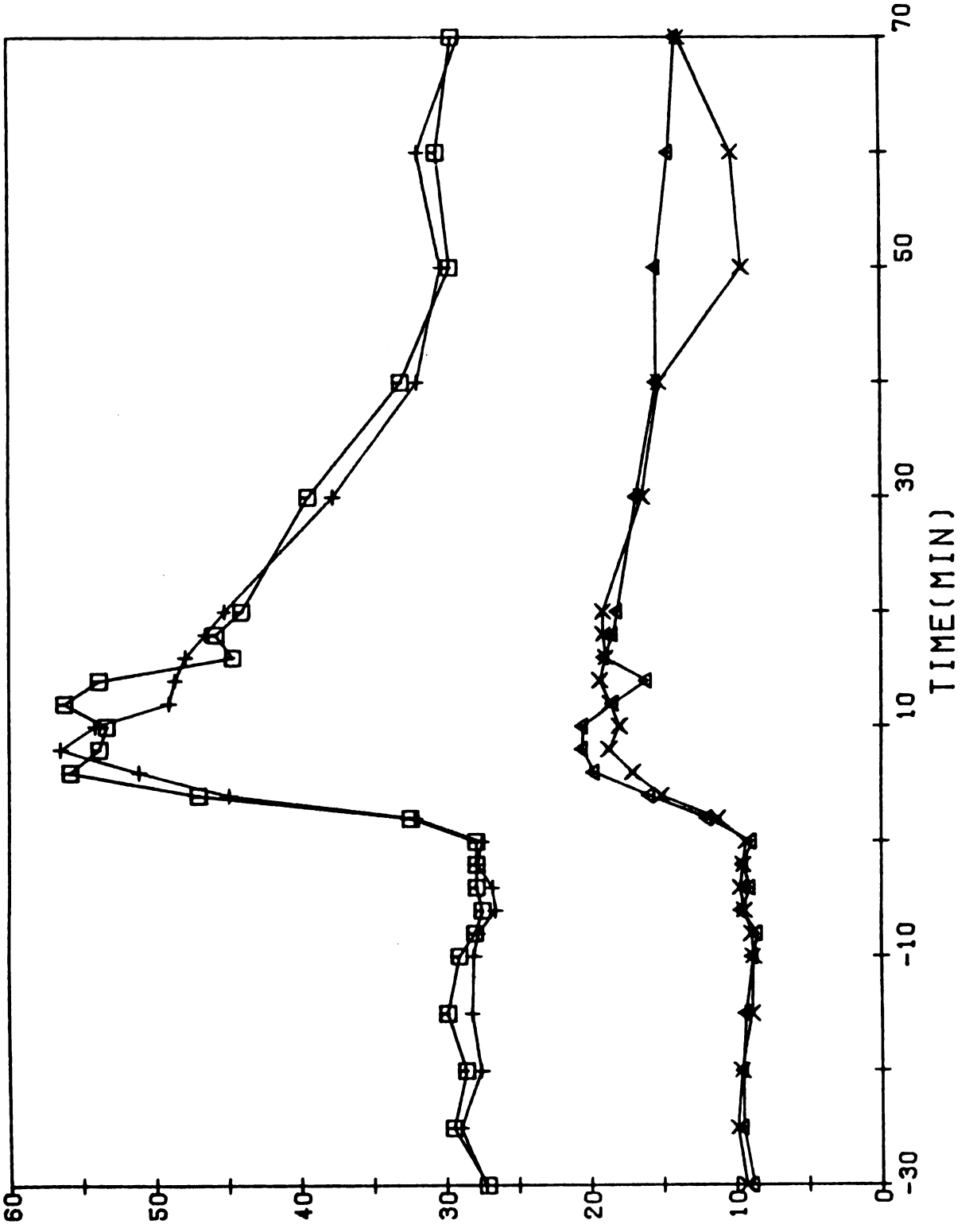
Fig. 1.--Prolactin concentrations in arterial and venous serum samples before, during, and after milking which began at 0 min and lasted 3 to 5 min.

□ - □ Mean prolactin concentrations of arterial serum samples from three replicates in each of six early lactating cows.

\+ - + Mean prolactin concentrations of venous serum samples from three replicates in each of six early lactating cows.

Δ - Δ Mean prolactin concentrations of arterial serum samples from three replicates in each of six late lactating cows.

x - x Mean prolactin concentrations of venous serum samples from three replicates of each of six late lactating cows.


SERUM PROLACTIN (NG ML)
60
50
40
30
20
10
0
-30
-10
10
30
50
70
TIME(MIN)

for the six late lactating cows (Figure 1). Following application of the milking machine at time 0, serum prolactin concentrations increased, reaching maximum concentrations of 19.7 ± 3.4 to 20.5 ± 4.1 ng/ml in arterial samples 6 to 10 min after the start of milking, and 18.7 ± 3.1 to 19.3 ± 5.5 ng/ml in venous samples 8 to 14 min after milking was initiated ($P < .01$). Thereafter serum arterial and venous prolactin concentrations declined to values of 14.0 ± 4.7 and 13.8 ± 4.1 ng/ml, 70 min post milking. Throughout the experiment, late lactating cows had lower serum prolactin concentrations ($P < .05$) than early lactating cows.

Prior to milking, the overall mean arteriovenous difference for serum prolactin concentrations was .68 ± .53 ng/ml for the early lactating cows and -.16 ± .14 ng/ml for late lactating cows. Neither of those values was different from zero ($P > .10$). From 0 to 20 min post milking, the mean arteriovenous difference in serum prolactin concentrations in early lactating cows (1.05 ± .65 ng/ml) approached statistical significance ($P \simeq .10$), whereas in late lactating cows the arteriovenous difference was .35 ± .37 ng/ml ($P > .10$). From 30 to 70 min post milking the mean arteriovenous difference decreased to .25 ± .57 in early lactating and increased to 2.18 ± 2.28 ng/ml in late lactating cows.

The apparent increase in arteriovenous differences in serum prolactin of late lactating cows at 50 and 60 min post milking was due to two of the six cows releasing a large quantity of prolactin on one of the three days of the experiment for unknown reasons. This large increase in serum prolactin resulted in an abnormally large arteriovenous difference.

B. Insulin

Serum insulin concentrations were relatively stable prior to milking in early lactating cows (Figure 2). During this period overall mean serum arterial and venous insulin concentrations were 2.6 ± .65 and 2.5 ± .61 ng/ml, respectively. Serum insulin concentrations remained constant during and after milking. For the 20-min period beginning at milking serum arterial and venous insulin concentrations ranged from 2.6 ± .61 to 3.0 ± .71 ng/ml and 2.4 ± .57 to 3.0 ± .77 ng/ml, respectively. Overall mean serum insulin concentrations from 30 to 70 min post milking were 2.6 ± .66 for arterial and 2.4 ± .64 ng/ml for venous samples.

Prior to milking the six late lactating cows, overall mean serum concentrations of insulin were 3.7 ± .28 and 3.5 ± .21 ng/ml for arterial and venous samples, respectively (Figure 2). During the 20-min period beginning at milking, serum arterial and venous concentrations averaged 3.6 ± .26 and 3.4 ± .27 ng/ml. Thus,

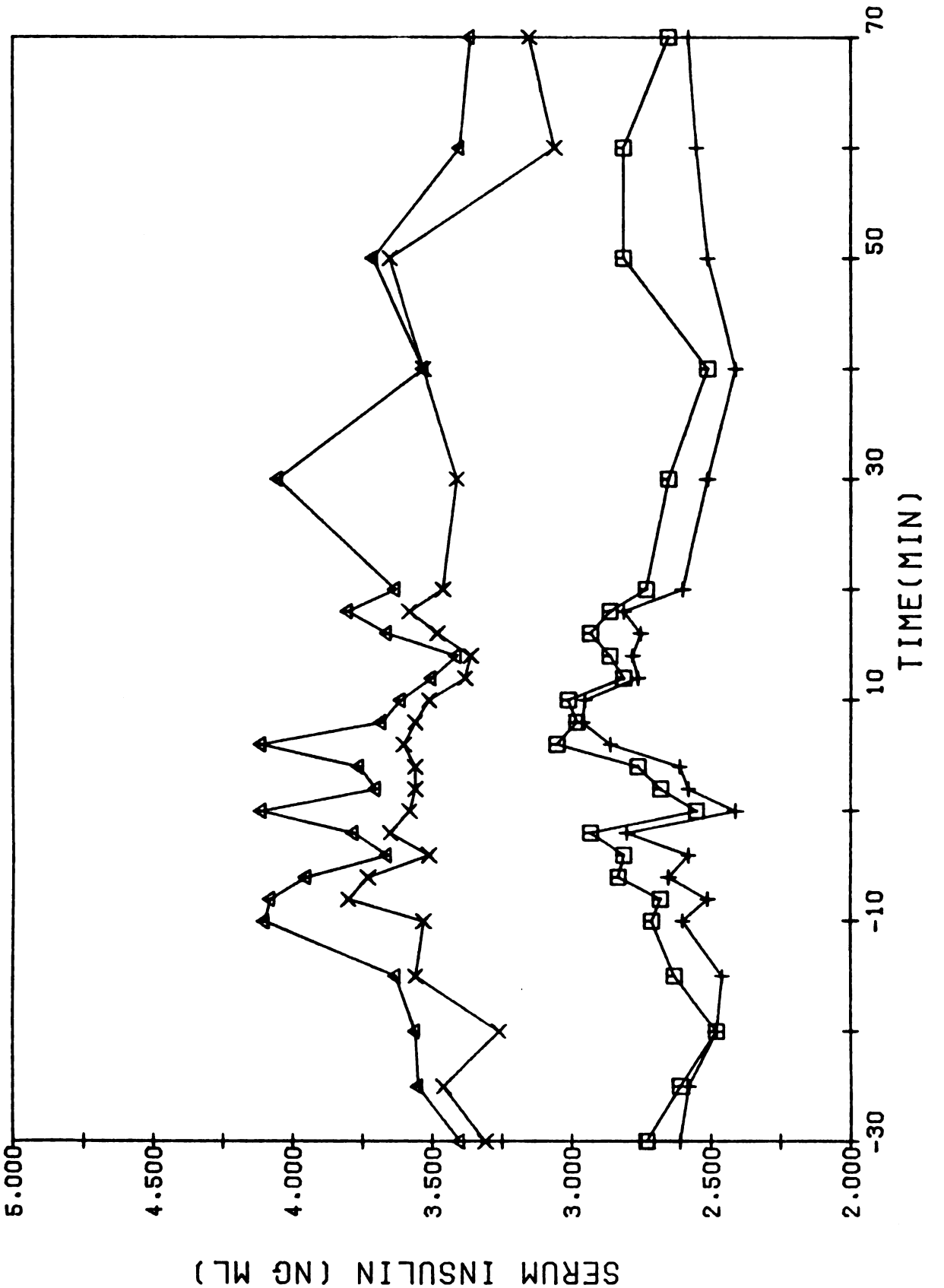
Fig. 2.--Insulin concentrations in arterial and venous serum samples before, during and after milking which began at 0 min and lasted 3 to 5 min.

□ - □ Mean insulin concentrations of arterial serum samples from three replicates in each of six early lactating cows.

\+ - + Mean insulin concentrations of venous serum samples from three replicates in each of six early lactating cows.

Δ - Δ Mean insulin concentrations of arterial serum samples from three replicates in each of six late lactating cows.

x - x Mean insulin concentrations of venous serum samples from three replicates in each of six late lactating cows.


SERUM INSULIN (NG ML)
5.000
4.500
4.000
3.500
3.000
2.500
2.000
-30
-10
10
30
50
70
TIME(MIN)

milking did not affect serum insulin concentrations. From 30 to 70 min post milking, mean arterial and venous serum insulin concentrations were 3.5 ± .40 and 3.3 ± .30 ng/ml. Overall concentrations of serum insulin throughout the entire experiment were greater in late than in early lactating animals ($P < .10$).

For the 30 min prior to milking, the mean arteriovenous difference for serum insulin was .13 ± .04 ng/ml for the six cows in early lactation. This value was different from zero ($P < .05$). However, during this period in late lactating cows, the arteriovenous difference (.21 ± .08 ng/ml) was not different from zero ($P > .05$). During the 20-min period beginning at milking, arteriovenous differences in serum insulin concentration in both early (.11 ± .03 ng/ml) and late (.16 ± .04 ng/ml) lactating cows were different from zero ($P < .05$). Between 30 and 70 min post milking arteriovenous differences in serum insulin concentrations in early (.20 ± .09 ng/ml) and late (.23 ± .14 ng/ml) were not different from zero ($P > .05$). A split plot analysis of variance of the data revealed that neither stage of lactation nor the period during the experiment when the samples were collected significantly affected arteriovenous differences of insulin. Overall arteriovenous differences from -30 to 70 min after milking averaged .13 ng/ml but this value was not different from zero ($P > .05$).

Experiment 2. Arterial and Venous Concentrations of Prolactin and Growth Hormone After the Administration of TRH Prior to Milking

A. Prolactin

During the 30-min period prior to injection of 200 μg TRH, overall mean prolactin concentrations in arterial and venous serum samples from the six early lactating cows were 29.0 ± 9.8 and 28.8 ± 10.3 ng/ml, respectively (Figure 3). Following administration of 200 μg TRH 30 min before milking, concentrations of arterial serum prolactin increased to a maximum 210 ± 52 ng/ml after 12 min ($P < .01$). Concentrations of prolactin in venous serum samples increased to a maximum of 212 ± 51 ng/ml at 14 min after injection of TRH ($P < .01$). Thereafter serum prolactin concentrations remained relatively constant until milking. Overall mean prolactin concentrations for the 30-min period beginning with the injection of TRH and ending at milking were 179.0 ± 43.0 for arterial samples and 171.0 ± 40.0 ng/ml for venous samples. Following initiation of milking at time 0, there was a further increase in serum prolactin concentrations until 10 to 12 min after milking commenced. Maximum post milking concentrations of serum prolactin were 269.0 ± 60.0 ng/ml in arterial samples and 264.0 ± 63.0 ng/ml in venous samples. Overall mean serum prolactin concentrations for the 20-min post-milking period were 248.0 ± 55.0 and

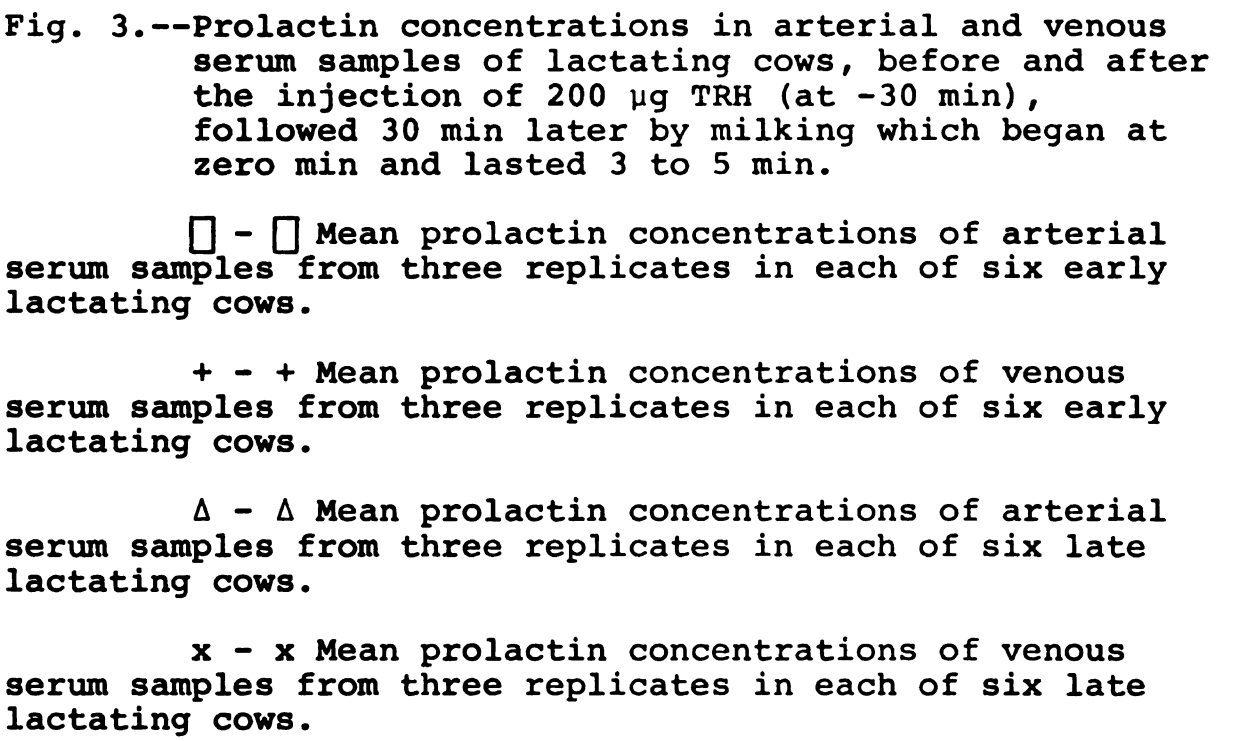


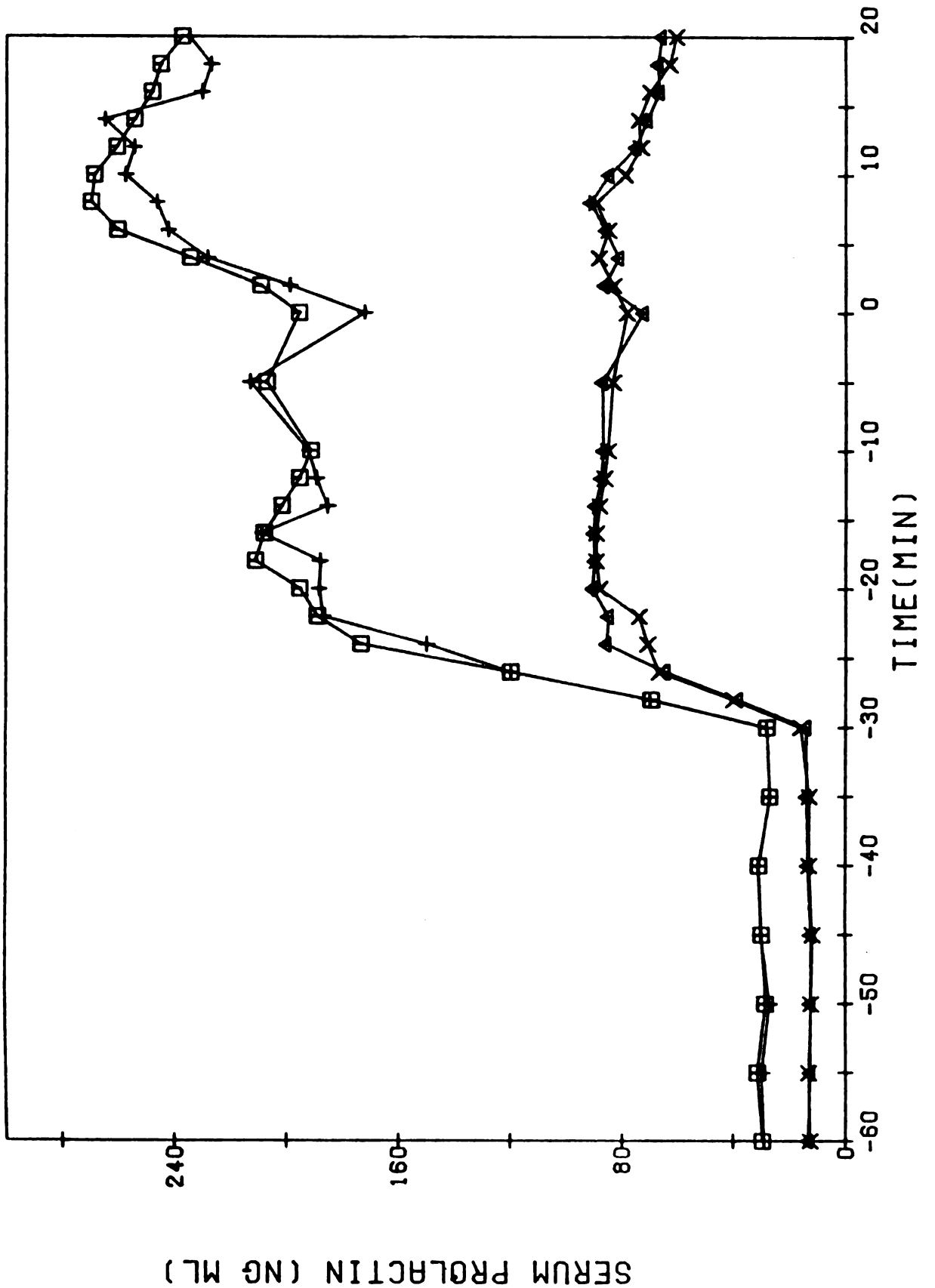
Fig. 3.--Prolactin concentrations in arterial and venous serum samples of lactating cows, before and after the injection of 200 µg TRH (at -30 min), followed 30 min later by milking which began at zero min and lasted 3 to 5 min.

□ - □ Mean prolactin concentrations of arterial serum samples from three replicates in each of six early lactating cows.

\+ - + Mean prolactin concentrations of venous serum samples from three replicates in each of six early lactating cows.

Δ - Δ Mean prolactin concentrations of arterial serum samples from three replicates in each of six late lactating cows.

x - x Mean prolactin concentrations of venous serum samples from three replicates in each of six late lactating cows.


SERUM PROLACTIN (NG ML)
240
160
80
0
-60
-50
-40
-30
-20
-10
0
10
20
TIME(MIN)

238.0 ± 51.0 ng/ml for arterial and venous samples, respectively.

Overall mean serum prolactin concentrations during the 30-min pre-injection period were 9.1 ± .6 ng/ml in arterial samples and 9.3 ± .6 ng/ml in venous samples from six late lactating cows (Figure 3). Following administration of 200 μg TRH 30 min before milking, serum prolactin concentrations increased to a maximum prolactin of 90.0 ± 25.0 ($P < .01$) in arterial samples and 89.0 ± 28.0 ($P < .01$) ng/ml in venous samples. These peaks occurred 10 to 12 min post injection, respectively. Overall mean serum prolactin concentration for the 30 min between injection of TRH and milking were 80.0 ± 24.0 ng/ml in arterial samples and 77.0 ± 23.0 ng/ml in venous samples. After attaining maximum concentrations, serum arterial and venous prolactin concentrations remained relatively constant until milking when a slight increase (18%) in serum prolactin concentrations occurred. Serum prolactin concentrations during the 20 min after the start of milking averaged 77.0 ± 20.0 ng/ml in arterial samples and 76.0 ± 20.0 ng/ml in venous samples.

Split plot analysis of variance indicated that the six early lactating cows had greater serum prolactin concentrations prior to TRH injection ($P < .05$) and released a greater amount of prolactin ($P < .05$) to the TRH stimulus, compared with late lactating cows. Furthermore, after

injection of 200 μg TRH, early lactating cows released more prolactin to the milking stimulus than did late lactating cows ($P < .01$).

Prior to injection to TRH, mean arteriovenous differences in prolactin concentrations for early (.40 ± .77 ng/ml) and late (-.01 ± .20 ng/ml) lactating cows were small and not different from zero ($P > .10$). However, mean arteriovenous differences during the 30-min post TRH injection period increased to 7.7 ± 3.1 ng/ml for early lactating and 2.6 ± 1.0 ng/ml for late lactating cows. These values were greater ($P < .05$) than zero, but stage of lactation did not affect these arteriovenous differences. During the 20 min beginning with milking the mean arteriovenous difference increased slightly in the early lactating cows to 10.3 ± 6.7 ng/ml. Conversely, in the six late lactating cows the mean arteriovenous difference decreased from 2.6 ± 1.0 to 1.0 ± 1.1 ng/ml in the 20-min post-milking period. However, neither of the mean arteriovenous differences in serum prolactin during the post-milking period were different from zero ($P > .05$).

B. Growth Hormone

Overall mean serum growth hormone concentrations in arterial and venous samples from six early lactating cows collected during the 30-min pre-injection period were 4.0 ± .2 and 4.0 ± .2 ng/ml, respectively (Figure 4).

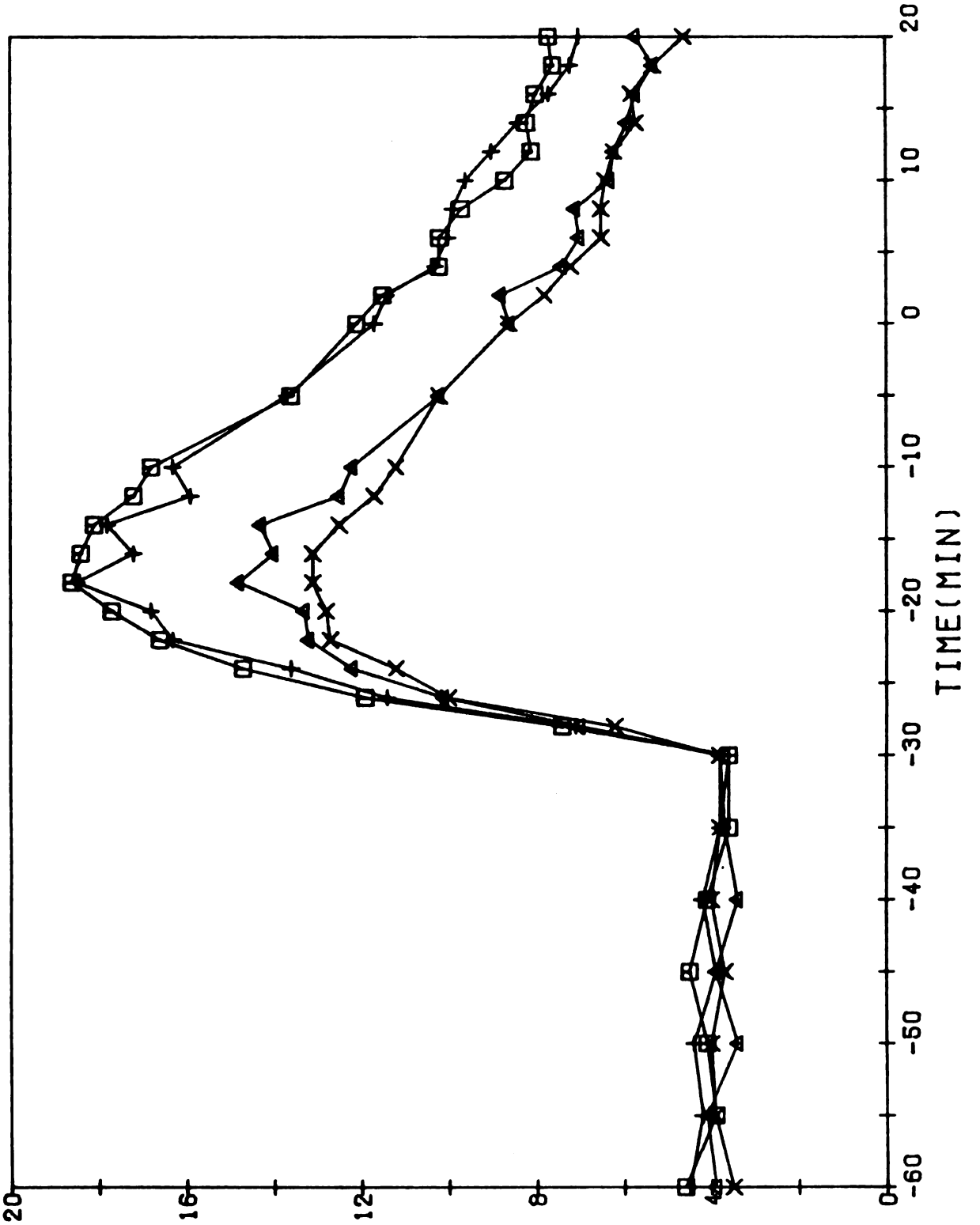
Fig. 4.--Growth hormone concentrations in arterial and venous serum samples of lactating cows, before and after the injection of 200 µg TRH (at -30 min) followed 30 min later by milking which began at zero min and lasted 3 to 5 min.

□ - □ Mean growth hormone concentrations of arterial serum samples from three replicates in each of six early lactating cows.

\+ - + Mean growth hormone concentrations of venous serum samples from three replicates in each of six early lactating cows.

Δ - Δ Mean growth hormone concentrations of arterial serum samples from three replicates in each of six late lactating cows.

x - x Mean growth hormone concentrations in venous serum samples from three replicates in each of six late lactating cows.


SERUM GROWTH HORMONE (NG ML)
20
16
12
8
4
0
-60
-50
-40
-30
-20
-10
0
10
20
TIME(MIN)

Serum growth hormone concentrations increased ($P < .01$) to maximum concentrations of 18.6 ± 3.1 in arterial and 18.5 ± 3.3 ng/ml in venous samples 12 min after intravenous injection of 200 μg TRH ($P < .01$). Thereafter, serum growth hormone concentrations declined in arterial and venous samples to values of 12.1 ± 1.4 and 11.7 ± 1.7 ng/ml at 30 min after injection of TRH, respectively. Overall mean arterial and venous concentrations of growth hormone during the 30-min post TRH injection period were 15.2 ± 2.3 and 14.6 ± 2.4 ng/ml. Milking was without effect on serum growth hormone concentrations and they continued to decline to values of 7.7 ± .8 (arterial) and 7.0 ± .6 ng/ml (venous) at 20 min after milking.

Before injection of TRH in six late lactating cows, mean serum concentrations of growth hormone in arterial and venous samples were 3.7 ± .6 and 3.8 ± .7 ng/ml (Figure 4). Injection of 200 μg TRH increased markedly ($P < .01$) serum growth hormone concentrations. Maximum arterial (14.8 ± 2.7 ng/ml) and venous (13.1 ± 2.3 ng/ml) concentrations occurred 12 min after injection of TRH. Overall mean arterial and venous concentrations of growth hormone during the 30-min post TRH injection period were 11.8 ± 2.0 and 11.1 ± 1.9 ng/ml, respectively. Although the late lactating cows appeared to release less growth hormone in response to TRH than early lactating cows, the difference was not significant ($P > .05$). Milking

these cows at 30 min after injection of TRH did not affect serum growth hormone concentrations. Serum arterial and venous growth hormone concentrations continued to decline from values of 8.6 ± 1.9 and 8.6 ± 1.6 ng/ml at milking (time 0) to 5.7 ± .81 and 4.6 ± .81 ng/ml 20 min after milking. The overall mean arteriovenous difference in serum growth hormone concentration during the 30-min pre-TRH injection period was -.03 ± .08 ng/ml for early and -.05 ± .10 ng/ml for late lactating cows. These values were not different from zero ($P > .05$). During the 30-min post TRH injection period, the mean arteriovenous difference was .58 ± .22 ng/ml in early and .73 ± .18 in late lactating cows. These values were different from zero ($P < .05$) and greater than arteriovenous differences in any other period. The overall mean arteriovenous difference (-.06 ± .20) during the 20-min period beginning at milking was not different from zero in early lactating cows ($P < .05$). However, during this period in late lactating cows the mean arteriovenous difference was significant (.33 ± .12 ng/ml; $P < .05$). Stage of lactation did not affect arteriovenous differences during any experimental time period.

Experiment 3. Arterial and Venous Concentrations of Prolactin After Milking in Cows Treated with Ergocryptine

The administration of 80 mg CB-154 to five early lactating cows reduced ($P < .01$) serum prolactin concentrations from an average of 28.0 ng/ml just prior to treatment to 7.0 ng/ml 24 hr later. Similarly, administration of 80 mg ergocryptine to five late lactating cows reduced serum prolactin concentrations from an average of 9.0 ng/ml immediately prior to treatment to 2.0 ng/ml 24 hr later ($P < .01$).

Overall mean serum prolactin concentrations in five early lactating cows averaged 7.0 ± 2.0 ng/ml for arterial and 7.4 ± 2.1 ng/ml for venous samples during the 30-min period prior to milking (Figure 5). After application of the milking stimulus maximal serum prolactin concentrations were 9.1 ± 3.2 ng/ml in arterial and 10.0 ± 2.5 ng/ml in venous samples. However, the mean arterial (7.7 ± 2.4 ng/ml) and venous (8.0 ± 2.5 ng/ml) prolactin concentrations were not different from pre-milking serum prolactin concentrations ($P > .05$). Between 30 and 70 min after milking mean serum prolactin concentrations changed little, averaging 7.2 ± 1.8 and 6.9 ± 1.7 ng/ml for arterial and venous samples, respectively.

During the 30-min pre-milking period of five late lactating cows serum prolactin concentrations were 1.7 ± .2 ng/ml in arterial samples and 1.7 ± .2 ng/ml in venous

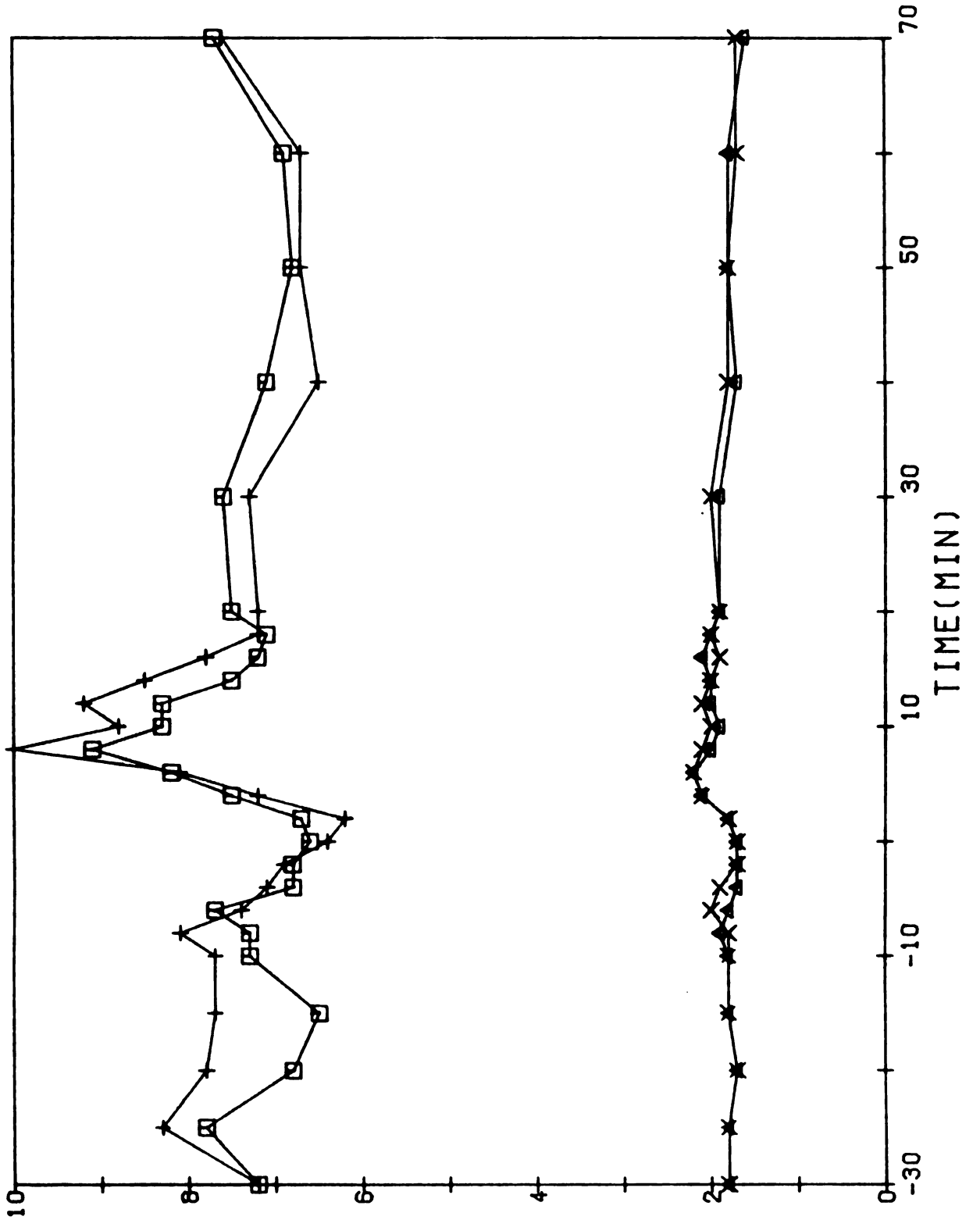
Fig. 5.--Serum prolactin concentrations in arterial and venous samples collected before, during and after milking in five early lactating cows treated with 80 mg CB-154, 24 hr prior to experimentation and five late lactating cows treated with 80 mg ergocryptine, 24 hr prior to experimentation. The animals were milked for 3 to 5 min beginning at 0 min.

□ - □ Mean prolactin concentrations in arterial samples from three replicates in each of five early lactating cows.

\+ - + Mean prolactin concentrations in venous samples from three replicates in each of five early lactating cows.

Δ - Δ Mean prolactin concentrations in arterial samples from three replicates in each of five late lactating cows.

x - x Mean prolactin concentrations in venous samples from three replicates in each of five late lactating cows.


SERUM PROLACTIN (NG ML)
10
8
6
4
2
0
-30
-10
10
30
50
70
TIME(MIN)

samples (Figure 5). Milking did not affect serum prolactin concentrations in arterial or venous samples. During the 20-min post-milking period serum prolactin concentrations averaged 2.0 ± .3 ng/ml in arterial and 2.0 ± .4 ng/ml in venous samples. From 30 to 70 min after milking, prolactin concentrations were 1.7 ± .20 ng/ml in arterial serum samples and 1.7 ± 2.3 ng/ml in venous serum samples. Prolactin concentrations were greater ($P < .05$) in early lactating compared with late lactating cows.

Arteriovenous differences in serum prolactin concentrations were small and not different from zero in either early or late lactating cows. During the 30-min pre-milking period mean arteriovenous difference in serum prolactin concentration was -.38 ± .11 ng/ml in early and -.02 ± .03 ng/ml in late lactating cows. The mean arteriovenous difference from 0 to 20 min post milking was -.28 ± .18 in early and 0.02 ± .03 ng/ml in late lactating cows. Thereafter, the mean arteriovenous difference in serum prolactin from 30 to 70 min post milking was .34 ± .21 and .00 ± .06 ng/ml for early and late lactating cows, respectively.

Experiment 4. Arterial and Venous Concentrations of Prolactin and Growth Hormone After Administration of TRH in Cows Treated With Ergocryptine

A. Prolactin

Serum prolactin concentrations remained depressed during this experiment as a result of the 80 mg of CB-154 given to the five early lactating cows, and the 80 mg of ergocryptine given to the five late lactating cows, just prior to the start of Experiment 3.

Serum prolactin concentrations during the 30-min pre-injection period averaged 9.2 ± 1.5 in arterial and 9.4 ± 1.5 ng/ml in venous samples from five early lactating cows (Figure 6). Serum prolactin concentrations increased ($P < .01$) rapidly following administration of 500 µg TRH to maximum values of 154 ± 29 ng/ml in arterial and 143 ± 25 ng/ml in venous samples at 12 to 14 min. Thereafter, serum prolactin concentration declined. Overall mean serum prolactin concentrations were 116 ± 25 and 110 ± 21 ng/ml, respectively, in arterial and venous samples during the 20-min post-TRH injection period. From 30 to 70 min after injection of TRH prolactin concentrations in arterial and venous samples continued to decline; overall mean concentrations during this period were 91 ± 12 and 89 ± 11 ng/ml.

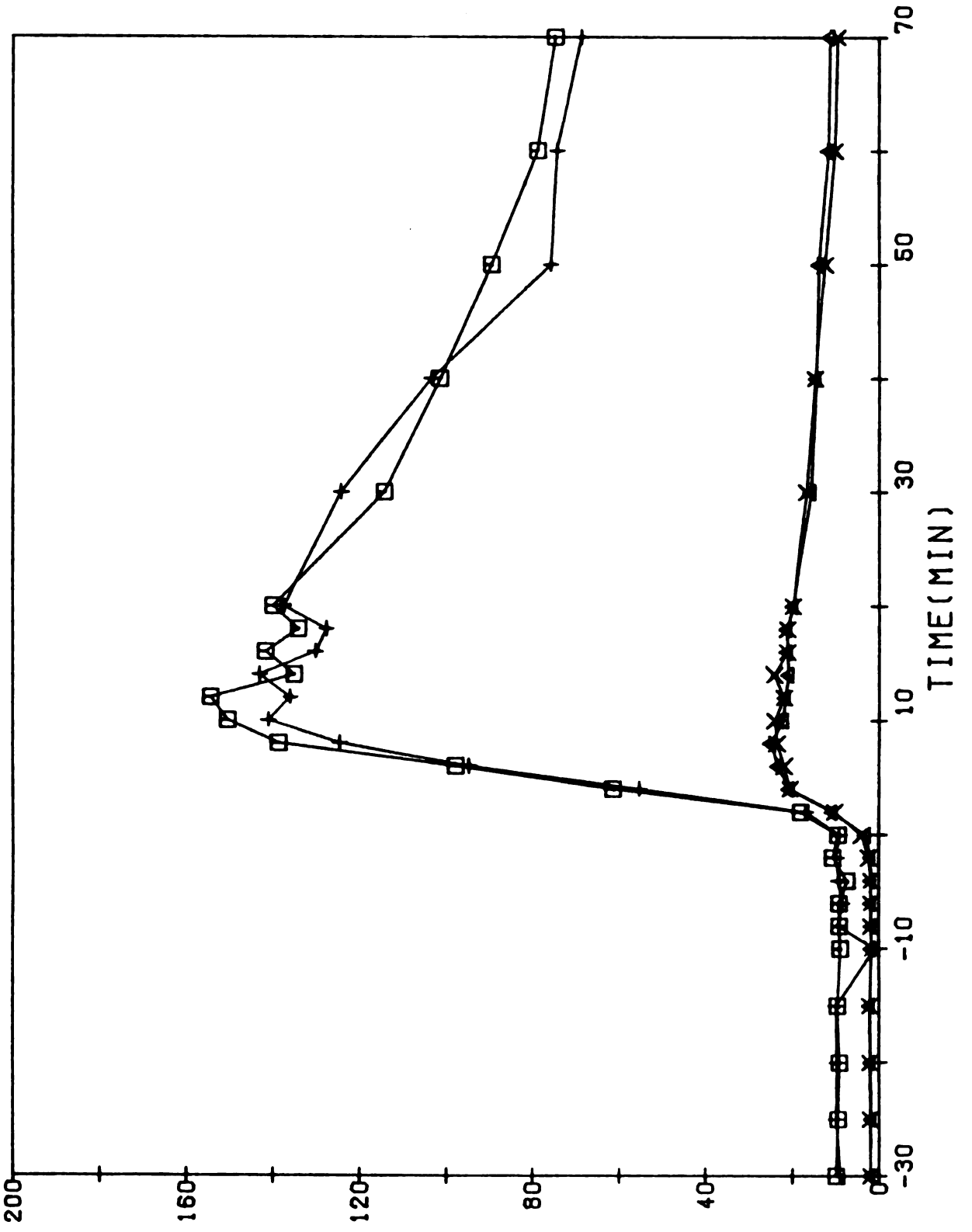
Fig. 6.--Prolactin concentrations in arterial and venous serum samples before and after injection of 500 µg TRH at 0 min to five early lactating cows treated with 80 mg CB-154 and five late lactating cows treated with 80 mg ergocryptine.

□ - □ Mean prolactin concentrations in arterial samples from three replicates in five early lactating cows.

\+ - + Mean prolactin concentrations in venous samples from three replicates in five late lactating cows.

Δ - Δ Mean prolactin concentrations in arterial samples from three replicates in five late lactating cows.

x - x Mean prolactin concentrations in venous samples from three replicates in five late lactating cows.


SERUM PROLACTIN (NG ML)
200
160
120
80
40
0
-30
-10
10
30
50
70
TIME(MIN)

Serum prolactin concentrations averaged 1.9 ± .2 for arterial and 2.1 ± .2 ng/ml for venous samples in five late lactating cows during the 30-min pre-injection period (Figure 6). Following injection of TRH, serum prolactin concentrations increased to maximum values of 24.9 ± 8.7 ng/ml in arterial and 24.0 ± 8.3 ng/ml in venous samples 8 to 10 min after injection of TRH ($P < .01$). Between 0 and 20 min post-TRH injection, mean serum prolactin concentrations were 20.5 ± 6.7 in arterial and 20.8 ± 6.7 ng/ml in venous samples. Serum prolactin concentrations averaged 13.3 ± 3.9 in arterial and 12.6 ± 3.7 ng/ml in venous samples collected between 30 and 70 min after injection of TRH. Serum prolactin concentrations in serum samples collected in each period from early lactating cows were greater ($P < .01$) than from late lactating cows.

Mean arteriovenous differences for serum prolactin were -.14 ± .18 for early lactating and -.18 ± .16 ng/ml for late lactating cows, prior to injection of TRH. From 0 to 20 min after injection of TRH, arteriovenous differences averaged 6.3 ± 4.5 ng/ml in early lactating and -.24 ± .40 ng/ml in late lactating cows. Prolactin arteriovenous differences averaged 2.4 ± 1.5 ng/ml in early and .68 ± .27 ng/ml in late lactating cows between 30 and 70 min after TRH injection. None of the above mean arteriovenous differences were different from zero ($P > .10$).

B. Growth Hormone

Mean serum growth hormone concentrations during the pre-TRH injection period averaged 4.9 ± .9 ng/ml in arterial samples and 5.0 ± .9 ng/ml in venous samples collected from cows treated with CB-154 (Figure 7). Injection of 500 μg TRH caused an increase in arterial and venous serum growth hormone concentrations to peaks of 25.2 ± 4.5 and 24.7 ± 4.5 ng/ml 12 min after injection of TRH, respectively ($P < .01$). During the 20-min post-TRH injection period arterial and venous concentrations of serum growth hormone averaged 20.6 ± 3.6 and 19.9 ± 3.5 ng/ml. Between 20 and 70 min after TRH injection, serum arterial and venous growth hormone concentrations declined from 24.1 ± 3.8 and 21.5 ± 2.9 to 7.1 ± 1.1 and 7.7 ± 1.8 ng/ml, respectively.

Serum growth hormone concentrations before injection of TRH in arterial (3.6 ± .7) and venous (3.4 ± .7) samples of late lactating cows were not different from those of early lactating cows ($P > .05$). Injection of 500 μg of TRH increased serum growth hormone concentrations to maximum values in arterial (15.7 ± 2.6) and venous (15.1 ± 2.7) samples within 12 min ($P < .01$). Overall mean concentrations of serum growth hormone for arterial (13.1 ± 2.0 ng/ml) and venous (12.6 ± 2.1 ng/ml) samples during the 20-min post-injection period were lower ($P < 0.01$) than those obtained during the same period in early

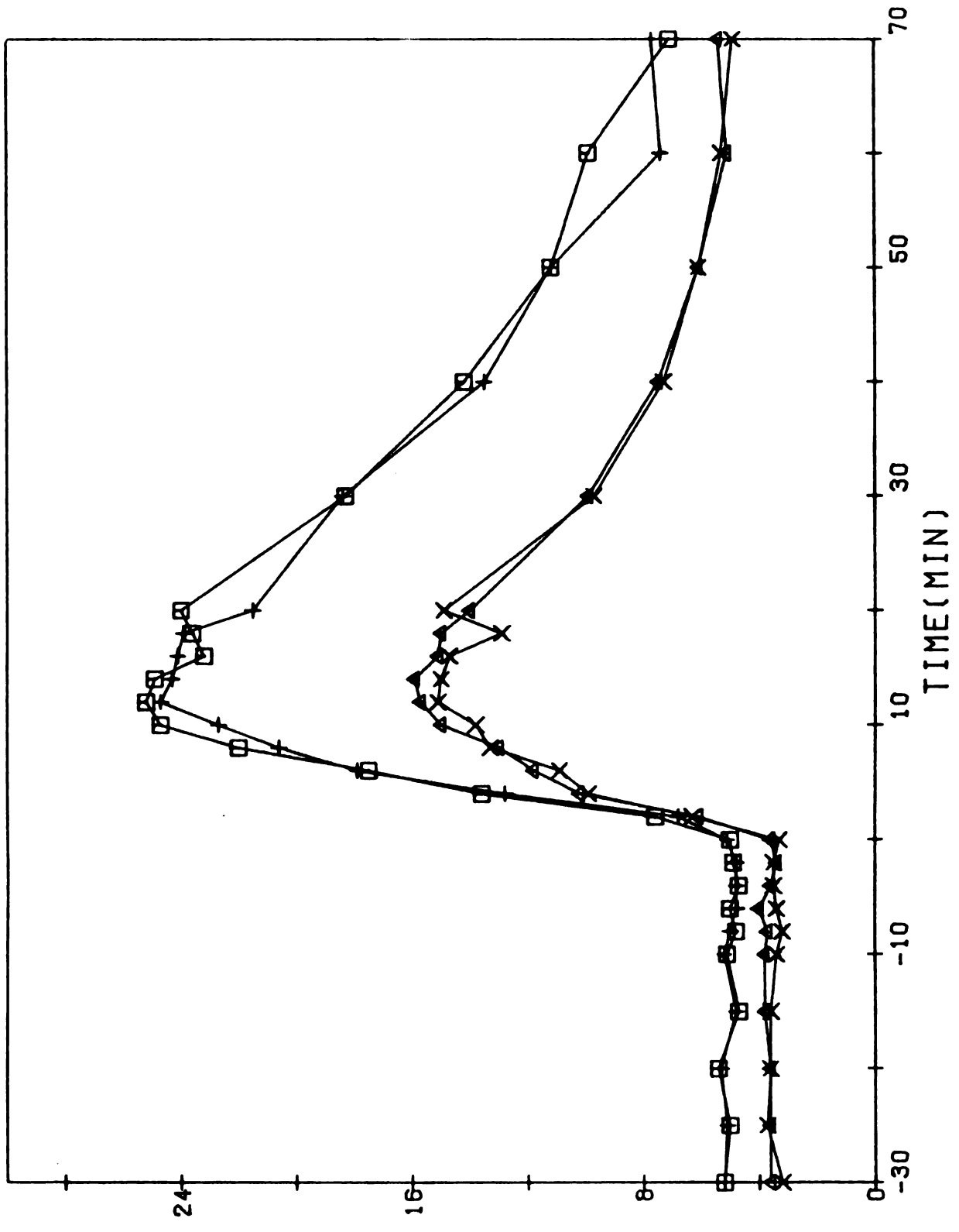
Fig. 7.--Serum growth hormone concentrations in arterial and venous serum samples before and after injection of 500 µg TRH at 0 min to five early lactating cows treated with 80 mg CB-154 and five late lactating cows treated with 80 mg ergocryptine.

□ - □ Mean growth hormone concentrations in arterial samples from three replicates in five early lactating cows.

\+ - + Mean growth hormone concentrations in venous samples from three replicates in five early lactating animals.

Δ - Δ Mean growth hormone concentrations in arterial samples from three replicates in five late lactating cows.

x - x Mean growth hormone concentrations in venous samples from three replicates in five late lactating cows.


SERUM GROWTH HORMONE (NG ML)
24
16
8
0
-30
-10
10
30
50
70
TIME(MIN)

lactating cows. From 30 to 70 min post TRH injection, serum concentrations of growth hormone in arterial and venous samples declined from 14.0 ± 2.0 and 14.9 ± 2.4 ng/ml to 5.4 ± .8 and 4.9 ± .7 ng/ml, respectively.

During the 30-min pre-TRH injection period arteriovenous differences for both early (-.04 ± .14) and late (.20 ± .11 ng/ml) lactating cows were small and not different from zero. In the 20-min period immediately following administration of TRH arteriovenous differences increased to .7 ± .36 ($P > .10$ relative to zero) in early lactating cows and .5 ± .12 ng/ml in late lactating cows; however, those arteriovenous differences were not different from each other ($P > .10$). Arteriovenous differences in serum growth hormone concentration from 30 to 70 min post-TRH injection decreased to .52 ± .29 in early and .16 ± .30 in late lactating cows but these values were not different from zero ($P > .10$).

Experiment 5. Milk Concentrations of Prolactin

In neither early nor late lactating cows was milk production affected by experimental treatment ($P > .10$; Table 1). Early lactating cows produced approximately 92% more milk than late lactating cows throughout Experiments 1 to 4.

The data given in Table 1 are mean values for prolactin concentrations in skim milk samples collected over

Table 1.--Milk weights and prolactin concentrations in skim milk samples obtained from the AM and PM milking of early and late lactating cows throughout experiments 1 to 4.

		Experiment 1[a,c]	Experiment 2[a,d]	Experiment 3[b,e]	Experiment 4[b,f]
Early Lactating Cows					
Mean Yield (kg)	AM	11.9 ± 1.4	13.3 ± 1.9	13.4 ± 1.8	13.4 ± 2.5
	PM	11.0 ± 1.8	11.2 ± 1.5	11.4 ± 1.5	9.7 ± 1.3
Mean Prolactin (ng/ml)	AM	25.6 ± 8.7	20.4 ± 4.6	11.6 ± 2.5	9.9 ± 1.5
	PM	23.9 ± 7.4	25.4 ± 7.2	12.3 ± 3.3	12.2 ± 2.9
Late Lactating Cows					
Mean Yield (kg)	AM	7.3 ± 0.6	7.3 ± 0.7	7.0 ± 0.7	7.0 ± 0.7
	PM	5.7 ± 0.9	6.1 ± 0.8	4.3 ± 1.0	4.6 ± 1.3
Mean Prolactin (ng/ml)	AM	10.4 ± 0.8	12.0 ± 1.1	7.8 ± 1.6	5.2 ± 1.1
	PM	12.4 ± 1.2	12.0 ± 1.4	6.3 ± 1.0	4.7 ± 0.8

[a]n = six cows.

[b]n = five cows.

[c]In this experiment six early and six late lactating cows were milked.

[d]In this experiment six early and six late lactating cows were given 200 µg TRH, 30 min prior to milking.

[e]In this experiment five early lactating cows were treated with CB-154 and five late lactating cows were treated with ergocryptine 24 hr before milking.

[f]In this experiment five early lactating cows treated with CB-154 and five late lactating cows treated with ergocryptine were given 500 µg TRH approximately 70 min before milking.

each experimental period. Prolactin concentrations in skim milk samples from early lactating (AM = 25.6 ± 8.7 and PM = 23.9 ± 7.4 ng/ml) and late lactating cows (AM = 10.4 ± .8 and PM = 12.4 ± 1.2 ng/ml) collected during Experiment 1, were quantitatively similar to serum concentrations (27.6 and 9.3 ng/ml, respectively) of prolactin collected before milking (Table 1 and Figure 1).

Since any increase in prolactin concentrations in skim milk following the administration of TRH may not be apparent in the overall means given in Table 1, specific means for the prolactin concentrations in skim milk samples collected immediately after and 12 hrs after TRH were calculated and are given below.

The elevation of serum prolactin in Experiment 2 in early lactating cows as a result of injecting TRH (Figure 3) did not increase skim milk concentrations of prolactin approximately 30 min later in the PM milking (19.8 ± 3.6 ng/ml), above those found in the milk during Experiment 2 ($\bar{x}$ = 24.8 ng/ml). Similarly, in late lactating cows, concentrations of prolactin in skim milk collected 30 min after injection of TRH (12.8 ± 2.1 ng/ml) were no different from concentrations found in those cows during Experiment 1 ($\bar{x}$ = 11.4 ng/ml).

The administration of 80 mg CB-154 to five early lactating cows and 80 mg of ergocryptine to five late lactating cows reduced basal serum prolactin concentrations

from 28 and 9 ng/ml, respectively, in Experiment 2 (Figure 3), to 7 and 2 ng/ml in Experiment 3 (Figure 5). Prolactin concentrations in skim milk samples were also reduced following the administration of CB-154 to early lactating cows or ergocryptine to late lactating cows (Table 1).

Administration of 500 µg TRH to five early lactating cows treated with CB-154 and to five late lactating cows treated with ergocryptine increased serum prolactin concentrations from 9.0 to 113.0 and 2.0 to 20.0 ng/ml, respectively. Approximately 70 min later the cows were milked. Similar to the results obtained in Experiment 3, CB-154 and ergocryptine reduced skim milk concentrations relative to those observed in Experiment 1. Moreover, prolactin concentrations in skim milk samples collected from that PM milking, were similar in both early (11.9 ± 2.9), and late (4.7 ± .08) lactating cows to the prolactin concentrations in the skim milk samples collected during Experiment 3 in which no TRH was given (Table 1).

Experiment 6. Serum Concentration of Prolactin in the Mammary Vein Following Intramammary Infusion of Prolactin

Concentrations of serum prolactin in the mammary vein before injection of saline or 50 mg prolactin ranged from 10.0 ± 1.1 to 13.2 ± 4.0 ng/ml in the five early lactating cows (Figure 8). The apparent increase in serum

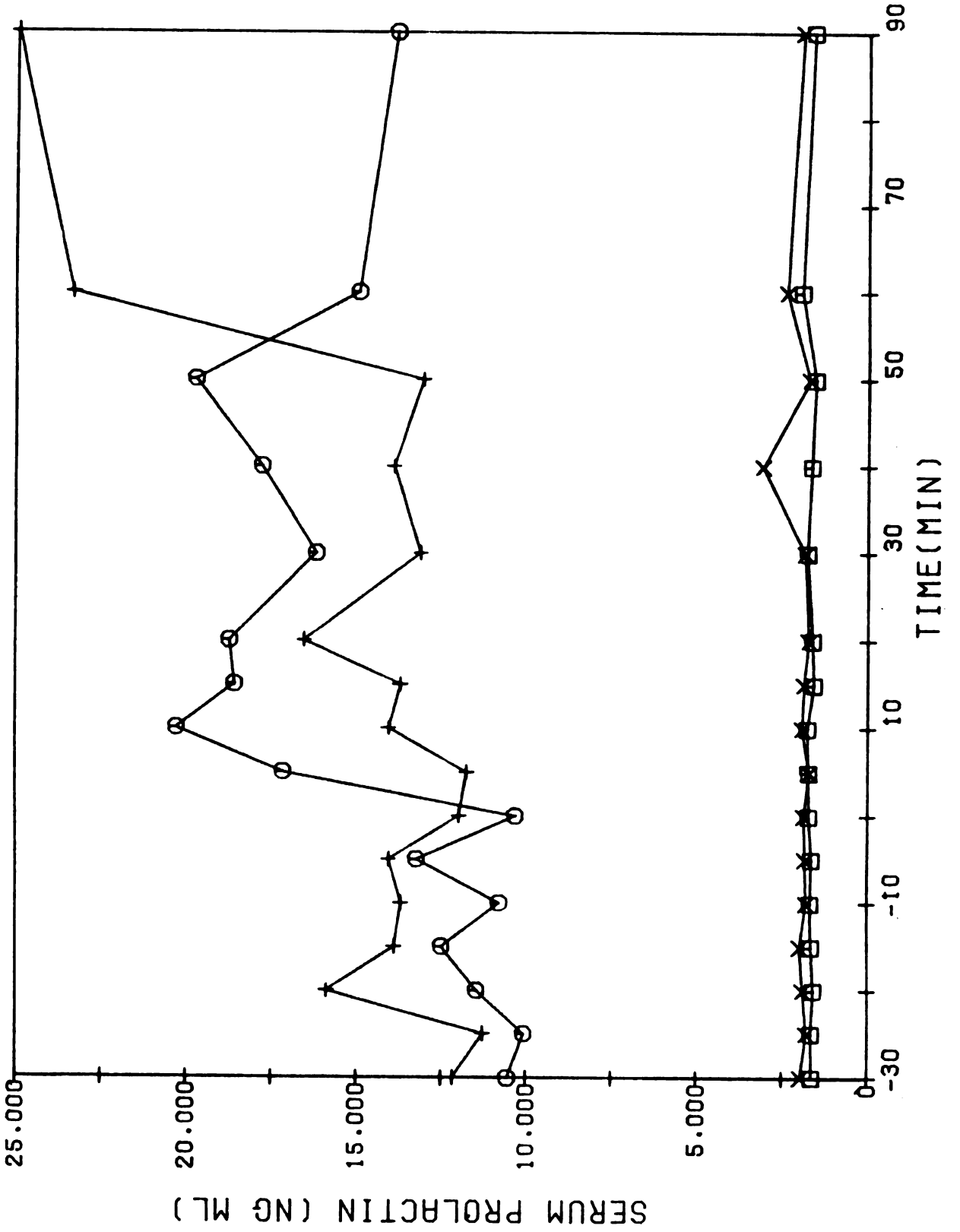
Fig. 8.--Serum prolactin concentrations in venous samples collected before and after intramammary infusion of 50 mg prolactin or saline at time 0.

o - o Mean prolactin concentrations of five early lactating cows before and after the injection of 50 mg prolactin.

+ - + Mean prolactin concentrations of five early lactating cows before and after the injection of saline.

□ - □ Mean prolactin concentrations of five late lactating cows before and after the injection of prolactin.

x - x Mean prolactin concentrations of five late lactating cows before and after the injection of saline.


SERUM PROLACTIN (NG ML)
25.000
20.000
15.000
10.000
5.000
0
-30
-10
10
30
50
70
90
TIME(MIN)

prolactin concentrations to 20.3 ± 9.4 ng/ml, 10 min post injection was not significant ($P > .05$). Thereafter serum prolactin concentration varied between 19.7 ± 8.1 and 13.8 ± 4.3 ng/ml for the remainder of the experiment. There was a great deal of variation in serum prolactin between individual animals during the post-injection period. Prior to the injection of saline, serum prolactin concentrations ranged from 11.2 ± 4.0 to 15.8 ± 5.4 ng/ml (Figure 5). From 5 to 50 min post injection serum prolactin concentrations ranged from 11.7 ± 4.3 to 16.5 ± 8.3 ng/ml. However, at 60 and 90 min post injection serum prolactin concentrations increased unexpectedly to 23.3 ± 14.2 and 24.9 ± 13.5 ng/ml.

Prior to intramammary injection of 50 mg prolactin, serum prolactin concentrations ranged from 1.5 ± .17 to 1.7 ± .21 ng/ml in five late lactating cows (Figure 8). During the 90 min post-injection period serum prolactin concentrations remained constant ranging from 1.7 ± 2.5 to 3.0 ± 1.3 ng/ml.

Experiment 7. The Effect of Ambient Temperature on Serum Prolactin Concentrations Before and After Milking or TRH

Blood samples were collected from three early lactating cows when the ambient temperature was high (20 to 28 C) and from three early lactating cows when the ambient temperature was low (3 to 8 C) before, during and

after milking. In the higher ambient temperature group, concentrations of serum prolactin in arterial and venous samples were 41.2 and 40.8 ng/ml, 30 min before milking (Table 2). From 0 to 20 min after milking, serum prolactin concentrations were 63.5 in arterial and 61.8 ng/ml in venous samples, thereafter declining (between 30 to 70 min after milking) to 44.5 ng/ml in arterial and venous samples. In the low temperature group, serum prolactin concentration averaged 15.6 in arterial and 14.5 ng/ml in venous serum samples, 30 min before milking (Table 2). For 20 min after milking serum prolactin concentrations averaged 33.6 in arterial and 33.0 ng/ml in venous serum samples. From 30 to 70 min after milking serum prolactin concentrations were 20.1 and 19.3 ng/ml, respectively, in arterial and venous samples.

The ambient temperature was high (21 to 26 C) when three late lactating cows were sampled, and low (1 to 17 C) when three other late lactating cows were sampled before, during and after milking. In the higher ambient temperature group, serum prolactin concentrations averaged 9.6 ng/ml in both arterial and venous samples 30 min before milking, increasing to 16.2 and 15.2 ng/ml, respectively, during the 20 min after milking (Table 2). From 30 to 70 min after milking, serum prolactin concentrations averaged 11.2 ng/ml in arterial and 10.9 ng/ml in venous samples. For 30 min before milking serum

Table 2.--Arterial and venous serum prolactin concentrations before and after milking at high and low ambient temperatures in early and late lactating cows.

	High ambient temperature (> 18 C)			Low ambient temperature (< 18 C)		
	30 to 0 min before milking	0 to 20 min after milking	30 to 70 min after milking	30 to 0 min before milking	0 to 20 min after milking	30 to 70 min after milking
Early Lactating Cows	41.2 ± 19.0[a]	63.5 ± 37.0[a]	44.5 ± 15.0[a]	15.6 ± 4.6[a]	33.6 ± 12.2[a]	20.1 ± 6.8[a]
	40.8 ± 18.2[b]	61.8 ± 15.6[b]	44.5 ± 15.0[b]	14.5 ± 4.1[b]	33.0 ± 12.0[b]	19.3 ± 6.7[b]
Late Lactating Cows	9.6 ± .6[a]	16.2 ± 3.1[a]	11.2 ± 1.68[a]	8.7 ± 1.5[a]	20.0 ± 10.1[a]	19.3 ± 17.8[a]
	9.6 ± .5[b]	15.2 ± 3.0[b]	10.9 ± 1.33[b]	9.1 ± 1.4[b]	19.7 ± 10.1[b]	16.6 ± 12.2[b]

[a]overall mean arterial serum concentrations of prolactin ng/ml, n = 3.

[b]overall mean venous serum concentrations of prolactin ng/ml, n = 3.

prolactin concentrations averaged 8.7 in arterial and 9.1 ng/ml in venous samples, in the low ambient temperature group. During the 20 min after milking, serum prolactin concentrations averaged 20.0 in arterial and 19.7 ng/ml in venous blood samples. From 30 to 70 min post milking, serum prolactin concentrations were 19.3 in arterial and 16.6 ng/ml in venous blood samples.

The differences in serum prolactin concentrations between high and low temperature groups, within a stage of lactation were not significant ($P > .05$). This is probably due to a large amount of variation associated with the small number of animals within each temperature group.

Blood samples were collected from three early lactating cows when the ambient temperature was high (20 to 29 C) and from three early lactating cows when the ambient temperature was low (11 to 12 C) before and after TRH, which was followed 30 min later by milking. In the high temperature group, serum prolactin concentrations were 43.8 in arterial and 43.6 ng/ml in venous samples, 30 min before administration of 200 μg TRH (Table 3). For 30 min after 200 μg TRH serum prolactin concentrations were 243 in arterial and 233 ng/ml in venous samples. The milking stimulus applied 30 min after TRH increased serum prolactin concentrations to 286 in arterial and 268 ng/ml in venous samples. In the lower ambient

Table 3.--Arterial and venous serum prolactin concentrations before and after 200 μg TRH, followed by milking at high and low ambient temperatures in early and late lactating cows.

	High ambient temperature (> 18 C)			Low ambient temperature (< 18 C)		
	30 to 0 min before TRH	0 to 30 min after TRH	0 to 20 min after milking	30 to 0 min before TRH	0 to 30 min after TRH	0 to 20 min after milking
Early Lactating Cows	43.8 ± 16.0[a]	243 ± 62[a]	286 ± 65[a]	14.6 ± 3.4[a]	115 ± 29.0[a]	209 ± 89.0[a]
	43.6 ± 16.7[b]	233 ± 57[b]	268 ± 54[b]	13.9 ± 3.3[b]	110 ± 28.0[b]	205 ± 88.0[b]
Late Lactating Cows	17.1 ± 8.0[a]	119 ± 35[a]	104 ± 28[a]	9.0 ± 2.0[a]	41.7 ± 19.0[a]	50.2 ± 25.2[a]
	17.4 ± 8.7[b]	115 ± 33[b]	101 ± 29[b]	8.8 ± 1.8[b]	40.7 ± 18.2[b]	50.8 ± 24.8[b]

[a]overall mean arterial serum concentrations of prolactin, n = 3.

[b]overall venous serum concentrations of prolactin, n = 3.

temperature group, serum prolactin concentrations were 14.6 in arterial and 13.9 ng/ml in venous serum samples, 30 min before 200 μg TRH. After TRH serum prolactin concentrations increased to 115 in arterial and 110 ng/ml in venous blood samples. Milking, 30 min after TRH, increased serum prolactin concentrations to 209 in arterial and 205 ng/ml in venous samples.

In three late lactating cows, from which blood samples were collected when the ambient temperature was high (18 to 23 C), serum prolactin concentrations averaged 17.1 in arterial and 17.4 ng/ml in venous samples before TRH (Table 3). For 30 min after 200 μg TRH, serum prolactin concentrations averaged 119 in arterial and 115 ng/ml in venous samples. For 20 min after milking, which occurred 30 min after TRH, serum prolactin concentrations averaged 104 in arterial and 101 ng/ml in venous samples. In three late lactating cows, from which blood samples were collected when the ambient temperature was low (1 to 9 C), serum prolactin concentrations were 9.0 in arterial and 8.8 ng/ml in venous samples. For 30 min after TRH, serum prolactin concentrations averaged 41.7 in arterial and 40.7 ng/ml in venous samples. For 20 min after milking, serum prolactin concentrations were 50.2 in arterial and 50.8 ng/ml in venous samples.

Within a stage of lactation, serum prolactin concentrations were not statistically different ($P > .05$)

between the high and low temperature groups, again because of a large amount of variation and small number of animals within a group.

The ambient temperature was high (22 and 23 C), when samples were collected from two early lactating cows treated with CB-154, before and after 500 µg TRH. For 30 min before TRH, serum prolactin concentrations were 7.7 in arterial and 7.6 ng/ml in venous samples (Table 4). For 20 min after TRH, serum prolactin concentrations in arterial and venous samples were 75.5 and 76.9 ng/ml, respectively. Serum prolactin concentrations averaged 65.7 in arterial and 66.8 ng/ml in venous samples 30 to 70 min post injection. In two early lactating cows treated with CB-154, from which blood samples were collected when the ambient temperature was low (6 and 7 C), serum prolactin concentrations were 10.6 in arterial and 11.1 ng/ml in venous samples 30 min before 500 µg TRH (Table 4). For 20 min following 500 µg TRH, serum prolactin concentrations were 171 in arterial and 155 ng/ml in venous blood samples. Thirty to 70 min after TRH serum prolactin concentrations were 113 in arterial and 107 ng/ml in venous blood samples.

The ambient temperature was high (19 C) when blood samples were collected before and after 500 µg TRH in two late lactating cows treated with ergocryptine. Thirty min before TRH, serum prolactin concentrations

Table 4.--Arterial and venous serum prolactin concentrations before and after 500 µg TRH, at high and low ambient temperatures in early lactating cows treated with 80 mg CB-154 and late lactating cows treated with 80 mg ergocryptine.

	High ambient temperature (> 18 C)			Low ambient temperature (< 18 C)		
	0 to 30 min before TRH	0 to 20 min after TRH	30 to 70 min after TRH	0 to 30 min before TRH	0 to 20 min after TRH	30 to 70 min after TRH
Early Lactating Cows	7.7 ± 1.7[a]	75.5 ± 6.7[a]	65.7 ± 16.4[a]	10.6 ± 4.1[a]	171 ± 39.0[a]	113 ± 33.0[a]
	7.6 ± 2.0[b]	76.9 ± 14.1[b]	66.8 ± 16.7[b]	11.1 ± 4.3[b]	155 ± 32.0[b]	107 ± 27.0[b]
Late Lactating Cows	2.0 ± .2[a]	35.9 ± 6.2[a]	22.3 ± 3.7[a]	1.9 ± .49[a]	6.6 ± 1.80[a]	5.0 ± 1.0[a]
	2.4 ± .5[b]	36.1 ± 8.8[b]	21.1 ± 5.0[b]	2.1 ± .50[b]	6.3 ± 1.79[b]	4.5 ± 1.0[b]

[a]overall mean arterial serum prolactin concentrations, n = 2.

[b]overall mean venous serum prolactin concentrations, n = 2.

(ng/ml) were 2.0 in arterial and 2.4 ng/ml in late lactating cows. Following TRH, serum prolactin concentrations increased to 35.9 in arterial and 36.1 ng/ml in venous samples. For 30 to 70 min after TRH, serum prolactin concentrations were 22.3 in arterial and 21.1 ng/ml in venous samples. Ambient temperature was low (2 and 5 C) in two late lactating cows treated with ergocryptine when blood samples were collected before and after the administrations of 500 μg TRH (Table 4). Serum prolactin concentrations were 1.9 in arterial and 2.1 ng/ml in venous samples before 500 μg TRH. Following TRH, serum prolactin concentrations increased to 6.6 in arterial and 6.3 ng/ml in venous samples. Thirty to 70 min after TRH serum prolactin concentrations were 5.0 in arterial and 4.5 ng/ml in venous samples.

Within the early stage of lactation, the two animals treated with CB-154 and sampled during the higher ambient temperatures had significantly lower serum prolactin concentrations after 500 μg TRH, compared with the two animals sampled at the higher ambient temperature ($P < .05$). Conversely, within the late stage of lactation, animals treated with ergocryptine and sampled at the lower temperature had significantly greater serum prolactin concentration following the administration of 500 μg TRH ($P < .05$).

DISCUSSION

In the present study basal serum prolactin concentrations before application of the milking stimulus or administration of TRH were approximately 300% greater in early lactating than in late lactating cows. Throughout this series of experiments early lactating cows produced approximately twice as much milk per day as did late lactating animals. In lactating ewes, basal serum prolactin concentrations were greater than in pregnant ewes (Arai and Lee, 1967; MacNeilly, 1971; Borger and Davis, 1973). Similarly, lactating pigs had greater basal concentrations of serum prolactin than agalactic sows at the same stage post partum (Threfall et al., 1974). Koprowski and Tucker (1973b) obtained estimates of basal serum prolactin concentrations in lactating cows from single samples, collected once a month, 2 to 4 hr before milking. Basal serum prolactin concentrations increased for the first 3 months of lactation, after which they remained relatively constant. Basal serum prolactin concentrations were not associated with milk yield for the first 3 months, but they were consistently and positively correlated in later lactation.

Vines (1976) observed in cows that basal serum prolactin concentrations declined from 22 ng/ml at the 2nd month of lactation to 12 ng/ml at the 8th month of lactation, after which they increased to 16 ng/ml at the 10th month of lactation. Collectively, greater basal concentrations of serum prolactin may be associated with greater intensity of milk production.

Administration of CB-154 to early lactating cows, or ergocryptine to late lactating cows caused a precipitous decline in basal prolactin concentrations. Similar observations have been recorded in rats (Nagasawa and Meites, 1970), sheep (Niswender, 1972), goats (Hart, 1973; McMurtry and Malven, 1974a), cows (Karg et al., 1972; Fell et al., 1974; Smith et al., 1974), and women (Brun del Re et al., 1973). However, in my study ergocryptine was somewhat more effective in depressing basal serum prolactin concentrations in late lactating cows (90% reduction) than was CB-154 in depressing basal prolactin concentrations in early lactating cows (75% reduction). After ergot treatment, serum prolactin concentrations in late lactating cows averaged less than 2 ng/ml whereas early lactating cows averaged approximately 7 ng/ml. It is possible that the difference is pharmacological since the two compounds are chemically different and may have different potencies. However, the pituitaries of early lactating, higher milk-producing cows contain greater quantities of prolactin compared with pituitaries of lower-producing animals (Reece and Turner,

1937). Furthermore, pituitaries of lactating sheep secrete prolactin at a faster rate than those of non-lactating sheep (Borger and Davis, 1973). Therefore, on the assumption that CB-154 and ergocryptine have similar potencies in cattle, then CB-154 may have been less effective in reducing basal serum prolactin concentrations because of physiological differences between early and late lactating cows. Coincident with the decrease in basal serum prolactin concentration there is also a decrease in the quantity of prolactin released to the milking or TRH stimulus with advancing stage of lactation. There are a number of possible explanations for this, for example the pituitary may gradually become refactory with advancing lactation, gradually releasing less prolactin both before and after the milking or TRH stimulus. Alternatively, as lactation advances, the quantity of prolactin synthesized by the pituitary may decline with advancing lactation, so that there is less prolactin available for release either before or after stimulation. A third possibility would be that the nerve tracts conveying the impulses to the brain, become less sensitive to the milking stimulus with advancing lactation. Obviously, further research is needed to solve this problem.

The milking or suckling stimulus releases prolactin not only in cows (Johke, 1969 and 1970; Tucker, 1971; Schams, 1972a; Koprowski and Tucker, 1973a) but in rats (Amenomori et al., 1968), goats (Bryant et al., 1968; Hart,

1975a; Bryant and Chameley, 1976), and sheep (Lamming et al., 1974). These observations were confirmed in Experiment 1 of the present study. Quantitatively serum prolactin concentrations of cows during early lactation increased an average of 20.3 ng/ml whereas late lactators increased 8.7 ng/ml. The greater release of prolactin in early as compared with late lactating cows agrees with the work of Koprowski and Tucker (1973a) in cows and that of Hart (1975a) in goats.

Vines (1976) observed that cows at two months of lactation tended to release more prolactin to the TRH stimulus than cows in later stages of lactation. In the present study, I observed that early lactating cows increased 150 ng/ml and late lactating cows increased 67 ng/ml following 200 µg of TRH. In cows pretreated with ergots early lactators increased 108 ng/ml whereas late lactators increased 19 ng/ml following 500 µg TRH.

Since milking and TRH released greater quantities of prolactin in early as compared with late lactators, this may suggest that the absolute quantity of prolactin released may be associated with lactational intensity. Indeed, Convey et al., (1973a,b) observed that TRH not only stimulated prolactin release from the pituitary but also caused a small but significant increase in milk yield. The increase in milk yield may well have been due partially to increased prolactin concentrations.

Although the quantities of prolactin released to milking or TRH were different between stages of lactation, the percentage differences between mean basal concentrations and mean post-milking or post-TRH concentrations were very similar in early and late lactating cows. Since milk yields were about 100% more in early as compared with late lactators, it seems doubtful that percent change in serum prolactin after milking or TRH is related to milk production.

The milking and TRH stimuli probably act to release pituitary prolactin by different mechanisms. Support for this concept is evident by comparing the results of Experiments 3 and 4. In cows treated with CB-154 or ergocryptine, milking did not release prolacting, whereas TRH did release prolactin. Ergot inhibition of milking-induced release of prolactin has been reported previously in goats (Hart, 1973) and cows (Smith et al., 1974). That TRH will overcome ergot inhibition was reported previously in goats (McMurtry and Malven, 1974a) and cows (Schams, 1972b). Collective evidence from the literature suggests that prolactin secretion from the pituitary is under chronic inhibition from release inhibiting factor(s) secreted from the hypothalamus (Meites and Clemens, 1972). The milking stimulus probably acts to reduce this inhibition on the pituitary, thus, allowing prolacting to be released. TRH acts directly on the pituitary to release prolactin (Smith and Convey, 1975). Furthermore, Hill-Salmi and MacLeod (1975) observed that ergocryptine blocked release of

prolactin from rat pituitaries *in vitro*, but TRH would overcome this inhibition. I postulate, in explaining the results of Experiments 3 and 4, that the milking stimulus in cattle may block release of the physiological prolactin release inhibiting factor(s) of hypothalamic origin, in Experiment 3, but because of the presence of an exogenous prolactin release inhibiting factor (CB-154 or ergocryptine) prolactin is not released. However, in Experiment 4, administration of a prolactin releasing factor, TRH, overcomes the inhibition of prolactin secretion by CB-154 or ergocryptine and releases prolactin.

Further evidence that TRH and the milking stimulus act by different mechanisms to release pituitary prolactin is provided in Experiment 2. Application of the milking stimulus following administration of 200 μg TRH increased serum prolactin concentrations an additional 39% in early lactating cows, and an additional transitory 18% in late lactating cows. A similar observation was reported by Tucker et al. (1975), who were able to obtain additional releases of prolactin by application of the milking stimulus to lactating cows being continuously infused intravenously with TRH. If in the above experiments, the pituitary is responding maximally to prolactin releasing factor stimulation (TRH), then the additional prolactin release following milking is probably caused by a different mechanism.

Although TRH released prolactin after ergot drug therapy and milking stimuli did not, the TRH-induced

release of prolactin was much reduced. For example, lactating cows treated with CB-154 and then given 500 µg TRH (Experiment 3) released only 65% as much prolactin as a lower dose (200 µg TRH) released in the same animals in the absence of CB-154 (Experiment 4). Similarly in late lactating cows treated with ergocryptine, 500 µg of TRH released only 25% as much prolactin as was released by 200 µg TRH in the same animals before ergocryptine treatment. These results agree with those of Schams (1972b) who observed a marked reduction in the ability of TRH to release prolactin in lactating cows pretreated with CB-154. Thus, whether the failure of the milking stimulus to release prolactin in the face of ergots truly represents a difference with TRH in mechanism of action, or merely a difference in potency of these two prolactin-releasing stimuli will require additional research.

The rate of disappearance of prolactin from the blood, following milking or TRH stimulus was slower especially in late lactating cows than would have been expected on the basis of past reports. Other workers determined the half life of serum prolactin in lactating cows to be 25 to 29 min (Johke, 1969; Schams and Karg, 1970; and Tucker et al., 1972). Tucker (1971) determined that serum prolactin concentrations post milking returned to pre-milking values within approximately 35 min. In the present study, following milking in early lactating cows, serum prolactin concentrations returned to pre-milking

values 50 min later. In late lactating cows prolactin concentrations had not returned to their pre-milking values 70 min after milking, and serum prolactin concentrations only declined 12% during the 70 min post-milking period. Davis and Borger (1973) found that lactating sheep had greater pituitary secretion rates and metabolic clearance rates for prolactin compared with non-lactating animals. Since early lactating cows were producing twice as much milk as cows during late lactation, perhaps the secretion and clearance rates of prolactin were reduced during late lactation in my study. Therefore, greater serum concentrations of prolactin, resulting from milking in the early lactating cows, would decline at a faster rate compared with those of the late lactating cows.

A rather slow disappearance rate for serum prolactin, following the administration of TRH, is evident in early lactating cows treated with CB-154 and late lactating cows treated with ergocryptine. For example, administration of TRH caused a 10 to 13-fold increase in serum prolactin concentrations, however, during the 70 min post-injection period, serum prolactin concentrations only declined 21 to 35%. Malven and McMurtry (1974a) observed in two goats pretreated with CB-154 that serum prolactin concentrations following TRH remained elevated for 6 hr. These slow disappearance rates during ergot therapy are consistent with reduced clearance rates for serum prolactin. Furthermore, since absolute quantities of serum prolactin are

also reduced, I suggest that ergots also may reduce secretion rates of prolactin.

The results of Experiment 7 suggest that, in addition to stage of lactation, another important factor which affects serum prolactin concentrations in cows is ambient temperature. Within a stage of lactation, basal (before milking or TRH) prolactin concentrations of serum in cows were on average 270% greater, but not significantly different, during high (>18 C) ambient temperatures compared with low (<18 C) temperatures. Although on average there was little difference in basal prolactin concentrations before milking between the high and low ambient temperature groups during late lactation (Table 2), this may be explained by one cow in the "low" temperature group being sampled during a relatively "high" (17 C) ambient temperature.

Within a stage of lactation, the groups subjected to high ambient temperature had quantitatively greater serum prolactin concentrations following milking or TRH plus milking. Serum prolactin concentrations were 100% greater after milking in early lactating cows milked at higher ambient temperatures compared with early lactating cows milked at lower ambient temperatures. Similarly, serum prolactin concentrations were respectively 211% and 175% greater in groups during high temperatures compared with groups during low temperatures after administration of 200 μg TRH followed by milking. That serum prolactin

concentrations are quantitatively greater in lactating cows at higher ambient temperatures, before and after TRH agree with previous reports in heifers (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976), lactating cows (Vines, 1976) and rats (Mueller et al., 1974). Furthermore, serum prolactin concentrations were greater before and after milking in goats (Hart, 1975b) and cows (Schams, 1972a; Koprowski and Tucker, 1973b) during warmer months.

Although absolute serum prolactin concentrations were generally greater before and after milking in the higher temperature groups the quantity of prolactin released after the milking stimulus (post-milking concentrations minus pre-milking concentrations) was not markedly affected by ambient temperature. In contrast, Koprowski and Tucker (1973b) observed greater quantities of prolactin released after milking during low temperatures as compared with high temperatures. However, in their study prolactin estimates were determined on single serum samples collected 2 to 4 hr before milking, 5 min after or 1 hr after milking. The design of the present study was based on multiple samples collected before and after milking and is in my opinion more representative of the changes which occur in serum prolactin at milking during high versus low ambient temperatures.

A discrepancy was observed in cows pretreated with ergots. Within a stage of lactation, basal serum prolactin concentrations were similar at either high or low ambient

temperatures. However, following 500 μg TRH, serum prolactin concentrations were 230% greater in the low temperature group compared with high temperature in early lactators pretreated with CB-154. In contrast, in the late lactating cows pretreated with ergocryptine serum prolactin concentrations following 500 μg TRH were 540% greater during low ambient temperatures compared with high ambient temperature group. Furthermore, changes in the quantities of prolactin released after TRH (post TRH minus pre TRH concentrations) parallelled the absolute concentrations. A possible explanation for the difference in response between stages of lactation at the same ambient temperature may be pharmalogical, since CB-154 and ergocryptine are chemically different.

I conclude that within a stage of lactation, higher ambient temperatures are associated with greater serum prolactin concentrations, both before and after milking or TRH. Furthermore, since greater prolactin concentrations are associated with increased water movement across membranes in lower animals, it may be speculated that greater serum prolactin concentrations in lactating cows during the warmer, drier summer months are associated with water conservation by the animal.

Prolactin binds to mammary glands of laboratory species <u>in vitro</u> (Frantz et al., 1974; Shiu and Friesen, 1974; Holocomb et al., 1976). Considering that mammary glands of lactating cows are very large target organs for

protein hormones, it seemed reasonable to suppose that binding of prolactin to bovine mammary cells might be apparent as a difference in serum hormone concentrations before and after blood has passed through the gland. Furthermore, since early lactating cows quantitatively released more prolactin to TRH and milking, one might suspect that mammary utilization or uptake would also be increased compared with that found in late lactating cows. Therefore, the arteriovenous differences in hormone concentration reported here are assumed to be representative of mammary binding or uptake of prolactin.

Arteriovenous differences tended to be small and not different from zero when serum prolactin was at basal concentrations. The average arteriovenous difference for basal serum prolactin concentrations was 0.1 ng/ml for all cows across all experiments. However, following TRH or the milking stimulus, average arteriovenous differences increased to approximately 3.0 ng/ml, and on average remained at this value for at least 50 min. However, the increases in arteriovenous differences for serum prolactin in Experiments 1 and 4 were not significant, although the increase in the arteriovenous difference for early lactating cows in Experiment 1 following milking approached significance. The absence of significant arteriovenous differences in Experiments 1 and 4 was caused by a large amount of between-animal variation. A particularly obvious example of this occurred in late lactating cows 30 to 70 min post milking

in Experiment 1. Here, arteriovenous differences were large and positive as the result of a large, unexplained release of prolactin in two of the six cows. This occurred after the milking-induced release of prolactin in one particular replicate of that experiment. Arteriovenous differences tended to be small and negative throughout Experiment 3 in which serum prolactin concentrations remained constant and markedly depressed because of ergot treatment. For 30 min after TRH in Experiment 2, arteriovenous differences for prolactin became large and positive for both early (7.7 ng/ml) and late (2.6 ng/ml) lactating cows.

The increase in arteriovenous differences associated with increased serum prolactin concentration may be indicative of prolactin binding to the mammary gland. This contention is supported by _in vitro_ studies, in which the specific mammary binding of prolactin increased with increasing concentrations of prolactin, until all the binding sites are saturated (Frantz et al., 1974; Shiu and Friesen, 1974; Holcomb et al., 1976). Possibly, as serum prolactin concentrations increase _in vivo_, more prolactin binds to specific mammary receptors, and in order to saturate the binding sites large serum concentrations are required. This contention is supported by the results of Experiment 2 where concentrations of serum prolactin were greatest and arteriovenous differences, which were different from zero, were largest. Furthermore, in cows treated

with CB-154 or ergocryptine, in which serum prolactin concentrations remained low, arteriovenous differences also remained small and never differed from zero.

Early lactating cows tended to have larger arteriovenous differences in serum prolactin after application of TRH or milking stimuli than late lactating cows. This may have been due to the early lactating cows releasing larger quantities of prolactin which may have resulted in more mammary binding sites taking up prolactin. Birkinshaw and Falconer (1972) showed that in rabbits, mammary receptors for prolactin are located on the epithelial cell membrane. If this is also true for cows, the mammary glands of cows during early lactation should have a larger number of prolactin receptors compared with late lactating cows, since they have a larger number of epithelial cells. The possibly larger number of prolactin receptors may explain the tendency for the mammary glands of early lactating cows to take up greater quantities of prolactin following milking or TRH.

The approximate blood flow, estimated from milk yield (using a 500:1 multiplication factor) through the mammary glands of early lactating cows is 8 l/min and in late lactating cows is 4 l/min. Therefore, mammary uptake of prolactin, calculated from blood flow times arteriovenous difference of prolactin concentrations could be at most approximately 9 µg/min in early and 1.5 µg/min for late lactating cows for the 20 min beginning at milking.

Similarly, during the 30 min after 200 μg TRH, maximum mammary uptake of prolactin could be 64 μg/min in early and 11 μg/min in late lactating cows. Therefore the mammary glands of the early lactating cows could be taking up substantially greater quantities of prolactin compared with those of late lactating cows, even though arteriovenous differences are not markedly different between the two stages of lactation. I speculate the possible increased uptake of prolactin in early lactating cows may be associated with their greater milk production.

In vitro studies have indicated that the number of specific binding sites for prolactin in the mammary gland is limited, and becomes saturated at higher concentrations of prolactin (Frantz et al., 1974; Shiu and Freisen, 1974; Holcomb, 1976). For 30 min following the TRH stimulus, arteriovenous differences were large and positive in both early and late lactating cows. When the milking stimulus was applied 30 min after TRH, serum prolactin concentrations increased 39 and 18% in early and late lactating cows. However, the mean arteriovenous differences after the application of the milking stimulus were not different from zero. This may indicate that mammary binding became saturated within 30 min of elevated serum prolactin concentrations.

It has been observed in rats (McMurtry and Malven, 1974b; Grosvenor and Whitworth, 1976) and goats (McMurtry and Malven, 1974a) that increased in plasma prolactin

concentrations are rapidly followed by increased in concentrations of prolactin in milk. This may imply that passage of prolactin from blood into milk is unrestricted. It is therefore pertinent to consider whether arteriovenous differences in serum prolactin concentrations reflect active uptake and specific binding of prolactin by the mammary gland or is indicative of a simple diffusion process in which prolactin leaks from blood into milk.

Following administration of TRH, serum prolactin concentrations were elevated for 30 to 70 min immediately before the PM milking. However, prolactin concentrations in skim milk samples collected at this milking or at the AM milking 12 hr later were not different from the prolactin concentrations in skim milk samples collected before administration of TRH. These results argue against the concept that simple diffusion is the mechanism responsible for the passage of prolactin from blood into milk. If simple diffusion was the mechanism responsible, one would expect prompt elevation in milk prolactin concentrations following TRH. Although administration of CB-154 or ergocryptine reduced milk concentration of prolactin 53% in comparison with milk from untreated cows, prolactin concentrations in skim milk samples remained elevated 71% above serum prolactin concentrations. McMurtry and Malven (1974a) observed that prolactin concentrations in whole milk samples from goats treated with CB-154, were greater than in plasma samples. Following intravenous infusion of

exogenous prolactin in rats, milk prolactin concentration remain elevated after serum prolactin returned in pre-infusion concentrations. Collectively, the above results support the concept of an active transport system which moves prolactin molecules from lower concentrations in the blood to higher concentrations in the milk.

Further evidence that passage of prolactin from blood into milk is not a simple process is provided by the results of the intramammary injections of saline and prolactin. Intramammary injection of 50 mg prolactin into late lactating cows did not increase blood serum concentrations of prolactin. Therefore, even when large quantities of prolactin are present in milk, there was no measurable passage of prolactin into the blood. The results of the intramammary injection of prolactin or saline in early lactating cows is more complex. Following intramammary injection of prolactin in early lactating cows there was a 50% increase in serum prolactin concentrations 10 min later. However, this was the result of increases in serum prolactin concentrations in two of five cows. I speculate that the increase in serum prolactin in these two cows was not the result of intramammary injection of prolactin, but caused possibly by the manipulation of the udder or possibly a non-specific release of prolactin from the pituitary. The possibility of a non-specific increase in serum prolactin concentration is likely since, following intramammary injections of saline, serum prolactin

concentrations remained low and relatively constant until 50 to 60 min post injection when they increased substantially for no apparent reason. I, therefore, suggest that the arteriovenous differences observed in the above experiments are probably a reflection of active transport of prolactin from blood to milk. Whether this process is part of the mechanism of action of prolactin on mammary cells is questionable, but after binding to a receptor at the surface of the cell, the prolactin molecule may be transported through the cell and excreted on the luminal side into the milk. Evidence to support this hypothesis has been presented by Nolin and Witorsh (1976) who found prolactin inside rat mammary epithelial cells.

On average the quantity of prolactin contained in the milk removed at one milking was approximately 288 μg for early and 72 μg in late lactating cows. Theoretically, in both early and late lactating cows, the mammary uptake of prolactin during the 30 min after milking or 6 min after TRH could account for all the prolactin found in milk. However, mammary uptake of prolactin continued for 30 min following TRH, and was in excess of the total amount of prolactin found in milk. This may indicate that a certain proportion of the prolactin taken up by the gland, is not immediately transferred to the milk or is metabolized in the mammary cells <u>en route</u> to the milk. The large arteriovenous differences which occurred in two late lactating cows following an unexplained increase in serum prolactin

concentrations, 40 to 50 min after milking, suggests that there may be a greater uptake of prolactin when the mammary gland is empty, after milking.

It is pertinent to consider that there are certain limitations in using the arteriovenous difference technique to determine mammary utilization of protein hormones. For example, not all the hormone passing from the blood capillaries into the extracellular fluid may be bound by the mammary cells. Excess hormone might be transported away by the lymphatic system, which would mean that the arteriovenous differences would yield an exaggerated estimate of hormone utilization. Conversely, the arteriovenous difference technique might underestimate mammary uptake of hormone. This is possible since in binding to a mammary receptor, a hormone may be competitively displacing a molecule of the same hormone already occupying the receptor. If the displaced molecules after binding to the receptor are biologically inactive, but still immunologically active, then any mammary uptake of hormone will be masked, since the radioimmunoassays used to determine serum hormone cannot distinguish between biologically active or inactive hormone.

The observations that basal concentrations of serum growth hormone are neither affected by stage of lactation, CB-154 nor ergocryptine, agree with previous reports (Oxender et al., 1972; Koprowski and Tucker, 1973b; Smith et al., 1974). Following administration of 200 μg TRH,

serum growth hormone concentrations increased 250% in early and 192% in late lactating cows. Injection of 500 µg TRH into early lactating cows or late lactating cows treated with ergot increased serum growth hormone approximately 280%. Thus, I conclude that ergot drugs did not effect markedly the TRH-induced release of growth hormone. This conclusion agrees with the finding of Benker et al. (1976) who observed that CB-154 did not effect TRH-stimulated release of growth hormone in humans.

The larger dose of 500 µg TRH released more growth hormone than the 200 µg dose. Convey et al. (1973a) observed that the quantity of growth hormone released in lactating cows was proportional to dose. Following 200 µg and 500 µg TRH growth hormone concentrations in serum increased 32 and 58% more in early than in late lactating cows. Vines (1976) found that cows in their second month of lactation released more growth hormone to the TRH stimulus than cows at any other stage of lactation. The greater quantity of growth hormone released to TRH by the early lactating cows of my study, may indicate that at the earlier stages of lactation, the pituitary is capable of releasing more growth hormone to other physiological stimuli. Furthermore, since growth hormone is galactopoeitic in cows (Hutton, 1957; Shaw et al., 1955; Brumby and Hancock, 1955; Bullis et al., 1965; Machlin, 1973), the larger quantities of growth hormone available for release in the pituitaries of early lactating cows may be

associated with the greater intensity of lactation at this stage.

That growth hormone plays an important role in milk production is suggested by the arteriovenous difference data. Prior to administration of TRH, arteriovenous differences in growth hormone were small (0.1) and not different from zero. However, associated with increased serum growth hormone concentrations after TRH, arteriovenous differences became larger (0.6 ng/ml), positive and significant. This may suggest that at higher serum concentrations, growth hormone binds to the mammary gland. Furthermore, since the estimated blood flow through the mammary glands of the early lactating cows is approximately twice that of the late lactating cows, mammary uptake of growth hormone after TRH would be approximately 5 μg/min in early and 2.6 μg/min in late lactating cows. Suggesting a greater mammary utilization of growth hormone in early lactating cows. In lactating cows growth hormone concentrations are remarkably constant. For example in the present study the milking stimulus did not increase serum growth hormone after TRH administration. This finding is in agreement with those of Reynaert and Peters (1972) and Koprowski and Tucker (1973b). However, growth hormone is responsive to changes in blood metabolite concentrations (Reynaert et al., 1975a). Therefore, mammary uptake of serum growth hormone may be dependent upon increased

growth hormone concentrations resulting from changes in blood metabolite concentrations.

Serum insulin concentrations were greater in the late lactating cows compared with the early lactating cows. This agrees with previous reports in which serum insulin concentrations increased with advancing lactation (Koprowski and Tucker, 1973b; Smith et al., 1976). Serum insulin concentrations are negatively correlated with milk yield (Koprowski and Tucker, 1973b; Hart et al., 1975). My observation of greater serum insulin concentrations in cows in late lactation which are producing 50% less milk compared with the early lactating cows would agree with these reports.

Serum insulin concentrations in both early and late lactating cows remained constant for a period of time beginning 30 min prior to milking until 70 min post milking. In other words, milking did not cause release of insulin in my cows. In contrast, Koprowski and Tucker (1973b) observed greater serum concentrations of insulin in samples collected within 5 min of milking, compared with samples collected 2 to 4 hr before or 1 hr after milking. However, in the experiment of Koprowski and Tucker (1973b) serum insulin concentrations were determined in single samples, collected at monthly intervals, which may not have been truly representative of the actual serum insulin concentrations during that period of time.

Although serum insulin concentrations tended to be greater in late lactating cows, arteriovenous differences for early and late lactating cows were not significantly different from each other. However, from 30 min before milking until 20 min post milking in early lactating cows and for the 20-min period beginning at milking for the late lactating cows, arteriovenous differences for insulin were greater than zero. I speculate that the arteriovenous differences for insulin observed during and after milking are associated with an increased uptake of glucose and other metabolites at this time, since removal of milk probably stimulates uptake of metabolites by the mammary cells for the further synthesis of milk products. However, the mammary glands of early lactating, higher producing cows probably utilize more glucose and other metabolites than those of late lactating cows. Therefore, it is surprising that the arteriovenous differences for insulin were similar at both stages of lactation. However, estimated mammary blood flow is approximately twice as great in early compared with late lactating cows. Therefore, from 30 min before, until 70 min after milking, mammary uptake of insulin would approximate .10 μg/min in early and .05 μg/min in late lactating cows. This suggests that early lactating cows would have a greater overall mammary uptake of insulin. Plasma insulin concentrations increase with plasma glucose concentrations (McAtee and Trenkle, 1971; Hove, 1975) and when administered simultaneously with

glucose, insulin is galactopoeitic in cows (Cowie, 1961). Perhaps a better time to determine the mammary utilization of insulin would have been obtained when both insulin and glucose concentration were increasing.

I conclude that greater intensity of lactation in cows is associated with greater serum prolactin concentration, and lower serum insulin concentrations. Also, that greater mammary uptake of prolactin and growth hormone occurred at intervals when these hormones were acutely increased in serum. The uptake of insulin by the mammary gland which occurs at milking may be associated with increased mammary uptake of metabolities.

SUMMARY AND CONCLUSIONS

1. In six early (5 to 12 weeks post partum) lactating cows, serum prolactin concentrations in arterial and venous samples, collected simultaneously, averaged 28 ng/ml for 30 min before milking, 48 ng/ml for 20 min beginning at milking and 32 ng/ml for 30 to 70 min after milking. For the same three periods, serum prolactin concentrations averaged 9, 17, and 14 ng/ml in six late (37 to 57 weeks post partum) lactating cows; values which were less than corresponding averages in early lactating cows. Arteriovenous differences in serum prolactin concentration were .68 in early and -.16 ng/ml in late lactating cows during the 30 min pre-milking period. For the 20 min beginning at milking, arteriovenous differences increased to 1.05 in early and .35 ng/ml in late lactating cows. None of the arteriovenous differences were different from zero but the arteriovenous difference during the 20 min beginning at milking approached significance ($P \simeq .1$). The generally greater concentrations of serum prolactin and the greater arteriovenous differences following milking in early lactating cows may be associated with greater intensity of lactation characteristic of this physiological state.

2. Serum prolactin concentrations in arterial and venous samples collected 30 min before administration of 200 μg TRH averaged 29 ng/ml in six early and 13 ng/ml in six late lactating cows. Following administration of 200 μg TRH, serum prolactin concentrations averaged 175 ng/ml in the early lactating and 79 ng/ml in late lactating cows. Application of the milking stimulus 30 min later, caused a further increase in serum prolactin concentrations to 243 ng/ml in the early lactating cows, but had little effect on serum prolactin concentrations in late lactating cows (77 ng/ml). Arteriovenous differences, 30 min prior to administration of TRH were not different from zero in early (.40 ng/ml) or late lactating cows (-.01 ng/ml). However, during the 30 min after TRH, arteriovenous differences in serum prolactin increased to 7.7 ng/ml in early and 2.6 ng/ml in late lactating cows ($P<.05$). The greater concentrations of serum prolactin and greater arteriovenous differences after TRH in early lactating, as compared with late lactating cows may be reflective of greater intensity of lactation.

3. Twenty-four hr following administration of 80 mg CB-154 to five early lactating and 80 mg ergocryptine to five late lactating cows, serum prolactin concentrations declined from 29.0 to 7.2 and 13.0 to 1.7 ng/ml, respectively. Thirty min prior to milking, serum prolactin concentrations in arterial and venous blood samples averaged 7.2 in early and 1.7 ng/ml in late lactating cows.

Seventy min following milking, serum prolactin concentrations remained constant, averaging 7.5 and 1.8 ng/ml, respectively. Overall mean arteriovenous differences from 30 min before, until 70 min after milking, averaged -.1 ng/ml in early, and -.02 in late lactating cows.

4. Prior to administration of 500 µg TRH, serum prolactin concentrations in arterial and venous blood samples averaged 9.3 ng/ml in five early lactating cows pre-treated with CB-154, and 2.0 ng/ml in five late lactating cows pre-treated with ergocryptine. For 20 min after injection of 500 µg TRH serum prolactin concentrations averaged 113 ng/ml in early and 20.7 ng/ml in late lactating cows. Thereafter, serum prolactin concentrations declined, averaging 90 ng/ml in early and 12.9 ng/ml in late lactating cows 30 to 70 min post TRH injection. During the 30 min pre-injection period arteriovenous differences in serum prolactin concentration were -.14 in early and -.18 ng/ml in late lactating cows. Arteriovenous differences increased to 6.3 ng/ml in early and averaged -.24 ng/ml in late lactating cows during the 20 min post-injection period. I speculate that greater serum prolactin concentrations and arteriovenous differences following administration of TRH in early lactating cows was associated with greater intensity of lactation during this physiological state.

5. Prolactin concentrations in skim milk samples, collected at milking, averaged 24.6 ng/ml in five early lactating and 11.4 ng/ml in five late lactating cows.

These values were similar to basal serum concentrations of prolactin in early (27.6 ng/ml) and in late (9.3 ng/ml) lactating cows. Administration of CB-154 to early or ergocryptine to late lactating cows reduced prolactin concentrations in skim milk samples from 22.9 to 11.9 and from 12.0 to 7.0 ng/ml, respectively. However, the decrease in prolactin concentrations in skim milk was not as great as in serum, in which prolactin concentrations decreased from 28.8 to 7.4 in early and 13.0 to 1.7 ng/ml in late lactating cows. Thus, prolactin is transported from blood into milk against a concentration gradient.

6. Five early lactating cows, pre-treated with CB-154 were given intramammary injections of 50 mg prolactin. Prior to injection, serum prolactin concentrations in mammary vein samples averaged 11 ng/ml. Serum prolactin concentrations increased to 20 ng/ml, 10 min post injection, but because of a large amount of between animal variation this change was not significant. In control experiments, in which an equal volume of vehicle was injected, serum prolactin concentrations averaged 13.2 ng/ml from 30 min before until 50 min after injection. At 60 and 90 min post injection, serum prolactin concentrations increased unexpectedly to 23 and 24 ng/ml, respectively.

For 30 min prior to intramammary injection of 50 mg prolactin or saline serum prolactin concentrations averaged 1.7 ng/ml in five late lactating cows treated with ergocryptine. During the 90 min after injection, serum

concentrations remained constant at 1.7 ng/ml. I conclude that prolactin passes from blood into milk but not from milk to blood.

7. Serum prolactin concentrations tended to be greater, within a stage of lactation, before and after milking or TRH, when blood samples were collected during high ambient temperatures in comparison with serum collected at low ambient temperatures.

8. Serum growth hormone concentrations in arterial and venous samples collected 30 min before administration of 200 μg TRH averaged 4.0 ng/ml in six early lactating cows and 3.8 ng/ml in six late lactating cows. For 30 min after TRH, serum growth hormone concentrations averaged 14.9 in early and 11.4 ng/ml in late lactating cows. Application of the milking stimulus 30 min after TRH did not affect serum growth hormone concentrations, and 20 min post milking serum growth hormone concentrations averaged 7.3 ng/ml in early and 5.1 ng/ml in late lactating cows. Arteriovenous differences in early (-.03 ng/ml) and late (-.05 ng/ml) lactating cows were not different from zero 30 min before TRH. For 30 min following administration of TRH, arteriovenous differences were different from zero increasing to .58 ng/ml in early and .73 ng/ml in late lactating cows. The significant arteriovenous differences following administration of TRH may be indicative of mammary binding of growth hormone.

9. Serum growth hormone concentrations in arterial and venous blood samples averaged 5 ng/ml in five early lactating cows pre-treated with CB-154, and 3.5 ng/ml in five late lactating cows pre-treated with ergocryptine, 30 min prior to administration of 500 μg TRH. For 20 min after TRH, serum growth hormone concentrations averaged 20.3 ng/ml in early lactating cows. During the same period, serum growth hormone concentrations were significantly lower in late lactating cows averaging 12.8 ng/ml. Seventy min after TRH, serum growth hormone concentrations declined to 7.4 in early and 5.2 ng/ml in late lactating cows. Arteriovenous differences were -.04 in early and .20 ng/ml in late lactating cows before administration of TRH. After TRH, arteriovenous differences increased to .7 in early and .5 ng/ml in late lactating cows. Whether the greater serum growth hormone concentrations in early lactating cows following TRH was associated with a greater responsiveness of the anterior pituitary in general must await further investigation.

10. From 30 min before until 70 min after milking, serum insulin concentrations remained constant averaging 2.6 in six early and 3.5 ng/ml in six late lactating cows. Serum insulin concentrations in arterial and venous samples were greater in late lactating cows ($P<.10$) compared with early lactating cows. During the 20 min post-milking period, arteriovenous differences were different from zero in both early (.11 ng/ml) and late (.16 ng/ml) lactating

cows. The greater concentrations of serum insulin in late lactating cows, compared with early lactating cows, may be associated with a lower intensity of lactation.

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