Growth and acyltransferase activity of rabbit mammary gland during pregnancy and lactation

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Abstract A bimodal change in yield and microsomal protein content of rabbit mammary gland was observed with the progress of pregnancy and lactation. The initial stimulus took place on day 22 of pregnancy and the second during early lactation. Palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase activity was monitored concurrently. This enzyme in rabbit mammary microsomes is composed of two isoenzymic species that differ with respect to the physical nature of the substrates with which each interacts. The activities of the two isoenzymes were recorded at progressive stages of pregnancy, lactation, and involution to determine if a regulatory role could be assigned to either or both species. Although the patterns were indefinite, total transacylase activity did increase over this period, i.e., the specific activity of LPAT-α was 12 and 24 nmoles/mg protein per min in pregnancy and lactation, respectively, while that of LPAT-β rose from zero to 90 nmoles/mg protein per min over the same period. The time of harvesting in relation to the interval between nursing periods is discussed in the light of these results.

Supplementary key words milk · circadian rhythms · optimum assay conditions · transacylases · isoenzymes · monomers and micelles · microsomes · biphasic substrate saturation kinetics · nonspecific substrate binding

In order to satisfy the nutrient and energy demands of the young, a pregnant mammal must acquire sufficient functional mammary tissue prior to the onset of lactation. Since the ability of the animal to produce milk is ultimately limited by the activity of rate-limiting enzyme(s) in the mammary cell, the temporal acquisition of these functionalities and their correlation with overall biosynthetic activity of the mammary gland are currently under investigation in a number of species. In the rat, for example, milk-synthesizing ability is acquired within 24 hr of parturition as evidenced by the significant rise in enzymatic activity of the mammary gland (1–5). In the cow, this capacity is developed within 4 weeks of parturition (6–8).

Two phases are apparent in the development of lactogenesis in the rabbit (New Zealand White variety). The first stimulus takes place in the third and beginning of the fourth week of pregnancy while the second occurs at parturition. These periods are characterized by changes in the weight and the DNA, RNA (9), and lactose contents (10) of the mammary gland, by changes in the specific activities (11, 12) and amounts (13) of certain mammary enzymes, and by a changing pattern of fatty acids synthesized by the gland (11–13).

The present investigation concerned identification of rate-limiting enzyme(s) in the glycerol 3-phosphate pathway of glycerolipid biosynthesis in rabbit mammary gland. Preliminary findings with rabbits of the Dutch Belted variety (14) indicated that the second enzyme in this pathway, i.e., palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase [EC 2.3.1.-] (lysophosphatidic acid acyltransferase or LPAT) was composed of two isoenzymic species, an α form, active with monomeric substrates but inhibited by micelles, and a β form, active only with micelles. It was postulated then (14) that these isoenzymes may somehow be involved in the regulation of the lipogenic activity of rabbit mammary gland. This possibility was investigated in the present study by following the activities of the LPAT isoenzymes as a function of time during pregnancy, lactation, and involution. Furthermore, as an index of changes in gland cellularity and particulate–enzyme content of the mammary cell, respectively, fresh gland weight and yield of microsomal protein were concurrently recorded.

MATERIALS AND METHODS

Materials

The 4,4′-dithiodipyridine (Aldrithiol-4) was obtained from Aldrich Chemical Co., Inc. (Milwaukee,

Abbreviations: LPAT, lysophosphatidic acid acyltransferase; LPA, lysophosphatidic acid; Aldrithiol-4, 4,4′-dithiodipyridine; cmc, critical micelle concentration.

WI) and lysophosphatidic acid (palmitoyl) from Serdary Research Laboratories (Ontario, Canada). Palmitoyl-CoA was from P-L Biochemicals, Inc., (Milwaukee, WI) and bovine serum albumin (fraction V powder) from Sigma Chemical Co., (St. Louis, MO). Pituitary luteinizing hormone was obtained from Armour-Baldwin Laboratories (Omaha, NE).

Animals

Virgin rabbits of the Dutch Belted variety were obtained from Rockland Farms, Inc., Gilbertsville, PA, at 6 months of age. Each was given pituitary luteinizing hormone (0.5 mg/kg body weight) intravenously via the marginal vein of the ear to stimulate ovulation and was immediately bred by artificial insemination. The animals, caged individually in a thermostatically controlled room, were fed Rabbit Chow (Agway, Ithaca, NY) ad libitum throughout pregnancy (31 days), lactation, and involution. The average litter size was 9 (range 6–11).

Preparation of the microsomal fraction

The temporal dependence of enzymic activity on stage of pregnancy and lactation was investigated in two replicate series of experiments. In the first series, rabbits were killed on days 7, 16, 19, 22, and 29 of pregnancy and on days 1, 3, and 9 of lactation. In the second series, tissue was harvested on days 13 and 26 of pregnancy, on days 4, 20, and 34 of lactation, and on day 14 of involution.

Animals were killed by cervical dislocation and immediate decapitation. Mammary gland was excised carefully to avoid contamination with adipose, muscle, or connective tissue. All subsequent procedures were performed on ice. Mammary tissue was washed with 0.25 M sucrose, blotted dry with cheesecloth, and weighed. After chopping with scissors, the tissue was homogenized for 30 sec at high speed in 3–6 volumes (6 vols, up to day 22 of pregnancy and 3 vols thereafter, to facilitate grinding of the entire tissue in one vessel) of 0.25 M sucrose on a two-speed Waring Blendor drive unit with stainless steel semimicro jar and blade unit accessories. Tissue that had associated upon homogenization was dispersed by chopping with scissors and rehomogenizing for a further 30 sec. All remaining associated tissue was then removed from the homogenate, ground in a mortar with glass beads (0.45–0.5 mm diameter, VWR. Scientific, Rochester, NY), returned to the homogenate, and rehomogenized for 60 sec at high speed. This crude homogenate was centrifuged at 15,000 *g* (11,250 rpm) for 20 min at 4°C in a Sorvall Superspeed RC2-B centrifuge with fixed angle rotor (Sorvall type SS-34, r_{av} 4.25″ Ivan Sorvall Inc., Norwalk, CT). The supernatant was carefully decanted through four layers of cheesecloth and centrifuged at 44,000 *g* (21,000 rpm) for 1 hr at 4°C in a Beckman L2-65 ultracentrifuge with fixed angle rotor (Beckman type 21, r_{av} 9 cm, Beckman Instruments, Spinco Div., Palo Alto, CA). The microsomal fraction was collected after careful decantation of the high speed supernatant and suspended in 0.25 M sucrose to 20–30 mg protein/ml using a Ten Broeck tissue homogenizer. After sonication for 1 min in a Model 8845-3 sonicator (Cole-Parmer Ultrasonic Cleaner, Cole-Parmer Instruments, Chicago, IL), aliquots of the microsomal suspension were analyzed for protein (14) and assayed for transacylase activity as below. The remainder of this suspension was stored at −25°C after lyophilization for 48 hr at 50–20 torr on a Model 10-100 Virtis Unitrap freeze-dryer (VWR Scientific).

Preparation of the microsomal suspension

When fresh microsomes were used as the enzyme source, the procedure outlined above was employed. With lyophilized microsomes, appropriate amounts were suspended in 0.25 M sucrose to give a final protein concentration of 20–30 mg/ml, using a Ten Broeck tissue homogenizer and Teflon pestle. Homogenization for approximately 1 min was carried out in an ice bath. Suspended microsomes were then sonicated for 1 min at 0°C as above and stored on ice. Protein determination was made prior to assay.

Acyltransferase assay

The spectrophotometric method for assaying transacylase activity in microsomes has been described previously (14). Unless otherwise stated, the standard reaction mixture contained Tris-HCl buffer, 70 mM, pH 7.4; 4,4′-dithiodipyridine, 0.3 mM; palmitoyl-CoA, 18 μM; microsomal protein in 0.25 M sucrose, 0.23 mg/ml; and monopalmitoyl-*sn*-glycerol 3-phosphate.

The 4,4′-dithiodipyridine solution was prepared to 6 mM in water and solubilized by sonicating for 1–2 min at 50–60°C. A concentration of 12 mM 4,4′-dithiodipyridine could be solubilized by this procedure but precipitated upon standing in ice. The 6 mM solution was stable on ice for up to 7 days.

Computer analysis

A Hewlett-Packard (Palo Alto, CA) calculator (Model 9820A) with cassette memory (Model 9865A) and X–Y plotter (Model 9862A) was used to plot the kinetic functions, the parameters of which were determined by trial and error.

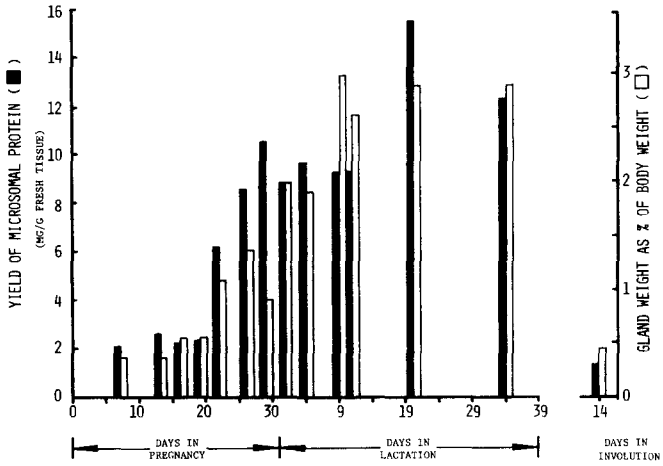

Fig. 1. Yield of fresh mammary gland (g/100g body weight, □) and mammary gland microsomal protein (mg/g fresh gland, ■) from rabbits of the Dutch Belted breed during pregnancy, lactation, and involution.

RESULTS

Mammary gland growth

Because Lu and Anderson (9) reported that approximately two-thirds of the total mammary growth in rabbits of the New Zealand White breed occurred between days 16 and 26 of pregnancy and that postparturient growth accounted for the additional weight increase, it was of interest to determine the mammary gland growth profile in rabbits of the Dutch Belted variety. Thus, at each harvesting date during pregnancy, lactation, and involution, weight of freshly harvested mammary gland was recorded along with body weight of the animal immediately before it was killed. In all cases, the yield of mammary gland was expressed as a percentage of body weight since Linzell (15) has reported that mammary gland weight increases with body weight.

The results (open bars, **Fig. 1**) reveal that up to the 20th day of pregnancy and again on the 14th day of involution, gland weight as a percentage of body weight was low at 0.5%. In between, the weight percentage increased in a biphasic manner. The first increase took place on day 22 of pregnancy and the second during early lactation. From day 9 to day 24 of lactation, gland weight as a percentage of body weight remained essentially constant. These results demonstrate that approximately 30–40% of total mammary gland growth takes place in pregnancy and the remaining 60–70% in lactation. Thus, our findings with Dutch Belted rabbits correlate qualitatively but not quantitatively with those of Lu and Anderson (9) for rabbits of the New Zealand White breed.

As an index of mammary gland intracellular membrane proliferation (and presumably that of the complement of membrane-associated enzymes), the yield of microsomal protein/g fresh gland was recorded over the entire experimental period. The manner in which this characteristic changes as a function of time (closed bars, Fig. 1) is similar in outline to that observed above for mammary gland yield. A similar profile was obtained by Lu and Anderson (9) for New Zealand White rabbits when the RNA content of the tissue was monitored as an index of the metabolic activity of the mammary cell.

Transacylase activities

We have previously reported that LPAT in rabbit mammary microsomes is composed of two isoenzymic species, denoted α and β respectively (14). The existence of multiple LPAT forms is readily demonstrable in microsomes from animals in which both LPAT species are active, with $V_{max-\beta}/V_{max-\alpha} > 0.5$ and $K_{m-\alpha} <$ substrate cmc $<$ "K-β" (**Fig. 2**). Under these conditions, the dependence of LPAT initial velocity on acceptor substrate concentration (V/S_a) is characteristically biphasic at the substrate cmc (curve A, Fig. 2). In contrast, mammary microsomes containing LPAT-α alone exhibit simple hyperbolic

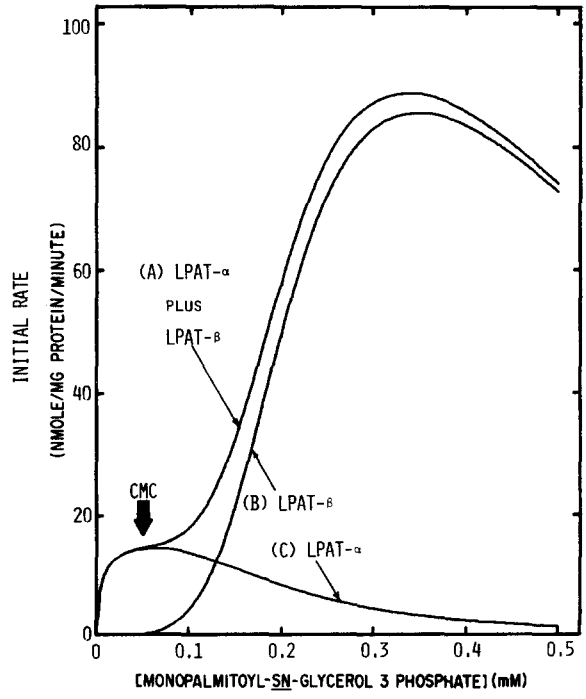

Fig. 2. Acceptor saturation kinetics for rabbit mammary microsomes containing the LPAT-α and/or LPAT-β isoenzymes. All curves were generated by computer according to the rate equations presented in Ref. 14.

kinetics, with pronounced substrate inhibition above the cmc (curve C, Fig. 2).

These data suggested that the two isoenzymic species differ with respect to the preferred physical state of the substrate, where LPAT-α is active with the monomeric form and inhibited by the corresponding micelles and LPAT-β is active with micellar substrates only. From a kinetic analysis of the experimental data in Fig. 2, it has been possible to define the V/S_a curve of LPAT-α (curve C, Fig. 2) and to graphically resolve that of LPAT-β (curve B, Fig. 2) from the composite biphasic V/S_a (curve A, Fig. 2). This theoretical description of the V/S_a relationship for LPAT-β was necessary since LPAT-β activity alone was not observed in any of the mammary microsomal samples examined.

In order to investigate the possibility that the LPAT isoenzymes are involved in regulating lipogenic activity of the rabbit mammary gland, it was proposed that changes in the specific activity of both LPAT-α and β in mammary microsomes be monitored as a function of time during pregnancy and lactation. Thus, optimum conditions of assay for the LPAT isoenzymes had to be established, and it was in the course of this exercise that difficulties were encountered. For example, it was found that when LPAT-α alone was active in the microsomes, the dependence of initial velocity of the transacylase reaction on enzyme concentration (V/E analysis) was linear in the range 0.01–0.34 mg/ml (closed circles, **Fig. 3**). However, when both isoenzymes were present the V/E relationship was biphasic (open circles, Fig. 3). This atypical behavior has been interpreted as reflecting a change in the physical state of the substrate as the level of protein increases and the subsequent appearance of two distinct LPAT activities in response to this change (14). Taken together the data in Fig. 3 indicated nonspecific substrate binding and it was therefore suggested that microsomal protein concentration might alter the shape of the V/S_a curve. This was indeed verified as shown in **Fig. 4** where it can be seen that reducing protein concentration by 50% resulted in a significant displacement to the left of the V/S_a curve on the abscissa. This shift clearly demonstrates nonspecific substrate binding.

In view of these findings it was apparent that any attempt to follow changes in LPAT-α and β activities through pregnancy and lactation must neces-

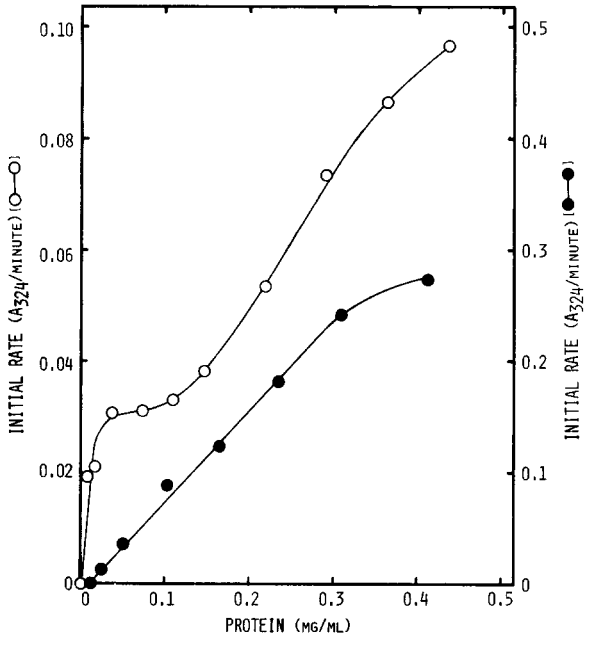

Fig. 3. Dependence of palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase activity on microsomal protein concentration. Microsomes were isolated from the mammary gland of a rabbit 9 days (○—○) and 34 days (●—●) in lactation. Acceptor saturation analysis revealed both LPAT-α and LPAT-β activities and only LPAT-α activity in microsomes isolated from the mammary gland of rabbits 9 and 34 days in lactation, respectively. The assay mixture contained Tris-HCl buffer, 70 mM, pH 7.4; 4,4′-dithiodipyridine, 0.3 mM; monopalmitoyl-*sn*-glycerol 3-phosphate, 40 μM; palmitoyl-CoA, 18 μM; and microsomal protein as indicated.

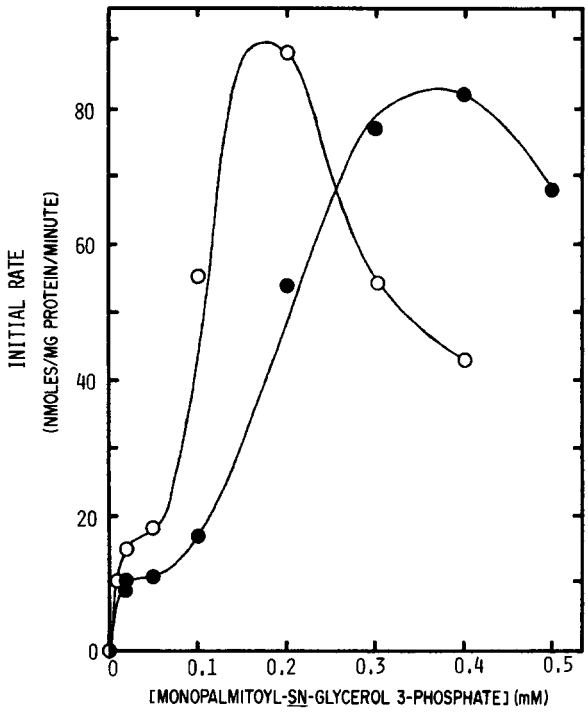

Fig. 4. Effect of microsomal protein concentration on the acceptor saturation kinetics of palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase. Reaction mixtures as specified in Fig. 3 with monopalmitoyl-*sn*-glycerol 3-phosphate as indicated; and microsomal protein, 0.11 (○—○) and 0.23 (●—●) mg/ml. Microsomes were isolated from the mammary gland of a rabbit 9 days in lactation.

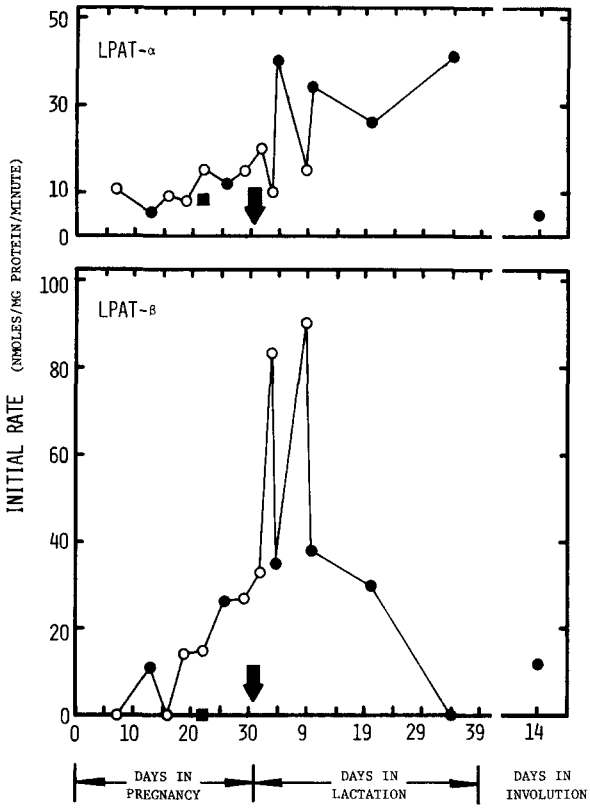

Fig. 5. Changes in the maximum specific activity of rabbit mammary gland palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase-α and β isoenzymes as a function of stage in pregnancy, lactation and involution. Assay conditions were as outlined in Fig. 4 with microsomal protein, 0.23 mg/ml. Harvesting dates in series 1 and 2 are denoted by (○) and (●), respectively. The arrow indicates parturition date. (■) Represents data obtained using microsomes isolated from the mammary gland of an animal about to abort. Each data point represents the result obtained using a single animal.

sarily involve an analysis of the entire V/S_a curve at each harvesting date, with rigid control over the microsomal protein concentration at which acyltransferase assays were performed. These recommendations were followed in the present survey of LPAT activity throughout the experimental period and 0.23 mg/ml was selected as the microsomal protein concentration of choice. This level of protein provided good resolution between the respective V/S_a curves of the two LPAT isoenzymes (see, for example, Fig. 2), and sufficiently high acylation rates for initial velocity measurements to be made in a reasonable time period with microsomal preparations exhibiting low acyltransferase activity.

As noted under Materials and Methods, LPAT-α and β activities were surveyed throughout pregnancy, lactation, and involution in two replicate series of experiments. In the first series, mammary tissue was harvested at five stages in pregnancy and three in lactation. The observed maximum LPAT-α and β specific activities are plotted (open circles) as a function of time in **Fig. 5.** Since LPAT-α activity varied little with harvesting data in the experimental period, it was suggested that this species represented a constitutive enzyme in rabbit mammary gland. In contrast, LPAT-β activity was not evident until mid-pregnancy (Fig. 5). From day 16 to 20 of pregnancy, however, the activity of this isoenzyme increased from zero to 30 nmoles/mg protein per min and from day 1 to day 9 of lactation a 3-fold increase was observed. This 2-step increase in LPAT-β activity correlated well with the bimodal changes in rabbit mammary gland composition (9, 10) and enzymic activity (11–13) as noted above. On the basis of these results it was suggested that perhaps the LPAT-α and β isoenzymes reflect the normal constitutive and the much higher activity required in lactation, respectively.

In the second series of experiments, designed to confirm and extend these findings, additional data on changes in maximum LPAT-α and β specific activity were obtained during pregnancy, lactation, and involution. These results are presented as closed circles in Fig. 5. When the results obtained in the first and second series of experiments are viewed simultaneously (Fig. 5), it is apparent that substantial variation exists in the data, particularly for the β isoenzyme. In pregnancy the average LPAT-α activity was 12 nmoles/mg protein per min whereas in lactation the corresponding value was 24 nmoles/mg protein per min. In the case of LPAT-β, the general trend was for β activity to increase from the second week in pregnancy, to reach a maximum in early lactation, and to fall with time during lactation. However, because of variation in the data, the significance of these changes cannot be assessed. Possible sources of variability are discussed later.

Depending on the relative activities of the two LPAT isoenzymes, a variety of V/S_a curves were obtained as evidenced in **Fig. 6.** A marked similarity exists between these and the individuals of a family of theoretical V/S_a curves (**Fig. 7**) generated by computer according to the equations presented in Ref. 14 at different $V_{max-\alpha}/V_{max-\beta}$ ratios. This compatibility of experimental observation and theory lends credence to the proposal that LPAT in rabbit mammary gland microsomes is composed of two isoenzymic species.

DISCUSSION

The capacity of the mammary gland for milk biosynthesis is a function of the number of mammary

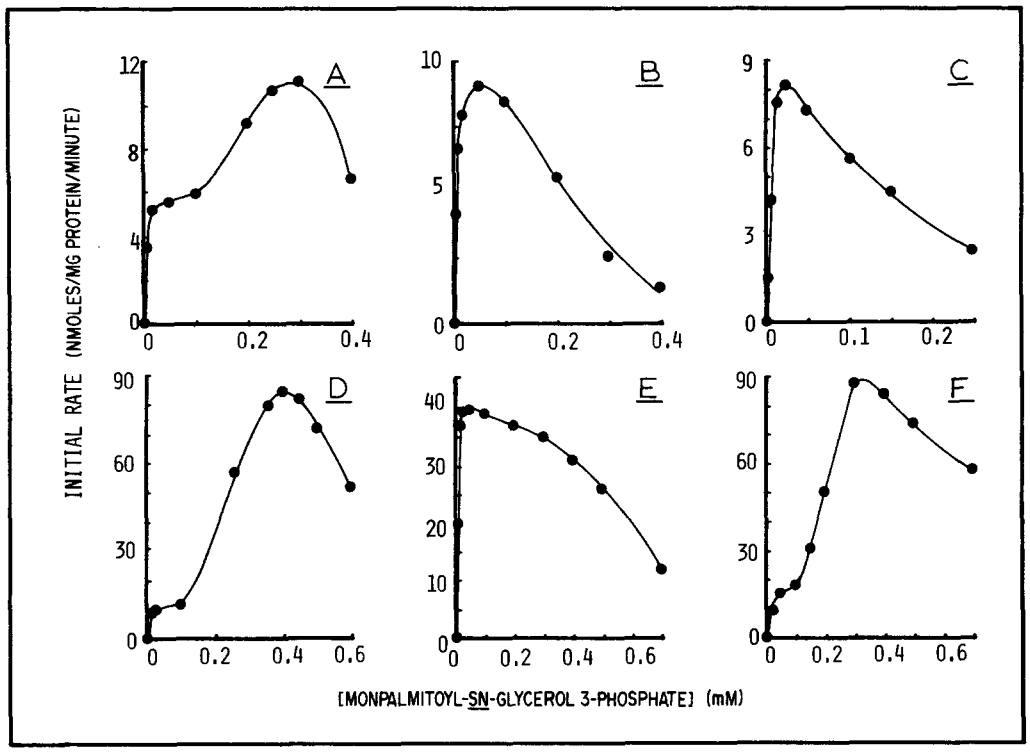

Fig. 6. Acceptor saturation kinetics of palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase in microsomes isolated from the mammary glands of rabbits at different stages in pregnancy and lactation. Assay conditions as specified in Fig. 5. Harvesting dates in pregnancy were (A) 13 days, (B) 16 days, and (C) 22 days and in lactation (D) 3 days, (E) 4 days, and (F) 9 days.

cells per gland and of the complement and activity of rate-limiting enzymes in each cell. As an index of gland cellularity, the fresh weight of mammary tissue (per 100g body weight) from rabbits of the Dutch Belted variety was recorded throughout pregnancy and lactation. After the third week in pregnancy, this parameter increased in a biphasic manner, an observation that is in agreement with the results of Lu and Anderson (9) for New Zealand White rabbits. The initial increase took place on day 22 of pregnancy, coinciding with a time of extensive cellular development (16) and a period of increased lipogenic activity (11, 12). The second increase occurred in early lactation and correlated with the second lipogenic response observed by Mellenberger and Bauman (11) and Strong and Dils (12).

As an indication of enzyme complement per cell, a record was made of the yield of mammary gland microsomal protein (mg/g fresh gland) at different stages of pregnancy and lactation. The first marked increase in this parameter correlated temporally with that for gland cellularity, occurring on day 22 of pregnancy. However, the second increase during the nursing period was less well-defined than in the case of gland cellularity and a significant rise was not observed until late in the second week of lactation. This profile correlates reasonably well with Lu and Anderson's (9) index of cellular activity, viz., mg mammary gland RNA/100g body weight.

As animals passed from early pregnancy to mid-lactation the recorded indices of gland cellularity and enzyme complement per mammary cell increased some 6- and 7-fold, respectively. Thus, the capacity of the gland to synthesize the various constituents of milk must rise as a result of the combined increases in number of cells/gland and enzyme complement/cell. In addition, selective synthesis and activation of the regulatory enzymes presumably render the mammary gland in its fully functional state capable of milk synthesis at a rate of 140g/day (15).

In the present study, the existence of at least one of the enzymes of the α-glycerolphosphate pathway (17), namely palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase (LPAT), in rabbit mammary gland is demonstrated. Evidence has been obtained that this transacylase is composed of two isoenzymic species that differ with respect to the

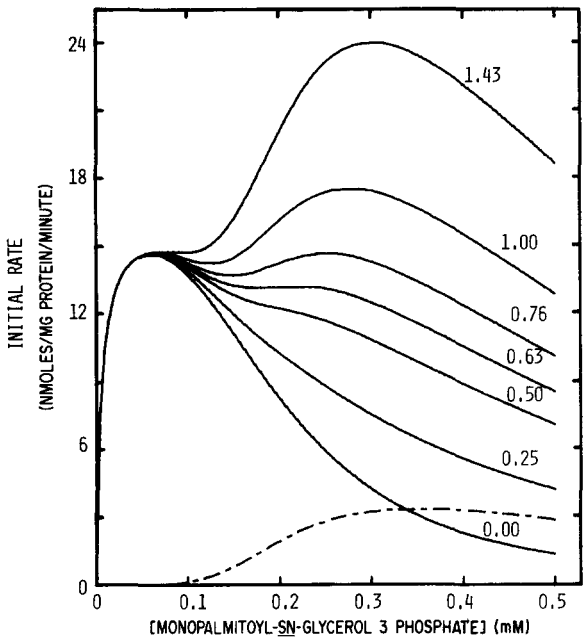

Fig. 7. Acceptor saturation kinetics for palmitoyl-CoA:monopalmitoyl-*sn*-glycerol 3-phosphate palmitoyltransferase at different $V_{max-\beta}$ to $V_{max-\alpha}$ ratios. All curves were generated by computer according to the summation rate equation presented in Ref. 14, with $V_{max-\alpha}$ = 16.8 nmoles/mg protein per min. Also included is the acceptor saturation curve for LPAT-β in the absence of LPAT-α activity when $V_{max-\beta}$ = 4.2 nmoles/mg protein per min (— - — -).

physical nature of the substrates with which each interacts (14). Like LPAT in most other tissues (18–21) both isoenzymes appear to be membrane bound.

Results obtained pertaining to changes in the activity of the LPAT-α and β isoenzymes through pregnancy, lactation, and involution proved inconclusive as a result of variability in the data. The general trend, however, was for LPAT-α activity to undergo a 2-fold increase during lactation, as compared to pregnancy, and for β activity to increase in the second week of pregnancy, attaining maximum levels in early lactation and then decreasing as lactation progressed. After 14 days in involution, both α and β activities had returned to the levels observed in early pregnancy.

The observed variations in specific activities of LPAT-α and β indicate that factors other than physiological stage of pregnancy, lactation, and involution influenced the activity of the two isoenzymes and that these were not controlled throughout the experimental period. The source of this variability is unknown since conditions such as housing, feeding, and killing the rabbits and harvesting and fractionation of the mammary gland were held constant at all harvesting dates. A possible source of variation worthy of consideration is the method of assaying transacylase activity. The LPAT assay system is an extremely complex one and problems inherent in the system have been examined in considerable detail (22, 23). Despite this complexity, consistently reproducible V/S_a curves were obtained with freshly prepared suspensions of freeze-dried microsomes. Thus, the likelihood that the assay method constitutes a source of variability in the data was eliminated.

The time at which mammary tissue was harvested in relation to the interval between nursing periods may have influenced LPAT activity. Rabbits nurse their young only once every 24 hr (24) and as nursing time is approached, the gland becomes increasingly engorged with milk. Jones (25) and Miller, Geroch, and Levy (26) have shown that certain enzymes are repressed as a result of milk accumulation in the mammary gland. In the present investigation, the time of day at which nursing took place was not recorded. It is possible, therefore, that tissue was harvested from animals at different stages in the post-nursing period, where milk accumulation influences transacylase activity, and that the variability in the data reflects this variable more so than it does stage of lactation. Obviously, this source of variation applies only to the results obtained post-partum which, incidentally, coincides with the region of maximum variability in the data. In view of our proposal (27) that the relative activities of the LPAT isoenzymes are regulated by the level and degree of self-association of physiological surfactants, this appears to be a plausible explanation of the observed variability and will be tested presently.

The LPA concentration at which maximum LPAT-β activity was observed varied from 300 to 400 μM, depending on the date on which the microsomal samples were harvested (Fig. 6). This was shown to occur even though microsomal protein concentration in the assay medium was held constant at 0.23 mg/ml on all sampling dates. The observed shift may be accounted for by a time-dependent change in the Hill constant of LPAT-β (K_β) for LPA. Alternatively, the nonspecific binding properties of the microsomes may have changed with harvesting date. Thus, although microsomal protein concentration in the assay medium was fixed throughout the experimental period, the observed displacement of the V/S_a curve on the abscissa may be accounted for by correlative changes in the density of nonspecific binding sites on the microsomes and the stage of pregnancy or lactation. Conceivably, changes may also be taking place in the degree to which these sites are embedded

in the microsome matrix and are thus available for nonspecific binding of the LPAT substrates.

During peak lactation rabbits of the Dutch Belted breed produce up to 140g milk/day containing 16% fat, 11% protein, and 2% lactose (15). The composition of the lipid fraction has not been reported but presumably resembles that of the bovine and rat with 98% glyceride (28). On the 9th day of lactation a maximum LPAT-β specific activity of 90 nmoles/mg protein per minute was recorded. The mammary gland of this animal weighed 72g and had a microsomal protein content of 10 mg/g fresh tissue. Given these values and assuming *1*) that LPAT catalyzes a rate-limiting step in glycerolipid biosynthesis, *2*) that synthesis of all milk glycerides requires mediation of LPAT, *3*) that the glyceride fraction of rabbit milk is composed mainly of triacylglycerides with molecular weights of 720 (based on a fatty acid composition of C16-C18-C8 (12, 29), and *4*) that conditions in vivo are such as to maximize LPAT-β activity, it was calculated that in order to provide enough glyceride for maximum milk production, LPAT-β need only function for 9 of the 24 hours in the interval between nursing periods. This compatibility between in vitro and in vivo activities engenders confidence that the present acyltransferase assay system reflects physiological conditions.

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