

The Importance of Prolactin for Initiation of Lactation in the Pregnant Ewe

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Abstract

A single injection of ergocryptine (0.5 mg/kg liveweight) given to ewes 0.5-20 days prepartum or two injections (0.5 mg/kg liveweight per injection) given *c.* 30 and 10 days prepartum reduced concentrations of plasma prolactin to negligible (<5 ng/ml) values for 4 weeks after parturition, but did not affect concentrations of growth hormone and placental lactogen.

Milking of treated ewes had no effect on concentrations of plasma prolactin during the first 4 weeks of lactation, but concentrations of growth hormone were increased during the 10-20 min period after milking. The half-life of prolactin in plasma was estimated as 21 min.

In spite of the dramatic effect of ergocryptine on plasma prolactin all treated ewes secreted copious quantities of milk of normal composition. Mean daily yields of ewes treated with ergocryptine were not significantly different ($P > 0.05$) from those of untreated control ewes, but the mean \pm s.e.m. of total milk production over the first 3 weeks of lactation for ergocryptine-treated ewes was significantly lower ($P < 0.05$) than that of control ewes (9.5 ± 1.11 v. 14.1 ± 1.20 kg milk).

The results suggest that prolactin is not an essential component of the lactogenic and galactopoietic complexes of hormones in the ewe.

Introduction

Prolactin is regarded as an essential component of the hormonal complex necessary for lactogenesis in many animal species (Denamur 1971). In the ewe, concentrations of plasma prolactin increase shortly before parturition to reach maximum values at term, then decrease during the post partum period (Davis *et al.* 1971; Lamming *et al.* 1974).

In previous studies with ewes induced to lactate artificially and treated with the prolactin inhibitor ergocryptine, lactogenesis occurred in the absence of detectable levels of prolactin in plasma (Fulkerson *et al.* 1975; Field *et al.* 1979). This finding led to the suggestion that prolactin may not be an essential component of the lactogenic complex of hormones in the ewe. However, in both studies, ergocryptine was administered immediately before lactation was triggered artificially in ewes with developed mammary glands. Thus it was possible that the requirement for prolactin had been met prior to ergocryptine treatment.

In the present studies the effects of treatment of pregnant ewes with ergocryptine during the last 30 days of pregnancy on subsequent milk yield and composition were determined. In addition, studies were conducted to determine whether prolactin was released in response to the milking stimulus during lactation in ewes treated before parturition with ergocryptine. Further, concentrations of growth hormone and placental lactogen before and after parturition, and of growth hormone following milking were measured.

Materials and Methods

Sheep

Thirty pregnant crossbred ewes (Border Leicester \times Merino), free of obvious abnormalities of the mammary glands, were used. Service dates were recorded and it was assumed that parturition would occur 148 days after mating. At the time the experiment commenced, liveweights of the pregnant ewes ranged from 47 to 66 kg. All ewes were accustomed to handling and could be blood sampled and milked with minimum restraint. They were housed indoors for at least 10 days before commencement of experiments, which were conducted during the winter months (June and July). Lucerne chaff was fed *ad libitum*.

Ergocryptine

Ergocryptine (α -ergocryptine; Sigma Chemical Co., St Louis, U.S.A.) was dissolved in ethanol. Saline (9 g NaCl/l) was then added to give a solution containing 60:40 (v/v), saline:ethanol, and a final concentration of 5 mg ergocryptine/ml.

Experimental Procedure

All ewes were fitted with indwelling jugular catheters (1.0 mm i.d., 1.5 mm o.d.; Dural Plastics, Sydney) 7 days before the experiments began. Ten control ewes were injected subcutaneously with saline:ethanol (60:40 v/v) (group 1) and 20 were injected subcutaneously with, ergocryptine (0.5 mg/kg liveweight per injection) as follows: a single injection <1 day (group 2, two ewes), 2–5 days (group 3, seven ewes), 6–10 days (group 4, two ewes), 11–20 days (group 5, five ewes) or c. 30 and 10 days (group 6, four ewes) before the time of parturition. Ewes treated 30 days before the expected time of parturition (group 6) were given a second subcutaneous injection of ergocryptine exactly 20 days after the first injection. Lambs were removed from their dams immediately after birth. All ewes were hand-milked immediately after parturition, and then at 0800 and 1700 hours each day. Milk yields were recorded daily and subsamples were kept at -16°C pending analyses for lactose by the method of Cowie *et al.* (1969).

Blood samples were collected prior to and after injection of saline:ethanol or ergocryptine until 30 days post partum. During the first to fifth weeks of lactation blood samples were collected before and at frequent intervals after milking, which was performed over exactly 2 min. Plasma samples were stored at -16°C pending radioimmunoassay for prolactin, growth hormone and placental lactogen.

Hormone Assays

In order to reduce variability of measurements stemming from between-assay variation, whenever possible, samples which were to be compared were assayed in a single batch. The radioimmunoassays were considered to be valid if intra- and interassay coefficients of variation were less than 10%. The sensitivity of the assay procedure for each hormone was determined by the method of Burger *et al.* (1972).

Prolactin

The talc radioimmunoassay described by Gow (1980) was used. Briefly, antiserum to ovine prolactin was prepared by immunization of turkeys with ovine prolactin (NIH-P-S12) coupled to bovine serum albumin emulsified with Freund's complete adjuvant. Cross reaction of the antiserum

with ovine growth hormone (NIH-GH-S11) was negligible ($<2\%$). Concentrations of prolactin were expressed in terms of the standard NIH-P-S12. The sensitivity of the assay was 1.0 ng/ml .

Growth Hormone

Concentrations of growth hormone were determined using the talc radioimmunoassay described by Wallace and Bassett (1970). Antiserum specific for ovine growth hormone was obtained from Mr A. L. C. Wallace, Division of Animal Production, CSIRO, Prospect, N.S.W. Concentrations were expressed in terms of the standard preparation NIH-GH-S11 and the sensitivity of the assay was 1.0 ng/ml .

Placental Lactogen

The radioimmunoassay described by Taylor *et al.* (1980) was used to measure ovine placental lactogen. Antiserum to placental lactogen was a gift from Dr J. S. D. Chan, University of Manitoba, Winnipeg, Canada. Iodination of placental lactogen was performed using the lactoperoxidase method of Thorell and Johansson (1971). There was no significant cross-reaction ($<1\%$) of the antiserum with ovine pituitary hormones (luteinizing hormone, follicle-stimulating hormone, prolactin, growth hormone and thyroid-stimulating hormone) or ergocryptine.

The recovery of ovine placental lactogen, when added to plasma from non-pregnant sheep was $96.5 \pm 3.9\%$ of the added hormone. The minimum dilution of plasma in the samples measured, was 1:10, and at this dilution the assay sensitivity was equivalent to 40 ng/ml plasma.

Statistical Analyses

The significance of differences between mean values for the parameters measured at given times were computed using the Student's *t*-test (Steel and Torrie 1960).

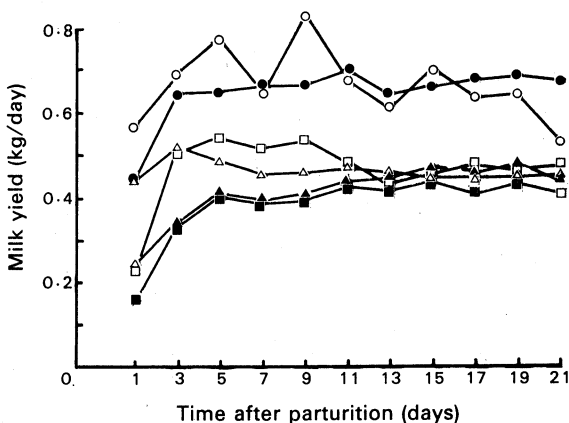


Fig. 1. Daily yields of milk from ewes given a control injection (●); a single injection of ergocryptine <1 day (○), 2–5 days (▲), 6–10 days (△) or 11–20 days (■); or injections of ergocryptine c. 30 and 10 days (□) before parturition. Plotted points represent mean values.

Results

Milk Yield and Composition

Mean milk yields are shown in Fig. 1. Except for ewes in group 2, which were treated with ergocryptine <1 day prepartum, milk yields of treated ewes (groups 3–6) were lower than those of control ewes. Between days 3 and 10 of lactation, mean daily yields of group 3 ewes (treated 2–5 days prepartum) were significantly lower ($P < 0.05$) than those of control ewes. For the other groups, mean daily milk yields did not differ significantly ($P > 0.10$) from those of the control group throughout the first 3 weeks of lactation. On day 21 of lactation, mean yields of ewes treated with ergocryptine were 78, 65, 67, 61 and 70% of those of control ewes, for groups 2–6 respectively.

When comparisons of total milk yield over the 21 days of the experiment were made, differences between total yields of control ewes (14.1 ± 1.20 kg, mean \pm s.e.m.) and total yields of groups treated with ergocryptine (14.4 ± 1.26 kg, 8.7 ± 1.20 kg, 9.6 ± 0.32 kg, 8.3 ± 1.49 kg and 9.9 ± 0.66 kg—groups 2–6 respectively) were not significant ($P > 0.05$). If, however, the mean total yield of all treated ewes (9.5 ± 1.11 kg) was compared with the mean total yield of control ewes, the difference was significant ($P < 0.05$).

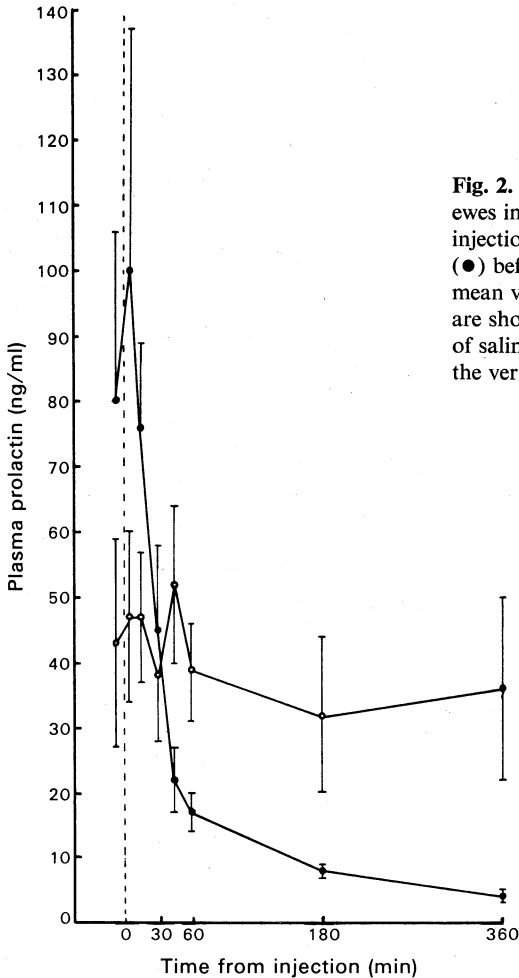


Fig. 2. Concentrations of prolactin in plasma of ewes immediately before and after a single injection of saline: ethanol (○) or of ergocryptine (●) before parturition. Plotted points represent mean values for seven ewes, and standard errors are shown as vertical bars. The time of injection of saline: ethanol or ergocryptine is indicated by the vertical dotted line.

No significant differences ($P > 0.10$) were detected, at any stage over the first 3 weeks of lactation, between lactose contents of mammary secretion from control and treated ewes. On day 21 of lactation, mean \pm s.e. lactose contents of milk were 4.36 ± 0.17 , 4.95 ± 0.17 , 4.54 ± 0.21 , 4.01 ± 0.01 , 4.66 ± 0.27 and 4.90 ± 0.20 g/100 ml for ewes in groups 1–6 respectively.

Plasma Prolactin

Concentrations of prolactin in plasma of control ewes (group 1) rose from basal values of *c.* 50 ng/ml, 1–2 days before parturition to a peak of *c.* 1000 ng/ml at the

time of parturition. Concentrations then fell during the first day post partum and remained relatively stable between 100 and 200 ng/ml thereafter.

Treatment with ergocryptine resulted in a rapid decrease in circulating prolactin to negligible values (<5 ng/ml) in all treated ewes. By 12 h after injection of ergocryptine, concentrations of prolactin in plasma were reduced to values between <1 and 4 ng/ml. This even occurred in group 2 ewes which were treated <1 day prepartum and at a time when the concentrations of prolactin had commenced to increase. Concentrations of prolactin remained low (<1 –4 ng/ml) in plasma from all ewes treated with ergocryptine throughout at least the first 4 weeks of lactation.

Changes in the concentrations of prolactin immediately after injection of ergocryptine are shown in Fig. 2. It can be seen that the concentrations of prolactin in ewes given control injections (group 1) did not change significantly throughout the first 3 h after injection. In contrast, for ewes injected with ergocryptine, concentrations of prolactin decreased from *c.* 100 ng/ml 5 min after injection to *c.* 45 ng/ml by 30 min and had fallen to <5 ng/ml by 3 h.

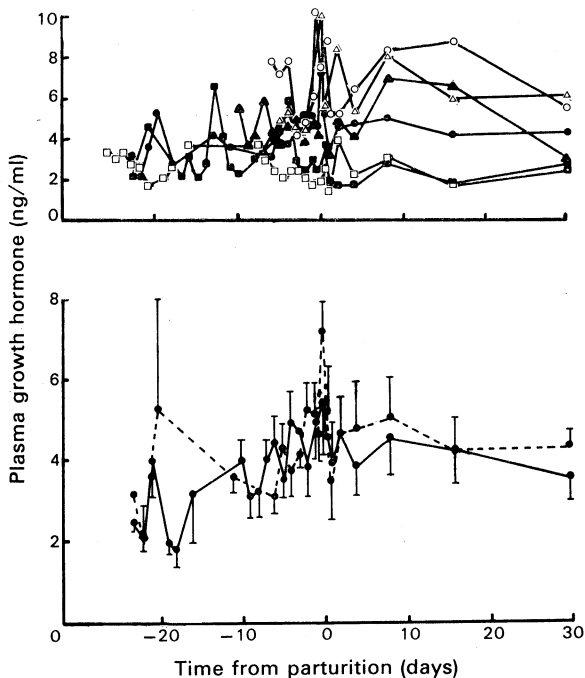


Fig. 3. Concentrations of growth hormone in plasma before and after parturition for groups of ewes treated with ergocryptine at various times before parturition. In the top portion of the figure mean values are presented for ewes injected with saline: ethanol (●); a single injection of ergocryptine <1 day (○), 2–5 days (▲), 6–10 days (△) or 11–20 days (■); or injections of ergocryptine *c.* 30 and 10 days (□) before parturition. The lower portion of the figure depicts mean values for control ewes (---) and all ewes given injections of ergocryptine (—). Standard errors are shown as vertical bars.

In response to milking there were marked changes in the concentrations of prolactin in plasma of control (group 1) ewes. During the first and second weeks of lactation, when responses of these ewes were measured, concentrations of prolactin increased from basal values of *c.* 200 ng/ml to reach peak values of 500–800 ng/ml within the first 10 min after milking commenced. These changes were significant ($P < 0.05$). By 15–20 min after milking had commenced plasma prolactin concentration began to decrease and reached basal values 50–60 min after the start of milking.

In contrast, there were no changes in concentrations of prolactin following milking during the first 4 weeks of lactation, for ewes treated with one (groups 2–5) or two

injections (group 6) of ergocryptine before parturition. However, by the fifth week of lactation basal concentrations of prolactin had increased from negligible values to *c.* 140 ng/ml for ewes given a single injection of ergocryptine prepartum. During the fifth week of lactation in these ewes there was a significant increase ($P < 0.05$) in plasma prolactin following milking. Concentrations increased from basal values to *c.* 350 ng/ml by 10 min after milking commenced, and then decreased to reach basal values 30–40 min later.

Plasma Growth Hormone

Changes in concentrations of growth hormone in plasma for the six groups of ewes are shown in Fig. 3. Differences between values for the different groups were not significant ($P > 0.10$). Treatment with ergocryptine had no effect on concentrations of growth hormone which increased from 2–8 ng/ml (range) to peak values of 4–13 ng/ml (range) at parturition in all ewes. After parturition, concentrations of growth hormone remained higher (2–12 ng/ml, range) than before parturition. The above differences were, however, not significant ($P > 0.10$).

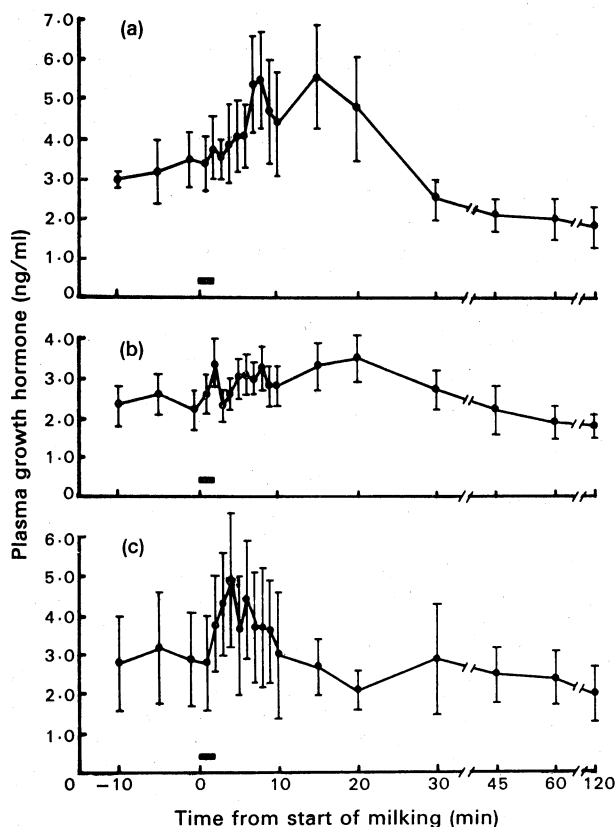


Fig. 4. Plasma growth hormone before and after milking for ewes given (a) injections of ergocryptine *c.* 30 and 10 days ($n = 9$), (b) a single injection of ergocryptine $< 1-20$ days ($n = 14$), and (c) a single injection of saline: ethanol ($n = 7$) before parturition. Values presented are means for measurements made during the first 4 weeks of lactation and standard errors are shown as vertical bars. The time of milking is indicated by the horizontal bar.

Changes in concentrations of growth hormone following milking are shown in Fig. 4. In all groups, concentrations of growth hormone were significantly higher ($P < 0.05$) than before milking at various times during the 10–20 min after milking commenced. By 30 min after milking concentrations had returned to basal values.

Plasma Placental Lactogen

Concentrations of placental lactogen were measured in plasma samples collected at times varying from 25 days before to 8 days after parturition for two ewes in each of the six groups. Treatment with ergocryptine had no apparent effect on plasma placental lactogen.

Substantial between- and within-animal variation for concentrations of placental lactogen were observed in plasma samples collected from the two ewes in each group 7, 4 and 2 days before parturition. During this period values measured for individual ewes varied as follows; 420–800 and 230–450 ng/ml (group 1), 680–900 and 205–1075 ng/ml (group 2), 750–1000 and 4600–8500 ng/ml (group 3), 200–850 and 2700–6000 ng/ml (group 4), 60–100 and 1800–2400 ng/ml (group 5) and 190–940 and 60–1000 ng/ml (group 6).

Concentrations of placental lactogen fell below 40 ng/ml (the sensitivity of the assay) during the two days before parturition and in most cases concentrations fell to these low values during the day of lambing. Thereafter concentrations remained below this value for all ewes in which measurements were made.

Discussion

It has been reported that treatment with prolactin inhibitors during late pregnancy delays the onset of lactation and reduces milk production to trivial amounts in cattle (Karg *et al.* 1972; Schams *et al.* 1972; Johke and Hodate 1978) and lowers milk production by as much as 75% in sheep (Kann *et al.* 1978). These observations have led to the conclusion that prolactin is an essential component of the lactogenic complex of hormones in ruminants.

Results of previous studies with ewes induced to lactate artificially (Fulkerson *et al.* 1975; Field *et al.* 1979) provided evidence that prolactin was not essential for the initiation of lactation. The present results are consistent with these previous observations. Even though mean total yields of milk during the 21 days of the study were significantly lower ($P < 0.05$) for ewes treated with ergocryptine (groups 2–6) than for control ewes (group 1), all treated ewes secreted substantial quantities of milk of normal composition. Data consistent with our own have been obtained recently with goats treated during weeks 5–20 of pregnancy with the prolactin inhibitor bromocriptine (I. A. Forsyth, personal communication). In the latter study, milk yields of bromocriptine-treated and control goats were similar throughout the first 50 days of lactation.

It might be argued that failure to reduce milk yields to trivial amounts in the ewes in the present study was due to the presence of sufficient prolactin to allow lactation to occur. Although it must be conceded that this is possible, concentrations of prolactin were undetectable (< 1 ng/ml) in the great majority (90%) of samples assayed and never more than 4 ng/ml. The possibility also exists that prolactin receptors in the mammary gland become increasingly saturated as parturition approaches and that levels of prolactin in peripheral blood are not correlated with prolactin bound to receptors.

Alternatively, in the pregnant ewe other hormone(s) may have effected mammary development and lactogenesis. In this connection, there is substantial evidence that placental lactogen is an important hormone in sheep (Martal and Djiane 1975, 1977; Kelly *et al.* 1976) and goats (Kelly *et al.* 1976; Buttle *et al.* 1979). It is pertinent that

the blood concentration of prolactin of pregnant sheep, goats and cows is low until shortly before parturition (Johke *et al.* 1971; Fell *et al.* 1972; Lamming *et al.* 1974). In contrast, concentrations of placental lactogen are low during the first 6–7 weeks of pregnancy in the ewe, then increase to reach peak values 10–20 days prepartum (Kelly *et al.* 1976; Chan *et al.* 1978). Moreover, injections of prolactin inhibitor given to late-pregnant ewes fail to affect the plasma concentrations of placental lactogen (Martal and Lacroix 1978).

In the present studies, concentrations of placental lactogen in plasma apparently were not affected by ergocryptine treatment during late pregnancy. Although placental lactogen was only measured from two ewes in each group, and there was considerable within-group variation, it was apparent that concentrations remained high until shortly before parturition. The substantial variation in concentrations of placental lactogen between animals is similar to that reported previously in normal, chronically catheterized ewes (Taylor *et al.* 1980).

Results presented by Hooley *et al.* (1978) raised the possibility that prolactin may be more important for mammogenesis than lactogenesis. In their studies with ewes induced to lactate artificially, ewes treated with prolactin inhibitor during hormone-induced mammogenesis secreted only trivial quantities of milk (0.05 kg/day) by comparison with 'control' ewes (0.16 kg/day) or ewes treated after development of mammary glands (0.14 kg/day). Although this study with non-pregnant ewes points to an important role for prolactin in the absence of pregnancy, it should be borne in mind that there is now substantial evidence for placental lactogen participating in at least mammary development in the normal pregnant ewe.

Growth hormone may also be important for mammogenesis–lactogenesis in the ewe. In the present study concentrations of growth hormone tended to increase at the time of parturition and remain higher during lactation than during pregnancy (see Fig. 3). Increases in concentrations of growth hormone in the prepartum period have been reported previously (Bassett *et al.* 1970; Hove and Blom 1976) and it has been suggested that growth hormone may be lactogenic by virtue of its metabolic effects (Convey 1974; Erb 1976). The maintenance of high concentrations of growth hormone during lactation may be important for ensuring a supply of nutrients to the mammary gland. Certainly, exogenous growth hormone increases milk yield in cows (Machlin 1973) and sheep (Jordan and Shaffhausen 1954) and positive correlations between milk yield and plasma concentration of growth hormone have been recorded in cows (Hart *et al.* 1975, 1978).

The finding that concentrations of plasma prolactin, but not growth hormone, were reduced to negligible values (<5 ng/ml) for more than 20 days after a single subcutaneous injection of ergocryptine (0.5 mg/kg liveweight) is consistent with previous reports from studies with sheep (Fulkerson *et al.* 1975; Field *et al.* 1979). From the data presented in Fig. 2 the half life of prolactin was calculated as 21 min. This value is consistent with estimates previously reported for lactating sheep (19 min, Davis and Borger 1973), goats (19 min, Bryant *et al.* 1970) and cows (23–25 min, Tucker *et al.* 1973; 23 min, Goodman *et al.* 1979).

The changes in concentrations of plasma prolactin following milking observed in the present study are of interest in view of previous reports on the role of prolactin during lactation. Grosvenor (1971) reported that prolactin released at suckling directly influenced the rate of refilling of the mammary gland in the rat. Others have reported positive correlations between the amount of prolactin released after milking

and milk yield in the cow (Koprowski and Tucker 1973) and goat (Hart 1975a). Furthermore, there have been reports that administration of prolactin inhibitors during lactation severely suppresses milk production in ewes (Hooley *et al.* 1978; Kann *et al.* 1978).

On the other hand, Hart (1975b) pointed out that substantial variations in plasma prolactin occur during different seasons of the year, and concluded that the decline in plasma prolactin occurring in the autumn months in goats was not the major cause of the decline in milk production which occurred at the same time. Others have reported data which suggest that prolactin is not required for the maintenance of lactation in ruminants. Thus Smith *et al.* (1974) and Hart (1973) failed to suppress milk yields of lactating cows and goats respectively, by treatment with prolactin inhibitors. The present data are consistent with these latter observations.

Concentrations of growth hormone increased following milking of control (group 1) and ergocryptine-treated (groups 2–6) ewes. In this respect our data are consistent with those of others who have reported increases in plasma growth hormone following milking in goats (Hart and Flux 1973; Hart 1974; Martal 1975) and sheep (Martal 1975). However, Tucker (1971) failed to observe an increase in plasma growth hormone following milking in cows, and Reynaert and colleagues (Reynaert and Peeters 1971; Reynaert *et al.* 1972) measured increases in plasma growth hormone infrequently following milking. Our data show that growth hormone is released in response to milking and that ergocryptine does not affect the release of growth hormone in the ewe.

In conclusion, the results of the present study suggest that prolactin is not an essential component of lactogenic and galactopoietic complexes of hormones in the ewe. The release of ACTH, glucocorticoids, oxytocin (Tucker 1974) and growth hormone (Hart and Flux 1973; Hart 1974; Martal 1975) at milking may account for the galactopoietic effects which often have been attributed to prolactin. Notwithstanding, prolactin may be necessary for optimum milk production.

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