Metabolic Adaptations During Lactogenesis

FATTY ACID SYNTHESIS IN RABBIT MAMMARY TISSUE DURING PREGNANCY AND LACTATION

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1. Mammary tissue was obtained from rabbits at various stages of pregnancy and lactation and used for tissue-slice incubations (to measure the rate of fatty acid synthesis and CO₂ production) and to determine relevant enzymic activities. A biphasic adaptation in fatty acid synthetic capacity during lactogenesis was noted. 2. The first lactogenic response occurred between day 15 and 24 of pregnancy. Over this period fatty acid synthesis (from acetate) increased 14-fold and the proportions of fatty acids synthesized changed to those characteristic of milk fat (77–86% as C_{8:0}+C_{10:0} acids). 3. The second lactogenic response occurred *post partum* as indicated by increased rates of fatty acid synthesis and CO₂ production (from acetate and glucose) and increased enzymic activities. 4. Major increases in enzymic activities between mid-pregnancy and lactation were noted for ATP citrate lyase (EC 4.1.3.8), acetyl-CoA synthetase (EC 6.2.1.1), acetyl-CoA carboxylase (EC 6.4.1.2), fatty acid synthetase, glucose 6-phosphate dehydrogenase (EC 1.1.1.49), and 6-phosphogluconate dehydrogenase (EC 1.1.1.44). Smaller increases in activity occurred with glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) and NADP⁺-isocitrate dehydrogenase (EC 1.1.1.42) and the activity of NADP⁺-malate dehydrogenase (EC 1.1.1.40) was negligible at all periods tested. 5. During pregnancy and lactation there was a close temporal relationship between fatty acid synthetic capacity and the activities of ATP citrate lyase ($r = 0.94$) and acetyl-CoA carboxylase ($r = 0.90$).

The mammal's ability to produce milk may depend on the capacity to increase the availability of various rate-limiting metabolites within the mammary gland (Hartmann, 1969). It seems likely that the availability of these key metabolites is under the hormonal regulation of rate-limiting enzymes. The ability of rat mammary gland to synthesize milk components is acquired 12–24 h before parturition as indicated by the rapid increases in enzymic activities and biosynthetic capacity (Baldwin & Milligan, 1966; Kuhn & Lowenstein, 1967; Kuhn, 1968, 1969; Gul & Dils, 1969; McKenzie *et al.*, 1971). By contrast, the activities of a number of enzymes increase between 30 and 7 days *pre partum* in the cow mammary gland and there is a highly significant temporal relationship between enzymic activity and biosynthetic capacity (Mellenberger *et al.*, 1973).

Recent studies with rabbit mammary tissue have suggested two periods of lactogenic stimulation. The first lactogenic stimulus occurs by 21–22 days of pregnancy and is characterized by a substantial increase in both the lactose content (Denamur, 1969) and the rate of fatty acid synthesis (Strong & Dils,

1972). There are also major cytological changes in mammary tissue at this period (Bousquet *et al.*, 1969). The second lactogenic stimulus occurs near parturition as shown by an increased rate of fatty acid synthesis (Strong & Dils, 1972) and enzymic activities (Gul & Dils, 1969; Hartmann & Jones, 1970).

The present investigation was to determine the temporal relationship between fatty acid biosynthetic capacity and relevant enzymic activities during pregnancy and lactation, and to identify the rate-limiting step(s) of fatty acid biosynthesis. A comparison of substrate utilization (acetate and glucose) for fatty acid synthesis and CO₂ production was also investigated.

Materials and Methods

Cofactors

Cofactors for enzyme determinations were obtained from either Sigma Chemical Co. (St. Louis, Mo., U.S.A.) or P-L Laboratories (Milwaukee, Wis., U.S.A.). Fatty acid standards (C_{4:0} to C_{18:0}), human chorionic gonadotrophin and bovine serum albumin (fatty acid poor) were from Sigma Chemical Co. Sodium [¹⁴C]acetate and sodium [¹⁴C]bicarbonate

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were purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.). [^{14}C]Glucose was obtained from Amersham/Searle (Arlington Heights, Ill., U.S.A.).

Animals

Twenty-seven New Zealand White rabbits (primiparous and multiparous) were injected with human chorionic gonadotrophin (80 i.u.) to induce ovulation and then bred by artificial insemination. Purina rabbit chow was fed *ad libitum* during pregnancy and lactation. The average litter size maintained by the lactating rabbits was 7 (range 6–8).

Rabbits were killed on days 15, 24 and 29 of pregnancy and 2, 5, 8, 15 and 22 of lactation. Mammary tissue was excised to remove as much muscle, adipose and connective tissues as possible. The mammary tissue was kept in ice-cold 0.3M-sucrose for about 10 min before slicing (for incubations) and homogenization (for enzyme analysis).

Tissue incubations

Mammary-tissue slices were prepared with a Stadie Riggs hand microtome and 100–130 mg wet wt. of tissue was incubated in 3 ml of Krebs–Ringer bicarbonate buffer (pH 7.4; DeLuca & Cohen, 1964) containing 10 mM-[^{14}C]acetate (1 μCi /incubation) or 10 mM-[^{14}C]glucose, (1 μCi /incubation) and 133 munits/ml of insulin. The incubation mixture was gassed with $\text{O}_2 + \text{CO}_2$ (95:5) and incubated in a shaking water bath at 37°C for 3 h. Incubations were terminated, CO_2 was collected, and fatty acids were extracted as previously described (Bauman *et al.*, 1970). The pattern of fatty acids synthesized from radioactive substrate was determined by g.l.c. of the free fatty acids (Bauman *et al.*, 1972) and results were expressed as molar percentages of fatty acids synthesized by the mammary-tissue slices.

Preliminary studies established optimum concentrations of substrates and the linearity of fatty acid synthesis with incubation time. As with cow mammary-tissue slice incubations (D. E. Bauman, unpublished work), an acetate or glucose concentration of 10 mM or greater was necessary to maximize fatty acid synthesis under the conditions used in the present study (3 ml incubation volume with 100–130 mg wet wt. of tissue/incubation). With these conditions fatty acid synthesis by rabbit mammary-tissue slices was linear for 3 h.

Enzyme assays

The tissue was homogenized and cytosol was obtained as previously described (Mellenberger *et al.*, 1973). Cytosol was maintained at 4°C until all enzyme analyses were completed (approx. 6–8 h maximum). Enzymes were assayed at 37°C under condi-

tions where the activity was linearly related to protein and to the time of incubation. Enzyme activities were expressed per mg of cytosol protein (Gul & Dils, 1969). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), NADP⁺-isocitrate dehydrogenase (EC 1.1.1.42), ATP citrate lyase (EC 4.1.3.8), and NADP⁺-malate dehydrogenase (EC 1.1.1.40) were assayed as described by Bauman *et al.* (1970). The assays for acetyl-CoA synthetase (EC 6.2.1.1), acetyl-CoA carboxylase (EC 6.4.1.2), and fatty acid synthetase (multienzyme complex) were described by Mellenberger *et al.* (1973). Blank reactions for the radioactive assays of acetyl-CoA synthetase (omission of CoA) and acetyl-CoA carboxylase (omission of acetyl-CoA) equalled approx. 10% of the optimum reaction rate.

Glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) was assayed at pH 9.8 (Kennedy, 1962). The concentrations used were 333 mM-hydrazine, 66 mM-glycine, 1 mM-NAD⁺ and 20 mM-3-glycerol phosphate. Control incubations without 3-glycerol phosphate were used.

Results

The lipogenic capacity of rabbit mammary tissue during pregnancy and lactation is given in Table 1. No differences in lipogenic capacity of mammary-tissue slices were found at the respective time periods between primiparous and multiparous rabbits. Fatty acid synthesis from acetate in the presence of glucose increased 14-fold between day 15 and day 24 of pregnancy and then remained relatively constant until parturition. The *post partum* period was characterized by a continual increase in the mammary tissue's ability to synthesize fatty acids. The rate of acetate incorporation at 22 days *post partum* was fivefold greater ($P < 0.05$) than on day 29 of pregnancy. The lipogenic rate on day 22 of lactation (Table 1) would presumably represent the maximum synthesizing capacity of rabbit mammary tissue since studies *in vivo* have indicated that maximum milk-fat secretion (yield) occurs 3–4 weeks *post partum* (Hartmann & Jones, 1970; Hall, 1971).

The rate of glucose incorporation into fatty acids (either in the presence or absence of acetate) during pregnancy and subsequent lactation followed the same trends as acetate (Table 1). However, the rate of lipogenesis was substantially greater from acetate relative to glucose. On a carbon basis, glucose incorporation into fatty acids represented only 7–20% of the acetate rate during pregnancy, whereas the similar value during lactation was 35–45%. The preference for acetate as a substrate for lipogenesis was further

Table 1. *Fatty acid synthesis by rabbit mammary-tissue slices during pregnancy and lactation*

Values are expressed as nmol of radioactive substrate incorporated into fatty acids/3h per 100mg wet wt. of tissue and represent means $\pm$ S.E.M. Numbers of animals in each period are given in parentheses. Details of the incubations are given in the Materials and Methods section. Sodium acetate and glucose were each 10mM.

Incubation media	Days pregnant			Days lactating				
	15 (3)	24 (4)	29 (4)	2 (4)	5 (2)	8 (4)	15 (1)	22 (5)
[1- ¹⁴ C]Acetate+glucose	210 $\pm$ 70	2860 $\pm$ 580	2680 $\pm$ 290	3150 $\pm$ 280	5240 $\pm$ 320	7200 $\pm$ 870	12130	13910 $\pm$ 970
[U- ¹⁴ C]Glucose+acetate	10 $\pm$ 5	60 $\pm$ 20	80 $\pm$ 30	90 $\pm$ 40	650 $\pm$ 150	420 $\pm$ 70	820	1420 $\pm$ 260
[U- ¹⁴ C]Glucose	30 $\pm$ 15	180 $\pm$ 60	230 $\pm$ 60	120 $\pm$ 60	740 $\pm$ 50	1360 $\pm$ 290	3890	2020 $\pm$ 130

Table 2. *Proportions of fatty acids synthesized by rabbit mammary-tissue slices during pregnancy and lactation*

Results are expressed as molar percent and represent means from incubations detailed in Table 1 ([1-¹⁴C]-acetate+glucose media). Distribution of radioactive fatty acids synthesized by tissue slices was determined on duplicate samples from each rabbit with number of rabbits per period as indicated in Table 1. S.E.M. (not presented) represented less than 10% of the mean.

Fatty acid	Days pregnant			Days lactating				
	15	24	29	2	5	8	15	22
C _{4:0}	5	1	1	1	1	1	1	1
C _{6:0}	3	2	2	2	1	1	1	1
C _{8:0}	9	49	51	37	49	45	53	43
C _{10:0}	19	35	33	37	37	37	35	40
C _{12:0}	15	10	8	15	10	12	8	11
C _{14:0}	14	3	3	4	3	4	2	4
C _{16:0}	28	1	1	2	1	1	1	1
C _{18:0}	7	1	1	2	1	1	1	1

indicated by the decrease in glucose incorporation into fatty acids when acetate was present (Table 1).

The proportions of fatty acids synthesized *in vitro* by rabbit mammary-gland slices during the experimental period are given in Table 2. Lipogenesis on day 15 of pregnancy was characterized by a relatively large proportion of long-chain fatty acids (59% as C_{14:0}, C_{16:0} and C_{18:0}), whereas C_{8:0} and C_{10:0} comprised only 28% of the fatty acids on a molar basis. By day 24 of pregnancy a substantial change had occurred in the distribution of synthesized fatty acids which corresponded to the increased rate of fatty acid synthesis noted in Table 1. The C_{8:0}+C_{10:0} fatty acids accounted for 77–86% of the synthesized fatty acids from day 24 of pregnancy throughout the remainder of pregnancy and subsequent lactation, whereas the C_{14:0}, C_{16:0} and C_{18:0} fatty acids comprised approx. 6% of the total. Dodecanoic acid (C_{12:0}) comprised a relatively constant portion (8–15%) of the fatty acids synthesized during pregnancy and lactation.

The proportions of fatty acids synthesized were not affected by substrate. The data in Table 2 represent

the fatty acid pattern obtained by using [1-¹⁴C]acetate+glucose, but the patterns were similar when [U-¹⁴C]-glucose+acetate or [U-¹⁴C]glucose alone were used.

The extent of the oxidation of glucose and acetate to CO₂ during pregnancy and lactation is shown in Table 3. Acetate oxidation to CO₂ increased approximately three- to six-fold between day 15 of pregnancy and subsequent lactation, whereas glucose oxidation increased approximately tenfold over the same period. A comparison of the relative CO₂ production from acetate and glucose on a carbon basis indicated that acetate contributed 53, 60, 73, 65 and 60% of CO₂ production at 15, 24 and 29 days of pregnancy and 2 and 5 days of lactation respectively. However, acetate contributed only 35% of the CO₂ carbon through the remainder of lactation (8–22 days *post partum*).

The activities of several enzymes associated with fatty acid biosynthesis increased markedly with the onset of lactation (Table 4). No differences ($P < 0.05$) in activities of individual enzymes were noted at respective periods between primiparous and multiparous rabbits.

ATP citrate lyase activity on day 15 of pregnancy showed a twofold increase (not significant) in activity by day 24 of pregnancy. The first significant increase ($P < 0.05$) in ATP citrate lyase activity occurred by 2–5 days *post partum*. The fivefold increase in lyase activity from 2–5 days *post partum* temporally corresponded to a fivefold increase in glucose incorporation into fatty acids (Table 1, glucose medium). A maximum ATP citrate lyase activity of 34–37nmol of nucleotide oxidized/min per mg of cytosol protein (Table 4) was similar to the results of Gul & Dils (1969) who used rabbits at 10–14 days *post partum*.

The first significant increase ($P < 0.05$) in acetyl-CoA synthetase activity did not occur until day 29 of pregnancy with an overall sevenfold increase occurring from day 15 of pregnancy to day 22 of lactation (Table 4). By contrast, acetyl-CoA carboxylase activity was very low at day 15 of pregnancy and increased dramatically at subsequent time-periods which coincided with the increases in the rate of fatty acid synthesis (Table 1). A 21-fold increase in acetyl-CoA carboxylase activity occurred between day 15 of

Table 3. *CO₂ production by rabbit mammary-tissue slices during pregnancy and lactation*

Values are expressed as nmol of radioactive substrate oxidized to CO₂/3 h per 100mg wet wt. of tissue and represent means $\pm$ S.E.M. The CO₂ production data were obtained from the same incubations as fatty acid synthesis data given in Table 1. Sodium acetate and glucose were each 10mM.

Incubation media	Days pregnant			Days lactating				
	15	24	29	2	5	8	15	22
[1- ¹⁴ C]Acetate+glucose	530 $\pm$ 110	1800 $\pm$ 170	1170 $\pm$ 260	1370 $\pm$ 390	2650 $\pm$ 140	1680 $\pm$ 360	3180	1500 $\pm$ 160
[U- ¹⁴ C]Glucose+acetate	130 $\pm$ 50	540 $\pm$ 150	180 $\pm$ 40	300 $\pm$ 80	860 $\pm$ 140	1180 $\pm$ 110	1110	1720 $\pm$ 240
[U- ¹⁴ C]Glucose	150 $\pm$ 60	400 $\pm$ 110	150 $\pm$ 40	250 $\pm$ 50	440 $\pm$ 60	1260 $\pm$ 190	2150	1070 $\pm$ 90

Table 4. *Enzymes concerned with fatty acid biosynthesis in rabbit mammary-tissue during pregnancy and lactation*

ATP citrate lyase, fatty acid synthetase and 3-glycerol phosphate dehydrogenase activities are expressed as nmol of nucleotide reduced or oxidized/min per mg of cytosol protein. Acetyl-CoA synthetase activity is expressed as nmol of [1-¹⁴C]acetate incorporated into [1-¹⁴C]acetoxyhydroxymate/min per mg of cytosol protein. Acetyl-CoA carboxylase activity is expressed as nmol of [1-¹⁴C]bicarbonate incorporated into acid-stable products/min per mg of cytosol protein. The activities are means $\pm$ S.E.M. with numbers of rabbits in each period as indicated in Table 1.

Enzyme	Days pregnant			Days lactating				
	15	24	29	2	5	8	15	22
ATP citrate lyase	1.9 $\pm$ 0.7	3.9 $\pm$ 1.1	3.9 $\pm$ 0.6	3.2 $\pm$ 0.9	15.1 $\pm$ 1.7	20.2 $\pm$ 3.4	37.3	34.0 $\pm$ 3.6
Acetyl-CoA synthetase	8.2 $\pm$ 0.2	10.8 $\pm$ 1.6	21.9 $\pm$ 2.2	16.4 $\pm$ 2.8	36.3 $\pm$ 1.7	48.8 $\pm$ 9.0	54.4	59.8 $\pm$ 5.5
Acetyl-CoA carboxylase	0.6 $\pm$ 0.0	1.8 $\pm$ 0.3	2.7 $\pm$ 0.4	2.9 $\pm$ 0.4	7.6 $\pm$ 1.2	8.5 $\pm$ 1.5	9.8	12.9 $\pm$ 1.6
Fatty acid synthetase	8 $\pm$ 3	26 $\pm$ 6	30 $\pm$ 4	55 $\pm$ 6	96 $\pm$ 17	122 $\pm$ 14	250	241 $\pm$ 52
3-Glycerol phosphate dehydrogenase	18 $\pm$ 3	12 $\pm$ 2	33 $\pm$ 13	78 $\pm$ 20	116 $\pm$ 8	114 $\pm$ 12	96	92 $\pm$ 16

pregnancy and day 22 of lactation. This overall increase in acetyl-CoA carboxylase activity exceeded that previously reported by Hartmann & Jones (1970).

Investigations by Smith *et al.* (1966) and Easter & Dils (1968) indicated that in rabbit mammary tissue, acetyl-CoA carboxylase is present in both the cytosol and microsomal fractions. These investigators demonstrated that acetyl-CoA carboxylase was loosely associated with microsomal particles, since this enzyme was removed by successive washes with 0.3M-sucrose while microsomal-marker enzymes were not. Preliminary studies (data not presented) indicated that with the homogenization, subcellular-fractionation and assay techniques used in the present study approx. 95% of the rabbit mammary-tissue acetyl-CoA carboxylase activity was located in the cytosol.

Fatty acid synthetase activity was substantial in rabbit mammary tissue on day 15 of pregnancy (Table 4). The threefold increase in fatty acid synthetase activity by day 24 of pregnancy coincided with the overall increase in the rate of fatty acid synthesis (Table 1). Fatty acid synthetase activity increased significantly ($P < 0.05$) by 8 days *post partum*, and the twofold increase ($P < 0.05$) from 8 to 22 days *post*

partum corresponded to a twofold increase in fatty acid synthesis observed *in vitro* from acetate or glucose (Table 1).

The activity of glycerol 3-phosphate dehydrogenase was variable but did increase ($P < 0.05$) approximately sixfold from day 15 of pregnancy to day 22 of lactation (Table 4). However, the increase in glycerol 3-phosphate dehydrogenase activity did not closely parallel the increase in fatty acid biosynthetic capacity. In particular, glycerol 3-phosphate dehydrogenase activity remained relatively constant between 5 and 22 days *post partum* (Table 4), whereas lipogenic capacity (measured *in vitro*) increased threefold (Table 1).

Cytosol enzymes that are involved with NADPH production in rabbit mammary tissue are shown in Table 5. Glucose 6-phosphate dehydrogenase activity increased substantially at each time-period during pregnancy and lactation. The sixfold increase ($P < 0.05$) in glucose 6-phosphate dehydrogenase activity between 5 and 22 days *post partum* corresponded to the increase in CO₂ production from glucose (Table 3, glucose medium). The 125-fold increase in glucose 6-phosphate dehydrogenase activity during lactogenesis was much greater than the 12-fold increase noted for 6-phosphogluconate

Table 5. Enzymes concerned with NADPH production in rabbit mammary tissue cytosol during pregnancy and lactation

Enzyme activities are expressed as nmol of nucleotide reduced/min per mg of cytosol protein. Activities represent means $\pm$ S.E.M. with the numbers of rabbits per period as indicated in Table 1.

Enzyme	Days pregnant			Days lactating				
	15	24	29	2	5	8	15	22
Glucose 6-phosphate dehydrogenase	7 $\pm$ 1	20 $\pm$ 6	55 $\pm$ 14	77 $\pm$ 20	152 $\pm$ 3	280 $\pm$ 80	715	891 $\pm$ 162
6-Phosphogluconate dehydrogenase	23 $\pm$ 5	35 $\pm$ 6	80 $\pm$ 13	93 $\pm$ 17	85 $\pm$ 5	124 $\pm$ 27	154	272 $\pm$ 54
NADP ⁺ -isocitrate dehydrogenase	105 $\pm$ 18	75 $\pm$ 12	205 $\pm$ 13	160 $\pm$ 20	236 $\pm$ 27	196 $\pm$ 14	187	218 $\pm$ 23
NADP ⁺ -malate dehydrogenase	<1	<1	<1	<1	<1	<1	<1	<1

dehydrogenase activity (Table 5). NADP⁺-isocitrate dehydrogenase activity changed less dramatically, increasing approximately twofold from day 15 of pregnancy to day 22 of lactation.

NADP⁺-malate dehydrogenase was absent throughout pregnancy and lactation (Table 5) corroborating the findings of Gul & Dils (1969). Similarly, NADP⁺-malate dehydrogenase activity is very low or absent in lactating sow, cow, and sheep mammary tissue (Bauman *et al.*, 1970, 1973; Gumaa *et al.*, 1973). These results suggest that the malate-transhydrogenation cycle plays little part in the generation of reducing equivalents necessary to support fatty acid synthesis in rabbit mammary tissue.

Discussion

Results of the present investigation coupled with the studies of Strong & Dils (1972) clearly indicate that rabbit mammary tissue undergoes a biphasic adaptation in fatty acid synthesis during pregnancy and subsequent lactation.

The first lactogenic stimulus occurs approx. 10 days *pre partum* (21st day of pregnancy) in the rabbit and is characterized by a dramatic increase in the rate of fatty acid biosynthesis (Strong & Dils, 1972; Table 1 of this paper). This period of increased biosynthetic capacity also coincides with the time of extensive cellular development (Bousquet *et al.*, 1969). Before days 20–22 of pregnancy, the fatty acids synthesized by rabbit mammary tissue are predominantly long-chain fatty acids (C_{14:0}, C_{16:0} and C_{18:0}) and therefore are characteristic of cellular membrane lipids (Strong & Dils, 1972; Table 2 of this paper). Subsequent to the first lactogenic stimulus, the predominant fatty acids synthesized are C_{8:0} and C_{10:0}. These fatty acids comprised 74–88% of the fatty acids synthesized during late pregnancy and throughout lactation (Table 2 of this paper; Strong & Dils, 1972). Studies *in vivo* have indicated that C_{8:0} and C_{10:0} acids normally comprise 85–87% of milk fatty acids synthesized by the rabbit mammary gland (Carey & Dils, 1972).

The present investigation showed that marked increases in enzyme activities associated with fatty acid

synthesis also occurred during the first lactogenic stimulus (Table 4). In particular, dramatic increases were noted in the activities of ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthetase. However, a portion of the increases in ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthetase activities could be explained by the increase in cell numbers as estimated from DNA concentration (Naito, 1958; Hartmann & Jones, 1970; Mellenberger, 1973). In contrast with bovine mammary tissue (Mellenberger *et al.*, 1973), the rabbit mammary gland contains detectable acetyl-CoA carboxylase and ATP citrate lyase activities before the first lactogenic stimulus occurs.

After the first lactogenic response, fatty acid synthetic capacity remained constant during the remainder of pregnancy (Table 1). Enzymic activities also remained relatively constant between 22 and 30 days of pregnancy (Hartmann, 1969; Hartmann & Jones, 1970; Table 4 of this paper). Subcellular development appears complete before parturition (Bousquet *et al.*, 1969) and therefore does not appear to be responsible for the low potential to synthesize fatty acids during late pregnancy.

Strong & Dils (1972) have suggested that a second lipogenic stimulus occurred 2–4 days *pre partum* in the rabbit. However, our data indicate that a second significant increase in fatty acid synthesis from acetate and glucose did not occur until 2–5 days *post partum* (Table 1). This suggestion was supported by the increases in CO₂ production, acetyl-CoA carboxylase activity and ATP citrate lyase activity which occurred 2–5 days *post partum* (Tables 3 and 4). Previous reports indicated that increased milk production (Cowie, 1969) and enzyme activities (ATP citrate lyase and acetyl-CoA carboxylase) commenced 24h *post partum* in the rabbit mammary gland (Hartmann & Jones, 1970). Increases in DNA and RNA concentrations (–1 to +1 days from parturition) appear to either precede or coincide with the *post partum* increase in enzymic activities (Hartmann & Jones, 1970; Mellenberger, 1973). Therefore our data substantiate previous results which indicated a second

Fig. 1. Comparison of acetyl-CoA carboxylase activity ($\blacktriangle$) and ATP citrate lyase activity ($\blacksquare$) with fatty acid biosynthetic capacity ($\bullet$) in rabbit mammary tissue during pregnancy and lactation

lactogenic stimulus approx. 1–2 days *post partum* (Cowie, 1969; Hartmann & Jones, 1970).

Most enzymes directly concerned with fatty acid synthesis increase throughout lactation (Tables 4 and 5 of this paper; Gul & Dils, 1969; Hartmann & Jones, 1970). However, the activities of ATP citrate lyase and acetyl-CoA carboxylase most closely paralleled the fatty acid synthetic capacity of rabbit mammary tissue (Fig. 1). The correlation coefficient for individual animals between the rate of fatty acid synthesis and ATP citrate lyase activity over the time-periods measured was $+0.94$ ($P < 0.01$), whereas the similar correlation coefficient between fatty acid synthetase and acetyl-CoA carboxylase activity was $+0.90$ ($P < 0.01$). A similar high correlation coefficient ($+0.97$) was previously shown between acetyl-CoA carboxylase activity and fatty acid synthesis in bovine mammary tissue during lactogenesis (Mellenberger *et al.*, 1973).

It has been suggested that in the rat and rabbit progesterone acts as a repressor of milk biosynthesis (Kuhn, 1969; Denamur, 1971). Denamur (1971) has summarized several investigations and notes that the concentration of rabbit plasma progesterone is maximum on the 15–20th days of pregnancy with a linear decline in the concentration of progesterone from day 21 to day 30 of pregnancy. The depressed progesterone concentration on day 21 of pregnancy combined with a rise in the concentration of free glucocorticoids (Denamur, 1971) coincided with the first lactogenic stimulus in rabbit mammary gland. Prolactin undoubtedly has a role in lactogenesis since injections of prolactin are capable of inducing lactation in rabbits (Chadwick, 1962; Delouis &

Denamur, 1972). In addition, culturing of mammary explants from 16 day pseudopregnant rabbits with prolactin for 2–4 days resulted in a 15-fold increase in fatty acid synthesis, and the production of fatty acids characteristic of rabbit milk fat (Strong *et al.*, 1972). The influence of prolactin in co-ordination with insulin and cortisol after the progesterone decrease (18–21 days *pre partum*) is probably responsible for the increased fatty acid synthesis by day 24 of pregnancy.

Other factors in the control of milk biosynthesis are the effect of suckling stimulus or the removal of milk from the gland or both. The second lactational stimulus may be related to these phenomena since the stimulus occurs after parturition (Fig. 1). Studies which indicate that milk accumulation in mammary tissue represses enzymic activity (Jones, 1968; Miller *et al.*, 1970) would support this concept. The increase in fatty acid synthetic capacity occurring during lactation (Table 1) parallels the milk fat production rate (Hartmann & Jones, 1970; Hall, 1971) and, presumably, the demand for milk by the suckling young.

Finally, there are interesting species differences concerning the timing of lactogenesis. Rabbit mammary tissue has acquired the ability to synthesize milk fatty acids (Table 1; Strong & Dils, 1972) and lactose (Mellenberger, 1973; Denamur, 1969) approx. 9 days *pre partum*. Similarly, cow (Mellenberger *et al.*, 1973) and sheep (Denamur, 1969) mammary tissue first acquire the capacity to synthesize milk components several days *pre partum*. Cytological investigations (Hollmann, 1969) coupled with the presence of α -lactalbumin (McKenzie *et al.*, 1971; Jones, 1972)

suggest that mouse mammary tissue is capable of milk synthesis several days *pre partum*. In this respect, the rat appears to be the exception. Rat mammary tissue acquires the ability to synthesize milk components 12–24 h before parturition (Kuhn, 1968; Kuhn, 1969; McKenzie *et al.*, 1971). Further knowledge concerning the mode of action of regulatory mechanisms may be obtained by comparisons of the activity of metabolically important enzymes both between and within species.

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