In Vitro Regulation of Mammary Glucose-6-Phosphate Dehydrogenase Activity by Palmitoyl Coenzyme A, Acetate, and Polyamines[1] (43035)

A. J. YOUNG, W. B. SCHMOTZER, AND L. V. SWANSON[2]
Department of Animal Science and College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331

Abstract. **An *in vitro* study was conducted to determine whether bovine mammary glucose-6-phosphate dehydrogenase (G6PD) activity was regulated by palmitoyl coenzyme A (CoA), acetate, spermidine, and putrescine and whether these effects were dependent upon stage of lactation. Early lactation explants incubated in media containing palmitoyl CoA or acetate had reduced ($P < 0.01$) G6PD activity compared with incubated control explants. G6PD activity in early lactation explants was reduced ($P < 0.05$) when incubated with 5 μM palmitoyl CoA or 1 mM acetate compared with 25 μM palmitoyl CoA or 10 mM acetate. Spermidine (0.4 mM) reversed ($P < 0.05$) palmitoyl CoA-induced inhibition of early lactation G6PD activity at 5 μM, but not at 25 μM palmitoyl CoA. G6PD activity in early lactation explants was decreased ($P < 0.05$) when treated with putrescine (0.4 mM) compared with explants treated with spermidine. Addition of acetate in combination with 5 μM palmitoyl CoA reversed G6PD inhibition ($P < 0.05$ for 1 mM and $P < 0.01$ for 10 mM) while addition of either level of acetate in combination with 25 μM palmitoyl CoA failed to reverse G6PD inhibition. G6PD activity was higher ($P < 0.01$) in early lactation than mid-lactation explants. No statistical differences ($P > 0.1$) were found among any treatments in explants from mid-lactation cows. We conclude that palmitoyl CoA and acetate will inhibit G6PD activity in early lactation, but not mid-lactation explants; addition of spermidine will reverse this inhibition.**

[P.S.E.B.M. 1990, Vol 193]

In ruminants, mammary secretion of fatty acids is primarily in the form of triacylglycerides, with palmitic acid being the primary fatty acid (1–4). Palmitic acid can be derived from mammary uptake of plasma-free fatty acids (5, 6) or synthesized within the tissue from acetate (7). Synthesis of mammary fatty acids requires a supply of reducing equivalents in the form of NADPH (1–4). In ruminants, approximately 30–50% of the NADPH for fatty acid synthesis is derived from the hexose monophosphate pathway, of which glucose-6-phosphate dehydrogenase (G6PD) is the rate-controlling enzyme (8–11), and the remainder from isocitrate dehydrogenase (9, 12–15). Newly synthesized or preformed palmitic acid must be activated to palmitoyl coenzyme A (CoA) before being attached to the glycerol phosphate backbone and subsequently secreted as triglycerides (16).

It is known that palmitoyl CoA can affect fatty acid synthesis by acting as an inhibitor of enzymes involved with fatty acid synthesis, such as acetyl CoA carboxylase (16) and G6PD (17, 18). It causes dissociation of the latter enzyme into inactive subunits (17; K. L. Barker, personal communication).

Polyamines (putrescine, spermidine, and spermine) are long-chain aliphatic amines which stimulate protein synthesis (19–23), triglyceride synthesis (24), and cell division (19–23). Polyamine levels increase in mammary tissue during lactation (25, 26) and appear to stimulate or maintain G6PD activity in mouse mammary tissue (27). Spermidine, spermine, and, to a lesser

[1] Technical Paper No. 8192, Oregon Argiculture Experiment Station. Partial results were presented at the 81st Annual American Dairy Science Association Meeting, June 1986 (Abstract 339).

[2] To whom requests for reprints should be addressed at Department of Animal Science, Oregon State University, Corvallis, OR 97331.

Received May 5, 1988. [P.S.E.B.M. 1990, Vol 193]
Accepted December 11, 1989.

0037-9727/90/1934-0274$2.00/0

extent, putrescine prevent or reverse palmitoyl CoA-induced inhibition of G6PD (28, 29).

If intramammary levels of NADPH for fatty acid synthesis are regulated by substrate availability or end product removal, acetate or palmitoyl coenzyme A, respectively, could act as regulators of G6PD. We also suggest that polyamines may be involved in the control of mammary fatty acid synthesis by exerting a permissive effect on G6PD activity by removal of palmitoyl CoA and/or a direct stimulation for increased nucleic acid needs (protein synthesis) (30).

Therefore, our objectives were (i) to determine whether mammary G6PD activity was regulated *in vitro* by palmitoyl coenzyme A and acetate levels, (ii) that if such a regulation were found, could polyamines reverse this inhibition, and (iii) to determine if this regulation changed during lactation.

Materials and Methods

Animals. Lactating Holstein dairy cows were selected for mammary biopsy from the Oregon State University herd. The cows selected were in their second or greater lactation, had no history of mastitis during their current lactation, and were assigned to two stage of lactation groups. Cows selected for the early lactation group (n = 2) were 33–38 days postpartum and the mid-lactation group (n = 4) ranged from 96–128 days postpartum. Mammary biopsies (approximately 3–5 g) were removed under aseptic conditions (15) using a local anesthetic. Milk production was monitored before and after surgery; in general, milk production decreased for one or two milkings before returning to presurgery levels.

Materials. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) with the exception of sodium bicarbonate, sodium acetate, KCl, $MgCl_2 \cdot 6H_2O$, and 2-mercaptoethanol from VWR Scientific (Akron, OH), streptomycin from Grand Island Biological Co. (Grand Island, NY), and prolactin (NIH-bPrl-B-1) as a gift from the National Hormone and Pituitary Program.

Tissue Incubation. Explant preparation and incubation protocol were based upon the procedures of Collier *et al.* (31) and Goodman *et al.* (32) with minor modifications. Media for transporting the biopsy and subsequent incubation periods contained the following materials: M-199 medium with Hanks' salts and L-glutamine without bicarbonate, 4.167 mM sodium bicarbonate, 20 mM Hepes, and bovine insulin (5 μg/ml); hydrocortisone (5 μg/ml); prolactin (1 μg/ml); penicillin G (100 units/ml); streptomycin (100 units/ml); and Amphotericin B (Fungizone) (2.5 μg/ml).

Upon removal of the biopsy, mammary tissue was placed in ice-cold media and transported to the laboratory on ice (approximately 10 min) where it was diced into 1- to 2-mm^3 explants, washed five to seven times in ice-cold media, and placed upon lens paper rafts (31). The raft and explants (approximately 10–30 mg wet wt) were then placed in incubation wells (Falcon 24-well Multiwell tissue culture plate; VWR Scientific) containing 1 ml of medium and placed in a humidified Modular Incubation Chamber (Billups-Rothenberg, Del Mar, CA), gassed with 95% O_2-5% CO_2, and incubated for 1.5–2 hr at 37°C. After this equilibration period, the following treatment or treatment combinations were given: 5 μM palmitoyl CoA alone or in combination with 1 mM acetate, 10 mM acetate, or 0.4 mM spermidine; 25 μM palmitoyl CoA alone or in combination with 1 mM acetate, 10 mM acetate, or 0.4 mM spermidine; 1 mM acetate alone or in combination with 0.4 mM spermidine; 10 mM acetate alone or in combination with 0.4 mM spermidine; and 0.4 mM spermidine or 0.4 mM putrescine given alone. A 10-μl aliquot of each treatment stock solution gave the desired final concentration in 1 ml of incubation medium. At 30, 60 or 120 min after addition of the treatments, the contents from duplicate wells of each treatment were removed, frozen on dry ice and stored until assayed for G6PD activity and protein content.

Enzyme and Protein Assays. Homogenization buffer consisted of 0.052 M Tris base, 0.1 M KCl, 7.2 mM $MgCl_2 \cdot 6H_2O$, 10 mM 2-mercaptoethanol, and 20% glycerol (pH = 7.4). Frozen explants were prepared for enzyme assay by homogenization (Tekmar Tissumizer, model SDT, setting 30; Tekmar Co., Cincinnati, OH) in 1.5 ml of cold homogenization buffer for two bursts of 15-sec each with a 15-sec rest between. Homogenates were centrifuged at 85,000g for 35 min in a Beckman Ultracentrifuge (model L5-50B; Palo Alto, CA). After centrifugation, samples were kept on ice and assayed immediately for G6PD activity. Assay buffer consisted of 0.0466 M Tris base and 7.14 mM $MgCl_2 \cdot 6H_2O$ (pH = 7.4).

G6PD activity was determined as described by Smith and Barker (33) with 1 enzyme unit equal to the amount of activity that reduces 1 μM $NADP^+$/min. Briefly, the assay consists of simultaneously running two cuvettes in which one cuvette measures combined NADPH formation from both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase whereas the second cuvette measures only 6-phosphogluconate dehydrogenase activity. The difference between the two slopes represents G6PD activity/ml. The reaction mixture for the first cuvette contained 2.7 ml of assay buffer, 50 μl of $NADP^+$ (11.1 mM), 50 μl of solution containing glucose-6-phosphate (41.8 mM) plus 6-phosphogluconate (22.9 mM) and 200 μl of supernatant containing the enzyme. The second cuvette contained identical ingredients except for glucose-6-phosphate. The change in formation of NADPH from $NADP^+$ was measured spectrophotometrically (model DU; Beckman Instruments, Palo Alto, CA) at 340 nm.

Protein determination was according to the method of Lowry *et al.* (34). Enzyme activity was expressed per mg protein.

Statistical analysis (35) was carried out by a square-root transformation of the data (average of duplicate samples for each time period per treatment), then analyzed by split-plot analysis of variance with treatments within stage of lactation as factor A and incubation time periods as factor B. Time of incubation was not significant ($P > 0.1$) for G6PD activity in early or mid-lactation explants; therefore, treatment means were averaged over all time periods (six values for early lactation and eight values for mid-lactation explants) and analyzed for differences among treatments. Differences among treatments were determined by orthogonal contrasts. Differences between early and mid-lactation control means were determined by Student's t test.

Results

Treatment means for G6PD activity in explants from early lactation cows are listed in Table I. The main effects due to treatments and animal variation, as measured by analysis of variance, were significant ($P < 0.05$) in early lactation. However, no differences ($P > 0.1$) in G6PD activity were found among any of the treatments in mid-lactation explants although animal variations did differ ($P < 0.005$). Control values for G6PD activity were significantly ($P < 0.01$) higher in early lactation explants than in explants taken from mid-lactation cows (7.4 ± 1.2 units $\times 10^{-3}$ vs $3.6 \pm .46$ units $\times 10^{-3}$/mg protein).

Early Lactation Effects. Palmitoyl CoA and acetate decreased ($P < 0.01$) G6PD activity during early lactation (Table II). Mammary G6PD activity was decreased ($P < 0.05$) when treated with 5 μM palmitoyl CoA or 1 mM acetate when compared with 25 μM palmitoyl CoA or 10 mM acetate. Addition of spermidine reversed ($P < 0.05$) palmitoyl CoA-induced inhibition of G6PD activity when treated with 5 μM palmitoyl CoA. Putrescine alone reduced ($P < 0.05$) enzyme activity while spermidine alone had no effect. The addition of acetate to palmitoyl CoA (5 μM) increased ($P < 0.05$ for 1 mM acetate and $P < 0.01$ for 10 mM acetate) G6PD activity compared with each given alone. Neither of the two acetate levels, when combined with the higher level of palmitoyl CoA (25 μM), affected the depressed G6PD activity. Although it is obvious that palmitoyl CoA had an effect on G6PD activity, we do not know how much was taken up by the cell (intracellular effect) or if it bound to the cell surface and had an effect after homogenization.

Discussion

Early Lactation. In mammary explants from early lactation cows, palmitoyl CoA decreased G6PD activity and spermidine reversed the inhibition, in agreement with changes in yeast G6PD activity (28, 29). Contrary to previous studies (17), however, we found that 5 μM palmitoyl CoA caused a greater inhibition of G6PD activity than did 25 μM palmitoyl CoA. Addition of 0.4 mM spermidine to the incubation media prevented G6PD inhibition by 5 μM palmitoyl CoA, but not by 25 μM palmitoyl CoA. This may be due to cationic interactions of spermidine and palmitoyl CoA which prevents binding of palmitoyl CoA with G6PD. The reduced effect with 25 μM may be due to more available palmitoyl CoA than can be bound by the fixed level of spermidine.

Acetate (1 mM, but not 10 mM) also reduced ($P < 0.025$) G6PD activity, similar to the effects of pal-

Table I. G6PD Activity in Mammary Explants Taken from Cows in Early Lactation[a]

Treatment[a]	Units/mg protein ($\times 10^{-3}$)	% Control
Control	7.4 ± 1.2	100
Palmitoyl CoA (5 μM)	5.2 ± 0.6	70
Palmitoyl CoA (25 μM)	6.0 ± 0.7	81
Acetate (1 mM)	5.4 ± 1.2	73
Acetate (10 mM)	6.3 ± 1.2	85
Spermidine (0.4 mM)	7.0 ± 0.9	95
Spermidine (0.4 mM) + palmitoyl CoA (5 μM)	6.7 ± 1.1	91
Spermidine (0.4 mM) + palmitoyl CoA (25 μM)	6.4 ± 1.3	87
Spermidine (0.4 mM) + acetate (1 mM)	5.9 ± 1.4[b]	80
Spermidine (0.4 mM) + acetate (10 mM)	7.3 ± 3.6[b]	99
Putrescine (0.4 mM)	5.4 ± 0.9	73
Palmitoyl CoA (5 μM) + acetate (1 mM)	6.6 ± 1.2	89
Palmitoyl CoA (5 μM) + acetate (10 mM)	7.5 ± 1.1	102
Palmitoyl CoA (25 μM) + acetate (1 mM)	6.1 ± 1.0	83
Palmitoyl CoA (25 μM) + acetate (10 mM)	5.9 ± 0.7	80

[a] Activity for each treatment is averaged over all incubation times (mean ± SE) and is computed from actual values. See table of orthogonal contrasts for comparison of treatment effects.
[b] These treatments were averaged for the +120-min incubation period only.

Table II. Table of Orthogonal Contrasts for Differences between G6PD Activity in Early Lactation Mammary Explants when Treated Alone or in Combination ( ) with 5 or 25 μM Palmitoyl CoA (P5 or P25), 1 or 10 mM Acetate (A1 or A10), Spermidine (S), and Putrescine[a]

Contrast[b]	*F* value	*P* value
1: Control vs P5 + P25 + A1 + A10	64.5	<0.01
2: S versus putrescine	6.9	<0.025
3: P5 vs (P5 + S) + S	8.5	<0.025
4: P25 vs (P25 + S) + S	1.3	NS
5: P5, P25 vs A1, A10	<1	NS
6: P5, A1 vs P25, A10	8.8	<0.025
7: P25, A10 vs (P25 + A10)	<1	NS
8: P25, A1 vs (P25 + A1)	<1	NS
9: P5, A10 vs (P5 + A10)	10.0	<0.01
10: P5, A1 vs (P5 + A1)	5.6	<0.05

[a] Mean SE = 0.000049; six observations/mean. *F* value (1,12 *df*) *P* (0.05) = 4.75; *P* (0.025) = 6.55; *P* (0.01) = 9.65.
[b] Contrast 1—Enzyme activity in control tissue compared with mean of enzyme activities in treatments P5, P25, A1, and A10, each given alone. Contrast 3—mean enzyme activity in treatment P5 compared with mean of enzyme activities in treatment S (given alone) and treatment P5 + S. Contrast 5—mean enzyme activity in treatment P5 and treatment P25 compared with mean enzyme activity in treatment A1 and treatment A10, each given alone.

mitoyl CoA. Addition of spermidine prevented G6PD inhibition by 10 mM acetate. These data suggest that polyamine regulation of protein synthesis (22, 25) may have a permissive effect on fatty acid synthesis by preventing negative feedback on G6PD activity and hence decreased NADPH formation. This is consistent with data which demonstrate that polyamines increase triglyceride synthesis in adipose tissue (24). These data are surprising considering that maximal mammary fatty acid synthesis occurs at 10 mM acetate (36, 37).

Our results also suggest a coordinated effect between *de novo* fatty acyl synthesis and palmitoyl CoA levels in control of G6PD activity. Acetate (fatty acid synthesis) alone is inhibitory, but addition of 5 μM palmitoyl CoA to 10 mM acetate totally abolishes G6PD inhibition. Addition of increasing levels of acetate stimulates fatty acid synthesis and the need for NADPH subsequently overrides the inhibitory effect of 5 μM palmitoyl CoA. The coordinated effect may be that removal of long-chain free fatty acids by attachment to the glycerol backbone acts to inhibit G6PD activity in the presence of only 5 μM palmitoyl CoA (38, 39), but G6PD levels return to normal when increasing acetate levels are given in combination with 5 μM palmitoyl CoA to stimulate *de novo* fatty acid synthesis (need for NADPH). At higher palmitoyl CoA levels the activity of glycerolphosphate acyl transferase is decreased (40), and long-chain fatty acyl CoA build-up could act as direct negative feedback signal on G6PD or acetyl CoA carboxylase levels. This direct effect cannot be overcome regardless of acetate level. Regardless of whether palmitoyl CoA acts directly on G6PD or on acetyl CoA carboxylase to reduce fatty acid synthesis, NADPH production via G6PD will decrease in the mammary gland. In addition, it may be advantageous to reduce the glucose-6-phosphate drain through the pentose phosphate pathway and redirect it through glycolysis for glycerolphosphate synthesis (41). Under these situations, NADPH production by isocitrate dehydrogenase, the other principle NADPH generating enzyme in ruminant mammary tissue, may be adequate to supply cellular needs *and* possibly to spare glucose for glycerol production. Finally, the ruminant has devised a method for sparing glucose by recycling it through the pentose phosphate pathway (9, 11). This has the effect of obtaining a greater quantity of NADPH from the same quantity of glucose. This also suggests that small changes in G6PD activity (i.e., 30%) may be magnified when coupled with recycling.

Mid-Lactation. Contrary to results found in early lactation explants, no differences were found among any treatments in explants from mid-lactation. It is possible that because the nutritional status of the cow has reached a point where food intake is equal to or greater than mammary gland needs, the uptake of long-chain fatty acids more closely matches an optimal balance with cellular *de novo* fatty acid synthesis. This may also reflect changes in how the cell controls G6PD activity. Furthermore, G6PD activity may already have been maximally inhibited at mid-lactation and therefore nothing else could happen when compared with control values.

In early lactation explants, addition of putrescine significantly decreased G6PD activity compared with control values or explants treated with spermidine. The reason for this decrease is unknown and requires further investigation.

We conclude that palmitoyl CoA and acetate will inhibit G6PD activity in bovine mammary explants from cows in early lactation, but not mid-lactation. The level of inhibition was greater after addition of 5 μM palmitoyl CoA or 1 mM acetate than after 25 μM palmitoyl CoA or 10 mM acetate. We also conclude that treatment with spermidine will reverse palmitoyl CoA or acetate inhibition. Our data suggest that reversal of palmitoyl CoA-induced G6PD inhibition (5 μM, but not 25 μM palmitoyl CoA) by addition of acetate may have a role in regulation of fatty acid and triglyceride synthesis in early lactation bovine explants. These results may contribute toward understanding the relationship of long-chain fatty acids and NADPH synthesis in the development of fatty acid-related metabolic disorders, such as bovine ketosis (42–44). We suggest that there may be other factors which regulate G6PD activity in the cell in addition to NADPH levels. If NADP/NADPH ratios are the driving force behind G6PD activity, then the total amount of both together should

remain constant while their ratio changes. This clearly does not happen (43, 45). Other metabolites such as glucose-6-phosphate, as well as the factors we have looked at in this study, are also important. It is obvious that many factors regulate G6PD activity in the mammary gland; our results suggest that there is still much to learn regarding this important enzyme in the mammary gland.

We wish to thank the National Hormone and Pituitary Program, Baltimore, MD, for supplying the gift of prolactin. We would also like to thank the staff at the Oregon State University Dairy Center for their help in caring for the animals.

1. Christie WW. The effects of diet and other factors on the lipid composition of ruminant tissues and milk. Prog Lipid Res **17**:111–206, 1979.
2. Christie WW. The composition, structure and function of lipids in the tissues of ruminant animals. Prog Lipid Res **17**: 245–277, 1979.
3. Moore JH, Christie WW. Lipid metabolism in the mammary gland of ruminant animals. Prog Lipid Res **17**:347–395, 1979.
4. Vernon RG, Flint DJ. Control of fatty acid synthesis in lactation. Proc Nutr Soc **42**:315–331, 1983.
5. Kinsella JE. The absorption of fatty acids by functional bovine mammary cells. Lipids **5**:892–895, 1970.
6. Kinsella JE. Coincident synthesis of fatty acids and secretory triglycerides in bovine mammary tissue. Int J Biochem **6**:65–67, 1975.
7. Smith S. Mechanism of chain length determination in biosynthesis of milk fatty acids. J Dairy Sci **63**:337–352, 1980.
8. Black AL, Kleiber M, Butterworth EM, Brubacher GB, Kaneko JJ. The pentose cycle as a pathway for glucose metabolism in intact lactating dairy cows. J Biol Chem **227**:537–550, 1957.
9. Smith GH, Crabtree B, Smith RA. Energy metabolism in the mammary gland. In: Mepham TB, Ed. Biochemistry of Lactation. New York: Elsevier, pp121–140, 1983.
10. Waghorn GC, Baldwin RD. Model of metabolite flux within mammary gland of the lactating cow. J Dairy Sci **67**:531–544, 1984.
11. Wood HG, Peeters GJ, Verbeke R, Lauryssens M, Jacobson B. Estimation of the pentose cycle in the perfused cow's udder. Biochem J **96**:607–615, 1965.
12. Baldwin RL. Enzymatic activities in mammary glands of several species. J Dairy Sci **49**:1533–1542, 1966.
13. Bauman DE, Brown RE, Davis CL. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow and cow. Arch Biochem Biophys **140**:237–244, 1970.
14. Gumaa KA, Greenbaum AL, McLean P. Adaptive changes in satellite systems related to lipogenesis in rat and sheep mammary gland and in adipose tissue. Eur J Biochem **34**:188–198, 1973.
15. Mellenberger RW, Bauman DE, Nelson DR. Metabolic adaptations during lactogenesis. Fatty acid and lactose synthesis in cow mammary tissue. Biochem J **136**: 741–748, 1973.
16. Bloch K. Control mechanisms in the synthesis of saturated fatty acids. Annu Rev Biochem **46**:263–298, 1977.
17. Kawaguchi A, Bloch K. Inhibition of glucose 6-phosphate dehydrogenase by palmitoyl coenzyme A. J Biol Chem **249**:5793–5800, 1974.
18. Taketa K, Pogell BM. The effect of palmitoyl coenzyme A on glucose 6-phosphate dehydrogenase and other enzymes. J Biol Chem **241**:720–726, 1966.
19. Tabor CW, Tabor H. 1,4-Diaminobutane (putrescine), spermidine, and spermine. Annu Rev Biochem **45**:285–306, 1976.
20. Tabor CW, Tabor H. Polyamines. Annu Rev Biochem **53**:749–790, 1984.
21. Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. Biochim Biophys Acta **473**:241–293, 1978.
22. Pegg AE, McCann PP. Polyamine metabolism and function. Am J Physiol **243**:C212–C221, 1982.
23. Pegg AE, Hibasami H, Matsui I, Bethell DR. Formation and interconversions of putrescine and spermidine in mammalian cells. Adv Enzyme Regul **19**:427–451, 1981.
24. Jamdar SC. Polyamines and triglyceride synthesis. In: Campbell RA, Ed. Advances in Polyamine Research. New York: Raven Press, Vol **2**: pp139–144, 1978.
25. Oka T, Perry JW, Takemoto T, Sakai T, Terada N, Inoue H. The multiple regulatory roles of polyamines in the hormonal induction of mammary gland development. In: Caldarera CM, Ed. Advances in Polyamine Research. New York: Raven Press, Vol **3**: pp309–320, 1981.
26. Russell DH, McVicker TA. Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. Biochem J **130**:71–76, 1972.
27. Oka T, Perry JW. Studies on the function of glucocorticoid in mouse mammary epithelial cell differentiation in vitro. J Biol Chem **249**:3586–3591, 1974.
28. Mita M, Yasumasu I. Reversal of palmitoyl coenzyme A-caused inhibition of glucose-6-phosphate dehydrogenase by polyamines. Biochem Biophys Res Commun **86**:961–967, 1979.
29. Mita M, Yasumasu I. Effect of polyamines on palmitoyl-coenzyme A-caused inhibition of glucose-6-phosphate dehydrogenase from Baker's yeast. Arch Biochem Biophys **226**:19–26, 1983.
30. Kunjara S, Sochor M, Salih N, McLean P, Greenbaum AL. Phosphoribosyl pyrophosphate and phosphoribosyl pyrophosphate synthetase in rat mammary gland. Biochem J **238**:553–559, 1986.
31. Collier RJ, Bauman DE, Hays RL. Lactogenesis in explant cultures of mammary tissue from pregnant cows. Endocrinology **100**:1192–1200, 1977.
32. Goodman GT, Akers RM, Friderici KH, Tucker HA. Hormonal regulation of α-lactalbumin secretion from bovine mammary tissue cultured in vitro. Endocrinology **112**:1324–1330, 1983.
33. Smith ER, Barker KL. Effects of estradiol and nicotinamide adenine dinucleotide phosphate in the rate of synthesis of uterine glucose-6-phosphate dehydrogenase. J Biol Chem **249**:6541–6547, 1974.
34. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
35. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York: McGraw Hill, 1980.
36. Bauman DE, Ingle DL, Mellenberger RW, Davis CL. Factors affecting in vitro lipogenesis by bovine mammary tissue slices. J Dairy Sci **56**:1520–1525, 1973.
37. Forsberg NE, Baldwin RL, Smith NE. Roles of acetate and its interactions with glucose and lactate in cow mammary tissue. J Dairy Sci **67**:2247–2254, 1984.
38. Gross MJ, Kinsella JE. Properties of palmitoyl-CoA:L-glycerol-phosphate acyl transferase from bovine mammary microsomes. Lipids **9**:905–912, 1974.
39. Hibbitt KG. Some factors involved in the control of fatty acid synthesis in the lactating bovine mammary gland. Biochim Biophys Acta **116**:56–66, 1966.
40. Marshall MO, Knudsen J. The specificity of 1-acyl-*sn*-glycerol 3-phosphate acyltransferase in microsomal fractions from lactating cow mammary gland towards short, medium and long chain acyl-CoA esters. Biochim Biophys Acta **489**:236–241, 1977.
41. Hansen HO, Jensen SS, Knudsen K. Absence of monoacylgly-

cerol pathway for triacylglycerol synthesis in goat mammary gland. Biochem J **238**:173–176, 1986.
42. Kronfeld DS. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. J Dairy Sci **65**:2204–2212, 1982.
43. Kronfeld DS, Raggi F. Nicotinamide coenzyme concentrations in mammary biopsy samples from ketotic cows. Biochem J **90**:219–224, 1964.
44. Tombropoulos EG, Kleiber M. The metabolism of specifically labelled glucose in bovine ketosis. Biochem J **80**:414–420, 1961.
45. McLean P. Carbohydrate metabolism of mammary tissue. II. Levels of oxidised and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide in the rat mammary gland. Biochim Biophys Acta **30**:316–324, 1958.