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Interrelationship of glycogen metabolism and lactose synthesis in mammary epithelial cells of mice

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Glycogen metabolism in mammary epithelial cells was investigated (i) by studying the conversion of glucose into glycogen and other cellular products in these cells from virgin, pregnant and lactating mice and (ii) by assaying the enzymes directly involved with glycogen metabolism. We find that: (1) mammary epithelial cells synthesized glycogen at rates up to over 60% that of the whole gland; (2) the rate of this synthesis was modulated greatly during the reproductive cycle, reaching a peak in late pregnancy and decreasing rapidly at parturition, when abundant synthesis of lactose was initiated; (3) glycogen synthase and phosphorylase activities reflected this modulation in glycogen metabolism; (4) lactose synthesis reached a plateau during late pregnancy, even though lactose synthase is reported to increase in the mouse mammary gland at this time. We propose that glycogen synthesis restricts lactose synthesis during late pregnancy by competing successfully for the shared UDP-glucose pool. The physiological advantage of glycogen accumulation during late pregnancy is discussed.

The significance of glycogen metabolism in mammary epithelial cells has not been considered seriously. Putative glycogen granules have been identified in these cells in the dog (Sekri & Faulkin, 1967), cow (Reid & Chandler, 1973) and human (Sterling & Chandler, 1977). Studies published some time ago revealed that pieces of bovine mammary gland (Petersen & Shaw, 1938) and homogenates of guinea-pig gland (Kittinger & Reithel, 1953) synthesize glycogen. However, because of the presence of several cell types in the preparations, these studies did not establish that the parenchymal cells were synthesizing glycogen. Ebner *et al.* (1961) reported glycogen synthesis from glucose in cultures of bovine mammary cells. This finding was extended by Twarog & Larson (1964), who also found a reciprocal relationship between glycogen and lactose synthesis in these cultures. 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.

In a preliminary study on the metabolic patterns of the mammary gland at different stages of the reproductive cycle, we found that glands from non-lactating mice synthesized considerably more glycogen than did those from lactating mice (Emerman & Bissell, 1979*a*). Glycogen formation was maximal during late pregnancy, when the mouse mammary epithelial cell develops the capacity for lactose synthesis (McKenzie *et al.*, 1971; Jones, 1972). Encouraged by the modulation of glycogen synthesis in the mammary gland, we undertook the studies reported here to establish (*a*) whether or not mammary epithelial cells could synthesize glycogen, and (*b*) what role glycogen metabolism may play in the physiology of the gland, especially with regard to lactose synthesis. We investigated glycogen metabolism in epithelial cells isolated from the mammary glands of mature virgin, pregnant and lactating mice. We present evidence that mouse mammary epithelial cells are capable of glycogen synthesis. The rate of glycogen synthesis is modulated and can be related to changes in the activities of the enzymes responsible for the synthesis and breakdown of glycogen and for lactose synthesis. Because glycogen metabolism and lactose synthesis have common intermediates, we propose that synthesis of glycogen during

Abbreviation used: SDS, sodium dodecyl sulphate.

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pregnancy restricts lactose accumulation by diverting UDP-4-glucose to glycogen. An abstract of this work has appeared (Bartley *et al.*, 1980).

Experimental

Animals

Balb/cCrgl mice were purchased from the Cancer Research Laboratory at the University of California, Berkeley, CA, U.S.A. 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 before nursing (designated day 0 of lactation), until day 10 of lactation.

Dissociation and incubation procedures

The mice were killed by cervical dislocation and the five pairs of mammary glands were removed. The glands were either cut into 1–2 mm^3 pieces or dissociated to single cells with the aid of collagenase (Lasfargues & Moore, 1971). The dissociating medium was Medium 199 (10 ml/g of tissue; Grand Island Biological Co., Grand Island, NY, U.S.A.) supplemented with glucose to a final concentration of 11 mM, 5 μg/ml each of insulin (Calbiochem, La Jolla, CA, U.S.A.; bovine pancreas, B grade), cortisol (Sigma Chemical Co., St. Louis, MO, U.S.A.), and prolactin (Sigma; 32 international units/mg), 0.12% collagenase (Worthington Biochemical Corp., Freehold, NJ, U.S.A.; CLS II, 135 units/mg), and 4% bovine serum albumin (Sigma). Dissociation was carried out in an Erlenmeyer flask at 37°C on a gyrator shaker at 125 rev./min for 1 h. The material was first passed through Dacron cloth, then through a 50 μm Nitex filter (Tetko, Elmsford, NY, U.S.A.) to break up clumps of cells (Emerman & Pitelka, 1977). The resulting cell suspension was centrifuged for 3 min at 80 *g*. The fat-cells and fibroblasts floated in the supernatant fraction, leaving a pellet which was composed mostly of epithelial cells. The viability of cells isolated by this procedure, as gauged by Trypan Blue exclusion, was over 90%. They reacted with the specific antibody for mouse mammary epithelial cells (Thompson *et al.*, 1976) and showed no reaction to an antibody made against mammary adipocytes (Thompson & Abraham, 1979). Less than 10% of the cells in the epithelial fraction were stained by the fluorescent antibody against fibroblasts (Thompson *et al.*, 1976).

Pieces of mammary-gland tissue (averaging 1 mg of protein) or 2×10^6 cells of the cell suspension were put into 5 ml tubes with 0.5 ml of Medium 199 containing [U-^{14}C]glucose (New England Nuclear, Boston, MA, U.S.A.; final sp. radioactivity 30 Ci/mol) and hormones as described above. The medium with samples was equilibrated with air/CO_2 (19:1), then put on a gyrator shaker at 125 rev./min and incubated at 37°C in air/CO_2 (19:1) for 1 and 2 h periods. The epithelial cell suspensions were centrifuged at 100 *g* for 3 min to pellet the cells. The medium was removed from all samples and frozen for later analysis. The cells and tissues were rapidly washed twice in Hanks' balanced salt solution containing unlabelled glucose, once with diluted Hanks' solution (1:2 with water), and killed with 3 ml of 80% (v/v) methanol in 0.01 M-NaOH containing 0.1% SDS as described previously (Emerman & Bissell, 1979*b*). The tissues were homogenized with a Tissumizer (Tekmar Corp., Cincinnati, OH, U.S.A.). The methanol was then evaporated under a stream of nitrogen.

Separation and identification of metabolites

The labelled metabolites were separated by two-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 metabolites was determined by radioautography with X-ray film, and the amount of ^{14}C in each metabolite was quantified.

The spots were identified by elution and rechromatography with pure standards. In all cases tested, the spot and the standard were superimposable in position and shape (Emerman & Bissell, 1979*b*).

Determination of the rate of glycogen synthesis

Glycogen synthesis was determined by two independent methods. Glycogen and other macromolecules remain at the origin after paper chromatography as described above. To determine the labelled glycogen, the origins were cut into small pieces and hydrolysed in 1 ml of 1 M-trifluoroacetic acid for 1 h at 200°C (Bissell *et al.*, 1973). The samples were evaporated under nitrogen and resuspended in 80% (v/v) methanol. The extract was again subjected to two-dimensional paper chromatography and the amount of [^{14}C]glucose released was considered to represent the amount of glycogen synthesized from glucose, since the amount of glycolipid and glycoprotein synthesized during this time period is negligible (Ceriani *et al.*, 1978). Because glycogen can be synthesized and degraded simultaneously, the rate of glycogen formation is not due to synthesis alone, but is the net result of these two processes. Nonetheless, for the sake of simplicity, we will refer to this accumulation as the rate of glycogen synthesis.

Glycogen was also isolated by ethanol precipitation (Good *et al.*, 1933). Rabbit liver glycogen (3 mg) was added as carrier to a portion of the

concentrated cellular extract. After delipidation (see below), ethanol was added to a final concentration of 66% (v/v). The samples were cooled to 0–4°C and the precipitate was allowed to collect for at least 2h. After centrifugation (800*g* for 3min) at 4°C, the supernatant material was carefully decanted. The remaining precipitate was dissolved in distilled water and a portion assayed for radioactive content in a liquid-scintillation spectrometer.

Determination of glycogen content

The glycogen content of mammary epithelial cells was determined by the procedure of Solling & Esmann (1975). The A_{340} of assay mixtures was read at room temperature on a recording spectrophotometer. Controls using purified labelled liver glycogen indicated better than 95% recovery.

Determination of the rate of lipid synthesis

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 was subjected to the procedure for glycogen precipitation with ethanol (see above).

Enzyme assays

The samples for enzymic assays were prepared as described above, except that after the final wash the cells were collected again by centrifugation as described above and taken up in 3vol. of homogenizing buffer (Golden *et al.*, 1977). The cells were totally disrupted at 4°C by three 20-s homogenizations with a Tissumizer (Tekmar Corp.). The total homogenates were used for assay because some glycogen synthase and phosphorylase activities are lost during centrifugation (Golden *et al.*, 1977). Total phosphorylase (EC 2.4.1.1) activity was assayed with a recording spectrophotometer as described by Mendocino *et al.* (1975). Total glycogen synthase (EC 2.4.1.11) was measured as described by Golden *et al.* (1977), except that glycogen was isolated chromatographically on thin layers of powdered cellulose. This modification was necessary because lactose, in preparations from late-pregnant and lactating mice, overlapped with glycogen in the column effluent. In a preliminary study with liver homogenates, this modification had no effect on the glycogen synthase activity recovered. Protein content of the homogenates was determined by the Lowry method as described below.

Measurement of radioactivity

The ^{14}C-labelled areas on the chromatograms were cut from the paper and their radioactive content was quantified with an automated Geiger-Muller apparatus (Moses & Lonberg-Holm, 1963). The radioactive content of glycogen precipitated with ethanol and the lipid were measured by using liquid-scintillatin spectrometry (Beckman Instruments, Fullerton, CA, U.S.A.). The scintillation mixture was 3a70b, purchased from Research Products International (Elk Grove, IL, U.S.A.). A portion of each sample was removed for protein determination by the method of Lowry *et al.* (1951) in an Autoanalyzer II system (Technicon Instruments Corp., Tarytown, NY, U.S.A.). The results are expressed as nmol of ^{14}C per mg of protein, which in this case is equivalent to ng-atoms of ^{14}C per mg of protein.

Results

When a portion of the extract from tissue that had been incubated with [^{14}C]glucose was subjected to two-dimensional paper chromatography, the amount of radioactivity remaining at the origin varied with 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 virgin, and reflected a rate of synthesis over 200nmol of ^{14}C/h per mg of protein. Total incorporation into the material at the origin fell to a very low value by mid-lactation. We concluded that of all the possible molecules held at the origin, glycogen would be the most likely intracellular metabolite to be formed from glucose to this extent.

To test for glycogen, the paper containing the material at the origin was hydrolysed as described in the Experimental section, and the labelled material in the hydrolysate was identified by paper chromatography. Some 75–87% of the ^{14}C was found to rechromatograph with glucose (Table 1). The only other source of glucose from the origin could be the direct incorporation of this sugar into glycolipid and glycoprotein (Speake & White, 1978). Ceriani *et al.* (1978) measured synthesis of glycoproteins by mammary epithelial cells and pieces of mammary tissue from late-pregnant mice by using labelled fucose. It can be calculated from their studies that the incorporation into macromolecules was infinitesimal compared with the amount of glucose released during hydrolysis of material at the origin in our studies. Furthermore, the presence of glycogen was confirmed by precipitation with ethanol (see below). Therefore the released glucose from the origins was taken as a measure of labelled glycogen in these cells.

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. 1*a*). The rate of glycogen synthesis in the mammary tissue of mature virgin mice increased with increas-

Table 1. *Rate of glycogen synthesis by pieces of mammary-gland tissue and isolated mammary epithelial cells from virgin, pregnant and lactating mice*

Pieces of tissue (1–2 mm^3) or 2×10^6 cells were incubated in 0.5 ml of Medium 199 containing 11 mM-[U-^{14}C]-glucose (final sp. radioactivity 30 Ci/mol) for 1 and 2 h. Samples of tissue were subjected to two-dimensional paper chromatography, and the material at the origin was hydrolysed as described in the Experimental section. The amount of [^{14}C]glucose released from the origins was taken as a measure of glucose carbon incorporated into glycogen. The radioactive glycogen in the cells was determined by two methods: (1) Hydrolysis after chromatography, as described above and in the Experimental section; (2) ethanol precipitation (Good *et al.*, 1933), as described in the Experimental section. Each value is the mean ± S.E.M. for at least three experiments for tissue pieces and at least six experiments for isolated cells. Results are expressed as nmol of ^{14}C/h per mg of protein.

		Mammary epithelial cells	
Physiological state of mice	Mammary tissue Origin hydrolysis	Origin hydrolysis	Ethanol precipitation
Virgin	107.0 ± 22.6	26.0 ± 7.8	11.5 ± 2.3
Pregnant (16–18 days)	202 ± 1	124.0 ± 3.4	53.0 ± 6.3
Lactating (7–10 days)	3.24 ± 0.04	1.9 ± 0.02	2.5 ± 0.5

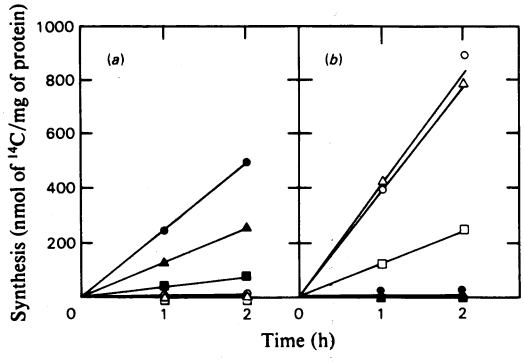

Fig. 1. *Effect of glucose concentration on the rate of glycogen (a) and lactose (b) synthesis by pieces of mammary gland from mature virgin and lactating mice*

The means of three experiments are presented for each concentration. Squares, 5.5 mM-glucose; triangles, 11 mM-glucose; circles, 27 mM-glucose. Closed symbols, mature virgin mice; open symbols, lactating mice.

ing glucose concentration in the medium. Glycogen synthesis by tissue from lactating mice was virtually unresponsive to the changes in glucose concentrations. In contrast with glycogen synthesis in tissue from lactating mice, the rate of lactose synthesis increased linearly with increases in glucose concentration up to 11 mM (Fig. 1*b*). Subsequent increases in glucose concentrations had no effect on lactose synthesis. This response in lactose synthesis by mouse mammary gland is in agreement with previous reports in the rat (Bartley *et al.*, 1966).

Approx. 80% of the gland during the quiescent state is composed of adipocytes, and these cells are known to synthesize glycogen (Vaughan & Korn, 1962). Therefore glycogen synthesis in the mammary gland, particularly of mature virgin mice, is not surprising. As the epithelial cell population becomes predominant during pregnancy, however, one might expect that glycogen synthesis would decrease. Nevertheless, the rate of glycogen synthesis by mammary-tissue pieces from late-pregnant mice was greater than that by the virgin gland (Table 1). This result led us to consider two possibilities: glycogen synthesis by adipocytes was increased during pregnancy, or the mammary epithelial cells were capable of glycogen synthesis.

To test the latter possibility, the mammary tissue was dissociated and the epithelial cells were isolated. The incorporation of ^{14}C from glucose into glycogen by epithelial cells is shown in Table 1. The mammary epithelial cells were clearly responsible for a large portion of the glycogen synthesized by the gland in all three physiological states. The rate of glycogen synthesis was highest during pregnancy and lowest during lactation. The glycogen is most probably derived from exogenous glucose, since the mammary gland is not capable of forming glucose or glucose 6-phosphate from lactate (Bartley *et al.*, 1966).

When glycogen was isolated by precipitation with 66% ethanol (Good *et al.*, 1933), the overall relationship between the physiological states was comparable with those obtained by hydrolysing the material at the origin after chromatography (Table 1). The radioactivity in precipitable material from epithelial cells derived from lactating mice was the same as that determined by chromatography. However, the rates of synthesis calculated from ethanol precipitation of glycogen from epithelial cells from glands of virgin and pregnant mice were less than that determined by the chromatographic method. The inability of ethanol to precipitate

shorter glycogen chains, particularly dextrins, has been noted previously (Good *et al.*, 1933; Golden *et al.*, 1977). The data would thus indicate that epithelial cells from mammary glands of virgin and pregnant mice incorporated the labelled glucose into smaller glycogen polymers. This conclusion has been confirmed by preliminary chromatographic studies (J. T. Emerman, J. C. Bartley & M. J. Bissell, unpublished work).

Assay of glycogen synthase and phosphorylase, the enzymes directly involved in glycogen synthesis and degradation, confirmed modulation of glycogen synthesis in mammary epithelial cells during pregnancy and lactation. The activities of both enzymes were present in epithelial cells from pregnant mice, but were barely detectable by day 7 of lactation (Table 2).

The rate of incorporation of ^{14}C from glucose into glycogen compared with that into other metabolites provides an indication of the importance of glycogen metabolism relative to the overall metabolism of glucose in the mammary epithelial cell. During the first 10 days of pregnancy, lipogenesis from glucose increased from 25 nmol of ^{14}C/h per mg of protein to over 90, but decreased from day 14 until just before parturition to a value one-quarter that of glucose incorporation into glycogen during this same state.

Table 2. *Specific activities of glycogen synthase and phosphorylase in mammary epithelial cells from pregnant and lactating mice.*

Activities are expressed as nmol of glucose incorporated into glycogen for glycogen synthase or nmol of glucose 1-phosphate released from glycogen for phosphorylase. Each value is the mean ± S.E.M. for eight experiments with pregnant mice and three experiments with lactating mice.

Physiological state of mice	Specific activity (nmol/min per mg of protein) of: Glycogen synthase	Phosphorylase
Pregnant	0.64 ± 0.12	3.08 ± 0.32
Lactating	0.06 ± 0.01	0.66 ± 0.40

When all intracellular metabolites identified by radioautography were considered, 8–15% of incorporated ^{14}C in the mammary epithelial cells of virgin and pregnant mice was found in glycogen (Table 3). By day 7 of lactation, however, glycogen synthesis was 0.2% of intracellular metabolite synthesis. This difference represents a significant change in the pattern of glucose utilization. The quantitative significance of glycogen synthesis during late pregnancy was substantiated by the fact that 50 μg of glycogen/mg of protein was found in mammary epithelial cells from late-pregnant mice and glycogen was undetectable in the cells from lactating mice.

Glycogen and lactose synthesis in mammary epithelial cells were measured throughout pregnancy and early lactation to determine at precisely what stages the changes in the rates of synthesis took place (Fig. 2). The highest rate of glycogen syn-

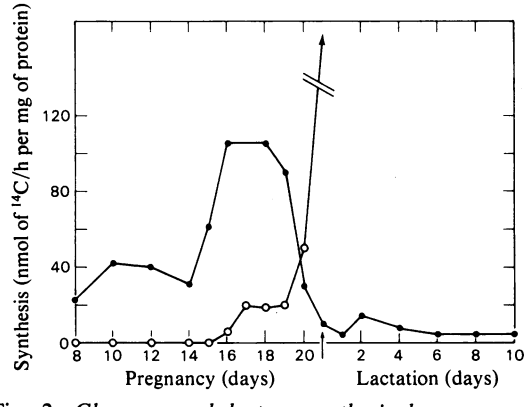

Fig. 2. *Glycogen and lactose synthesis by mammary epithelial cells from mice 8 days pregnant to 10 days lactating*

Each point represents the mean of the rate of glycogen or lactose synthesis from three experiments after a 1 h incubation with 11 mM-[U-^{14}C]glucose on the day indicated. ●, Rate of glycogen synthesis; ○, rate of lactose synthesis. Arrow on abscissa indicates time of parturition.

Table 3. *Formation of labelled intracellular metabolites and glycogen from [U-^{14}C]glucose in mammary epithelial cells*

Mammary epithelial cells were incubated for 1 and 2 h with [U-^{14}C]glucose as described in the Experimental section and Table 1. The labelled metabolites were isolated by two-dimensional paper chromatography and labelled lipids were extracted by the method of Slayback *et al.* (1977) as described in the Experimental section. The sum of the intracellular metabolites was determined by adding the labelled metabolites and labelled lipids recovered from the cell samples. The labelled glycogen was determined by hydrolysing the origin of samples applied to paper as described in the Experimental section. Each value is the mean ± S.E.M. of at least six experiments, and is expressed as nmol of ^{14}C/h per mg of protein.

Physiological state of mice	Intracellular metabolites	Glycogen
Virgin	314 ± 69	26.0 ± 7.8
Pregnant (16–18 days)	853 ± 222	124.0 ± 33.4
Lactating (7–10 days)	1094 ± 126	1.9 ± 0.2

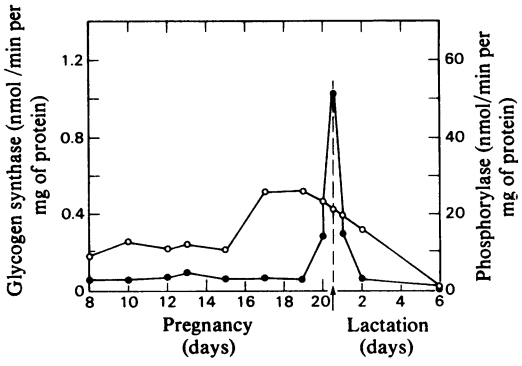

Fig. 3. *Total glycogen synthase and phosphorylase in mammary epithelial cells from mice 8 days pregnant to 6 days lactating*

Each point represents the mean of assays on at least two cell preparations. O, Activity of glycogen synthase; ●, activity of phosphorylase. Arrow indicates time of parturition.

thesis, over 120nmol of ^{14}C/h per mg of protein, occurred between days 16 and 18 of pregnancy; this rate fell steadily to reach a constant low value by day 6 of lactation. Lactose synthesis was initiated at day 16 of pregnancy, but the rate of synthesis remained low until just before parturition, when there was a sharp increase in the rate of synthesis corresponding to the decline in glycogen synthesis. Similar results were obtained by measuring the rates of glycogen and lactose synthesis in mammary-gland tissue at the time of parturition.

The enzymes of glycogen metabolism were assayed in the same epithelial-cell preparations used for the biosynthetic studies. The pattern of glycogen synthase activity was similar to that of glycogen synthesis (Fig. 3). Phosphorylase activity was little changed until 1 or 2 days before parturition, when it started to increase. Phosphorylase increased sharply to its maximal specific activity right at parturition, returned to the pre-partum value by day 3 of lactation, and declined to a barely detectable value by day 7. Phosphorylase activity, as measured under optimal conditions in the presence of AMP, always exceeded that of glycogen synthase. Since glycogen was much more abundant in the epithelial cells during late pregnancy than during lactation, phosphorylase activity must be limited somehow during this period. Because detection of phosphorylase activity was absolutely dependent on the addition of AMP, the intracellular concentration of this allosteric effector possibly modulates phosphorylase activity during pregnancy.

Discussion

In order to understand the metabolism of the lactating gland, it is important to investigate the metabolic relationships in the preceding stages of the reproductive cycle. 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. Furthermore, these experiments, conducted on populations of epithelial cells not contaminated with adipocytes (Thompson & Abraham, 1979) and fibroblasts (Thompson *et al.*, 1976), show that the glycogen accumulation is substantial in the epithelial cells. The parenchyma includes both secretory epithelium and myoepithelium. Because the population of secretory epithelial cells increases during pregnancy to become the predominant epithelial cell type, whereas the myoepithelial cell population increases very little, if at all (Hollmann, 1974), glycogen synthesis at this time is most likely attributed to the secretory epithelium.

If we compare the rates of glycogen synthesis in pieces of mammary tissue and isolated cells, it is clear that synthesis is much lower in the cells. This could be due to the dissociation procedure used to isolate the cells, even though cell viability exceeded 90%. Cell damage and the disruption of cell–cell interactions may adversely affect the maintenance of biosynthetic processes. On the other hand, it is more likely that other cell types in the mammary gland, particularly adipocytes, are responsible for some synthesis. However, this does not detract from the finding that the epithelial cells synthesize an appreciable amount of glycogen. The quantitative importance of glycogen synthesis in the mammary epithelial cell during late pregnancy is indicated by the facts that the maximum rate of glycogen synthesis exceeds that of lipid synthesis by almost 4-fold (Fig. 2 and the text) and that 15% of the ^{14}C from glucose is found in glycogen (Table 3).

The increase in glycogen synthesis at day 15 of pregnancy is of special interest because of its relationship to the initiation of lactose synthesis 1 day later (Fig. 2). From day 15 of pregnancy onward the mouse mammary gland contains an appreciable amount of lactose synthase (E.C. 2.4.1.22) activity (McKenzie *et al.*, 1971; Jones, 1972). Although lactose synthase activity continues to increase linearly until parturition (McKenzie *et al.*, 1971; Jones, 1972), the initial rate of increase of lactose synthesis does not continue, but attains a plateau between days 17 and 19 of pregnancy, when glycogen synthesis is maximal. At this stage, lactose synthase activity (McKenzie *et al.*, 1971; Jones, 1972), recognized as the rate-limiting step in lactose synthesis (Kuhn, 1968), is only slightly less than that

reported here for glycogen synthase, and yet the rate of incorporation of glucose into glycogen exceeds that of incorporation into lactose by 5-fold. We propose that as long as the activity of glycogen synthase equals or exceeds that of lactose synthase, the extent of lactose synthesis is limited by the availability of UDP-4-glucose, and thereby UDP-4-galactose. The enzyme catalysing the conversion of UDP-4-glucose into UDP-4-galactose, UDP-4-glucose epimerase (EC 5.1.3.2), is present and appears to be at equilibrium at this stage of gestation (Murphy *et al.*, 1973). Possible changes in activities of hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1) and UDP-glucose pyrophosphorylase (EC 2.7.7.9) are not a factor, because the two synthases share the pathway leading to the UDP-hexose pool, and glycogen phosphorylase activity is unchanged during this period. The K_m of each enzyme for its UDP-hexose substrate appears to be similar (Newsholme & Start, 1973; Kuhn, 1968), so that substrate affinity would not be a significant factor in this regulatory mechanism. Nevertheless, because the equilibrium of UDP-4-glucose epimerase lies toward UDP-glucose (Murphy *et al.*, 1973), glycogen synthesis would be strongly favoured over that of lactose until lactose synthase activity exceeded that of glycogen synthase.

By day 20 of pregnancy the situation is reversed. Glycogen synthase activity has not decreased sufficiently to account for the precipitous fall in the rate of glycogen synthesis, but lactose synthase activity by this time in mouse mammary-gland development (McKenzie *et al.*, 1971; Jones, 1972) far exceeds that of glycogen synthase, and thereby is the favoured recipient of the available UDP-derivatives. Furthermore, the rise in phosphorylase activity means that glycogen turnover time is decreased and would be perceived in our analyses as a decreased rate of synthesis. We propose, therefore, that the accepted pathway for lactose synthesis should be modified to include glycogen as shown in Scheme 1. In short, during quiescence and pregnancy UDP-4-glucose is converted into glycogen, thereby limiting lactose synthesis. At parturition, glycogen synthesis declines and breakdown of glycogen releases glucose 1-phosphate, which can supply glucose units for lactose synthesis.

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-chain triacylglycerols 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 & Peaker, 1971), at this time could destroy the integrity of the intracellular compartments and, eventually, the cell, owing 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.

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 & Bauman, 1974), yet 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. Our results indicate that glycogen synthesis should be considered as an integral and important pathway in the mammary gland in preventing lactose accumulation before lactation.

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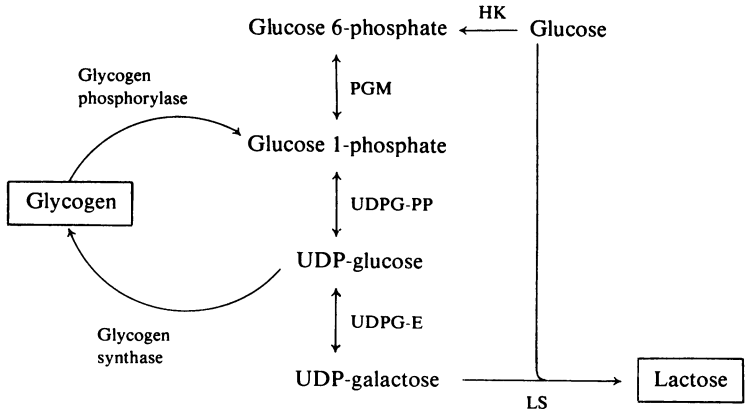

Scheme 1. *Interrelationship between glycogen metabolism and lactose synthesis in mammary epithelial cells*
Abbreviations: HK, hexokinase; PGM, phosphoglucomutase; UDPG-PP, UDP-glucose pyrophosphorylase; UDPG-E, UDP-glucose 4-epimerase; LS, lactose synthase.

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References

Bartley, J. C., Abraham, S. & Chaikoff, I. L. (1966) *J. Biol. Chem.* **241**, 1132–1137

Bartley, J. C., Emerman, J. T. & Bissell, M. J. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 954

Bassham, J. A., Bissell, M. J. & White, R. C. (1974) *Anal. Biochem.* **61**, 479–491

Bissell, M. J., White, R. C., Hatie, C. & Bassham, J. A. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2951–2955

Ceriani, R. L., Peterson, J. A. & Abraham, S. (1978) *In Vitro* **14**, 887–894

Denamur, R. (1974) in *Lactation—A Comprehensive Treatise* (Larson, B. L. & Smith, V. R., eds.), vol. 1, pp. 414–465, Academic Press, New York

Ebner, K. E., Hoover, C. R., Hageman, E. C. & Larson, B. L. (1961) *Exp. Cell Res.* **23**, 373–385

Emerman, J. T. & Bissell, M. J. (1979*a*) *Proc. Can. Fed. Biol. Soc.* **22**, 50

Emerman, J. T. & Bissell, M. J. (1979*b*) *Anal. Biochem.* **94**, 340–345

Emerman, J. T. & Pitelka, D. R. (1977) *In Vitro* **13**, 316–328

Golden, S., Wals, P. A. & Katz, J. (1977) *Anal. Biochem.* **77**, 436–445

Good, C. A., Kramer, H. & Somogyi, M. (1933) *J. Biol. Chem.* **100**, 485–491

Hollmann, K. H. (1974) in *Lactation—A Comprehensive Treatise* (Larson, B. L. & Smith, V. R., eds.), vol. 1, pp. 3–95, Academic Press, New York

Jones, E. A. (1972) *Biochem. J.* **126**, 67–78

Kittinger, G. W. & Reithel, F. J. (1953) *J. Biol. Chem.* **205**, 527–533

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

Larson, B. L. (1969) *J. Dairy Sci.* **52**, 737–747

Lasfargues, E. Y. & Moore, D. H. (1971) *In Vitro* **7**, 21–25

Linzell, J. L. & Peaker, M. (1971) *Physiol. Rev.* **51**, 564–597

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

McKenzie, L., Fitzgerald, D. K. & Ebner, K. E. (1971) *Biochim. Biophys. Acta* **230**, 526–530

Mellenberger, R. W. & Bauman, D. E. (1974) *Biochem. J.* **142**, 659–665

Mellenberger, R. W., Bauman, D. E. & Nelson, D. R. (1973) *Biochem. J.* **136**, 741–748

Mendocino, J., Abou-Issa, H., Medicus, R. & Fratowich, N. (1975) *Methods Enzymol.* **42c**, 389–390

Moses, V. & Lonberg-Holm, K. K. (1963) *Anal. Biochem.* **5**, 11–27

Murphy, G., Ariyanayagam, A. D. & Kuhn, N. J. (1973) *Biochem. J.* **165**, 1105–1120

Newsholme, E. A. & Start, C. (1973) *Regulation in Metabolism*, p. 187, John Wiley and Sons, New York

Petersen, W. E. & Shaw, J. C. (1938) *J. Dairy Sci.* **21**, 168

Reid, I. M. & Chandler, R. L. (1973) *Res. Vet. Sci.* **14**, 334–340

Sekri, K. K. & Faulkin, L. J., Jr. (1967) *J. Ultrastruct. Res.* **21**, 161

Slayback, J. R. B., Cheung, L. W. Y. & Geyer, R. P. (1977) *Anal. Biochem.* **83**, 372–384.

Solling, H. & Esmann, V. (1975) *Anal. Biochem.* **68**, 664–668

Speake, B. K. & White, D. A. (1978) *Biochem. J.* **176**, 993–1000

Sterling, J. W. & Chandler, J. A. (1977) *Virchows Arch. A* **373**, 119–132

Thompson, K. & Abraham, S. (1979) *In Vitro* **15**, 441–445

Thompson, K., Ceriani, R. L., Wong, D. & Abraham, S. (1976) *J. Natl. Cancer Inst.* **57**, 167–172

Twarog, J. M. & Larson, B. L. (1964) *Exp. Cell Res.* **34**, 88–99

Vaughan, M. & Korn, E. D. (1962) in *Adipose Tissue as an Organ* (Kinzell, L. W., ed.), pp. 173–235, Charles C. Thomas, Springfield, IL