Utilization of Volatile Fatty Acids in Ruminants. 8. Acetate Activation in Mammary Tissue

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Acetyl-CoA synthetase and acetyl-CoA hydrolase activity in mammary tissue was studied. The activity of both enzymes is low in the nonlactating Holstein gland and significantly increases in the lactating gland. Synthetase activity relative to hydrolase activity is high. Synthetase activity increases as parturition approaches, reaches a maximum level soon after parturition, and declines to low levels as lactation advances. Studies of the effects of various hormones on enzyme activity in goat mammary tissue when administered 1 month postpartum showed that thyroxine increases the activity of both enzymes by 50–100%. This stimulation is blocked by pretreatment with actinomycin D or puromycin. Acetyl-CoA synthetase activity can be partially reinstated in goats at 2 months postpartum by treatment with a combination of prolactin, dexamethasone, and growth hormone. The importance of acetyl-CoA synthetase to milk synthesis and the role that acetyl-CoA plays in this process are discussed.

It is important to understand the major biochemical mechanisms that regulate milk synthesis. Acetate is a major substrate for the lactating ruminant mammary gland. Factors that determine the amount of acetate that is taken up by the mammary gland no doubt are important in controlling the quantity of milk that is synthesized.

Acetyl-CoA synthetase and acetyl-CoA hydrolase are enzymes that activate and deactivate acetate. The acetate activation reaction is an important rate-limiting step in acetate utilization (Cook et al., 1969). We have shown that as lactation advances there is a decline in the activity of these enzymes and we have shown that certain hormones will increase the activity of the enzymes. For comparison similar studies were conducted using pigs and rats. The details of these experiments are reported in this paper.

EXPERIMENTAL SECTION

Enzyme Activity during Pregnancy and Lactation. Mammary tissue biopsies were taken from 8 multiparous Holstein cows starting 49 days prepartum and continuing at intervals through parturition to 10 months postpartum. The cows were bred at 4 months postpartum.

Mammary tissue from primiparous Holstein heifers was available from an experiment being conducted by Dr. Wayne Oxender of the Department of Large Animal Surgery and Medicine. The fetus was removed from 10 first calf heifers at either 180 or 265 days gestation. Mammary tissue was provided at the time of Caesarean section and 10 days later. The removal of the fetus initiated lactation. The results of the enzyme analysis were similar for the tissue taken at both 180 and 265 days gestation. The data represent the average for all 10 heifers.

Mammary tissue samples were taken from 4 dry goats and from 8 lactating goats at 2, 4, 6, and 8 weeks postpartum.

Mammary tissue was taken from primiparous Sprague Dawley (180–200 g) rats at 2 days prepartum, at parturition, and at 2 and 12 days postpartum.

Effects of Hormones on Enzyme Activity. Pairs of lactating grade goats were given daily injections of various hormones for 3 to 5 days immediately prior to mammary biopsy. The sequence of injections was such that biopsies were taken 1 month postpartum except in one experiment where they were taken 2 months postpartum. In one experiment the effect of amount and frequency of injection of thyroxine on enzyme activity was determined. In another experiment lactating goats were pretreated with actinomycin D or puromycin for 2 days before treatment with thyroxine.

Pairs of lactating Yorkshire pigs were treated with hormones such that mammary biopsies were taken 1 month postpartum.

Either 0.13 M KCl or corn oil was used as a vehicle for injecting the experimental materials. Controls were injected with 0.13 M KCl or corn oil.

Mammary Tissue Samples. Goats and pigs were anesthetized with sernylan. Cows were tranquilized with an intramuscular injection of sodium pentathol and restrained. Primiparous heifers were given a caudal block. Approximately 3 g of mammary tissue was taken.

Rats were decapitated and the mammary tissue removed by excision. Immediately after removal all tissue was blotted dry, weighed, and added to ice cold 0.13 M KCl. The tissue was minced and homogenized using 3 strokes with a glass Teflon homogenizer. The homogenate was centrifuged at 1000*g* for 10 min. The supernatant was then centrifuged for 20 min at 20000*g*. The 20000*g* supernatant is referred to as the cytosol fraction. The 20000*g* pellet was suspended in 0.13 M KCl and frozen and thawed three times to release the enzymes. After the last thaw the material was centrifuged for 20 min at 20000*g*. The supernatant (mitochondrial extract) was taken for enzyme assay.

Acyl-CoA synthetases, acetyl-CoA hydrolase, and palmitoyl-CoA hydrolase were determined in the cytosol and mitochondrial extract by methods previously described (Quraishi and Cook, 1972). DNA and RNA were determined by the procedure of Schmidt and Thannhauser (1945). Protein was measured by the method of Lowry et al. (1951).

Chemicals. Predef 2X (9-fluoroprednisolone acetate) was a gift from the Upjohn Company, Kalamazoo, Mich. Prolactin was a gift from the National Institutes of Health. The other hormones, actinomycin D, puromycin, DNA, RNA, acetyl-CoA, palmitoyl-CoA, coenzyme A, and ATP were purchased from Sigma Chemical Company, St. Louis, Mo.

RESULTS

Enzyme Activity during Pregnancy and Lactation. The activity of acetyl-CoA synthetase in the cytosol of goat mammary gland at different stages of lactation is shown in Table I. There is an eight- to ninefold decrease in enzyme activity between 2 and 8 weeks postpartum.

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Table I. Acetyl-CoA Synthetase Activity in the Cytosol of Lactating Goat Mammary Gland at Different Stages of Lactation

Stage of lactation, weeks	Enzyme act.,[a] units[b]/mg of protein
2	2.6
4	1.8
6	1.0
8	0.3
Dry	ND[c]

[a] Results are average of values obtained from five animals. [b] One unit is defined as 1 μmol of substrate reacting per hour. [c] ND, not detectable.

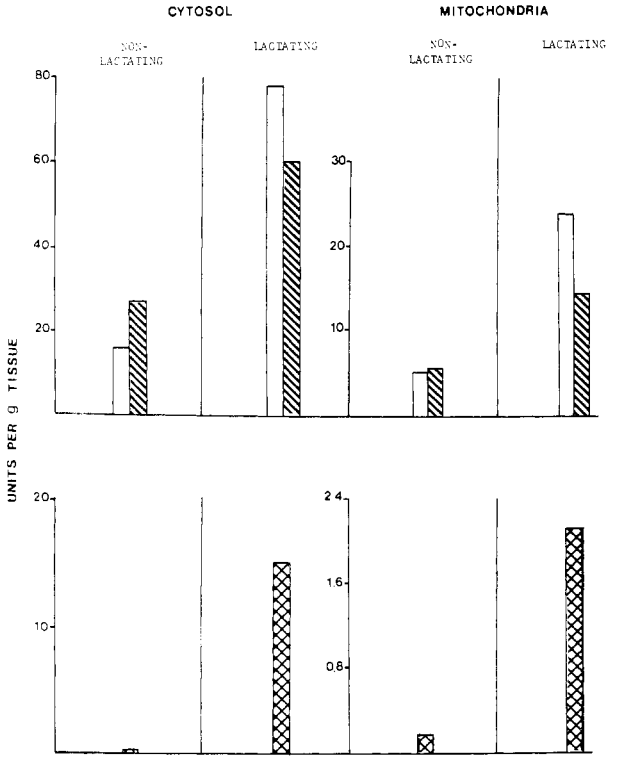

Figure 1. The effects of initiating lactation in Holstein heifers on the activity of mammary acetyl-CoA synthetase (upper graph) and mammary acetyl-CoA hydrolase (lower graph). One enzyme unit is defined as 1 μmol of substrate reacting per hour: (□) activity using acetate as the substrate; (▨) activity using propionate as the substrate; (▩) activity using acetyl-CoA as substrate.

Activity is rarely detected in the nonlactating mammary gland.

Both acetate and propionate activating abilities in the nonlactating cow mammary gland were low (Figure 1). The enzyme tends to be more active on propionate than acetate in the nonlactating gland. When lactation was initiated by removal of the fetus there was a substantial (fourfold) increase in acetate activating ability by 10 days after Caesarean section and this was true for both the mitochondrial and cytosol fractions. Propionate activating ability also increased (two- to threefold) but this was usually not as marked as the increase in acetate activating ability. Consequently, in the lactating gland the enzyme was usually more active on acetate than it was on propionate. A corresponding increase in acetyl-CoA hydrolase activity also occurred in the lactating gland (Figure 1).

The activity of acetyl-CoA synthetase in the cytosol during late pregnancy and throughout lactation of multiparous cows which had been allowed to calve normally is shown in Figures 2 and 3. Enzyme activity increased

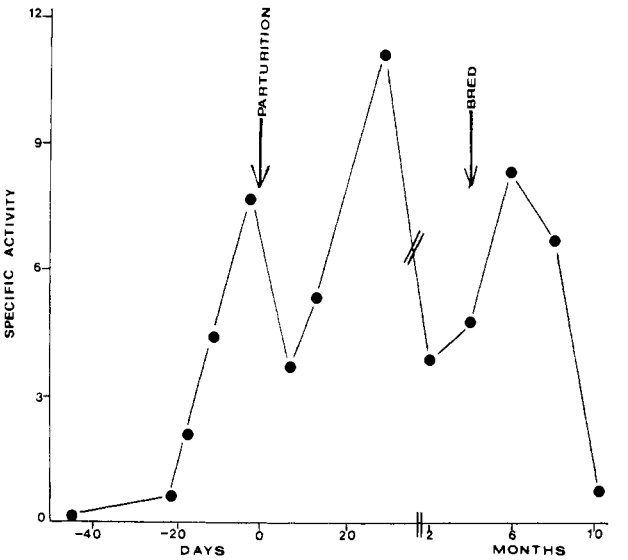

Figure 2. Specific activity of acetyl-CoA synthetase (micromoles per hour per milligram of protein) in mammary cytosol from Holstein cows before parturition and throughout lactation.

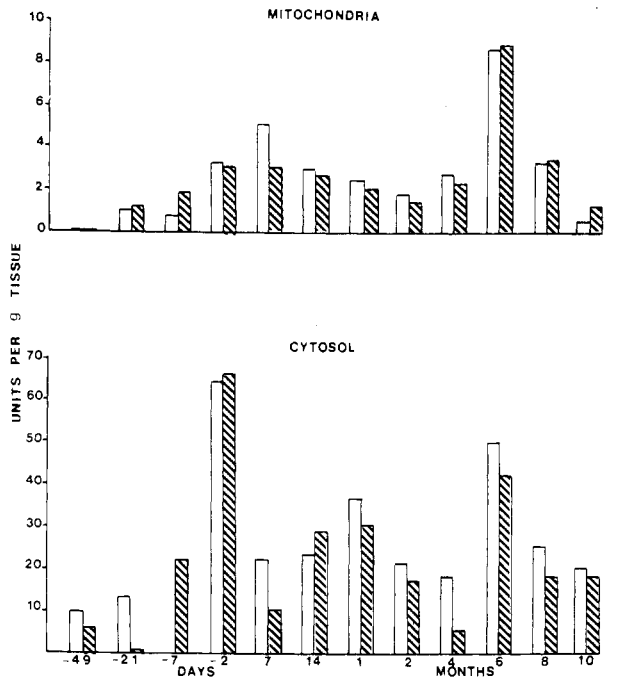

Figure 3. The activity of mammary acetyl-CoA synthetase from Holstein cows before parturition and throughout lactation. The activity is expressed as units (micromoles/hour) per gram of mammary tissue: (□) activity using acetate as substrate; (▨) activity using propionate as substrate.

sevenfold during the last 3 weeks of pregnancy, showed a brief decline following parturition, rose progressively as milk secretion increased, to reach a maximum approximately 4 weeks after parturition, and then fell as lactation declined. When the animals were bred a further increase in enzyme activity occurred, followed by a return to the low levels of early pregnancy. In this experiment acetate activating ability of the lactating gland was generally higher than propionate activating ability; however, there was some variation in this ratio especially during the early phases of lactation (Figure 3). A similar pattern was observed for mitochondrial acetyl-CoA synthetase as had been noted for the cytosol enzyme (Figure 3). In this case, however, the increase in acetate activating ability relative

Table II. Effects of Diet and Hormones on the Activity of Acetyl-CoA Synthetase and Acetyl-CoA Hydrolase in Goat Mammary Cytosol[a]

Treatment	Goat no.	Enzyme act., units/g of tissue: Acetyl-CoA synthetase	Acetyl-CoA hydrolase	Synthetase/ hydrolase	Nucleic acids, mg/g of tissue: DNA	RNA	RNA/ DNA
Control,	31	100.0	48.0	2.08	0.84	2.15	2.56
70% alfalfa,	7	100.0	41.5	2.40	0.63	2.80	4.44
30% grain							
Grain diet	18	102.5	49.5	2.07	3.50	7.30	2.08
	32	111.5	40.0	2.78			
Alfalfa meal	14	121.0	20.5	5.90	2.02	6.30	3.11
diet	23	112.0	50.0	2.24			
Insulin	30	72.5	40.0	1.81			
	17	72.5	34.0	2.13			
Epinephrine	19	120.0	39.0	3.07	2.81	8.80	3.13
	2	84.5	34.0	2.48	1.42	4.80	3.38
Predef	29	120.5	34.0	3.54	1.73	4.00	2.31
	7-2	122.0	50.0	2.44			
Thyroxine	15	159.5	80.0	1.98	1.78	5.70	3.20
	9	133.5	119.5	1.11			

[a] Daily injections of the hormones were given for 5 days. The treatments were such that mammary biopsies were taken 1-month postpartum. The first injection of insulin was at 0.25 mg/kg body weight followed by 4 more injections at 0.01 mg/kg body weight. Epinephrine, Predef, and thyroxine were injected at the level of 1 mg/kg body weight. One unit of enzyme activity equals 1 μmol of substrate reacting per hour.

to propionate activating ability subsequent to parturition was more marked.

In rat mammary tissue both acetate and propionate activating abilities of the cytosol and mitochondrial fractions increase during late pregnancy, show a sharp decline following parturition, and rise as lactation progresses (Figure 4). In both the mitochondrial and cytosol fractions propionate activating ability relative to acetate activating ability is greater prior to and just subsequent to parturition. As lactation progresses this ratio is maintained in the mitochondrial fractions but is reversed in the cytosol fractions, acetate becoming the major substrate (Figure 4). Enzyme activity is never as great as that found in the lactating cow or goat mammary gland.

Effect of Hormones on Enzyme Activity during Lactation. The effects of various hormones on acetyl-CoA synthetase and acetyl-CoA hydrolase activity in the lactating goat mammary gland are shown in Table II, and Figures 5, 6, 7, and 8. Table II shows the effect of five daily injections of various hormones on the acetyl-CoA synthetase and acetyl-CoA hydrolase activities of the cytosol. Thyroxine stimulated acetyl-CoA synthetase 30–60% and acetyl-CoA hydrolase 50–100%. Alfalfa meal gave a small increase in acetyl-CoA synthetase activity as did Predef. Insulin decreased the activity of both enzymes. The RNA/DNA ratio was of the same order of magnitude for all treatments.

Dose and time response effects of thyroxine on acetyl-CoA synthetase activity are shown in Figure 5. When thyroxine was injected daily for 2 days at 0.1 mg/kg body weight the activity of acetyl-CoA synthetase in the cytosol was doubled. The response was not as great when thyroxine was administered at higher levels and for shorter or longer times. In contrast with the cytosol enzyme, the stimulatory effect of thyroxine on mitochondrial acetyl-CoA synthetase when injected at the low level for 96 h or at the high level for 48 h was similar. In both the mitochondrial and cytosol fractions acetyl-CoA synthetase activity was less than control values 4 h after a single injection of thyroxine at 1.0 mg/kg body weight (Figure 5). Although the response of acetyl-CoA synthetase to the glucocorticoid Predef was small the cytosol enzyme responded at 16 h whereas the mitochondrial enzyme activity was highest at 48 h. Injections of Predef over longer periods of time decreased enzyme activity.

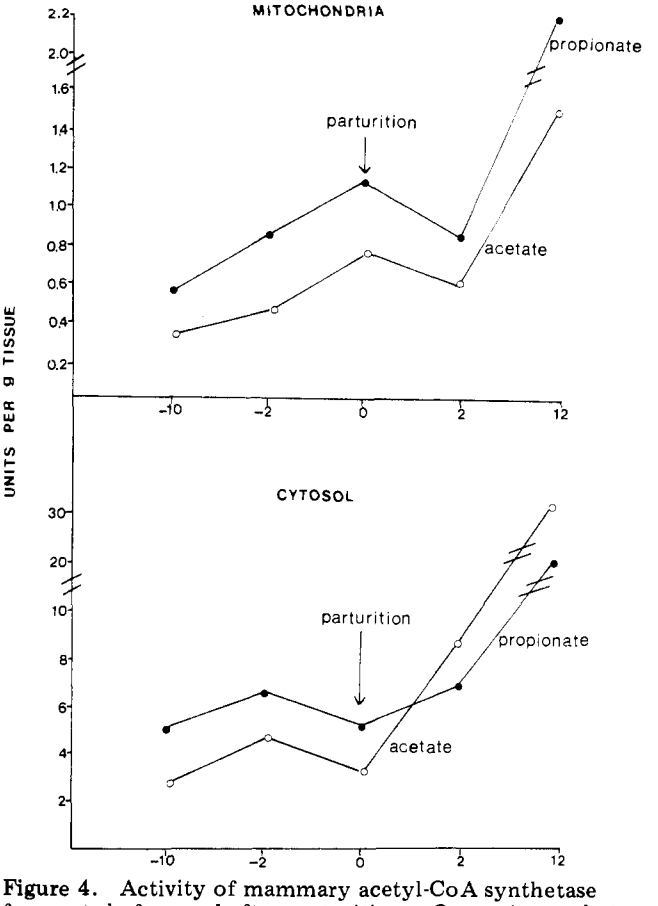

Figure 4. Activity of mammary acetyl-CoA synthetase from rats before and after parturition. One unit equals 1 μmol/h: (○) activity using acetate as substrate; (●) activity using propionate as substrate.

Mitochondrial acetyl-CoA hydrolase activity was stimulated by thyroxine injected at the lower levels (Figure 6). The stimulation was less when thyroxine was injected for longer periods of time. The high level of thyroxine decreases acetyl-CoA hydrolase activity 4 h after a single injection. There was a 70% increase in activity at 48 h but no effect at 96 h. Acetyl-CoA hydrolase activity in the cytosol increased by only 20% when thyroxine was injected at 0.1 mg/kg body weight for 48 h. However, the other

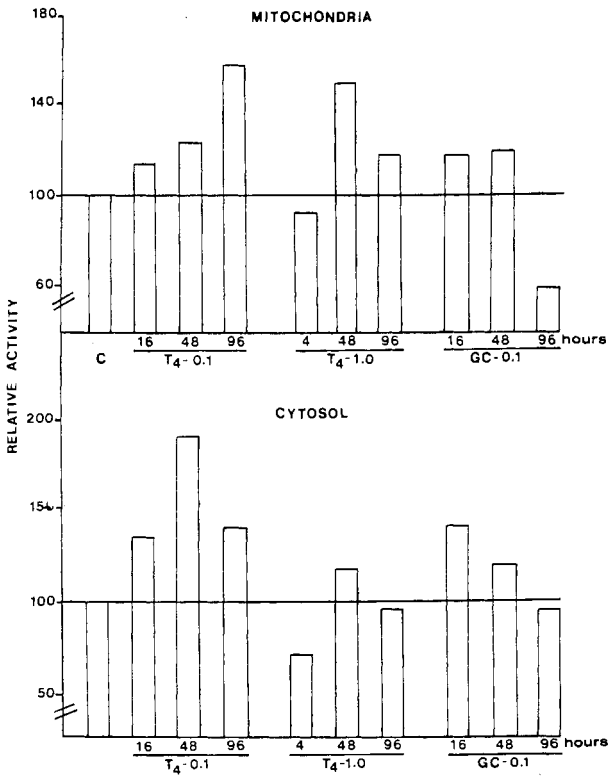

Figure 5. The effects of thyroxine (T_4) and the glucocorticoid, 9-fluoroprednisolone acetate (GC), on the activity of goat mammary acetyl-CoA synthetase. The enzyme activity was calculated as units per gram of tissue and then expressed relative to control animals. Thyroxine was injected at 0.1 mg/kg body weight, (T_4-0.1) and at 1.0 mg/kg body weight (T_4-1.0) and glucocorticoid was injected at the level of 0.1 mg/kg body weight (GC-0.1). Pairs of goats were biopsied at 16 h after a single hormone injection, after daily injections for 2 days (48), and after daily injections for 4 days (96). The treatments were scheduled such that the biopsies were taken 1 month postpartum.

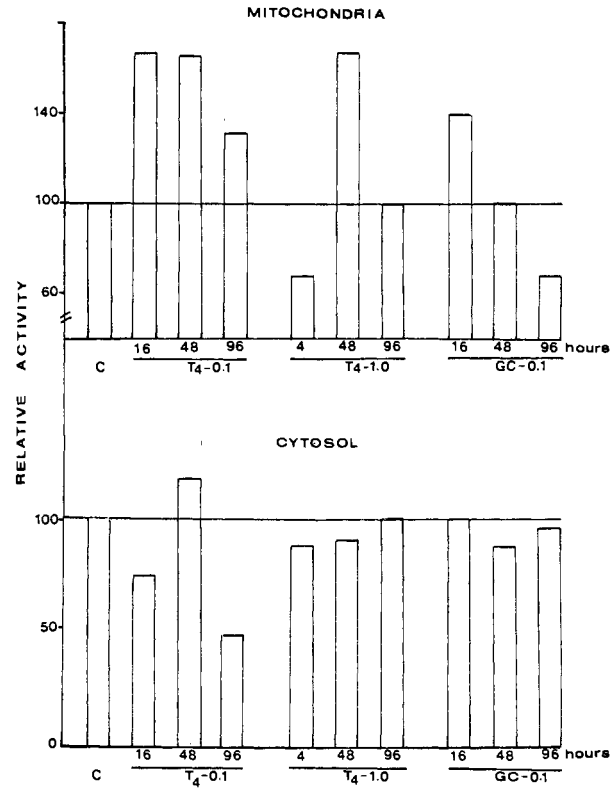

Figure 6. The effects of T_4 and the glucocorticoid, 9-fluoroprednisolone acetate, on the activity of acetyl-CoA hydrolase in goat mammary gland. The enzyme activity was calculated as units per gram of tissue and then expressed relative to control animals. Thyroxine was injected at 0.1 mg/kg body weight (T_4-0.1) and at 1.0 mg/kg body weight (T_4-1.0) and glucocorticoid was injected at the level of 0.1 mg/kg body weight (GC-0.1). Pairs of goats were biopsied at 16 h after a single hormone injection, after daily injections for 2 days (48), and after daily injections for 4 days (96). The treatments were scheduled such that the biopsies were taken 1 month postpartum.

thyroxine treatments either decreased or did not affect the cytosol enzyme. Predef did not have a major effect on the cytosol enzyme (Figure 6). Predef did increase the mitochondrial enzyme activity 16 h after a single injection. Hydrolase activity was depressed when Predef was given for 4 days.

The effects of dexamethasone and prolactin on acetyl-CoA synthetase in the cytosol are shown in Figure 7. Dexamethasone at 0.1 mg/kg body weight gave a 70% increase in enzyme activity where 10 times this level gave only a 20% increase. The lower dose of prolactin gave a 50% increase in enzyme activity and the high dose gave about a 65% increase in activity. Mitochondrial acetyl-CoA synthetase was stimulated 40%, and 37% when dexamethasone was injected at the low and high dose, respectively. Prolactin at 0.1 mg/kg body weight had little effect on mitochondrial acetyl-CoA synthetase activity. However, prolactin at the higher dose increased enzyme activity by 100%. Figure 7 also shows the effect of these hormones on the activity of acetyl-CoA hydrolase. Dexamethasone increased activity of the cytosol enzyme by 40% when injected at 0.1 mg/kg body weight. At the higher level activity was increased by only 6%. Mitochondrial acetyl-CoA hydrolase was not significantly altered by either dosage of dexamethasone. Prolactin increased both mitochondrial and cytosol acetyl-CoA hydrolase at both levels. It was proposed that there might be a synergistic effect between prolactin and dexamethasone. However, the results presented in Figure 7 show that this is not true for the concentrations used in our experiment. Moreover, for some doses the stimulatory effects of the individual hormones were not additive when administered in combination.

Since synthetase activity decreased in the later stages of lactation in the goat and cow mammary gland (Table I, Figure 3) and since stimulatory effects on enzyme activity had been observed in the goat mammary gland 1 month postpartum with hormone treatment it was decided to investigate whether enzyme activity could be reinstated in the late lactational goat gland (2 months postpartum) with various combinations of prolactin, dexamethasone, and growth hormone. The results of this experiment are presented in Table III. The most effective combination was dexamethasone, 0.2 mg/kg body weight, prolactin, 0.5 mg/kg body weight, and growth hormone, 0.2 mg/kg body weight. With this treatment acetate activating ability of the cytosol was more than doubled and propionate activating ability was substantially increased. Acetyl-CoA hydrolase activity was diminished. The stimulatory effects of prolactin on acetyl-CoA synthetase activity in the cytosol and mitochondria which we had observed in the 1-month postpartum gland were not apparent in this experiment. Indeed, prolactin exerted an inhibitory effect on the enzyme in the cytosol and mitochondria with the exception of the propionate activating enzyme of the mitochondria. Dexamethasone when administered alone did stimulate

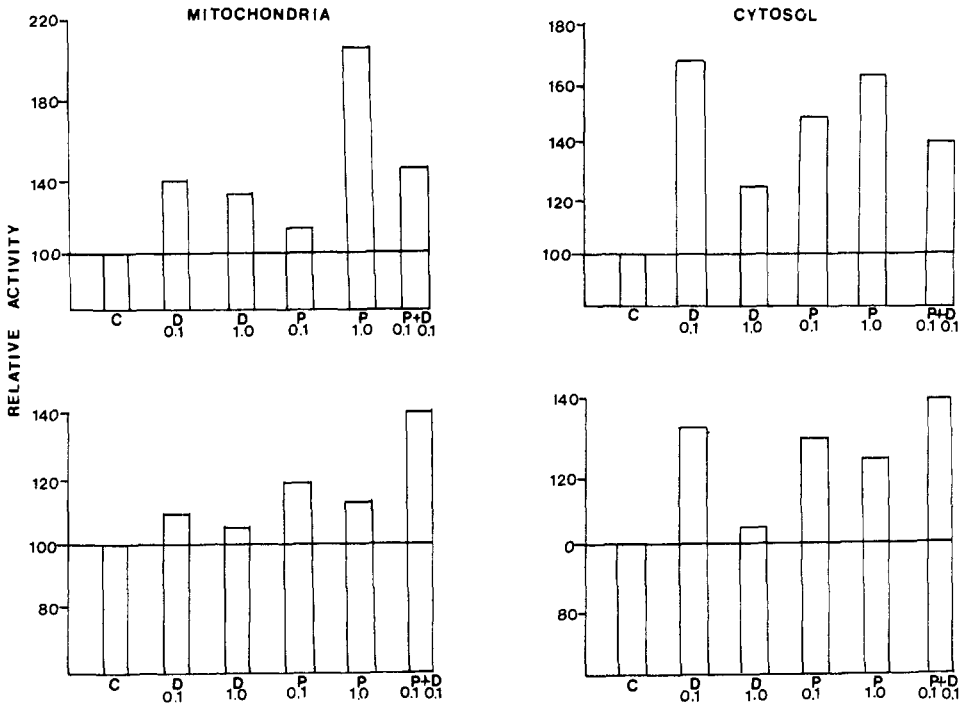

Figure 7. The effects of dexamethasone (D), prolactin (P), and dexamethasone plus prolactin (P+D) on the activity of goat mammary acetyl-CoA synthetase (upper graphs) and goat mammary acetyl-CoA hydrolase (lower graphs). The activity was calculated relative to control goats (C). Pairs of goats were injected daily for 5 days at two different levels of hormone, 0.1 mg/kg body weight or 1.0 mg/kg body weight.

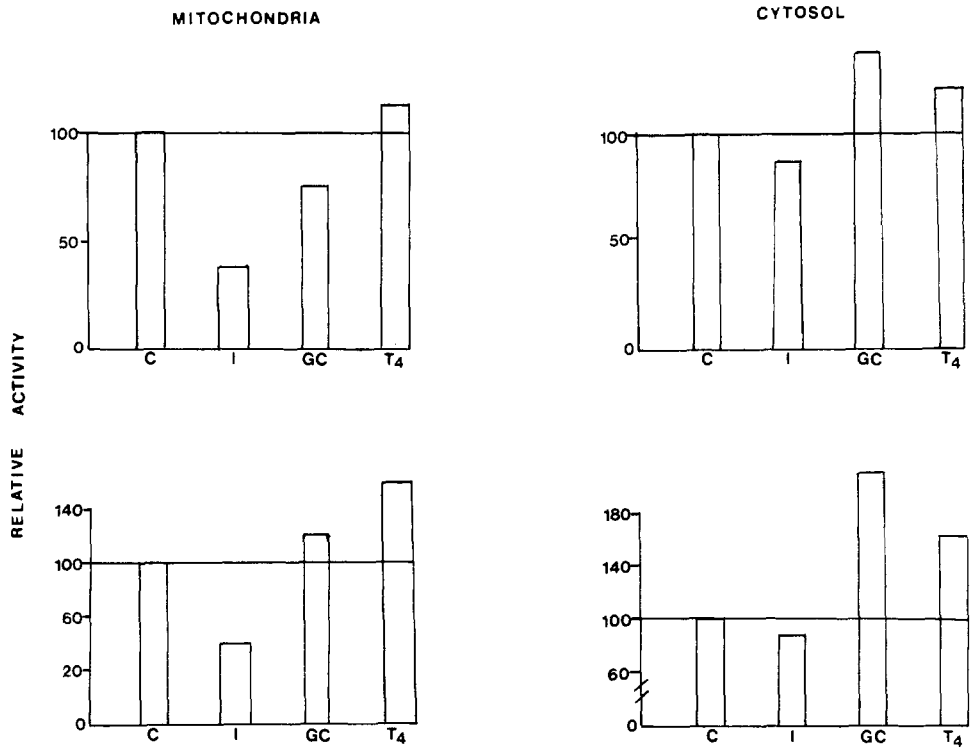

Figure 8. The effects of insulin (I), glucocorticoid (GC), and thyroxine (T_4) (see Figure 5) on the activity of acetyl-CoA synthetase (upper graphs) and acetyl-CoA hydrolase (lower graphs) in pig mammary gland. Pairs of lactating pigs were injected daily for 5 days with I (0.25 mg/kg body weight), GC (1 mg/kg body weight), and T_4 (1.0 mg/kg body weight). The treatments were scheduled such that the mammary biopsies were taken 1 month postpartum.

synthetase activity of the cytosol. However, mitochondrial synthetase activity was depressed. Dexamethasone stimulated acetyl-CoA hydrolase activity of the cytosol and mitochondria. Growth hormone inhibited both of these.

The effect of hormones on mitochondrial acetyl-CoA synthetase obtained from lactating sow mammary gland 4 weeks postpartum is shown in Figure 8. Thyroxine injected at 1 mg/kg body weight stimulated enzyme activity by 15%, whereas Predef at the same dose decreased enzyme activity 15%. Insulin had a major effect on enzyme activity. When injected at 0.25 mg/kg body weight enzyme activity in the mitochondria was depressed by 60%. Insulin did not have a major effect on the activity of acetyl-CoA synthetase in the cytosol (Figure 8). In contrast to the mitochondrial enzyme both thyroxine and Predef at 1 mg/kg body weight stimulated cytosol ace-

Table III. Effects of Daily Injections of Various Hormones for 5 Days on the Activity of Acetyl-CoA Synthetase and Acetyl-CoA Hydrolase in Lactating Goat Mammary Gland 8 Weeks Postpartum[a]

Treatment	Cytosol, substrate				Mitochondria, substrate			
	Acetate	Propionate	C_2/C_3	Acetyl-CoA	Acetate	Propionate	C_2/C_3	Acetyl-CoA
Control	292	377	0.77	958	175	130	1.51	25
Prolactin (0.5)	252	236	1.07		157	179	0.88	
Dexamethosone (0.2)	608	389	1.56	1103	133	100	1.33	52
Growth hormone (0.2)	313	355	0.88	864	113	100	1.13	11
Dexamethosone (0.2) + Prolactin (0.5)	446	396	1.20	631	93	100	0.93	55
Dexamethosone (0.2) + Prolactin (0.5) + growth hormone (0.2)	649	419	1.57	812				
Control (4 weeks postpartum)	1986	1697	1.17	1263				

[a] Enzyme activity is in nanomoles of substrate reacting per minute per gram of tissue. Numbers in parentheses represent the level of hormone injected in milligrams per kilogram body weight.

Table IV. Effects of Tris or Bicarbonate Buffer on the Specific Activity (Nanomoles per Minute per Milligram of Protein) of Acetyl-CoA Synthetase in the Cytosol from Lactating Goat and Pig Mammary Gland

Substrate	Bicarbonate		Tris	
	Pig	Goat	Pig	Goat
C_2	5.7	5.3	81.6	40.0
C_3	9.0	6.7	31.7	35.0
C_4	4.5	2.5	ND	ND
C_5	10.0	7.3	ND	ND
C_6	10.2	6.5	ND	ND
C_7	8.8	4;8	ND	ND
C_8	11.0	1.8	ND	ND

tyl-CoA synthetase by 20 and 30%, respectively. The effect of these hormones on acetyl-CoA hydrolase activity of the mitochondrial and cytosol fractions is also presented in Figure 8. Insulin affected acetyl-CoA hydrolase activity in exactly the same way as it had affected acetyl-CoA synthetase activity. This was true for both the mitochondrial and cytosol fractions. The same was true for thyroxine except that the stimulation of the cytosol acetyl-CoA hydrolase activity was much greater (60%). There was a major stimulation of acetyl-CoA hydrolase activity in the cytosol and mitochondrial fractions with Predef treatment. The cytosol enzyme was doubled in activity and the mitochondrial enzyme activity was increased by 20%.

Our standard procedures for measuring acetate and propionate activating enzymes involve the use of Tris buffer. C_4–C_8 fatty acid activating enzymes in the cytosol fraction of lactating goat or pig mammary gland 4 weeks postpartum are not active in Tris buffer (Table IV). However, these enzymes can be detected when potassium bicarbonate buffer (0.02 M, pH 8.5) is used (Table IV). By using the appropriate buffer system the effect of various hormone treatments on the C_4–C_8 fatty acid activation reaction in the cytosol of lactating sow mammary gland could be determined. The results are shown in Table V. When Predef or thyroxine were injected at 1 mg/kg body weight, there was a two- to threefold stimulation in activity. The most notable effect of Predef was a sixfold stimulation of C_4 activating ability, and the most marked effect of thyroxine was a fourfold stimulation in activity of the same enzyme. Insulin had little or no effect on C_3–C_6 fatty acid activating enzymes. In contrast, there was a major inhibition of enzyme activity by insulin on both C_2 and C_7–C_8 activating enzymes. The same general phenomena of stimulation by Predef or thyroxine and inhibition by insulin were observed for acetyl-CoA hydrolase activity (Table V). DNA and RNA levels were approximately the same for all treatments with the exception of insulin. There was a marked reduction in DNA and RNA when the animal was injected with insulin.

Palmitoyl-CoA hydrolase activity in both the cytosol and mitochondria of lactating pig mammary gland is not stimulated by either Predef or thyroxine when these are injected at 1 mg/kg body weight (Table VI). This is in contrast to the effect of these hormones on acetyl-CoA hydrolase activity.

Since thyroxine injected daily at 0.1 mg/kg body weight for 48 h consistently increased the activity of acetyl-CoA synthetase and acetyl-CoA hydrolase in both the cytosol and mitochondria of lactating goat mammary gland an experiment was conducted to determine whether or not the stimulation was due to an increase in protein synthesis. Pairs of lactating goats were treated with saline or thyroxine or pretreated with actinomycin D (0.1 mg/kg body weight) or puromycin (0.1 mg/kg body weight) before thyroxine treatment. The results are presented in Figure

Table V. Effects of Hormones and Buffers on the Activity of Acetyl-CoA Synthetases and Acetyl-CoA Hydrolase in Lactating Pig Mammary Gland

Treatment	Enzyme act., units/g of tissue[a]								Acetyl CoA hydrolase[a]	Acetyl-CoA synthetase[b]/acetyl-CoA hydrolase	Nucleic acid, mg/g of tissue		
	C_2[b]	C_2[c]	C_3[c]	C_4[c]	C_5[c]	C_6[c]	C_7[c]	C_8[c]			DNA	RNA	RNA/DNA
Control[d]	149	14	15	5	15	17	16	17	26	6	0.94	2.60	2.77
Insulin	64	14	11	6	14	14	7	7	22	3	0.48	1.88	3.92
Predef	176	19	30	30	49	47	39	34	44	4	1.24	3.10	2.50
Thyroxine	182	17	28	23	35	36	32	33	39	5	1.07	3.20	2.99

[a] Enzyme unit equals 1 μmol of substrate reacting per hour. [b] Assayed in 0.2 M Tris (pH 8.6). [c] Assayed in 0.02 M potassium bicarbonate (pH 8.5). [d] Two control animals were injected with saline and two with propylene glycol.

Table VI. Effects of Predef and Thyroxine on the Specific Activity (Nanomoles per Minute per Milligram of Protein) of Acetyl-CoA Hydrolase and Palmitoyl-CoA Hydrolase Activity from Pig Lactating Mammary Tissue

	Cytosol		Mitochondria	
Treatment	Palmitoyl-CoA hydrolase	Acetyl-CoA hydrolase	Palmitoyl-CoA hydrolase	Acetyl-CoA hydrolase
Control	13.0	10.5	10.0	20.0
Predef	16.0	16.0	9.5	30.0
Thyroxine	15.5	16.3	8.8	26.7

9. Both actinomycin and puromycin prevented the stimulation of acetyl-CoA synthetase in the cytosol. However, for the mitochondrial enzyme only actinomycin prevented the effects of thyroxine. The same general effects were observed for acetyl-CoA hydrolase.

DISCUSSION

The relative importance of glucose and acetate to the overall metabolism of the ruminant and nonruminant mammary gland has been well documented (Davis and Bauman, 1974; Bauman and Davis, 1974). Nonruminant mammary tissue preferentially utilizes glucose as a supply of carbon for de novo fatty acid synthesis and for generation of energy as ATP via glycolysis and mitochondrial oxidation. In ruminant mammary tissue, however, glucose has been excluded as a major source of energy for ATP synthesis and as a source of carbon for fatty acid synthesis because of lack of certain key enzymes (Bauman and Davis, 1974). Annison et al. (1967) have shown conclusively that acetate is the major precursor in ruminant mammary gland for synthesis of fatty acids of chain length C_4–C_{16}. Furthermore, these workers have shown that the mammary cell obtains a major part of its energy from acetate oxidation. It is well established that the enzymes necessary for the synthesis of the C_4–C_{16} fatty acids are located in the cytosol (Ganguly, 1960) whereas the Kreb's cycle enzymes are located in the mitochondria. The acetate activating enzyme acetyl-CoA synthetase which converts acetate to acetyl-CoA, a key intermediate of metabolism, is located predominantly in the cytosol of ruminant mammary tissue (Quraishi and Cook, 1972); however, some activity is also associated with the mitochondrial fraction. These workers also showed the same to be true for acetyl-CoA hydrolase which converts acetyl-CoA to acetate and CoA.

The work presented in this paper shows that major changes in acetyl-CoA synthetase activity occur as a result of pregnancy and lactation in multiparous cows (peak lactation approximately 6–7 weeks), primiparous cows in which lactation had been initiated by Caesarean section, goats (peak lactation approximately 4 weeks), and rats. The general pattern was a sharp increase in acetyl-CoA synthetase activity after parturition followed by a gradual decrease in enzyme activity as lactation progressed. Presumably, the lower levels of acetyl-CoA synthetase activity found in the rat compared to those found in the cow and goat reflect the greater significance of glucose as a metabolic substrate in this species.

Hydrolase activity in the 1-month postpartum goat mammary gland was greater than in the nonlactating gland, although in both cases this enzyme activity relative to the synthetase activity was much lower. Quraishi and Cook (1972) have shown that in the brain where glucose serves as the primary substrate under normal feeding conditions acetyl-CoA hydrolase activity is greater than acetyl-CoA synthetase activity. They suggested that

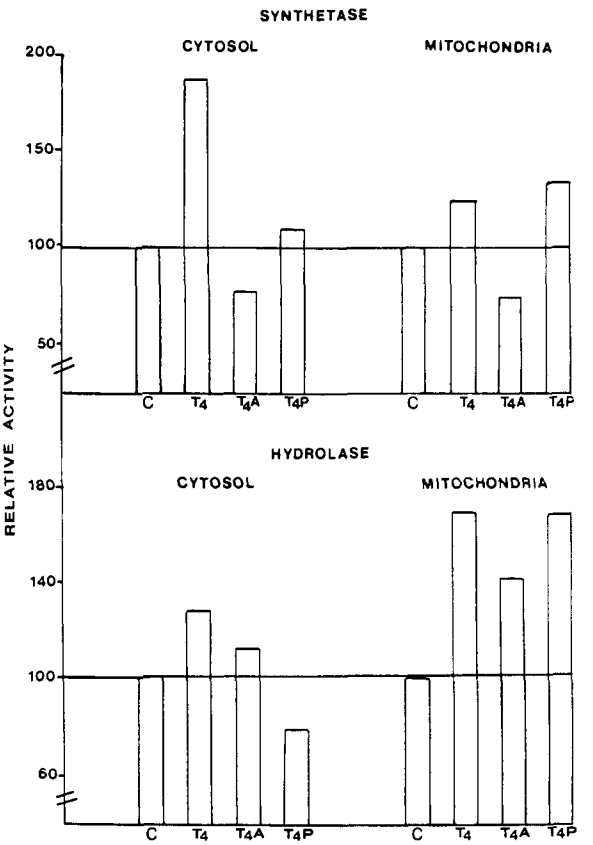

Figure 9. The effects of actinomycin D (A) and puromycin (P) on the induction by thyroxine of acetyl-CoA synthetase and acetyl-CoA hydrolase in goat mammary gland. Pairs of lactating goats were pretreated with actinomycin D (0.1 mg/kg body weight) or puromycin (0.1 mg/kg body weight) for 2 days before treatment with thyroxine (0.1 mg/kg body weight).

acetyl-CoA hydrolase in the brain might function to maintain an optimum level of CoA for glucose oxidation. In the mammary gland, where glucose serves a secondary role to acetate as a source of energy, a lower level of acetyl-CoA hydrolase would be desirable. It seems likely that with the onset of lactogenesis the metabolic pools of acetyl-CoA and/or flux of acetyl-CoA would increase. Presumably, the relative levels of acetyl-CoA hydrolase and acetyl-CoA synthetase are important in regulating the amount of acetyl-CoA in the cell. It should be pointed out that Costa and Snoswell (1975) have recently proposed that the acetyl-CoA hydrolase reaction may be catalyzed by two different enzymes, carnitine acetyltransferase and carnitine acetylhydrolase. Even so, the products of the reaction are still free acetate and free CoA.

Mellenberger et al. (1973) have also reported increases in cytosol acetyl-CoA synthetase activity as well as an increase in activity of acetyl-CoA carboxylase in the multiparous postpartum cow mammary gland which closely paralleled changes in the lipogenic capacity of mammary tissue slices. They suggested that acetyl-CoA carboxylase and perhaps acetyl-CoA synthetase represent important regulatory enzymes in fatty acid synthesis. Baldwin (1966) using a number of ruminant species found no significant increase in any of the other key enzymes of metabolism when the mammary gland became secretory. This was in direct contrast to the situation in nonruminants in which major changes in key enzymes occurred at parturition (Baldwin, 1966; Baldwin and Yang, 1974). Since we have observed sharp increases in synthetase activity coincident with parturition and lactation it seems

reasonable to suppose that acetyl-CoA synthetase and possibly acetyl-CoA hydrolase may be important control points of fatty acid synthesis and of energy metabolism. In this respect, it is perhaps significant that the acetate uptake by goat mammary gland at peak lactation calculated from data reported by Annison et al. (1967) is approximately 100 μmol of acetate/h per g of tissue. Specific activity of goat mammary acetyl-CoA synthetase at peak lactation in our experiments is approximately the same and therefore acetate uptake is about equivalent to the ability of the mammary gland to activate acetate. A high correlation between arterial acetate concentrations and acetate uptake and activation has also been noted by Baldwin and Smith (1971). It follows that as enzyme activity declines less acetate can be activated and therefore less acetate can be taken up by the mammary gland.

The significance of an increase in acetate activating ability in the mammary gland at parturition in terms of fatty acid synthesis and energy metabolism is obvious. However, it is not immediately clear why there is a propionate activating enzyme which also increases at parturition since the propionic acid produced by microbial fermentation of feed in the rumen is removed from the circulation by the liver, little appearing in peripheral blood (Cook and Miller, 1965). In the dry goat or cow mammary gland propionate activating ability marginally exceeds acetate activating ability and this ratio tends to be reversed in the lactating gland. The same was true also for the enzyme activity in the cytosol fraction of rats. This effect can be produced experimentally in goats in declining lactation by treatment with a combination of dexamethasone, prolactin, and growth hormone (Table III). The physiological significance of this is not known at this time although the fact that a constant ratio of acetate to propionate activating ability is not maintained during parturition and lactogenesis suggested that more than one enzyme might be involved. Recent work in our laboratory (Ricks and Cook, 1976) has in fact established that in the bovine there are at least two enzymes which can activate propionate. One, a C_2–C_3 enzyme, is characteristic of the heart (H enzyme), and the other, a C_3–C_8 activating enzyme, is characteristic of the liver (L enzyme).

The mammary gland contains predominantly the H enzyme but there is a small but signficant amount of L enzyme activity present also. This may account for the change in relative activity on acetate and propionate which occurs when the gland becomes secretory, or when acetyl-CoA synthetase activity is reinstated by hormone treatment, the H enzyme activity being stimulated and not the L enzyme. H enzyme has been purified and characterized (Ricks and Cook, 1975) and shown to consist of one polypeptide chain of apparent mol wt 67 000 which activates both acetate and propionate. Acetate activating ability slightly exceeds propionate activating ability. Since the enzyme activates both acetate and propionate an induction of acetate activating ability would result in a concomitant rise in propionate activating ability. This rise in propionate activating ability may not therefore be of physiological significance in the mammary cell, but is just a necessary consequence of induction of acetyl-CoA synthetase activity.

The initiation of lactation as well as various hormones stimulate the activity of the enzymes. Using hypophysectomized goats Cowie (1969) has shown that prolactin, glucocorticoids, growth hormone, and thyroxine are important for maintaining lactation. Others have reported that prolactin and growth hormone stimulate lactogenesis in the late lactational goat gland (Meites, 1961). Our work has shown that prolactin, growth hormone, thyroxine, and glucocorticoids (dexamethasone or Predef) generally stimulated acetyl-CoA synthetase activity in the goat mammary gland both in the mitochondrial and cytosol fractions (Tables II, III, and V; Figures 5, 7, and 8). Furthermore, acetyl-CoA synthetase activity in the cytosol could be partially reinstated in the late lactational goat gland by treatment with dexamethasone, prolactin, and growth hormone (Table III). Similar hormone treatments also stimulated acetyl-CoA hydrolase activity but the stimulation was never as great (with the exception of the thyroxine stimulation) as the stimulation of acetyl-CoA synthetase activity.

Since inhibitors of protein synthesis prevented the thyroxine stimulation of acetyl-CoA synthetase and acetyl-CoA hydrolase in 1-month postpartum goat mammary gland it seems probable that the synthesis of both enzymes was promoted with thyroxine treatment. Whether the stimulation in activities of these enzymes by other hormone treatments was due to an increase in enzyme synthesis has not been clarified although some increase in RNA/DNA ratio following such treatments was noted in goat mammary gland (Table II).

It is interesting that insulin decreased acetyl-CoA synthetase activity in goats and pigs (Table II; Figure 8). Rook and Hopwood (1970) have shown that intravenous infusion of insulin depressed plasma glucose concentration and this was associated with a decrease in milk volume and lactose yield and increases in content of fat. However, this increase in fat yield was due to an increased output of C_{12}–C_{18} fatty acids—the output of C_4–C_{10} acids fell. This would be consistent with the fall in cytosol acetyl-CoA synthetase which we have observed.

It is generally recognized that acetyl-CoA is an important regulator of metabolism. For example, acetyl-CoA is an allosteric inhibitor of hexokinase and glucokinase (Weber et al., 1967), an inhibitor of pyruvate dehydrogenase (Garland and Randle, 1964), and a positive modulator of pyruvate carboxylase (Utter and Keech, 1963). Furthermore, when intramitochondrial pools of acetyl-CoA are high citrate tends to accumulate. Citrate is freely permeable to the mitochondrial membrane and is known to inhibit a key enzyme of glycolysis, phosphofructokinase (Mansour, 1969) and pyruvate dehydrogenase (Weber et al., 1967). Therefore, when acetyl-CoA levels are high the rate of glycolysis tends to decrease. As a consequence less glucose is oxidized. Since there is a lack of citrate cleavage enzyme in ruminants, a possible shortage of oxaloacetate (OAA) necessary for the maintenance of the citric acid cycle could occur when acetyl-CoA levels are high. However, the OAA levels may be maintained by a combination of several factors. Acetyl-CoA may stimulate pyruvate carboxylase and inhibit pyruvate dehydrogenase. Chesworth and Smith (1971) have proposed that glucose enters the citric acid cycle as OAA rather than as acetyl-CoA. Thus, pyruvate is preferentially converted to OAA rather than acetyl-CoA. In addition, the recycling of citrate and α-ketoglutarate may serve as other sources for the refurbishing of the citric acid cycle intermediates.

Citrate is known to be an allosteric activator of acetyl-CoA carboxylase (Gregolin et al., 1968), one of the enzymes generally accepted to be of major importance in regulating fatty acid synthesis. It is possible that accumulation of citrate under high intracellular levels of acetyl-CoA may activate acetyl-CoA carboxylase and hence stimulate fatty acid synthesis. In addition these investigators have shown that acetyl-CoA stimulates the active polymeric form of acetyl-CoA carboxylase.

The decline in milk production in cattle with advancing lactation can be influenced by many factors. However, it is clear that one important factor is the level of acetyl-CoA synthetase and acetyl-CoA hydrolase, which in turn affects the level of acetyl-CoA. The data suggest that the activity of the enzymes is under the control of a complex system of hormones which are also intimately involved in the initiation and maintenance of lactation. Prolactin, growth hormone, thyroid hormone, and glucocorticoids are generally accepted as being of major significance in this respect.

We propose that as lactation advances and acetyl-CoA synthetase activity declines, there is a fall of acetyl-CoA levels in the cell in both the mitochondrial and extramitochondrial pools. As a consequence more glucose is oxidized via glycolysis and the citric acid cycle and so less is available for lactose synthesis. When lactose synthesis is inhibited, milk secretion declines (Rook and Hopwood, 1970). In this way acetyl-CoA synthetase plays a major role in the control of milk production.

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Mineral Composition of Liver and Kidney of Rats Fed Corn, Sorghum, and Soybean Grain Grown with Sewage Sludges and NPK Fertilizers

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Weanling rats were fed grains of corn, sorghum, and soybeans that were grown on soils treated with sewage sludge from an industrial area known to be high in heavy metals. The diets contained 80% grain and adequate quantities of essential nutrients. Grains and tissues were analyzed for P, K, Mg, Mn, Fe, Zn, Cu, Cr, Cd, Ni, and Pb. Of these, differences in tissue composition associated with type of soil treatment were found only for Mn, Cu, and Fe and even for these elements all values were within normal ranges. Measurable quantities of Cd, Ni, and Pb were not present in either grain or tissue samples. Cr was found in trace quantities in the grains but not in the animal tissues.

The disposal of sewage sludge is of great concern to municipalities because of limited areas available for disposal and current governmental regulations relative to incineration and/or discharge into surface waters. An alternative disposal operation, which is currently being considered, is recycling via land application. Possible problems that may arise from use of sewage sludge as a soil additive are that substances, such as heavy metals, present in the sludge may enter the food chain and become a hazard to animals consuming the plants grown on sludge treated soils (Somers, 1974). Numerous reports in the literature attest to the uptake of heavy metals by plants grown on soils treated with sludge (e.g., Kirkham, 1975) but few of these explore the eventual effects on animals consuming such plants. After a 3-year study of effects of

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