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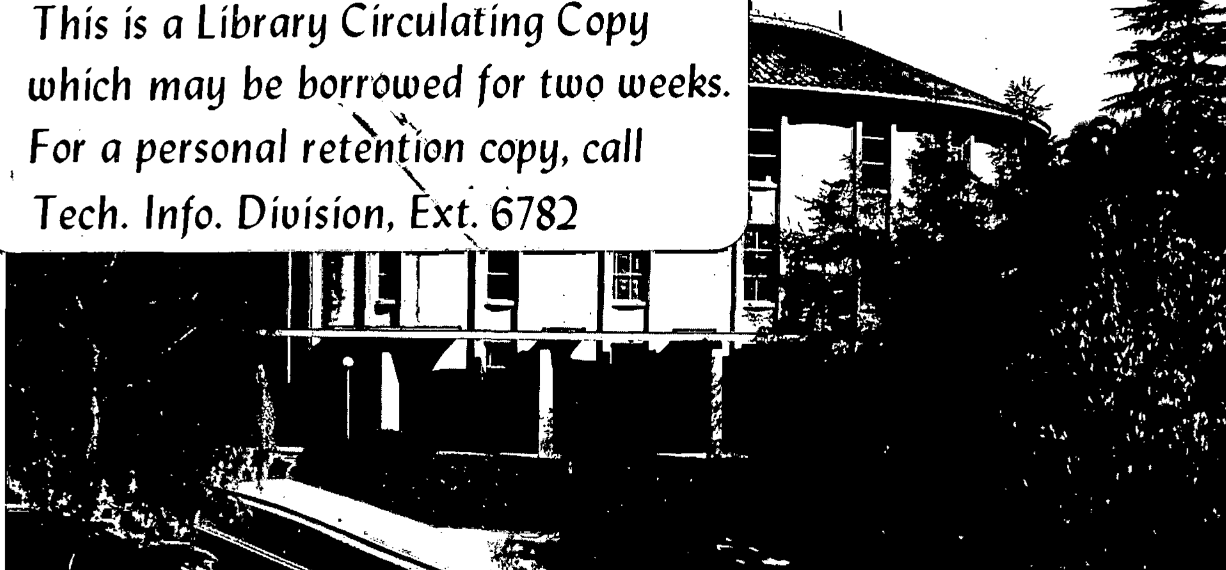

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INTERRELATIONSHIP OF GLYCOGEN METABOLISM AND LACTOSE SYNTHESIS IN MAMMARY EPITHELIAL CELLS OF MICE*

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Running title:

Glycogen and Lactose Synthesis in Mammary Epithelial Cells

SUMMARY

Evidence is presented for an interrelationship between glycogen metabolism and lactose synthesis in mammary epithelial cells. To investigate the role of glycogen in the mammary gland, we studied the conversion of glucose to glycogen and other cellular products in epithelial cells and in adipocytes isolated from glands of mature virgin, pregnant and lactating mice. The results of these experiments demonstrate that a large portion of glucose is converted to glycogen by mammary epithelial cells from mature virgin and pregnant mice. The rate of glycogen synthesis is maximal in the epithelial cell at late pregnancy but decreases rapidly at parturition when the abundant synthesis of lactose is initiated. In glands from mature virgin and pregnant mice, glycogen synthesis in the epithelial cell exceeds that in the adipocyte by over two-fold.

Since glycogen and lactose share a common synthetic pathway, in part, we propose that glycogen synthesis during late pregnancy restricts lactose build-up within the epithelial cell at this time. At parturition, mobilization of glycogen stored during pregnancy could provide substrate for the dramatic increase in lactose synthesis. The possible mechanisms for this mobilization and the physiological advantage of glycogen accumulation during late pregnancy are discussed.

INTRODUCTION

The significance of glycogen metabolism has not been seriously considered in mammary epithelial cells. The presence of glycogen in these cells has been reported in the dog (Sekri and Faulkin, 1967),cow (Reid and Chandler, 1973), and human (Sterling and Chandler, 1977). Studies published some time ago revealed that pieces of bovine mammary gland (Peterson & Shaw, 1938) and homogenates of guinea pig gland (Kittinger and Reithel, 1953) synthesize glycogen. These studies however, did not establish that the parenchymal cell was synthesizing glycogen because of the possibility of other cell types, particularly adiocytes, being responsible for this synthesis. Ebner and his colleagues (1961) reported glycogen synthesis from glucose by bovine mammary cells in culture. This finding was extended by Twarog and Larson (1964) who also found a reciprocal relationship between glycogen and lactose synthesis in these cells in culture. Nevertheless, Larson (1969) questioned the physiological significance of glycogen metabolism in mammary epithelial cells, possibly because the cultures were known to be contaminated with other types of cells.

We undertook the studies reported here to establish what role, if any, glycogen metabolism may play in the mammary epithelial cell. In a preliminary study, we found that glands from nonlactating mice synthesize considerably more glycogen than those from lactating mice. An abstract describing this work has appeared (Emerman and Bissell, 1979). Encouraged by the possibe modulation of glycogen synthesis in the mammary gland, we have expanded these investigations to include epithelial cells and adipocytes isolated

from the mammary glands of mature virgin, pregnant, and lactating mice. From these studies we obtained evidence that the mammary epithelial cell is responsible for most of the glycogen synthesized by the mouse mammary gland during the quiescent and pregnant stages of the reproductive cycle. We show further that glycogen formation is maximal during late pregnancy, when the mouse mammary epithelial cell develops the capacity for lactose synthesis (McKenzie et al., 1971; Jones, 1972). Because glycogen metabolism and lactose synthesis have intermediates in common, we propose that synthesis of glycogen restricts lactose accumulation at this time. As a corollary, we further propose that initiation of abundant synthesis of lactose at parturition is enhanced by degradation of glycogen synthesized by the epithelial cell during late pregnancy.

EXPERIMENTAL PROCEDURES

Animals. Balb/cCrgl mice were purchased from the Cancer Research Laboratory at the University of California at Berkeley. Mature virgin female mice ranged in age from 12 to 16 weeks. Pregnant mice were obtained by mating female mice with males for one night; the following day was designated day 1 of pregnancy. Lactating mice were used from the time they gave birth to their litter, but prior to nursing (designated day 0 of lactation) through the tenth day of lactation.

Dissociation and Incubation Procedures. The mice were killed by cervical dislocation and the 5 pairs of mammary glands were removed. The glands were either cut into 1 to 2 mm^2 pieces or dissociated to single cells with the aid of collagenase (Lasfargues & Moore, 1971). The dissociating medium was Medium 199 (10 ml/gm

tissue; Grand Island Biological Co.) supplemented with glucose to a final concentration of 11 mM, 5 μg/ml each of insulin (Calbiochem; bovine pancreas, B grade) cortisol (Sigma) and prolactin (Sigma; 32 I.U./mg), 0.12% collagenase (Worthington Biochemical Corp.; CLS II, 135 U/mg), and 4% bovine serum albumin (Sigma). Dissociation was carried out in an Erlenmeyer flask at 37°C on a gyrator shaker at 125 rpm for 1 h. The material was passed through dacron cloth then through a 50 μ Nitex filter (Tetko, Inc.) to break up clumps of cells (Emerman & Pitelka, 1977). The resulting cell suspension was centrifuged for 3 min at 800 rpm. The fat cells float to the top of the supernatant leaving a pellet which is mostly composed of epithelial cells. Both cell types were collected.

A series of experiments were conducted on mice which had their mammary epithelial anlage removed from 1 pair of glands when the mice were 3 weeks old (DeOme _et al._, 1959). These cleared fat pads were handled in the same manner as normal glands. In these experiments pieces of tissue from abdominal fat pads were also used.

Pieces of mammary gland tissue (averaging 100 mg protein) or 2×10^6 cells of the cell suspension were put into 5 ml tubes with 0.5 ml of Medium 199 containing high specific activity [U-^{14}C] glucose (New England Nuclear; final specific activity 30 Ci/mol). Insulin, cortisol, and prolactin were present in the medium at 5 μg/ml each. The glucose concentration was routinely 11 mM, but in one set of experiments 5.5 mM, 11 mM and 27 mM were used. [U-^{14}C] galactose (New England Nuclear; final specific activity 330 Ci/mol) was used in 1 set of experiments. The concentration of galactose

was 0.11 mM. The medium with samples was equilibrated with 95% air, 5% CO_2, then put on a gyrator shaker and incubated at 37°C in 95% air, 5% CO_2 for 1 and 2 h periods. The epithelial cell suspensions were centrifuged at 1000 rpm for 3 min to pellet the cells. The fat cells were concentrated by collecting the medium beneath the cells with a pipette. The medium was removed from all samples and frozen for later analysis. The cells and tissue were rapidly washed twice in Hanks' balanced salt solution (HBSS) containing unlabeled glucose, once with diluted HBSS (1:2 with H_2O), and killed with 3 ml of 80% methanol (v/v) in 0.01N NaOH containing 0.1% SDS[1] as described previously (Emerman & Bissell, 1979b). The tissue was homogenized. The methanol was then evaporated under a stream of nitrogen.

Separation and Identification of Lactose. Lactose was separated from other labeled metabolites by 2-dimensional paper chromatography. The processing of samples for paper chromatography and the chromatographic procedures have been described previously (Bissell *et al.*, 1973; Bassham *et al.*, 1974). The location of lactose was determined with autoradiography using X-ray film, and the amount of ^{14}C-lactose was counted.

The spot identified as lactose was eluted and rechromatographed with pure lactose standard. In all cases tested, the spot and the standard were superimposable in position and shape (Emerman & Bissell, 1979b).

Separation and Identification of Glycogen. Glycogen and other macromolecules remained at the origin after 2-dimensional paper chromatograhy using our solvent system. To determine the glycogen

content, the origins were cut into small pieces and hydrolyzed in 1 ml of 1N trifluoroacetic acid for 1 h at 200°C (Bissell et al., 1973). The samples were evaporated under nitrogen and resuspended in 80% methanol in H_2O. The extract was again subjected to 2-dimensional chromatography and the amount of ^{14}C-glucose was considered to represent the amount of ^{14}C-glucose incorporated into glycogen. To ensure that the labeled glucose was not incorporated to any significant degree into glycoproteins or glycolipids, we repeated the experiments using ^{14}C-galactose and determined the amount of label retained at the origin.

Glycogen was also isolated by ethanol precipitation (Good et al., 1933). Three mg of rabbit liver glycogen were added as carrier to a portion of the concentrated SDS extract. After delipidation, as described below, ethanol was added to a final concentration of 66% (v/v). The samples were cooled to 0-4°C and the precipitate allowed to collect for at least 2 h. After centrifugation at 4°C, the supernatant material was carefully decanted. The remaining precipitate was dissolved in distilled water and a portion assayed for radioactive content.

Lipid Determination. The lipid was extracted from the SDS extract by the method of Slayback et al. (1977). The organic extract was removed for assay of ^{14}C content and the remaining aqueous phase subjected to the procedure for glycogen precipitation with ethanol (see above).

Measurement of Radioactivity. The ^{14}C-labeled areas on the chromatograms were cut from the paper and their radioactive content was quantitated with an automated Geiger-Muller apparatus (Moses &

Lonberg-Holm, 1963). The radioactive content of glycogen precipitated with ethanol and the lipid was measured using liquid scintillation spectrometry (Beckman). A portion of each sample was removed for protein determination by the method of Lowry et al. (1951) using an Autoanalyzer II system (Technicon). The results are expressed as nanomoles of ^{14}C per milligram protein.

RESULTS

When a portion of tissue extract which had been incubated with ^{14}C-glucose was subjected to 2-dimensional paper chromatography, the amount of radioactivity remaining at the origin was different at each stage of the reproductive cycle. The amount of label at the origin in tissues from late pregnant mice was twice that of the mature virign, exceeding 200 nmol ^{14}C/mg protein/h. The level of incorporation in the origin material dropped to a very low level by midlactation. Of all the possible molecules held at the origin, we concluded that glycogen would be the most likely intracellular metabolite to be formed from glucose to this extent.

To confirm that the material at the origin was indeed glycogen, the origins were hydrolyzed as described in Experimental Procedures, and the labeled material in the hydrolysate was identified by paper chromatography. Approximately 75-87% of the ^{14}C was found to rechromatograph with glucose (Fig. 1). This value was taken as a measure of ^{14}C glucose incorporation into glycogen. Because glycogen can be synthesized and degraded simultaneously, we recognize that the rate of glycogen formation is not due to

synthesis alone but is the net of these two processes. Nonetheless, for the sake of simplicity, we will refer to this accumulation as the rate of glycogen synthesis.

Glucose has been shown to be a component of glycolipid and glycoprotein in the lactating mammary gland (Speake & White, 1978), as it is in other cell types. To eliminate the possibility that some of the material retained at the origin may be due to the synthesis of glycoproteins and glycolipids during the time course of these experiments, mammary tissue was exposed to ^{14}C-galactose, a major carbohydrate component of glycoproteins (see Parry, 1978). Less than 0.1 nmol ^{14}C/mg protein/h from this sugar was held at the origin of 2-dimensional chromatogrpahs regardless of the physiological state of the animal. This synthesis is negligible when compared to 10 nmol in lactating mice to over 200 nmol in pregnant mice of labeled carbon from glucose incorporated into macromolecules. Ceriani _et al._ (1978) also measured synthesis of glycoproteins by mammary epithelial cells and pieces of mammary tissue, using labeled fucose. It can be calculated from their studies that the level of incorporation into macromolecules was also infinitesimal compared to the glucose incorporation into glycogen observed here in a 2 h incubation period. These studies confirm that the activity retained at the origin was mainly in glycogen.

Glycogen was also identified by precipitation in ethanol according to the method of Good _et al._ (1933). The relationship between the physiological states were comparable to those obtained by hydrolyzing the origin of the paper after chromatography and measuring the released ^{14}C-glucose (Fig. 3). However, the values

for glycogen synthesized in the virgin gland and during pregnancy were only 1/3-1/2 of that determined by paper chromatography. The lower recovery was likely due to the inability of ethanol to cause of the shorter glycogen chains. The presence of short glycogen chains was verified by identification of material trailing from the origin as yielding solely glucose upon acid hydrolysis. In contrast, the ethanol precipitable material from samples derived from lactating mice was the same as that determined by paper chromatography. This is in agreement with our finding that shorter glycogen chains were not present in samples from lactating mice.

Not only was there a marked difference in the rate of ^{14}C incorporation into glycogen, but the response of the mammary tissue exposed to different glucose concentrations appeared to differ with the various stages of mammary gland development (Fig. 2a). The rate of glycogen synthesis in the mammary tissue of mature virgin mice increased with increasing glucose concentration in the medium. Glycogen synthesis by tissue from lactating mice was virtually unresponsive to the changes in glucose concentrations. In contrast to glycogen synthesis in tissue from lactating mice, the rate of lactose synthesis increased linearly with increases in glucose concentration up to 11 mM (Fig. 2b). Subsequent increases in glucose concentrations had no effect on lactose synthesis. This response by mouse mammary gland is in agreement with previous reports in the rat (Bartley et al, 1966).

Glycogen synthesis in the mammary gland, particularly of mature virgin mice, is not an unexpected finding. Approximately 80% of the gland during the quiescent state is composed of adipocytes and these cells synthesize glycogen (Vaughan and Korn, 1962). As the epithelial cell population becomes predominant during pregnancy and lactation, one might expect that glycogen synthesis would decrease. However, the rate of glycogen synthesis by mammary tissue pieces from late pregnant mice was greater than that synthesized by the virgin gland even though it was decreased during lactation (Fig. 1). This result led us to consider two possibilities; glycogen synthesis by adipocytes was increased during pregnancy, or the mammary epithelial cells were responsible for the synthesis of glycogen.

To test these possibilities, the mammary tissue was dissociated and the adipocytes and epithelial cells isolated separately. The incorporation of ^{14}C from glucose into glycogen by epithelial cells is shown in Fig. 3. The mammary epithelial cells were clearly responsible for most of the glycogen synthesized. The rate of glycogen synthesis increased during pregnancy reaching a peak between 16-18 days, then there was a decline in synthesis as the animal approached parturition. This decline continued until day 6 of lactation. The level of synthesis remained very low and constant thereafter. The adipocytes also synthesized glycogen but the rate of synthesis was approximately 1/2 that in the epithelial cells in the virgin and pregnant mouse. Synthesis decreased during lactation and was the same for both cell types during this period.

Glycogen synthesis was much lower in dissociated cells than in pieces of whole tissue. This could be due to a decrease in the level of ATP. A decline in ATP production after cell dissociation has been reported for liver cells (Bissell, et al., 1973). Furthermore, cell damage and the disruption of cell-cell interactions may adversely affect the maintenance of biosynthetic processes.

Since it is known that the presence of epithelial cells can influence lipid accumulation by adipocytes (Elias et al., 1973), glycogen storage might be similarly affected. To investigate this, we measured glycogen synthesis in pieces of cleared mammary fat pads and of abdominal fat pads. The rate of glycogen synthesis by abdominal fat pads from virgin and pregnant mice was the same, averaging 225 nmol ^{14}C/mg protein/h, but decreased during lactation to 5 nmol. In contrast, the level of synthesis in the cleared fat pads (averaging 300 nmol ^{14}C/mg protein/h) remained constant during all physiological states (additional results not shown).

To determine when changes in the rates of glycogen and lactose synthesis occurred, glycogen and lactose synthesis in mammary epithelial cells were measured at different times throughout pregnancy and early lactation. The highest rate of glycogen synthesis, over 100 nmol ^{14}C/mg protein/h, occurred between 16-18 day of pregnancy, the fell steadily to reach a constant low rate by the sixth day of lactation (Fig. 4). Lactose synthesis was initiated at day 16 of pregnancy, but the rate of synthesis remained low until just prior to parturition when there was a sharp increase in the rate of lactose synthesis corresponding

to the drop in glycogen synthesis. Similar results were obtained by measuring the rate of glycogen and lactose synthesis in mammary gland tissue at the time of parturition.

We also measured the rate of lipid synthesis during pregnancy. During the first 10 days of pregnancy, lipogenesis from glucose increased from 10 nmol ^{14}C/mg protein/h to over 60. By day 14 the rate was again around 10 nmol and remained at that level until just prior to parturition.

DISCUSSION

In order to understand the metabolism of the lactating gland, it is important to investigate the metabolic relationships in the preceeding stages of the reproductive cycle: quiesence and pregnancy. Certainly much of the activity in the epithelial cell during quiescence and, particularly, pregnancy is in preparation for lactation. By taking this approach, we have clearly shown that not only do the epithelial components of the mammary gland of the mouse synthesize glycogen at all stages of the reproductive cycle, but the activity is greatly modulated. The rate of glycogen synthesis increases during pregnancy and falls dramatically at lactation. Although the cell populations are not homogeneous, the experiments conducted on populations enriched either with epithelial cells or adipocytes show that the level of glycogen accumulation is greater in the epithelial cells. The parenchyma includes both secretory epithelium and myopithelium. Because the population of secretory epithelial cells increases during pregnancy

while the myoepithelial cell population increases very little if at all (Hollmann, 1974), the increased rate of glycogen synthesis at this time is more likely attributed to the secretory epithelium.

This decrease in glycogen synthesis at parturition is not limited to the mammary epithelial cell. At this time, there is a corresponding decrease in glycogen synthesis in the abdominal fat pad. The low rate of synthesis in these pads may be due to a general body response to lactation, a result of meeting the demand of the mammary epithelial cell for glucose and triglyceride substrates for synthesis of milk components. Mammary fat pads are not sensitive to this general response as the rate of glycogen synthesis of pregnancy is maintained throughout lactation in fat pads cleared of their epithelial elements. This lack of response may be due to the increase in blood flow to the mammary gland just prior to lactation (Linzell, 1974). This increased supply of nutrient would preferentially support the mammary fat pad during lactation just as it does the mammary gland. There is, in addition to the change in blood supply, a local effect of mammary epithelial cells on mammary adipocytes. If the epithelial components of the gland are not removed, the rate of glycogen synthesis is depressed in all mammary cells including fat cells (Fig. 3 and text). Elias et al., (1973) have reported a similar local inhibitory effect of mammary epithelial cells on lipid accumulation by adipocytes in the gland. This local effect could be due to the production of a metabolic inhibitor but is more likely attributable to the greater affinity of epithelial cell for glucose and triglyceride. Scow and his coworkers (Spooner et al., 1977) have established how the

epithelial cell gains an advantage over the surrounding adipocytes in lipid uptake by the specific, hormonally-induced increase in lipoprotein lipase activity.

Measurement of glycogen, lactose, and lipid synthesis throughout pregnancy reveals differing relationships between the biosynthetic rates of these cellular products and the stage of gestation. Lipid formation is maximal during midpregnancy, the period corresponding to maximal cell and membrane proliferation. Glycogen synthesis does not increase during that period, but increases later (day 15 on) as cell proliferation decreases and the epithelial cells prepare for production of milk. This conclusion is supported by the fact that a small accumulation of lactose is noted at this same time (Fig. 4), which corresponds to the onset of lactose synthase activity (McKenzie et al., 1971; Jones, 1972). The fact that the maximal rate of glycogen synthesis during gestation exceeds that of lipid synthesis by almost 2-fold (Fig. 3 and text) is indicative of the quantitative importance of glycogen synthesis in the mammary epithelial cell during pregnancy.

The increase in glycogen synthesis at day-15 of pregnancy is of special interest because of its relationship to the initiation of lactose synthesis a day later. From day-15 of pregnancy onward the mouse mammary gland contains lactose synthase, albeit not at lactational levels (McKenzie et al., 1971; Jones, 1972). The initial rate of increase of lactose synthesis does not continue but plateaus between days 17 and 19 of pregnancy, when glycogen synthesis is maximal. At this stage, the rate of incorporation of glucose into glycogen exceeds that into lactose by 5-fold. Based

on the enzymatic assays, the gland has the capacity to synthesize lactose at a higher rate during this time (McKenzie _et al_., 1971; Jones, 1972). We propose that the high rate of glycogen synthesis at this time restrict lactose synthesis by limiting the availability of UDP 4-glucose, and thereby, UDP 4-galactose. The enzyme catalyzing the conversion of UDP 4-glucose to UDP 4-galactose, UDP 4-glucose epimerase, is present and appears to be at equilibrium at this stage of gestation (Murphy _et al_., 1973). If UDP 4-glucose epimerase were inhibited during this period until just prior to parturition, formation of glycogen would be further favored over lactose. Whether such modulation of epimerase activity takes place is under investigation. Based on K_m values, the affinity of glycogen synthase for UDP 4-glucose (Newsholme and Start, 1973) and lactose synthase for UDP 4-glucose (Kuhn, 1968) are similar. Hence, neither biosynthetic pathway would be clearly favored on this basis. Nonetheless, lactose synthesis would be limited as long as the rate of glycogen synthesis is greater.

The accumulation of glycogen rather than lactose could have important consequences for the epithelial cell. Mammary epithelial cells do synthesize and store casein and medium change triglyceride during pregnancy in preparation of the onset of lactation (Denamur, 1974; Hollmann, 1974) but the accumulation of large quantities of lactose, the main osmole in milk (Linzell and Peaker, 1971), at this time could destroy the integrity of the intracellular compartments and, eventually, the cell due to osmotic pressure.

The storage of glycogen does not cause cell damage and glycogen can be readily mobilized as a substrate for lactose synthesis at parturition.

We propose that the accepted pathway for lactose synthesis should be modified to include glycogen as shown in Fig. 5. During quiescence and pregnancy, UDP 4-glucose is converted to glycogen. At parturition, breakdown of glycogen releases G-1-P which can be used preferentially for lactose synthesis.

Changes in glycogen metabolism could make glucose immediately available for lactose synthesis by several mechanisms: 1) a decrease in glycogen synthase activity which would make UDP 4-glucose more readily available for conversion to UDP 4-galactose and, thence, to lactose; 2) an increase in phosphorylase activity which would increase the rate of glycogen breakdown; and 3) a combination of these two. Our preliminary enzymatic studies (Bartley _et al._)[2] indicate that both processes 1 and 2 are operating but that the major factor responsible for the decrease in glycogen accumulation is a sharp increase in phosphorylase activity in the epithelial cells right at parturition. Of course, lactose synthesis is also favored at this time by the concurrent increase in lactose synthase activity in mouse mammary gland (McKenzie _et al._, 1971; Jones, 1972). This increase in lactose synthesizing capacity probably accounts for the lack of change in the concentration of intermediates of lactose synthesis (Baldwin and Cheng, 1969; Gumaa _et al._, 1971; Murphy _et al._, 1973) in the face of sudden influx of substrate from glycogen at the onset of abundant lactose production. Once lactose synthesis has been

initiated at parturition, the rate of lactose synthesis is probably regulated by the concentration of intermediates and enzymatic activities in the Golgi compartments, as described by Kuhn and White (1977).

This role for glycogen is most likely not limited to the mouse. Lactose synthase is active during pregnancy in the cow (Mellenberger _et al._, 1973) and rabbit (Mellenberger and Bauman, 1974) but the rate of lactose synthesis at this time is far less than it is during lactation. Lactose production could be curtailed by glycogen synthesis in these animals as well. The rat seems to be an exception in that both lactose synthase activity and lactose synthesis are not detected until parturition (Kuhn, 1968; McKenzie _et al._, 1971). Whether glycogen is present and serves as a substrate for lactose synthesis at parturition in mammary epithelial cells of the rat has yet to be demonstrated. Our results indicate that glycogen synthesis should be considered as an important pathway in the mammary gland in preparation for lactation and that glycogen breakdown is instrumental in initiating copious lactose synthesis at parturition.

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1. The abbreviation used is: SDS, sodium dodecyl sulfate.

2. Bartley, J.C., Emerman, J.T., and Bissell, M.J., manuscript in preparation.

FIGURE LEGENDS

Figure 1. Rate of glycogen synthesis by pieces of mammary gland from mature virgin, pregnant, and lactating mice. Tissues were incubated in 0.5 ml of 11 mM [U-^{14}C] glucose (final specific activity 30 Ci/mol) for 1 and 2 h periods. The radioactive content of glycogen was determined as described in Experimental Procedures. Samples were subjected to 2-dimensional paper chromatography and the origins were hydrolyzed. The amount of ^{14}C-glucose released from the origins was taken as a measure of glucose incorporated into glycogen. V, mature virgin mice; P, mid- to late pregnant mice; L, lactating mice. Standard error of each mean is indicated by the vertical lines.

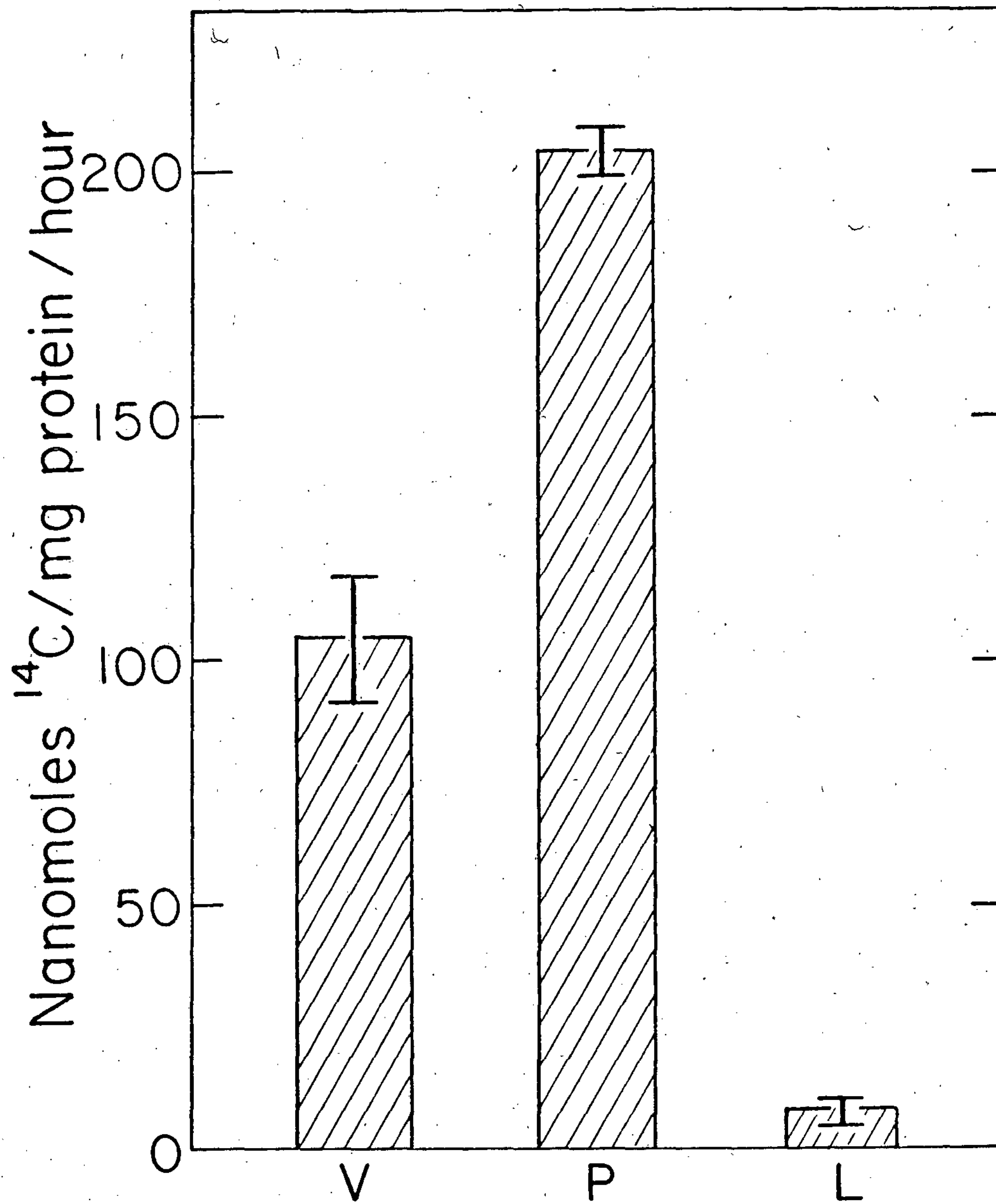
Figure 2. Effect of glucose concentration on the rate of glycogen (A) and lactose (B) synthesis by pieces of mammary gland from mature virgin and lactating mice. A - The radioactive content of glycogen was determined as described in Fig. 1. B - The radioactive content of lactose was determined as described in Experimental Procedures. Squares, 5.5 mM glucose; triangles, 11 mM glucose; circles, 27 mM glucose. Closed symbols, mature virgin mice; open symbols, lactating mice.

Figure 3. Rate of glycogen synthesis by mammary epithelial cells from mature virgin, pregnant, and lactating mice. Dissociation and incubation procedures are described in Experimental Procedures. Cells were incubated in 11 mM [U-^{14}C] glucose. The radioactive content of glycogen was determined by 2 methods: 1. As described in

Fig. 1. 2. By ethanol precipitation as described in Experimental Procedures. Standard error of each mean is indicated by the vertical lines.

Figure 4. Glycogen and lactose synthesis by mammary epithelial cells from mice 8 days pregnant to 10 days lactating. Cells were isolated and incubated in 11 mM [U-^{14}C] glucose as described in Experimental Procedures. Each point represents the rate of glycogen or lactose synthesis after a 1 h incubation in 11 mM [U-^{14}C] glucose on the day indicated. •--•, rate of glycogen synthesis; o--o rate of lactose synthesis. Arrow indicates time of parturition.

Figure 5. Interrelationship between glycogen metabolism and lactose synthesis in mammary epithelial cells. HK, hexokinase (ATP: D-hexose-6-phosphotransferase; EC 2.7.1.1); PGM, phosphoglucomutase (α-D-glucose-1-6-diphosphate; α-D-glucose-1-phosphate phosphotransferase; EC 2.7.5.1); UDPG-PP, UDPG pyrophosphorylase (UTP: α-D-glucose-1-phosphate uridyltransferase; EC 2.7.7.9); UDPG-E, UDP glucose-4-epimerase (EC 5.1.3.2); LS, lactose synthase (UDP galactose: D-glucose-1-galactosyltransferase; EC 2.4.1.22); glycogen synthase (uridine diphosphate glucose: glycogen 4- α-glucosyltransferase; EC 2.4.1.11); glycogen phosphorylase (1,4- -D-Glucan: orthophosphate -glucosyl-transferase; EC 2.4.1.1).



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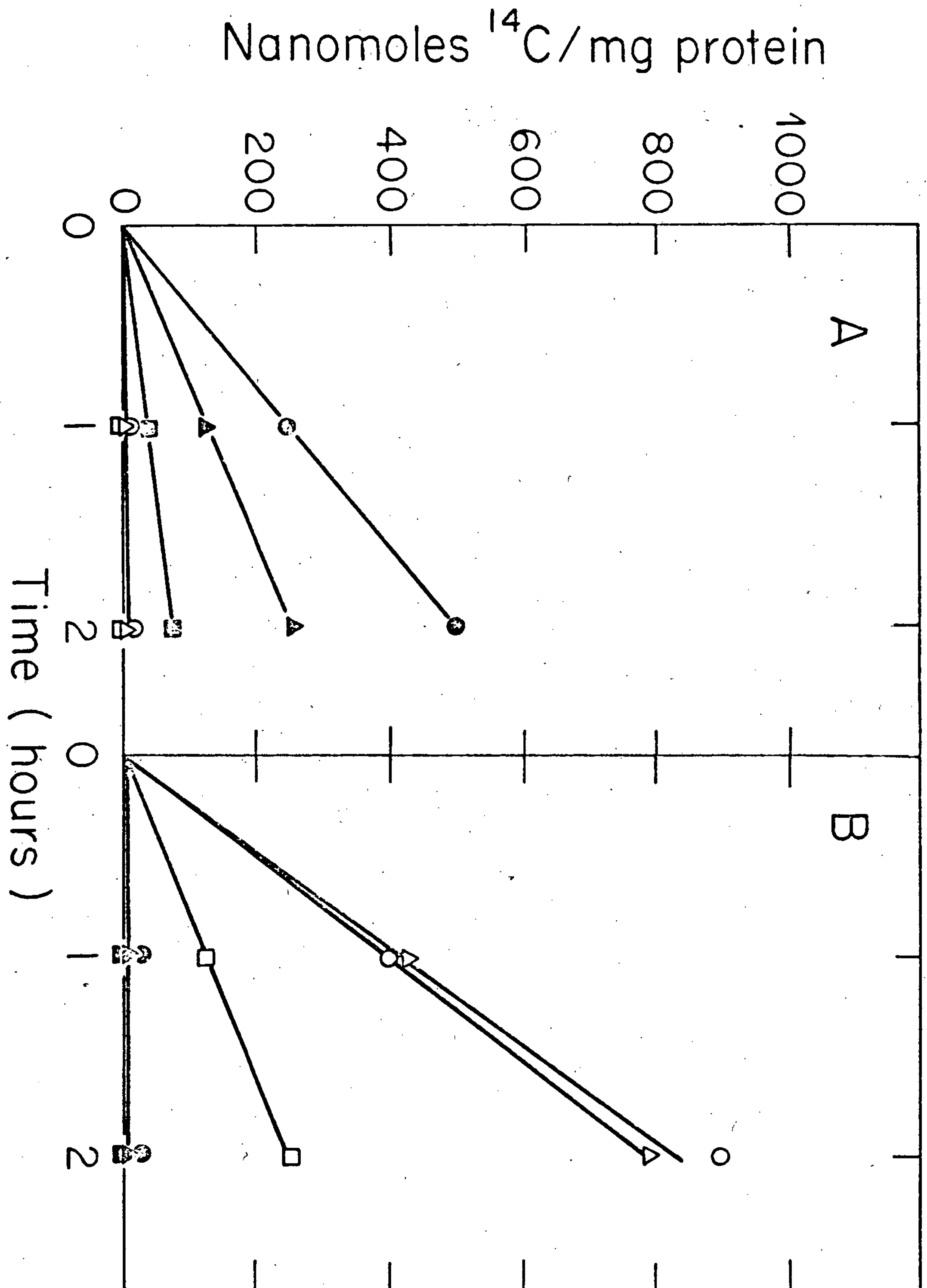
Nanomoles ^{14}C/mg protein
0
200
400
600
800
1000
A
B
0
1
2
0
1
2
Time (hours)
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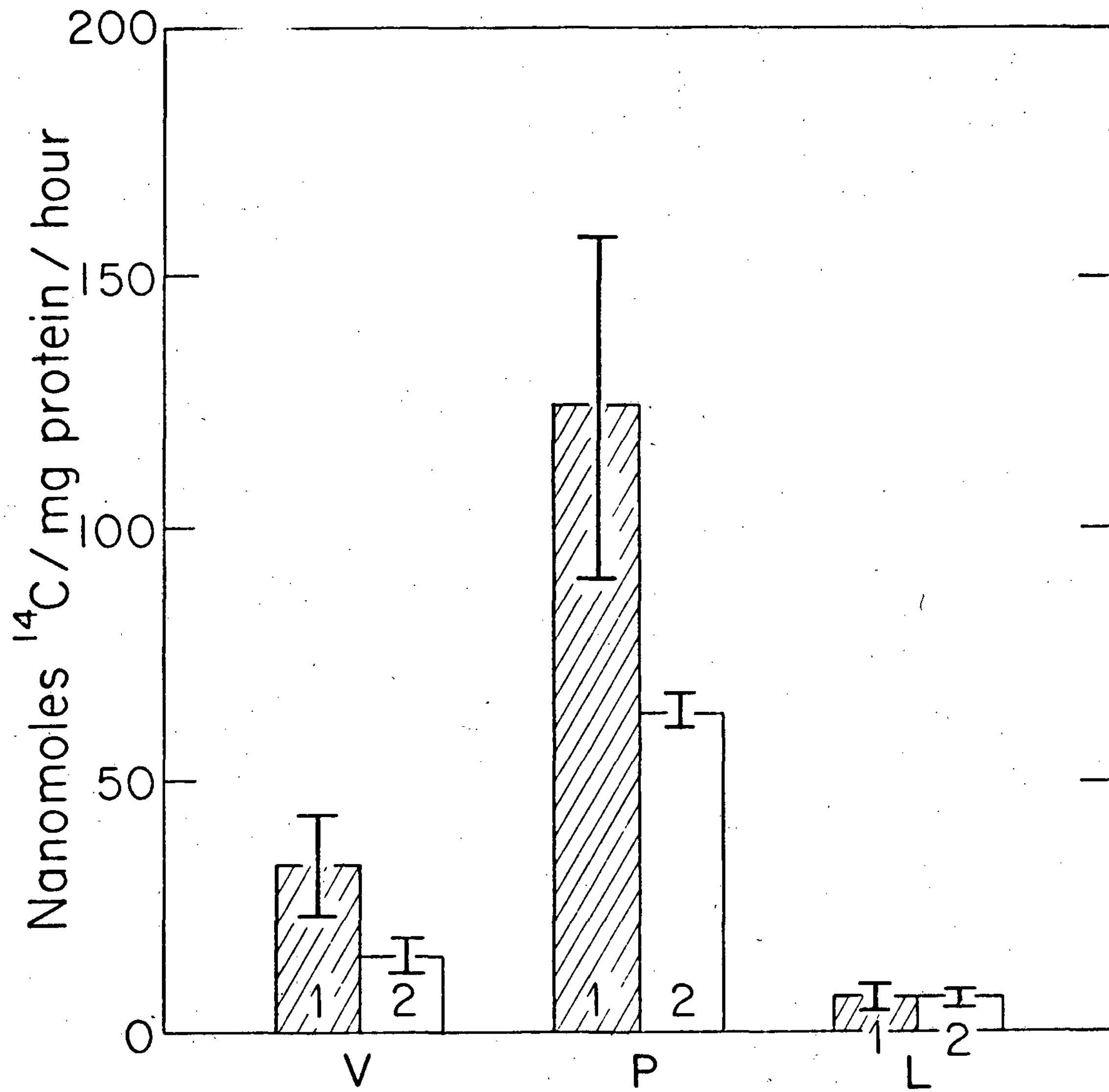

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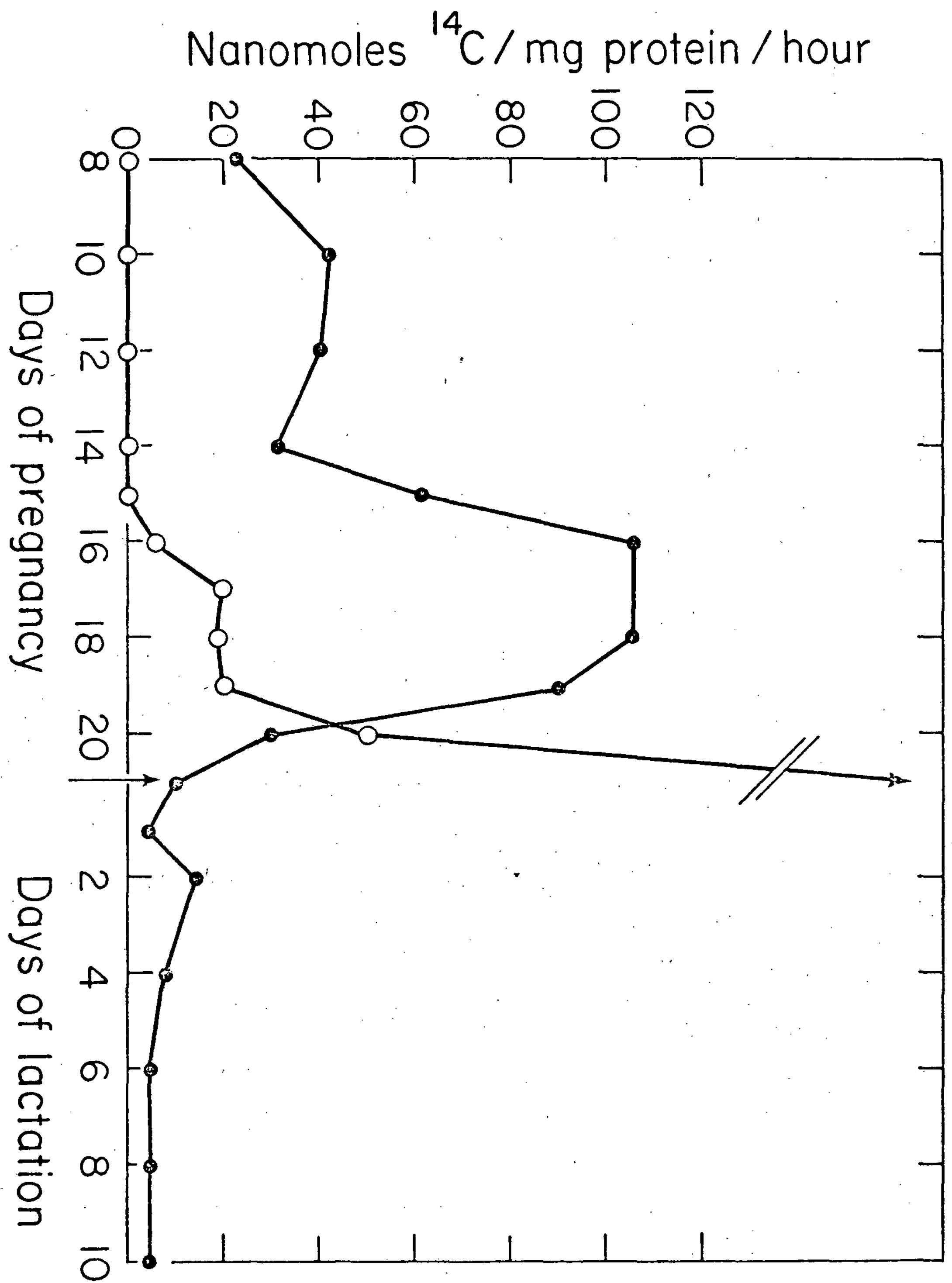

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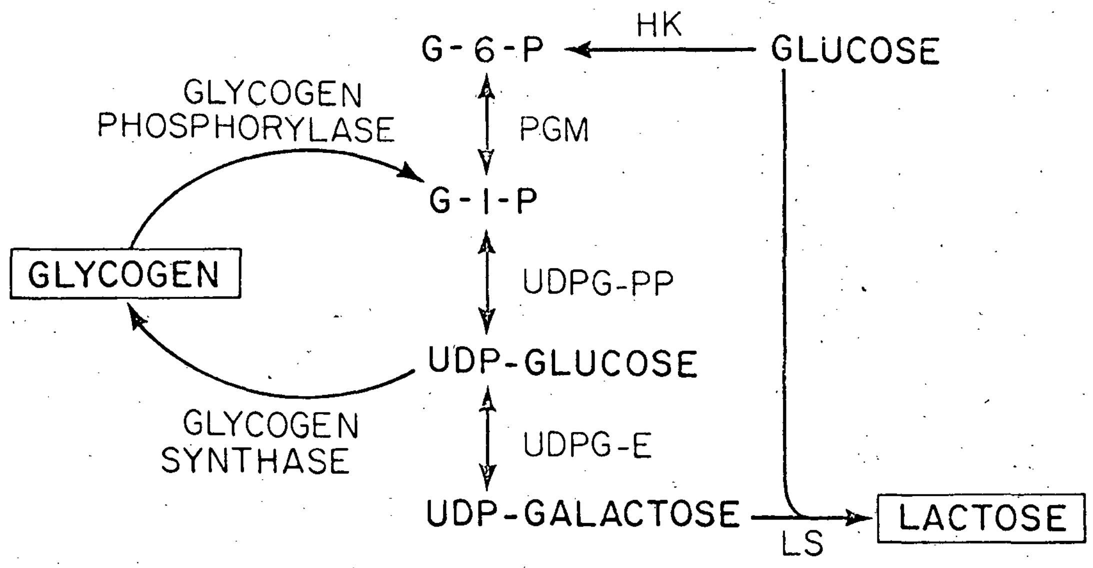

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