Metabolic Adaptations during Lactogenesis
LACTOSE SYNTHESIS IN RABBIT MAMMARY TISSUE DURING PREGNANCY AND LACTATION

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1. Mammary tissue, obtained from rabbits at various stages of pregnancy and lactation, was used for tissue-slice incubations to measure the rate of lactose biosynthesis and for relevant enzymic activity measurements. A biphasic adaptation in the rate of lactose synthesis and in the RNA concentration was noted during lactogenesis. 2. The first increase in the rate of lactose biosynthesis occurred between days 15 and 24 of pregnancy. A second substantial increase was noted immediately post partum. The overall rate of lactose biosynthesis increased 12-fold from day 24 of pregnancy to day 15 of lactation post partum, and then decreased from 15 to 22 days post partum. 3. The RNA concentration/g wet wt. of tissue and the ratio of RNA/DNA closely represented the biphasic ability of the mammary-tissue slice to synthesize lactose. 4. Increases in the activities of UDP-glucose 4-epimerase (EC 5.1.3.2) and lactose synthase (EC 2.4.1.22) were most closely correlated with increases in the rate of lactose biosynthesis. UDP-glucose pyrophosphorylase (EC 2.7.7.9) activity was not correlated with the ability to synthesize lactose, and hexokinase (EC 2.7.1.1) and phosphoglucomutase (EC 2.7.5.1) activities were variable during pregnancy and lactation. 5. Lactose synthase activity was present by day 15 of pregnancy, but the ability to synthesize lactose could not be detected until day 24 of pregnancy.

Lactogenesis, the initiation of milk synthesis and secretion, is under hormonal control but is covertly expressed in terms of the mammary gland’s metabolic activity. Investigations of fatty acid biosynthesis in rabbit mammary tissue during lactogenesis have suggested two lactogenic stimuli (Strong & Dils, 1972; Mellenberger & Bauman, 1974). However, there do not appear to have been any investigations with rabbit mammary tissue establishing the temporal relationships between lactose biosynthesis and the activity of relevant enzymes during pregnancy and lactation, although Danamur (1965) has reported the appearance of lactose in rabbit mammary tissue by day 22 of pregnancy. This would correspond to the first lactogenic stimulus given by fatty acid synthetic data (Strong & Dils, 1972; Mellenberger & Bauman, 1974) and by cytological investigations (Bousquet et al., 1969). Since lactose is synthesized exclusively by the mammary gland and is closely related to total milk production (Wheelock & Rook, 1966), lactose biosynthesis would be indicative of the functional state of the mammary gland.

There are a number of reports which suggest a species difference in the initiation of lactose synthesis in the mammary gland pre partum. Cow mammary tissue first acquires lactose synthase activity as well as the ability to synthesize lactose between 30 and 7 days pre partum (Mellenberger et al., 1973). Similarly, Danamur (1965) found that the first appearance of lactose in sheep mammary tissue occurred 3–4 weeks before parturition. This is in contrast with the rat, where significant lactose biosynthesis and lactose synthase activity are present only 12–24 h pre partum (Kuhn & Lowenstein, 1967; Kuhn, 1968; McKenzie et al., 1971).

The major objective of the present study was to investigate lactose biosynthesis by rabbit mammary tissue during various stages of pregnancy and lactation. Enzymes concerned with lactose synthesis were measured and their activity temporally related to the capacity of the tissue to synthesize lactose. In addition, RNA and DNA concentrations were determined as parameters of cellular development.

Materials and Methods

Chemicals

Cofactors used for enzyme determinations were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and P-L Laboratories (Milwaukee, Wis., U.S.A.). Human chorionic gonadotropin, bovine
serum albumin (fatty acid-poor), calf liver tRNA and calf thymus DNA were purchased from Sigma. [U-14C]Glucose and [1-14C]lactose were obtained from Amersham/Searle (Arlington Heights, Ill., U.S.A.).

Animals

New Zealand White rabbits (27; primiparous and multiparous) were injected with human chorionic gonadotropin (80 i.u.) to induce ovulation. They were then bred by artificial insemination. Rabbits were judged to be pregnant by manual palpation 14 days after insemination. The period of pregnancy in these rabbits was 31 days. Purina rabbit chow was fed ad libitum during pregnancy and lactation. An average litter of 7 (range 6–8) pups was maintained with the lactating rabbits to assure complete milk removal (Cowie, 1969).

Rabbits were killed on days 15, 24 and 29 of pregnancy and 2, 5, 8, 15 and 22 days post partum. Mammary tissue was excised so as to exclude as much muscle, adipose and connective tissue as possible. The portion of mammary tissue to be used for RNA and DNA determinations was placed in ice-cold iso-osmotic 0.3 m-mannitol. The remainder of the tissue was placed in ice-cold iso-osmotic Tris–sucrose buffer (pH 7.3; 30 mM-Tris-0.3 M-sucrose) (Mellenberger et al., 1973) before being sliced or homogenized (approx. 10 min). No significant differences between primiparous and multiparous rabbits were found in the parameters measured so the data were combined.

Determination of DNA and RNA

Nucleic acids were extracted by a modification of the method of Marmur (1961). Mammary-gland tissue was minced, and then rinsed four times with 0.3 M-mannitol to remove residual milk. Minced tissue (2 g wet wt.) was homogenized for two 45 s periods in 20 ml of 0.3 M-mannitol by using a Sorvall Omni-Mixer. The preparation was further homogenized with a Potter–Elvehjem homogenizer and then filtered through three layers of cheesecloth. Nucleic acids were extracted from triplicate 2 ml portions of homogenate by the addition of 4 ml of 0.5 M-HClO4 and heating for 1 h at 70°C. Samples were deproteinized and lipids were removed by the addition of 6 ml of chloroform–isoamyl alcohol (25:1, v/v). The supernatant was used for nucleic acid determinations.

The concentration of DNA was determined spectrophotometrically at 600 nm with diphenylamine reagent as described by Schneider (1957). The standard curve relating to colour intensity at 660 nm was prepared by using 25–400 μg of calf liver tRNA. The concentrations of DNA and RNA were calculated by the method of Schneider (1957).

Tissue incubations

Slices of mammary tissue were prepared with a Studie–Riggs hand microtome and rinsed with iso-osmotic Tris–sucrose buffer (pH 7.3; 30 mM-Tris-0.3 M-sucrose) to remove residual milk. They were then incubated (100–130 mg wet wt. of tissue/incubation) in 3 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (DeLuca & Cohen, 1964). The incubations contained 10 mM-[U-14C]glucose (1 μCi/ incubation) and 133 munits of insulin/ml. Where indicated, incubation solutions contained 10 mM-sodium acetate. The incubation mixtures were gassed with O2+CO2 (95:5) and incubated in a shaking water bath at 37°C for 3 h (Mellenberger et al., 1973).

Incubations were terminated by the addition of 0.25 ml of 1 M-HCl. To determine lactose synthesis quantitatively descending paper chromatography was used to separate the [14C]lactose from the unchanged radioactive glucose as described by Mellenberger et al. (1973). [1-14C]Lactose was used as the reference standard.

Extensive preliminary studies, which were similar to those reported by Bauman et al. (1973), were conducted with the tissue-slice system so as to ensure maximum rates of lactose synthesis. These investigations included the stability of the tissue and the best way of handling the mammary tissue before slicing, and the substrate concentrations required to ensure that the rate of lactose synthesis was linear during the 3 h period of incubation. The concentration of glucose required to obtain maximum rates of lactose synthesis by rabbit mammary-tissue slices over the 3 h incubation period was slightly higher than the rabbit plasma glucose concentration of approximately 6–7 mM (Dittmer, 1961). The insulin concentration required for maximum rates of lactose synthesis in the tissue-slice incubations was probably substantially greater than the physiological concentration in blood. The high insulin concentration required for incubations in vitro is presumably due to the insulin binding to the glass incubation vessel (Topper et al., 1972).

Enzyme analysis

Fresh mammary tissue was minced, rinsed with iso-osmotic Tris–sucrose buffer (pH 7.3; 30 mM-Tris-0.3 M-sucrose–1 mM-GSH–1 mM-EDTA) to remove residual milk, and homogenized at 4°C in 2 vol. of iso-osmotic Tris–sucrose buffer. The methods used for tissue homogenization and subcellular fractionation to obtain cytosol and particulate
Enzymes were assayed immediately after subcellular fractionation. All assays were done at 37°C in conditions where the activity was linearly related to the protein concentration and the time of incubation. Enzyme activities were expressed per mg of cytosol or particulate protein (Gul & Dils, 1969). Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

The activities of phosphoglucomutase (EC 2.7.5.1), UDP-glucose pyrophosphorylase (EC 2.7.7.9) and UDP-glucose 4-epimerase (EC 5.1.3.2) were determined as described by Kuhn & Lowenstein (1967). Lactate synthase (EC 2.4.1.22) was assayed as described by Mellenberger et al. (1973).

Hexokinase (EC 2.7.1.1) was assayed by a coupled reaction (Baldwin & Milligan, 1966) in the presence of excess of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.43). The concentrations of components in the 3 ml incubation were 50 mM-glycylglycine (pH 7.4), 8.33 mM-MgCl2, 0.5 mM-NADP+, 100 mM-KCl, 5 mM-ATP (sodium salt), 0.7 unit (μmol/min) of glucose 6-phosphate dehydrogenase/ml, 0.33 unit of 6-phosphogluconate dehydrogenase/ml and 0.05 mM-glucose. ATP was omitted from control reaction mixtures.

Gul & Dils (1969) have demonstrated the advantages of expressing the enzymic activities in mammary tissue per mg of protein of the subcellular fraction actually used in the enzymic assay. Our preliminary investigations, which give similar results to those of Gul & Dils (1969), indicated that expressing enzymic activities per g wet wt. of tissue was less satisfactory because techniques which are available lead to incomplete homogenization of the mammary tissue. Under the conditions used for homogenization and centrifugation of mammary tissue, the mg of cytosol protein per g wet wt. of tissue averaged (mean ± s.e.m.) 19.6 ± 4.0, 26.0 ± 4.0 and 23.2 ± 1.4 for days 15, 24 and 29 of pregnancy and 28.8 ± 4.2, 31.6 ± 1.0, 31.2 ± 3.8, 40.2 and 29.8 ± 1.0 for days 2, 5, 8, 15 and 22 of lactation respectively.

**Results**

Table 1 shows the effect of lactogenesis on the concentrations of DNA and of RNA in mammary gland. The concentration of DNA/g wet wt. of rabbit mammary tissue increased approx. 45% between day 15 and day 24 of pregnancy and then remained relatively constant until 15 days post partum. A constant concentration of DNA/g wet wt. of tissue throughout late pregnancy and early lactation in the rabbit has also been reported by Denamur (1963); however, Hartmann (1969) has reported a doubling in the concentration of DNA/g wet wt. of rabbit mammary tissue at the time of parturition.

The concentration of RNA/g wet wt. of tissue and the ratio of RNA/DNA also increased between day 15 and day 24 of pregnancy. Denamur (1969) has demonstrated that the major increase in the concentration of RNA/g wet wt. of tissue during this period occurred on day 21 of pregnancy. The RNA concentration/g wet wt. of tissue and the ratio of RNA/DNA remained constant from day 24 to day 29 of pregnancy, but increased by 2 days post partum (P < 0.05) and both continued to increase to a maximum at 15 days post partum (Table 1). The changes in the ratio of RNA/DNA in mammary tissue during pregnancy and lactation were similar to the results of Denamur (1963).

Table 2 shows the ability of rabbit mammary-tissue slices to synthesize lactose during pregnancy and lactation. Lactose synthesis was not detectable on day 15 of pregnancy, but the rate of incorporation of glucose into lactose was 125 nmol/3h per 100 mg wet wt. of tissue (approx. 0.3 mg of lactose/h per g dry wt. of tissue) by day 24 of pregnancy. The rate of lactose synthesis continued to increase to a maximum at 15 days post partum which was 15-fold greater than the rate of lactose synthesis on day 24 of pregnancy. Similar results are obtained if the lactose synthesis is expressed on a DNA basis since the DNA/g wet wt. of tissue remained relatively constant between day 24 of pregnancy and day 15 of lactation.

The rate of lactose synthesis decreased approximately twofold between 15 days and 22 days post partum.

### Table 1. Concentrations of DNA and RNA in rabbit mammary tissue during pregnancy and lactation

Concentrations are expressed as mg/g wet wt. of tissue. The number of rabbits used at each period is given in parentheses. Values represent means ± s.e.m. The tissue was minced, rinsed to remove residual milk, homogenized, and nucleic acids were extracted as indicated in the Materials and Methods section.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days pregnant</th>
<th>Days lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15(3)</td>
<td>24(4)</td>
</tr>
<tr>
<td>DNA</td>
<td>2.9 ± 0.5</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>RNA</td>
<td>3.8 ± 1.0</td>
<td>7.2 ± 0.6</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>1.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Vol. 142
Table 2. Synthesis of lactose by rabbit mammary-tissue slices during pregnancy and lactation

Results are expressed as nmol of [U-14C]glucose incorporated into lactose/3h per 100 mg wet wt. of tissue. Values represent means ± S.E.M. with the number of rabbits used at each period as indicated in Table 1. Mammary tissue was sliced, rinsed to remove as much residual milk as possible, and incubated as detailed in the Materials and Methods section.

<table>
<thead>
<tr>
<th>Incubation media</th>
<th>15</th>
<th>24</th>
<th>29</th>
<th>Days pregnant</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>15</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>[U-14C]Glucose</td>
<td>&lt;5</td>
<td>125±40</td>
<td>125±25</td>
<td></td>
<td>435±80</td>
<td>725±50</td>
<td>960±125</td>
<td>1500</td>
<td>665±200</td>
</tr>
<tr>
<td>[U-14C]Glucose + sodium acetate</td>
<td>&lt;5</td>
<td>100±35</td>
<td>170±65</td>
<td></td>
<td>385±90</td>
<td>795±70</td>
<td>1350±275</td>
<td>1515</td>
<td>635±95</td>
</tr>
</tbody>
</table>

Table 3. Activities of enzymes concerned with lactose biosynthesis in rabbit mammary tissue during pregnancy and lactation

Enzyme activities are means ± S.E.M. with the number of animals used at each period as indicated in Table 1. The activities of hexokinase, phosphoglucomutase, UDP-glucose 4-epimerase and UDP-glucose pyrophosphorylase are expressed as nmol/min per mg of cytosol protein. Lactose synthase activity is expressed as nmol/min per mg of particulate protein.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>15</th>
<th>24</th>
<th>29</th>
<th>Days pregnant</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>15</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>10.4±1.7</td>
<td>6.5±0.7</td>
<td>6.8±0.6</td>
<td></td>
<td>7.1±1.3</td>
<td>16.2±2.9</td>
<td>13.6±3.0</td>
<td>10.8</td>
<td>16.0±2.7</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>92±18</td>
<td>47±7</td>
<td>141±20</td>
<td></td>
<td>97±14</td>
<td>211±5</td>
<td>118±25</td>
<td>194</td>
<td>163±21</td>
</tr>
<tr>
<td>UDP-glucose pyrophosphorylase</td>
<td>25±5</td>
<td>32±6</td>
<td>74±14</td>
<td></td>
<td>288±71</td>
<td>441±16</td>
<td>590±74</td>
<td>957</td>
<td>1265±97</td>
</tr>
<tr>
<td>UDP-glucose 4-epimerase</td>
<td>8±3</td>
<td>8±2</td>
<td>9±2</td>
<td></td>
<td>54±13</td>
<td>132±3</td>
<td>182±17</td>
<td>140</td>
<td>103±9</td>
</tr>
<tr>
<td>Lactose synthase</td>
<td>2.3±0.2</td>
<td>4.2±0.3</td>
<td>4.8±0.3</td>
<td></td>
<td>5.8±0.8</td>
<td>5.0±0.6</td>
<td>7.7±0.6</td>
<td>7.2</td>
<td>7.2±1.0</td>
</tr>
</tbody>
</table>

*partum* whether expressed on a wet wt. of tissue basis or on a DNA basis. The present investigation is limited in that lactose synthesis on day 15 of lactation is given for only one rabbit (Table 2). However, this rate is similar to that reported for 16–19-day lactating rabbits by Martal (1970). In addition, Cowie (1969) and Hartmann & Jones (1970) have reported that the yield of lactose in *vivo* decreased approx. 50% from 15–24 days *post partum*.

The rabbit mammary-tissue weight can be calculated as a function of body weight at the various stages of lactation from the data of Lu & Anderson (1973). Applying these values to the present study the rate of lactose synthesis by the tissue slices (Table 2) is virtually identical with the yields of rabbit milk lactose at the various stages of lactation which have been reported by Cowie (1969) and by Hall (1971).

The rabbit mammary gland would presumably have substantial quantities of acetate available owing to caecal fermentation. Mellenberger & Bauman (1974) have reported that the addition of acetate decreases glucose utilization for fatty acid biosynthesis. In the case of lactose synthesis, the addition of acetate to glucose incubations could possibly increase the availability of glucose for lactose synthesis. However, the addition of acetate had no effect (*P* < 0.05) on the rate of incorporation of glucose into lactose (Table 2).

Table 3 shows the changes in activity of the enzymes involved with lactose synthesis. Hexokinase and phosphoglucomutase activities were variable during pregnancy with activity increasing twofold (*P* < 0.05) between day 2 and day 5 of lactation. Hexokinase and phosphoglucomutase activities did not change significantly (*P* < 0.05) after 5 days of lactation. Hartmann & Jones (1970) have shown similar variation in phosphoglucomutase activity during lactogenesis in the rabbit.

UDP-glucose pyrophosphorylase activity on day 15 of pregnancy (Table 3) was similar to the UDP-glucose pyrophosphorylase activity obtained in cow mammary tissue at 30 days *pre partum* (Mellenberger et al., 1973). The activity of this enzyme in rabbit mammary gland increased 11-fold by 2 days *post partum* with another fivefold increase occurring by 22 days of lactation (*P* < 0.05). It is noteworthy that UDP-glucose pyrophosphorylase activity was increasing at 22 days *post partum* (Table 3) when lactose biosynthesis by mammary slices was decreasing (Table 2). The 50-fold increase in UDP-glucose pyrophosphorylase activity during lactogenesis in this study was similar to the increase found in rat mammary tissue (Baldwin & Milligan, 1966) but was substantially greater than that found in cow mammary tissue (Mellenberger et al., 1973).

The activity of UDP-glucose 4-epimerase increased in a manner similar to lactose synthesis. UDP-
glucose 4-epimerase activity was relatively low during pregnancy but increased sixfold between 2 days prior to parturition (day 29 of pregnancy) and 2 days post partum. Maximum epimerase activity was observed at 8 days post partum with a gradual decrease in activity occurring between 8 and 22 days post partum. The correlation between epimerase activity and lactose synthesis in vitro was 0.76 (P < 0.01).

Lactose synthase activity (assayed in the absence of exogenous 0-lactalbumin) in rabbit mammary tissue was 2.3 units on day 15 of pregnancy (Table 3). The activity increased during pregnancy and lactation with maximum activity occurring by 8 days post partum. Between 8 and 22 days of lactation, lactose synthase activity remained relatively constant. The correlation across time periods between lactose synthase activity and the rate of lactose biosynthesis in mammary-tissue slices (Table 2) was 0.65 (P < 0.01).

Preliminary investigations indicated that the homogenization and subcellular-fractionation methods used in the present study result in lactose synthase activity being distributed in both the microsomal fraction (approx. 75% of total activity) and the mitochondrial fraction. Therefore lactose synthase activity was assayed on the combined fractions and expressed/unit of particulate protein. Investigations with more precise cellular fractionation methods have indicated that lactose synthase activity is associated with the Golgi apparatus (Coffey & Reithel, 1968; Brew, 1969; Keenan et al., 1970).

Expressing the activities of the enzymes on the basis of DNA leads to similar conclusions as those previously discussed where the activity was expressed on the protein concentration of the cellular fraction usually used in the assay. The cytosol protein/DNA ratio remained relatively constant during pregnancy and lactation averaging 6.7 ± 0.4 (mean ± S.E.M.) for the eight periods of pregnancy and lactation which were investigated. In addition a comparison of the activities of the enzymes involved in lactose biosynthesis (Table 3) with the rate of lactose synthesis by the tissue-slice preparations (Table 2) indicates that the activities of all five enzymes are greater than is necessary to account for the rate of synthesis in the tissue slices of lactose. This is presumably due to enzyme activities being determined by using optimum assay conditions so as to give maximum rates.

Discussion

The rabbit mammary gland first acquires the ability to synthesize detectable amounts of lactose (present study) and fatty acids (Strong & Dils, 1972; Mellenberger & Bauman, 1974) approximately 9–10 days prior to parturition. Similarly investigations with cow mammary tissue have indicated that lactose- and fatty acidbiosynthesis capacities are acquired before 7 days prior to parturition (Mellenberger et al., 1973).

The results of the present investigation indicated that rabbit mammary tissue responded to two lactogenic stimuli during lactogenesis. The first stimulus occurred approximately 10 days prior to parturition. Lactose synthesis increased 25-fold between day 15 and day 24 of pregnancy (Table 2). The studies of Denamur (1965) would suggest that the onset of lactogenesis occurred on day 22 of pregnancy, since this marked the first biochemical appearance of lactose in rabbit mammary tissue. An increase in the concentration of DNA and in the ratio of RNA/DNA also occurred (Table 1), which suggested an increase in protein synthesis by day 22 of pregnancy. Cellular maturation, as indicated by development of the endoplasmic reticulum and Golgi apparatus, is also accelerated between days 19 and 21 of pregnancy (Bousquet et al., 1969). In addition, the rate of synthesis of fatty acids increases at approximately day 21 of pregnancy in rabbit mammary tissue, and the pattern of lipids synthesized changes to that characteristic of rabbit milk lipids (Strong & Dils, 1972; Mellenberger & Bauman, 1974).

After the first lactogenic response, the capacity of rabbit mammary tissue for lactose (Table 2) and fatty acid (Mellenberger & Bauman, 1974) synthesis is constant during the remainder of pregnancy. The concentrations of DNA and RNA (Table 1 of the present study; Denamur, 1963) as well as enzymic activities (Table 3 of the present study; Hartmann, 1969; Hartmann & Jones, 1970; Mellenberger & Bauman, 1974) also remain relatively constant between day 22 and day 30 of pregnancy. The control of lactose synthesis during late pregnancy could possibly be end-product inhibition of lactose synthase activity. However, Palmiter (1969b) indicated that lactose did not repress the synthesis of the A-protein (galactosyl transferase) or B-protein (a-lactalbumin) components of the lactose synthase complex of mouse mammary tissue. Kuhn (1969) has also reported that lactose does not inhibit lactose synthase activity in rat mammary tissue.

The second lactogenic stimulus in rabbit mammary tissue occurred at or after parturition. Between day 29 of pregnancy and day 2 of lactation, a substantial increase occurred in RNA concentration and the ratio of RNA/DNA (Table 1 of the present study; Denamur, 1963). During this period lactose synthesis increased three- to four-fold (Table 2) and substantial increases occurred in UDP-glucose 4-epimerase and UDP-glucose pyrophosphorylase activities (Table 3). Lactose synthesis continued to increase throughout the first 2 weeks of lactation. Results of the present study on lactose synthesis are similar to those of fatty acid biosynthesis in rabbit mammary tissue.
(Mellenberger & Bauman, 1974) with regard to the timing of the second lactogenic stimulus and the magnitude of increases occurring in the 2-week period after parturition. Mellenberger & Bauman (1974) have suggested that the suckling stimulus or the removal of milk from the gland by the growing pups or both are possible controls of milk synthesis during the early post-partum period.

An asynchronous relationship exists in rabbit mammary tissue between lactose and fatty acid synthesis between day 15 and day 22 of lactation. During this period, the rate of lactose synthesis by mammary-tissue slices decreased approximately twofold (Table 2), whereas the rate of fatty acid biosynthesis continued to increase (Mellenberger & Bauman, 1974). The reasons for this asynchrony are unclear, although results from the studies in vitro are consistent with measurements in vivo. The daily yield of lactose in lactating rabbits decreased linearly with time from approximately 4.0 g on day 15 post partum to 0.6 g by 27 days post partum, whereas the yield of fat and percentage of fat in the milk continued to increase over the same period (Cowie, 1969; Hall, 1971).

The hormonal regulation of biosynthesis in mammary gland during lactogenesis remains unclear. Kuhn (1969) and Denamur (1971) have suggested that progesterone acts as a repressor of milk biosynthesis before the onset of lactation. In the rabbit, the initial decline in the concentration of plasma progesterone occurs by day 21 of pregnancy (Denamur, 1971; Challis et al., 1973) which coincides with the first lactogenic stimulus. There do not appear to be any reports of changes in the plasma concentration of prolactin in the rabbit during lactogenesis, but it seems likely that prolactin is involved as well as cortisol. Experiments both in vivo and in vitro indicate that prolactin is capable of inducing the synthesis of lactose and fatty acids in pseudopregnant rabbits (Palmiter, 1969a; DeLouis & Denamur, 1972; Forsyth et al., 1972).

The control of the initiation of lactose synthesis during lactogenesis appears to be different for various species. In the rat (Kuhn, 1968; McKenzie et al., 1971) and cow (Mellenberger et al., 1973) mammary gland, the appearance of lactose synthase activity, \( \alpha \)-lactalbumin and lactose-biosynthetic capacity are temporally related during lactogenesis. In contrast, lactose synthase activity is present by mid-pregnancy in rabbit (present study) and mouse (McKenzie et al., 1971; Jones, 1972) mammary tissues even though the mammary gland does not have the ability to synthesize detectable quantities of lactose. Mammary cellular development, rather than a specific enzyme, may be limiting lactose synthesis before the first lactogenic stimulus in rabbit mammary tissue. The appearance of the Golgi apparatus by day 20–21 of pregnancy (Bousquet et al., 1969) may be the last developmental step necessary for the acquisition of the capacity to synthesize lactose (Table 2) in the rabbit mammary gland pre partum.

References


Challis, J. R. G., Davies, I. J. & Ryan, K. J. (1973) *Endocrinology* 93, 971–976


Kuhn, N. J. (1968) *Biochem. J.* 106, 743–748


1974

Topper, Y. J., Oka, T., Owens, I. S. & Vonderhaar, B. K. (1972) In Vitro 8, 228-236