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SUMMARY

Pituitary prolactin has been quantified by radioimmunoassay in whole milk obtained from cattle, goats, sheep and rats. Prolactin concentrations in milk samples obtained following the completion of lactogenesis approximate concentrations of the hormone in blood plasma or serum. However, concentrations of prolactin in prepartum mammary secretions were much higher than plasma prolactin in prepartum dairy cows. This observation was consistent with the hypothesis that during mammary lactogenesis, endogenous milk prolactin in the alveolar lumen may be an additional source of biologically active prolactin.

The value of milk prolactin to neonatal animals remains unknown. Experiments with milk-fed calves and suckling rats failed to demonstrate absorption of the intact molecule into the neonate’s blood. Further research is needed to determine the role, if any, that maternal prolactin consumed in milk plays in neonatal physiology.

Measurements of milk prolactin seem to be highly predictive of the average blood prolactin concentration. Milk prolactin can probably be used in lactating females to predict average plasma prolactin in a manner that is relatively independent of stress- or milking-induced increases in pituitary release of the hormone.

(Key Words: Prolactin, Milk, Pituitary Hormones.)

INTRODUCTION

Proteins circulating in blood would be expected to pass in small amounts into milk of lactating females. This transfer has been demonstrated for serum albumin as well as for selected immunoglobulins in blood, but most of the proteins present in secreted milk appear to be synthesized from amino acids in the mammary cell and not received intact from blood (Larson and Jorgensen, 1974). Since only small quantities of blood proteins were known to be transferred intact into milk, the initial quantification by radioimmunoassay of pituitary prolactin (PRL) in milk yielded surprisingly high concentrations (Malven and McMurtry, 1974). In fact, PRL concentrations estimated in bovine milk approximated those present in bovine blood plasma. The initial research also demonstrated that radioimmunoassay procedures for samples of whole milk gave valid estimates of immunoreactive PRL as long as sample volumes were 50 μl or less.

Although radioimmunoassay techniques can accurately quantify immunoreactive PRL in milk, they cannot determine whether biologically active pituitary PRL is present. However, the literature does contain several reports of prolactin-like biological activity in mammary secretions. Geschickter and Lewis (1936) bioassayed bovine colostrum as well as fluid aspirated from human mammary cysts. Both materials were found to contain prolactin-like activity using the pigeon crop-sac bioassay. Iwamura (1943, 1949) also reported prolactin-like activity in bovine colostrum. Amino acid analyses of the protein responsible for this prolactin-like activity were used to estimate a molecular weight which was found to be similar to that for pituitary PRL (Iwamura, 1952).

In the present review current knowledge about protein hormones in milk will be summarized with most emphasis on PRL because more information is available. The review will go beyond knowledge about PRL’s presence in milk and consider in detail the possible significance of milk PRL. The areas of potential...
significance include: (a) the role of milk PRL in lactating females, (b) the value of milk PRL to the neonate consuming milk, and (c) the usefulness of milk PRL to researchers trying to estimate blood PRL concentrations in large groups of lactating females.

MEASUREMENT OF PROTEIN HORMONES IN MILK

Protein hormones are most readily quantified by immunological methods. These procedures require antibodies which contain hormone-specific sites capable of reversibly binding radiolabeled hormone. The basis of the hormone quantification is the competition for these specific binding sites between radiolabeled hormone and unlabeled hormone in either standards or unknowns. It is also necessary to demonstrate that factors in the unknown samples, other than the hormone to be quantified, do not under conditions of incubation during assay affect the competition between radiolabeled and unlabeled hormone. One of the methods used to satisfy this criterion of radioimmunoassay validation is to generate inhibition curves for increasing amounts of unlabeled standard hormone and for increasing volumes of milk. If inhibition curves are parallel when plotted as percentage of antibody-bound radiolabeled hormone vs log dosage of standard or of milk volume, it suggests, but does not prove, that nonhormonal factors in milk do not interfere with competition for binding sites.

While it is necessary to satisfy the criterion of parallel inhibition curves, additional criteria should also be satisfied (Malven and McMurtry, 1974). The criterion most difficult to satisfy for the radioimmunoassay of whole milk has been minimal incubation damage of the radiolabeled hormone. This term encompasses (a) actual damage of the radiolabeled hormone by milk making it unable to bind and (b) nonhormonal or nonspecific interference by milk with the binding of radiolabeled hormone. In order to quantify accurately the amount of incubation damage, one must have a large excess of hormone-binding sites and incubation mixtures with and without the biological fluid being tested. Incubation damage is revealed as a reduction in binding of radiolabeled hormone in the mixtures containing milk relative to those without milk. If there were not an excess of hormone-binding sites, competition between unlabeled hormone in the milk and radiolabeled hormone would itself decrease binding of the radiolabeled hormone. When significant incubation damage occurs under normal radioimmunoassay conditions (i.e., limited number of binding sites) and where competition exists for occupancy of those binding sites, the binding of radiolabeled hormone is, of course, decreased both by competition with unlabeled milk hormone and by incubation damage. The radioimmunoassay quantification then overestimates the true concentration of unlabeled hormone in the milk sample because of incubation damage. Malven and McMurtry (1974) quantified incubation damage of radiolabeled PRL using excess binding sites and reported 29% damage when subjected to incubation mixtures including 200 μl of whole milk. However, incubation damage was only 6% when 50 μl of whole milk was used, and this amount of damage was equivalent to that produced by incubation with blood plasma (50 and 100 μl).

Prolactin has also been quantified by radioimmunoassay in human milk (Gala et al., 1975). Inhibition curves for milk and standard were parallel. Concentrations of PRL in human milk were less than those in blood serum, but they did correlate well with serum concentrations.

Radiolabeled luteinizing hormone (LH) is also subject to considerable incubation damage by whole milk (P. V. Malven, unpublished data). Incubation under assay conditions with 80 μl and 160 μl volumes of whole milk produced incubation damage estimates of 34% and 44%, respectively. Since blood concentrations of LH are much lower than those of PRL, it was necessary to assay these larger volumes of milk when attempting to quantify LH in milk. Therefore, a large proportion of the apparent LH concentration that could be quantified in milk (1 to 6 ng/ml) was probably due to the incubation damage. Until this problem can be reduced or eliminated, it will not be possible to validly quantify LH in whole milk by the usual radioimmunoassay procedures.

PROTEIN HORMONES PRESENT IN MILK

The earliest reports of protein hormones present in milk were, of necessity, based on bioassay procedures for detection of the hormonal activity. Pregnant mare serum (PMS) gonadotropin was detected biologically in milk samples from four of seven pregnant mares (Cole et al., 1967). However, the concentra-
tions of PMS in mare milk were only .2% of those in blood serum. Erythropoietin, another protein hormone (Rambach et al., 1959), was detected in ovine milk using bioassay procedures. Furthermore, experimental anemia in the lactating ewes increased the concentration of erythropoietin in milk (Hyzy, 1969).

As mentioned earlier, biological assays also were used to detect lactogenic (prolactin-like) activity in bovine colostrum and in fluid aspirated from human mammary cysts (Iwamura, 1943; Geschickter and Lewis, 1936). Because these early reports were never confirmed with the subsequently improved bioassay procedures for PRL, one can only assume that most researchers believed that the bioassays used for the original work lacked specificity. Therefore, it was surprising to find high concentrations of PRL when radioimmunoassay procedures were first applied to samples of bovine milk at the suggestion of Dr. D. J. Bolt (Malven and McMurtry, 1974). These procedures have now been applied to other species, and Table 1 summarizes the published data on PRL concentrations in normal milk from four species. The data for cows, goats and sheep were all based on common radioimmunoassay procedures using antiserum against ovine PRL and radiolabeled ovine PRL with reference preparations of either bovine PRL (cow and goat milk) or ovine PRL (sheep milk). The estimates of PRL in rat milk had to be based on a totally different set of radioimmunoassay reagents because there was little crossreaction between rat PRL and bovine PRL (Malven and McMurtry, 1974). Nevertheless, rat milk has been found in two laboratories (Table 1) to contain very high concentrations of PRL. It is important to point out that the PRL concentrations for rat milk would be only 46% of the listed values if adjusted to a biological potency comparable with the other species (see footnote c to Table 1). Such an adjustment would reduce the PRL estimates for rat milk to 108 and 141 ng/ml and reduce the variation among species.

Inspection of the sheep data in Table 1 clearly reveals an influence of season. Milk PRL concentrations in the summer were twice as high as those in winter. However, this difference in milk PRL probably reflected only the effect of season on plasma PRL in these ewes (see footnotes a and b to Table 1). Seasonal influences also have been observed in lactating cows for concentrations of both blood PRL (Koprowski and Tucker, 1973; Thatcher, 1974) and milk PRL (McMurtry et al., 1975).

J. P. McMurtry and P. V. Malven (unpublished data) measured PRL in milk sampled from 78 Angus cows 21 to 50 days after parturition. The average PRL concentration was 21 ± 2 ng/ml as compared with 50 ± 1 ng/ml for dairy cows (Table 1). However, one should not conclude definitely that there is a physiological difference in milk PRL between dairy

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**TABLE 1. SPECIES COMPARISON OF AVERAGE (+ SE) PRL CONCENTRATIONS IN WHOLE MILK COLLECTED DURING NORMAL LACTATION**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>PRL concentration (ng/ml)</th>
<th>Stage of lactation</th>
<th>Literature citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cow</td>
<td>1078</td>
<td>50 ± 1</td>
<td>All stages</td>
<td>McMurtry et al., 1975</td>
</tr>
<tr>
<td>Dairy goat</td>
<td>42</td>
<td>44 ± 5</td>
<td>Mid to late</td>
<td>Malven and McMurtry, 1974</td>
</tr>
<tr>
<td>Sheep December</td>
<td>36</td>
<td>56 ± 5</td>
<td>Days 32 to 69</td>
<td>Erb et al., 1977a</td>
</tr>
<tr>
<td>Sheep June</td>
<td>23</td>
<td>116 ± 6</td>
<td>Days 49 to 65</td>
<td>Erb et al., 1977a</td>
</tr>
<tr>
<td>Rat 8 pups/litter</td>
<td>94</td>
<td>234 ± 9c</td>
<td>Days 2 to 22</td>
<td>McMurtry and Malven, 1974a</td>
</tr>
<tr>
<td>Rat 6 pups/litter</td>
<td>20</td>
<td>308 ± 22c</td>
<td>Days 14 to 15</td>
<td>Grosvenor and Whitworth, 1976</td>
</tr>
</tbody>
</table>

aPlasma concentrations of PRL averaged 61 ± 4 ng/ml in blood samples collected concurrently with the milk samples.
bPlasma concentrations of PRL averaged 125 ± 8 ng/ml in blood samples collected concurrently with the milk samples.
cThe reference preparation of rat PRL used to estimate the milk PRL concentration had only 46% of the biological potencies for the reference preparations of ovine and bovine PRL used to estimate the other values in this table.
and beef cows. There were many uncontrolled differences involved in the comparison. These included: (1) milk sampling methods, (2) time since suckled or milked when sampled, (3) season, and (4) stage of lactation. The hormone is clearly present in milk from beef cows, but it will require additional controlled research to determine whether milk from beef and dairy cows really differs in PRL concentration.

**ROLE OF MILK PROLACTIN IN LACTATING FEMALES**

One physiological role of milk PRL in lactating females is readily evident. Milk provides an additional route of excretion for PRL in the circulation. The metabolic clearance rate of blood PRL was significantly greater in lactating ewes than in nonlactating ewes (Davis and Borger, 1973). A similar difference was reported between lactating and nonlactating female rats (Whitworth and Grosvenor, 1975). While the difference was significant in both sheep and rats, PRL excretion in secreted milk probably represents only a small fraction of the total amount of PRL cleared from the circulation.

Another possible role of milk PRL in lactating females involves the initiation and maintenance of lactation. Pituitary PRL acts on mammary secretory cells as one essential component in a complex of hormones required for initiation and, in some species, for maintenance of lactation (Tucker, 1974). Blood PRL would appear to be the main source of the PRL which acts on the mammary cells. However, PRL present in secreted milk may also serve as a source of PRL for this purpose. This possibility is the basis for the following hypothesis: PRL in secreted milk contained in the alveolar lumen acts in vivo as a local source of the hormone in order to maximize its action on the adjacent mammary epithelial cells. Interestingly, there was support for this hypothesis even before endogenous PRL had been quantified in milk. Lyons (1942) pretreated ovariecetomized rabbits with ovarian steroids and then injected PRL locally into the mammary duct leading from one sector of one mammary gland. The PRL was lactogenic to only the injected sector of the gland, and milk secretion was clearly visible. Chadwick (1963) and others have confirmed the local action of intraductally administered PRL in pseudopregnant rabbits and have utilized it as the basis for a PRL bioassay. Intraductal injection of radiolabeled PRL in pseudopregnant rabbits also resulted in specific autoradiographic localization on or near the plasma membrane of the mammary alveolar cell, but opposite to the alveolar lumen and adjacent to the vascular supply (Birkinshaw and Falconer, 1972). It was noteworthy that the localization of radiolabeled PRL was the same following either intraductal or intravenous administration. It is well known that mammary cells possess PRL-concentrating mechanisms (i.e., receptors), and the results from intraductal administration of unlabeled and radiolabeled PRL suggest that PRL in secreted milk might be available to these PRL-concentrating mechanisms. The mammary gland’s lactogenic requirement for circulating PRL is greatest in cows just before parturition (Schams et al., 1972; Karg and Schams, 1974). The efficiency with which the goat mammary gland extracted PRL from its arterial blood supply was greater before than after parturition (Reynolds and Tucker, 1975). During lactogenesis, induced in nonpregnant ewes with exogenous estradiol-17β and progesterone, N. E. Sitarz and R. E. Erb (unpublished data) observed that increased concentrations of milk PRL immediately preceded periods of increased lactogenesis and larger milk yields. If PRL in secreted milk acts in vivo as a local source of the hormone, it should theoretically be most important during lactogenesis. Therefore, an experiment was conducted in May and June with periparturient dairy cows in which PRL was measured in both blood and milk (Erb et al., 1977b). Prepartum mammary secretions (milk) were sampled once daily from six cows by removing a small quantity of milk from one mammary gland (Group SM from Keller et al., 1977). Milk was also sampled in this manner within 4 hr after parturition. Thereafter, milk was removed normally from all glands twice daily, and a sample from the afternoon milking was taken for PRL quantification. Blood was also sampled by venipuncture of the tail vein just before the afternoon milking or prepartum sampling (Group C from Erb et al., 1977b).

Concentrations of PRL in blood plasma and milk are graphically presented in figure 1. Plasma PRL peaked 1 day before parturition as reported by others (see review by Convey, 1974). Milk concentrations of PRL were already high at least 6 days before parturition (173 ± 31 ng/ml). They increased throughout the prepartum period and reached a maximum (369 ± 56 ng/ml) in the samples collected.
within 4 hr after parturition (day 0). Milk PRL decreased sharply thereafter and 2 days later averaged only 74 ± 9 ng/ml. Sampling of blood and milk continued as described above until day 25 of lactation in these cows (Erb et al., 1977b). Since blood was collected just before the afternoon milking, plasma PRL measurements should not have reflected the pulsatile discharges induced by milking. Under these conditions, milk and plasma concentrations of PRL fluctuated between 20 and 40 ng/ml between days 5 and 25 (figure 1).

The relative concentrations of PRL in blood plasma and in milk vary greatly during the periparturient period when lactation is initiated (figure 1). The concentration gradients for different time periods are summarized in table 2, and they are based on the data plotted in figure 1. Concentration gradients for PRL were calculated as milk/plasma ratios and as milk minus plasma differences. During days -7 to -2, the PRL ratios were maximal. Beginning on day -1, when plasma PRL peaked (figure 1), and continuing through day 3, the ratios were lower and fluctuated between 3.4 and 5.8 with a mean of 4.3 ± .4. The PRL difference was decreased markedly on day 1 even though the PRL ratio was not. After day 3, the concentration gradient, measured as either ratio or difference, approached zero.

The data presented herein are consistent with our hypothesis that endogenous milk PRL in the alveolar lumen may serve in vivo as an additional source of biologically active PRL during lactogenesis. However, these results do not prove this hypothesis. The high concentrations of milk PRL during lactogenesis may be only coincidental and reflective of more efficient mechanisms for concentrating blood PRL.

### Table 2. Concentration Gradients of PRL Between Milk and Blood Plasma in Periparturient Cows

<table>
<thead>
<tr>
<th>Days from parturition</th>
<th>Milk/ Plasma ratio</th>
<th>Milk/ Plasma difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7 to -2</td>
<td>8.3 ± .8</td>
<td>191 ± 21</td>
</tr>
<tr>
<td>-1</td>
<td>3.5</td>
<td>229</td>
</tr>
<tr>
<td>0</td>
<td>5.8</td>
<td>305</td>
</tr>
<tr>
<td>1</td>
<td>4.2</td>
<td>113</td>
</tr>
<tr>
<td>2</td>
<td>3.4</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>35</td>
</tr>
<tr>
<td>4 to 11</td>
<td>1.5 ± .1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>13 to 25</td>
<td>1.1 ± .1</td>
<td>4 ± 2</td>
</tr>
</tbody>
</table>

*Daily averages, across cows, were used to calculate concentration gradients. Where presented, SE reflect variability among the days in that time period.

### Value of Milk PRL to Neonate Consuming Milk

The presence of maternal PRL in milk consumed by neonatal animals immediately raises the question of its value to the neonate. Carmichael and Gordon (1975) reported that maternal erythropoietin, also a protein hormone, was transmitted to neonatal rats via milk and that enough hormone apparently was absorbed by the neonate to stimulate erythropoiesis. As a first step toward elucidating a comparable action of maternal PRL in nursing neonates, Malven et al. (1976) investigated the possible absorption of orally ingested milk PRL into the blood of milk-fed calves or suckling rats. They failed to demonstrate significant absorption of the intact PRL molecule into the neonate's blood. Since the gastrointestinal tract of both species, at the ages studied, should have been permeable to macromolecules, these results suggested that maternal PRL contained in milk was utilized or degraded prior to reaching the site of intestinal absorption.

In contrast to the above conclusions of Malven et al. (1976), Whitworth and Grosvenor (1976) observed significant transfer of radioiodinated PRL from injected dams into suckling rat pups. The radioiodide present in the blood of the pups was immunoprecipitable with antisem against PRL. Lactating dams have also been injected with radioiodinated PRL in this laboratory (P. V. Malven, unpublished data). Large quantities of the radioiodide were trans-
mitted via milk to suckling pups, but it was not possible to accurately determine what proportion of the radioiodide found in the blood of the pups was maternally-derived PRL. In fact, a majority of the radioiodide could not be precipitated with antiserum against PRL. There actually may be absorption of maternal PRL into the blood of nursing neonates as reported by Whitworth and Grosvenor (1976), but studies using radioiodinated PRL must be carefully validated because radioiodide may be cleaved from the PRL molecules and enter the metabolism of either the lactating female or the nursing neonate. For example, the mammary gland itself readily accumulates nuclides of free iodide (Grosvenor, 1960; Reineke, 1961).

One possible action of maternal PRL in milk consumed by the neonate might not require absorption of the intact molecule into the blood. Circulating PRL exerts an action on the small intestine to promote the absorption of fluid and electrolytes from the lumen (Ramsey and Bern, 1972; Mainoya et al., 1974). It is possible that maternal PRL might act locally on the lumenal side of the intestine to promote transport of fluid and electrolytes out of the lumen. Such an action of PRL could theoretically foster intestinal absorption during normal conditions. During conditions of diarrhea, perhaps induced by viral or bacterial agents, this action of maternal PRL might reduce the secretion of fluid and electrolytes into the lumen and thereby minimize the severity of the neonate’s dehydration.

Additional research will obviously be needed to prove whether maternal PRL exerts any effect in neonatal animals. For adults of any species, the PRL in consumed milk is probably hydrolyzed to its constituent amino acids prior to absorption. The concentrations of PRL in bovine milk (table 1) represent an infinitely small fraction of the total protein in milk. Considering all of these facts, there is probably no situation in which oral ingestion of milk PRL by adult humans would constitute a health hazard.

VALUE OF MILK PRL FOR PREDICTING BLOOD PRL

Blood concentrations of PRL fluctuate episodically even in unstressed animals sampled via indwelling cannula (Butler et al., 1972). In several experiments, PRL concentrations in blood plasma and milk have been measured concurrently. In lactating goats, pharmacological methods were used to either decrease or increase plasma PRL (McMurtry and Malven, 1974b). Concentrations of milk PRL changed accordingly even though milk yield did not change. Lactating ewes, studied in summer and in winter, were found to have greatly different concentrations of plasma PRL. Milk PRL concentrations during both seasons corresponded closely to the plasma concentration for that season (table 1). McMurtry et al. (1975) also noted significant seasonal effects on milk PRL in cows. Although these authors did not quantify plasma PRL, there are reports in the literature documenting some of the same seasonal influences on plasma PRL in cows (Koprowski and Tucker, 1973; Thatcher, 1974). In addition, the data in figure 1 for 5 or more days after parturition show that milk PRL concentrations were roughly equivalent to basal levels of plasma PRL (excluding milking-induced surges).

In summary, the studies in sheep, goats and cattle indicate that sampling of milk and measurement of its PRL concentration probably approximates the plasma concentration of PRL averaged over time. Within animal correlation coefficients between plasma PRL and milk PRL were not uniformly high in experiments with ewes (Erb et al., 1977a). However, these modest correlations probably reflected the fact that the plasma measurement covered only one instant of time during a fluctuating profile. On theoretical grounds, milk PRL measurements should give a value highly predictive of the average blood concentration of PRL in an unstressed animal. This statement is based on the following two assumptions: (1) PRL passes into milk in proportion to its blood concentration during established lactation and (2) the act of milk collection may release PRL from the pituitary but the milk sample would be obtained before it could be affected by this PRL discharge. This second assumption cannot be made for blood sampling except under carefully controlled conditions.

The ease with which milk is sampled could make milk PRL a variable for genetic investigation. McMurtry et al. (1975) investigated whether individual dairy cows tended to have a characteristic milk PRL concentration when sampled repetitively. There was significantly greater variation among cows than within cows, but statistical adjustment of the data for environmental and lactational variables elmi-
nated this difference. These same authors showed that milk PRL was significantly related to either daily milk yield or stage of lactation, but that inclusion of either variable in the multivariate statistical analysis eliminated the significant relationship between milk PRL and the other variable. Measurement of milk PRL in dairy cows would be a poor indicator of either daily or lactation milk yields, especially since environment and stage of lactation also are related to milk PRL. It remains to be determined whether milk PRL might be predictive of milk yield in other domestic animals, especially those in which milk yields cannot be measured directly.

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