LBL-11054
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

Submitted to Experimental Cell Research

GLUCOSE METABOLITE PATTERNS AS MARKERS OF FUNCTIONAL DIFFERENTIATION IN FRESHLY ISOLATED AND CULTURED MOUSE MAMMARY EPITHELIAL CELLS

Joanne T. Emerman, Jack C. Bartley, and Mina J. Bissell

June 1980

Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

GLUCOSE METABOLITE PATTERNS AS MARKERS OF FUNCTIONAL DIFFERENTIATION IN FRESHLY ISOLATED AND CULTURED MOUSE MAMMARY EPITHELIAL CELLS

Joanne T. Emerman, Jack C. Bartley,* and Mina J. Bissell

Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

*Present Address: Peralta Cancer Research Institute
3023 Summit Street
Oakland, CA 94609

Running Title: Glucose metabolite patterns in mammary epithelial cells

Send proofs to: Joanne T. Emerman
Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

SUMMARY

In the mammary gland of nonruminant animals, glucose is utilized in a characteristic and unique way during lactation [11]. We have measured the incorporation of glucose carbon from [U-^{14}C] glucose into intermediary metabolites and metabolic products in mammary epithelial cells from virgin, pregnant, and lactating mice and demonstrate that glucose metabolite patterns can be used to recognize stages of differentiated function. For these cells, the rates of synthesis of glycogen and lactose, the ratio of lactate to alanine, and the ratio of citrate to malate were important parameters in identifying the degree of expression of differentiation. We further show that these patterns can be used as markers to determine the differentiated state of cultured mammary epithelial cells. Cells maintained on plastic substrates lose their distinctive glucose metabolite patterns while those on floating collagen gels do not. Cells from pregnant mice have a pattern similar to freshly isolated cells from pregnant mice. The pattern of cells from lactating mice is different from that of the cells of origin, and resembles that of the cells from pregnant mice. Our findings suggest that the floating collagen gels under the culture conditions used in these experiments provide an environment for the functional expression of the pregnant state, while additional factors are needed for the expression of the lactating state.

INTRODUCTION

In mammary epithelial cells of nonruminant animals, glucose is a major substrate for the synthesis of tissue-specific milk components, and the gland utilizes glucose in a characteristic manner during lactation (see [11]). Studies in the animal and on mammary gland tissue slices have shown that prepartum levels of substrate for energy and milk constituents are adequate to support lactation, but that the pattern of glucose utilization changes in the transition from the pregnant to lactating state [2,19,29]. However, the mammary gland is composed of a heterogeneous population of cells and the proportions of each cell type varies throughout the reproductive cycle [30,32]. Therefore, it is not possible to determine (a) how much a given cell type contributes to the overall metabolic activity, and (b) whether these changes are taking place due to an increase in the epithelial cell population or a shift in their metabolic pathways, or both.

In this report, we describe the glucose metabolite patterns of isolated mammary epithelial cells from virgin, pregnant, and lactating mice. Differing metabolite patterns do exist among the cells at different physiological states of the animal. We further show that these patterns can be used as markers to resolve the degree of differentiation expressed by mammary epithelial cells cultured on floating collagen gels.

MATERIALS AND METHODS

Dissociating and Culturing Procedures

Mature virgin (12-16 weeks old), pregnant (14-16 days), and lactating (7-10 days) Balb/c Crgl mice (Cancer Research Laboratory, University of California, Berkeley, CA) were killed by cervical dislocation. Several 1-2 mm^3 pieces of the mammary glands from lactating mice were removed, washed in Medium 199 (Grand Island Biolog. Co.) and added to the incubation medium (described below). The remainder of the glands was dissociated according to our modification [13] of the method of Lasfargues and Moore [24]. The dissociation medium consisted of Medium 199 (10 ml/gm tissue) 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). After passing the cell suspension through a 50 μm Nitex cloth (Tetko, Inc.) to disperse cell aggregates [16], the epithelial cells were pelleted by centrifugation at 800 rpm for 3 min. The cells were washed 3 times with Medium 199 and viable cells, as determined by trypan blue exclusion, were counted on a hemacytometer. Some of the cells were transferred to incubation medium, and the rest were cultured at 5×10^5 cells/cm^2 on 35 mm plastic Petri dishes or 35 mm collagen-gel-coated Petri dishes. Cultures were incubated in Medium 199 containing 50 μg/ml Gentamicin (Schering Corp), 11 mM glucose, 5% fetal calf serum (Grand Island Biolog. Co.), and 5 μg each of insulin, cortisol, and prolactin at 37°C in 95% air and 5% CO_2. Serum was eliminated from the medium after the first day in culture. The medium was changed daily.

Preparation of Floating Collagen Gels

Collagen gels were prepared from solubilized rat-tail collagen [9] as previously reported [16]. Briefly, 0.85 ml of collagen solution was mixed with 0.2 ml of a 2:1 mixture of 10X concentrated Medium 199: 0.34 N NaOH in a 35 mm Petri dish. Gelation occurred after several minutes. One day after cells were seeded on collagen-gel-coated Petri dishes, the gels were released from the plastic substrates to float beneath the medium surface. For experimentation, cells on plastic and gels were removed daily from 2-day to 5-day cultures by treatment with collagenase [16].

Labeling Procedures

Pieces of mammary gland tissue (averaging 1 mg protein), 2×10^6 freshly dissociated cells, or 2×10^6 cultured cells were incubated for 1 and 2 h in Medium 199 containing high specific activity [U-^{14}C] glucose (New England Nuclear; final specific activity 30 Ci/mol) and hormones as described above. The medium was then removed and analyzed separately. The cells and tissues were rapidly washed in Hanks' balanced salt solution containing unlabeled glucose, killed in 3 ml of 80% methanol (v/v) in 0.01 N NaOH containing 0.1% sodium dodecyl sulfate, homogenized, and sonicated [14].

Separation and Identification of Metabolites

The methanol was evaporated under a stream of nitrogen. A portion of each sample was applied to Whatman No. 1 paper (22 x 18 in) to separate the glucose metabolites by 2-dimensional paper chromatography. A portion of medium was applied to a second paper to analyze the metabolites secreted by the cells. The chromatography procedures have been reported previously [4,8]. Briefly, the papers were run in phenol: water: acetic acid (84:16:1) for 24 h. After drying, they were turned 90° and run

in butanol: water: propionic acid (50:28:22) for another 24 h. The labeled compounds were identified by autoradiography using X-ray film. Labeled metabolites were cut from the paper and their radioactive content was quantitated with an automated Geiger-Muller apparatus [28]. Labeled metabolites were identified by eluting the spots and rechromatographing the samples with pure standards [14].

Glycogen and other macromolecules are retained at the origin of the chromatograms using these chromatographic procedures. Radioactive glycogen was determined by hydrolyzing the origins and measuring the released ^{14}C glucose by chromatography and autoradiography as previously described [8,13].

α-glycerol phosphate is not separated from dihydroxyacetone phosphate in these solvent systems. It was necessary to elute the spot and rechromatograph the sample in one direction using phenol:water:bisulfite solution:acetic acid (76:13:10:1). The bisulfite solution consisted of 25% $NaHSO_3$ in water (w/v) [S. Chin & M.J. Bissell, unpublished].

Lipid Determination

The lipid was extracted from the cellular extract by the method of Slayback et al. [35]. The organic phase was removed for assay of ^{14}C content by liquid scintillation spectrometry.

Collection of CO_2

A modified procedure of Bissell et al. [7] was used to measure the production of CO_2 from [U-^{14}C] glucose. Tubes (5 ml) containing samples were placed in 25 ml Erlenmyer flasks. The flasks were gassed with 5% CO_2 and 95% air for 1 min. They were then sealed with rubber serum caps into which plastic center wells had been inserted (Kontes) and incubated at 37° C. At the end of the incubation period, 0.2 ml of 4 N HCl was

injected into the incubation medium to terminate the reaction and 0.2 ml of Nuclear Chicago Solubilizer (Packard) was injected into the center well to collect the released CO_2. The flasks were left at room temperature for 90 min. Control experiments indicated that this amount of time was sufficient to collect all the released CO_2. The center wells were then removed and each was immersed in 10 ml of aquasol and counted by liquid scintillation spectrometery.

Expression of Results

A portion of each sample was removed for protein determination by the method of Lowry et al. [25] using an Autoanalyzer II system (Technicon). Results of all experiments were expressed as nmol ^{14}C per mg protein [4,8,14].

RESULTS

Comparison of Glucose Utilization by Mammary Tissue Pieces and Mammary Epithelial Cells from Lactating Mice.

We compared glucose utilization by pieces of mammary tissue to that by mammary epithelial cells from lactating mice to determine if the pattern was altered by the dissociation procedure and/or loss of cell to cell interactions occurring during isolation (Table I). The comparison between tissues and cells is valid for lactating mice since the glands of these animals are composed mainly of epithelial cells, while significant amounts of other cell types are present in the glands of mice during quiescence and pregnancy [30,32]. The cells utilized more glucose on a protein basis than the tissues, but the percentage distribution of ^{14}C from glucose into lipid and lactose remained the same. Although the amount of ^{14}C from glucose that was converted to lactate was higher in

cells than in tissues, the ratio of lactate:CO_2 was comparable: 0.22 for tissues and 0.30 for cells. This result indicated that lactate production did not specifically increase after tissue dissociation but that the increase in lactate production was a reflection of a general increase in glucose utilization.

Glucose Metabolite Patterns of Freshly Isolated Mammary Epithelial Cells from Virgin, Pregnant, and Lactating Mice.

We define glucose metabolite patterns as the relative incorporation of glucose carbon into intermediary metabolites and biosynthetic products. Examination of these metabolite patterns indicated that ^{14}C incorporation into intermediary metabolites reached saturation after the first hour of incubation in [U-^{14}C] glucose. The steady-state conditions of glucose-derived metabolite pools made comparisons between pool sizes valid. Incorporation of glucose carbon into metabolic products increased linearly over the duration of the experiment (2 h), indicating that the cells in all 3 states remained functional during this time.

Distinct qualitative differences in glucose metabolic patterns were seen among cells from virgin, pregnant, and lactating mice (Fig. 1). We used such differences to select specific metabolites that might be used as markers for expression of functional differentiation. Conversions of glucose carbon into these selected metabolites are shown in Table 2. The major differences observed in the transitions from the virgin to lactating state in the steady-state levels of glucose-derived metabolic intermediates were as follows: (1) An increase in α-glycerol phosphate was associated with lactation. (2) The glucose-derived citrate pool increased progressively in cells as the mice went from the quiescent state, through pregnancy, to lactation. (3) The pregnant state was characterized by a

low citrate:malate ratio (Table 3). (4) ^{14}C incorporation into the amino acids that were selected for study--alanine, glutamate, and aspartate--were increased with pregnancy and decreased with lactation. The level of alanine was especially high during pregnancy. (5) A distinct difference between the pregnant state and the virgin and lactating states was the low lactate:alanine ratio in the pregnant animal (Table 3).

The increase in the levels of α-glycerol phosphate and citrate in cells from lactating mice may be related to the well-known high rate of lipogenesis at this stage [5]. The modulation of the ratio of citrate:malate at different stages of the reproductive cycle also correlated with the rate of lipogenesis (see below). In mid- to late pregnant mice, lipogenesis was low and the citrate:malate ratio was low; in virgin and lactating mice, lipogenesis was higher and a correspondingly higher ratio of citrate to malate was observed.

The major changes in the rate of incorporation of ^{14}C from glucose into metabolic products by mammary epithelial cells in the transitions from the virgin to lactating state were as follows: (1) The rate of lipid synthesis in cells from virgin mice decreased from 33 nmole ^{14}C/mg protein/h in the virgin to 10 nmol ^{14}C/mg protein/h in the mid- to late pregnant mouse and increased greatly with lactation to 651 nmol ^{14}C/mg protein/h. (2) Glycogen synthesis was detected in all 3 physiological states, but the maximal rate of synthesis occurred during late pregnancy and the lowest during lactation. There was a 40-fold difference in the rate of glycogen synthesis between cells from pregnant mice and those from lactating mice. (3) Lactose synthesis was not detected prior to mid-pregnancy and remained low until abundant lactose synthesis was detected at lactation. (4) CO_2 produced from glucose increased

progressively from virgin to pregnant to lactating state. (5) Conversion of glucose carbon to lactate also increased accordingly.

Glucose Metabolite Patterns of Mammary Epithelial Cells from Pregnant and Lactating Mice in Culture.

The unique glucose metabolite patterns identified in freshly isolated cells from virgin, pregnant, and lactating mice provided the basis for using these patterns as markers for the study of differentiated function in cell culture. It has been previously reported that morphological and biochemical characteristics related to milk synthesis are not retained by mammary epithelial cells cultured on plastic substrates (see for example [6,15,16]). The metabolite patterns of cells cultured on plastic substrates were altered also beyond identification as mammary epithelial cells (results not shown). On the other hand, the metabolite patterns of cells cultured on floating collagen gels were similar to those of freshly isolated mammary epithelial cells and revealed much information concerning the degree of expression of the differentiated state of these cells. Under the same conditions, these cells have been shown previously to maintain other mammary-specific characteristics [10,15,16].

The total glucose utilized by cells from both pregnant and lactating mice on gels was approximately 1/3 that of freshly isolated cells. Nevertheless, the incorporation of glucose carbon into intermediary metabolites reached a steady state after 1 h and that into metabolic products increased linearly over a 2 h period as was seen with freshly isolated cells, again making comparisons between pool sizes valid.

The steady-state levels of the metabolic intermediates and products derived from glucose in the cultured cells from pregnant mice were similar

to those of freshly isolated cells from the same source (Table 4). The glucose-derived alanine pool was lower in cells in culture and lactate production decreased, so that the ratio of lactate:alanine remained the same (Table 5).

Cells cultured from pregnant mice behaved metabolically much like their freshly isolated counterparts, but cells cultured from lactating mice did not behave like the cells of origin. In general, the cells cultured from lactating mice showed a glucose metabolite pattern similar to that of cells from pregnant mice (Table 4). This situation was seen in ^{14}C incorporation into glycolytic intermediates, amino acids, and metabolic products. The steady-state incorporation of ^{14}C from glucose into amino acids and the reduced lactate:alanine ratio (Table 5) were both commensurate with properties of the cells from pregnant mice. Similarly, the increase in glycogen synthesis over that in freshly isolated cells from lactating mice and the decrease in lactose synthesis suggested a functional state in culture more comparable to that of pregnancy. By measuring glucose incorporation into lactose and glycogen daily from day 2 to day 5 in culture, the precise time of the shift in the expression of these 2 products could be determined (Fig. 2). The crossover point opposite to that occurring on day 19 of pregnancy [13] occurred between the 2nd and 3rd day of culture. Lipid synthesis also decreased to low levels in cultured cells. The only exceptions were that the amounts of ^{14}C incorporated into TCA cycle intermediates and the values for the citrate:malate ratio were comparable in cultured cells and freshly isolated cells from lactating mice.

DISCUSSION

The pattern of incorporation of radioactive carbon from [U-^{14}C] glucose is very characteristic of the degree of expression of differentiated function in the mammary epithelial cells of mice. Intermediary processes, such as glucose metabolism, are generally not considered to be tissue-specific, since all cells metabolize glucose. However, the pattern of glucose utilization in several cell types has been shown to be unique [6]. We have demonstrated here that not only is the glucose metabolite pattern unique in the fully expressed differentiated state of cells from lactating mice, but that specific changes in the metabolite pattern correlate with the changing physiological state of the mice.

Comparison of Glucose Utilization by Mammary Tissue Pieces and Mammary Epithelial Cells from Lactating Mice.

Based on results obtained with cells from lactating mice, glucose utilization by isolated mammary epithelial cells does not appear to be altered by the dissociation procedure. Mammary cells have a capacity for aerobic lactate production [17,27,32], therefore, the increase in lactate production after cell dissociation raised the possibilities that (a) a more homogeneous population of mammary epithelial cells may be more glycolytic than the rest of the gland, or (b) the dissociation procedure or the isolated state of the cells may produce alterations in glucose metabolism. Based on the ratio of lactate produced to glucose utilized, Elkin and Kuhn [12] concluded that rat mammary tissue is more similar to the gland *in vivo* than are isolated cells. However, they did not take

into account the fact that the mammary gland is known to utilize lactate as a substrate [3,12,22,23,33]. Epithelial cells within tissues would have a better opportunity to utilize lactate than isolated cells since in tissues lactate does not diffuse from the vicinity of the epithelial cells and thus is not diluted in the rest of the medium. Our experiments have shown that the absolute increase in lactate production is most likely due to increased glucose catabolism in cells over that in tissues because the increased rate of lactate synthesis was paralleled by a comparable increase in the rate of CO_2 production.

Others have shown that isolated mammary epithelial cells provide a valid model for metabolic studies. Several workers have dissociated the mammary glands from lactating animals to study the metabolic activity of isolated epithelial cells and have shown that the metabolism reflects that of the gland in vivo for lactating rats [2,18,23,26,33,34,36] and for lactating mice [1]. The validity of using isolated mammary epithelial cells from lactating rats has been further confirmed by the demonstration that cells are more responsive to hormones than tissue pieces [18].

Glucose Metabolite Patterns of Mammary Epithelial Cells from Pregnant and Lactating Mice in Culture.

Two major observations of cells in culture are: (1) they become increasingly glycolytic and (2) they lose their differentiated functions [31]. It is possible that these two phenomena are related. This possibility is supported by our observation that when cells retain their specific functions, aerobic lactate production does not increase. Mammary epithelial cells from pregnant mice cultured on floating collagen gel substrates maintain mammary-specific characteristics [15,16], and the

amount of lactate produced relative to the total glucose utilized by cells under these conditions does not exceed that of freshly isolated cells.

Examination of the glucose metabolite patterns of cells on floating collagen gel cultures indicates the degree of differentiation these cells are expressing. The pattern of cells in culture from pregnant mice is similar to that of their freshly isolated counterparts. On the other hand, the results from cells cultured from lactating mice suggest that these cells have modulated to a prelactating state comparable for the most part to that of days 14-16 of pregnancy [13]. They have an intermediary metabolite pattern almost identical to that of freshly isolated cells from mice at this stage of pregnancy and the high rate of glycogen synthesis accompanied by the low rate of lactose synthesis are also indicative of the same stage of differentiated function.

The fact that lipogenesis is retarded in the cultured cells from lactating mice is consistent with a change in the differentiated state of the cells under these culture conditions. The protein kinase which maintains glycogen synthase activity depresses acetyl CoA carboxylase activity [20], thus one cannot expect high rates of lipogenesis at the same time that glycogen synthesis is increasing. The one exception to the altered metabolite pattern of cultured cells from lactating mice is that the citrate level remains as high in these cells as it does in the freshly isolated cells from lactating mice. This result would be consistent with a block in lipogenesis occurring after citrate formation in culture.

The metabolite patterns of the epithelial cells during pregnancy, as well as other mammary-specific characteristics, such as ultrastructural organization and casein production [15,16], are maintained on floating collagen gels. It has also been demonstrated that the cells from pregnant

rabbits maintain α-lactalbumin synthesis on the gels [21]. While it has been shown that cells from lactating mice cultured on floating collagen gels maintain morphological differentiation [10] there is little difference between the morphological characteristics of late pregnant and lactating mammary epithelial cells either *in vivo* or on the gels [10,16]. We have shown here that glucose metabolite patterns are distinctly different during pregnancy and lactation and the pattern of cells from lactating mice cultured on collagen gels indicates that these cells have modulated to the pregnant state.

The glucose metabolite patterns provide us with the opportunity to recognize more stages of differentiated function than does synthesis of other milk-related products. This is because these latter products are produced only during a specific phase of the reproductive cycle. The metabolite patterns should aid in identifying currently unrecognizable changes in differentiated function brought about by hormones and other factors in culture which will add to our understanding of the biochemical mechanisms involved in the expression of differentiation. The precise metabolite changes seen in the mammary epithelial cells of mice may not apply exactly to mammary epithelial cells from other sources and the pattern of glucose utilization will have to be mapped out for each species. The approach presented here could also be used to determine the functionally differentiated state of other cell types using glucose or other labeled substrates.

ACKNOWLEDGEMENT

This work was supported in part by the Division of Biomedical and Environmental Research of the U.S. Department of Energy under Contract No. W-7045-ENG-48, and in part by the National Institutes of Health Fellowship 1F32 CA06139-01 from the National Cancer Institute awarded to J.T.E.

REFERENCES

1. Abraham, S, Kerkof, PR & Smith, S, Biochim biophys acta 261 (1972) 205.
2. Baldwin, RL & Cheng, W, J dairy sci 52 (1969) 523.
3. Bartley, JC & Abraham, S, J lipid res 17 (1976) 467.
4. Bassham, JA, Bissell, MJ & White, RC, Anal biochem 61 (1974) 479.
5. Bauman, DE & Davis, CL, Lactation--a comprehensive treatise (eds BL Larson & VR Smith) vol. 2, p. 31. Academic Press, New York (1974).
6. Bissell, MJ, Intl rev cytol, in press.
7. Bissell, MJ, Hatie, C & Rubin, H, J natl cancer inst 49 (1972) 555.
8. Bissell, MJ, White, RC, Hatie, C & Bassham, JA, Proc natl acad sci USA 70 (1973) 2951.
9. Bornstein, MB, Lab invest 7 (1958) 134.
10. Burwen, SJ & Pitelka, DR, Exp cell res, in press.
11. Davis, CL & Bauman, DE, Lactation--a comprehensive treatise (eds BL Larson & VR Smith) vol. 2, p. 3. Academic Press, New York (1974).
12. Elkin, AR & Kuhn, NJ, Biochem j 146 (1975) 273.
13. Emerman, JT, Bartley, JC & Bissell, MJ, submitted.
14. Emerman, JT & Bissell, MJ, Anal biochem 94 (1979) 340.
15. Emerman, JT, Enami, J, Pitelka, DR & Nandi, S, Proc natl acad sci USA 74 (1977) 4466.
16. Emerman, JT & Pitelka, DR, In vitro 13 (1977) 316.
17. Folley, SJ & French, TH, Biochem j 45 (1949) 270.
18. Greenbaum, AL, Salam, A, Sochor, M & McLean, P, Eur j biochem 87 (1978) 505.

19. Gumaa, KA, Greenbaum, AL & McLean, P, Lactation (ed IA Falconer) p. 197. Butterworths, London (1971).

20. Hardie, DG & Cohen, P, FEBS letters 91 (1978)1.

21. Hauptle, MT, Suard, Y & Kraenhenbuhl, JP, J cell biol 83 (1979) 237a.

22. Hawkins, RA & Williamson, DH, Biochem j 129 (1972) 1171.

23. Katz, J, Wals, PA & Van de Velde, RL, J biol chem 249 (1974) 7348.

24. Lasfargues, EY & Moore, DH, In vitro 7 (1971) 21.

25. Lowry, OH, Rosebrough, NJ, Farr, AL & Randall, RJ, J biol chem 193 (1951) 265.

26. Martin, RJ & Baldwin, RL, Endocrinol 89 (1971) 1263.

27. Moretti, RL & Abraham, S, Biochim biophys acta 124 (1966) 280.

28. Moses, V & Lonberg-Holm, KK, Anal biochem 5 (1963) 11.

29. Murphy, G, Ariyanayagam, AD & Kuhn, NJ, Biochem j 136 (1973) 1105.

30. Nichol, CS & Tucker, HA, Life sci 4 (1965) 993.

31. Paul, J, Cell and tissue in culture (ed EN Willmer) p. 239. Academic Press, New York (1965).

32. Rees, ED & Eversole, A, Amer j physiol 207 (1964) 595.

33. Robinson, AM & Williamson, DH, Biochem j 164 (1977) 153.

34. _____ Biochem j 168 (1977) 465.

35. Slayback, JRB, Cheung, LWY & Geyer, RP, Anal biochem 83 (1977) 372.

36. Yang, YT & Baldwin, RL, J dairy sci 58 (1975) 337.

FIGURE LEGENDS

Fig. 1. Autoradiograms of labeled glucose metabolites separated by 2-dimensional paper chromatography as described in Materials and Methods. The mammary epithelial cells from virgin mice (A), pregnant mice (B), or lactating mice (C) were incubated in 0.5 ml of 11 mM [U-^{14}C] glucose (final specific activity 30 Ci/mol) for 1 h. Abbreviations: O, origin; FDP, fructose-1,6-diphosphate; HMP, hexose monophosphates; 6 PGA, 6-phosphogluconate; 3-PGA, 3-phosphoglycerate; GP, α-glycerol phosphate; ASP, aspartate; CIT, citrate; MAL, malate; FUM, fumerate; GLU, glutamate; GLUC, glucose; GLN, glutamine; S, sorbitol; F, fructose; ALA, alanine; LAC, lactate.

Fig. 2. Abscissa: time in culture (days); ordinate: (left) nmol ^{14}C incorporated into lactose/mg protein/h; (right) nmol ^{14}C incorporated into glycogen/mg protein/h. Glycogen and lactose synthesis by mammary epithelial cells from pregnant and lactating mice cultured on floating collagen gels. Cells were removed from collagen gels daily from day-2 to day-5 in culture as described by Emerman and Pitelka [16]. Cells (2 x 10^6) were incubated for 1 and 2 h with [U-^{14}C] glucose as described in Materials and Methods and Fig. 1. The radioactive content of glycogen and lactose was determined by 2-dimensional paper chromatography as described in Materials and Methods. Each point represents the mean of the amount of glycogen or lactose synthesis from 3 experiments after a 1 h incubation with [U-^{14}C] glucose on the day indicated. ●——●, rate of glycogen synthesis; ▲——▲, rate of lactose synthesis.

Table 1. Utilization of ^{14}C from [U-^{14}C] glucose by mammary tissue pieces and mammary epithelial cells from lactating mice.

Type of Preparation	nmole ^{14}C/mg protein/h					
	Intracellular Metabolites	Lipids	Lactose (Extracellular)	Lactate (Extracellular)	CO_2	Total Glucose Utilized
Tissues	480±171	420[1]	536±207	644±217	142± 34	2,102
Cells	443±126	651±30	845±341	4,834±1,520	1,450±462	8,223

1-2 mm^3 pieces of tissue or 2 x 10^6 cells were incubated in 0.5 ml of Medium 199 containing 11 mM [U-^{14}C] glucose (final specific activity 30 Ci/mol) for 1 and 2 h. The labeled intracellular metabolites and extracellular lactose and lactate were isolated by 2-dimensional paper chromatography, labeled lipids were extracted by the method of Slayback et al. [35], and $^{14}CO_2$ was collected according to Bissell et al. [7] as described in Materials and Methods. Each value is the mean ± S.E.M. of at least 6 experiments.

Table 2. Incorporation of ^{14}C from [U-^{14}C] glucose into selected metabolites in mammary epithelial cells from virgin, pregnant, and lactating mice.

Metabolites	nmol ^{14}C/mg protein/h		
	Virgin	Pregnant	Lactating
Metabolic Intermediates			
Hexose monophosphates	7.2±4.2	4.1±1.4	1.7±0.4
3-phosphoglycerate	1.4±0.5	2.1±0.9	0.9±0.2
α-glycerol phosphate	3.2±1.2	5.0±2.3	43.2±6.1
6-phosphogluconate	0.7±0.1	0.8±0.3	0.5±0.2
citrate	2.1±0.3	4.9±0.3	11.1±4.2
malate	2.5±0.2	12.0±2.6	8.8±2.0
alanine	9.5±1.2	89.3±1.9	23.2±7.7
glutamate	5.5±1.6	17.1±1.8	3.6±1.2
aspartate	2.5±1.2	6.4±0.6	2.7±0.9
Metabolic Products			
glycogen	26±7.8	80±22	1.9±0.2
lactose*	--	0.8±0.2	899 ±341
lactate*	2,661±462	3,740±1,078	5,014±1,520

*Values include intracellular and extracellular lactose and lactate. Cells were incubated for 1 and 2 h with [U-^{14}C] glucose as described in Materials and Methods and Table 1. The labeled metabolites were isolated by 2-dimensional paper chromatography as described in Materials and Methods. Each value is the mean ± S.E.M. of at least 6 experiments.

Table 3. Ratios of glucose carbon incorporation into citrate and malate, lactate and alanine in mammary epithelial cells from virgin, pregnant, and lactating mice.*

Source of Cells	Citrate:Malate	Lactate:Alanine
Virgin	0.8	280
Pregnant	0.4	42
Lactating	1.3	218

*Data derived from Table 2.

Table 4. Incorporation of ^{14}C from [U-^{14}C] glucose into selected metabolites in mammary epithelial cells from pregnant and lactating mice cultured on floating collagen gels.

Metabolites	nmol ^{14}C/mg protein/h	
	Pregnant	Lactating
Metabolic Intermediates		
hexose monophosphates	7.9±1.0	6.4±2.0
3-phosphoglycerate	3.0±1.4	1.4±0.2
α-glycerol phosphate	6.8±1.3	4.8±2.4
6-phosphogluconate	1.1±0.6	1.3±0.7
citrate	8.1±0.5	13.7±2.1
malate	16.7±5.6	8.7±1.4
alanine	31.9±5.8	29.9±3.6
glutamate	13.2±4.7	20.1±3.0
aspartate	5.7±2.1	7.2±1.2
Metabolic Products		
glycogen	42.4±6.4	39.1±5.4
lactose*	1.6±0.2	2.7±1.9
lactate*	2,198±541	1,507±558

*Values include intracellular and extracellular lactose and lactate.

Cells were removed from the collagen gels on day-5 in culture according to Emerman and Pitelka [16] and incubated for 1 and 2 h with [U-^{14}C] glucose as described in Materials and Methods and Table 1. The labeled metabolites were isolated by 2-dimensional paper chromatography as described in Materials and Methods. Each value is the mean ± S.E.M. of at least 3 experiments.

Table 5. Ratio of glucose carbon incorporation into lactate and alanine in freshly isolated cells and cultured cells from pregnant and lactating mice.*

	Lactate:Alanine	
Source of Cells	Freshly Isolated Cells	Cultured Cells
Pregnant	42	69
Lactating	218	50

*Data derived from Tables 2 and 4.

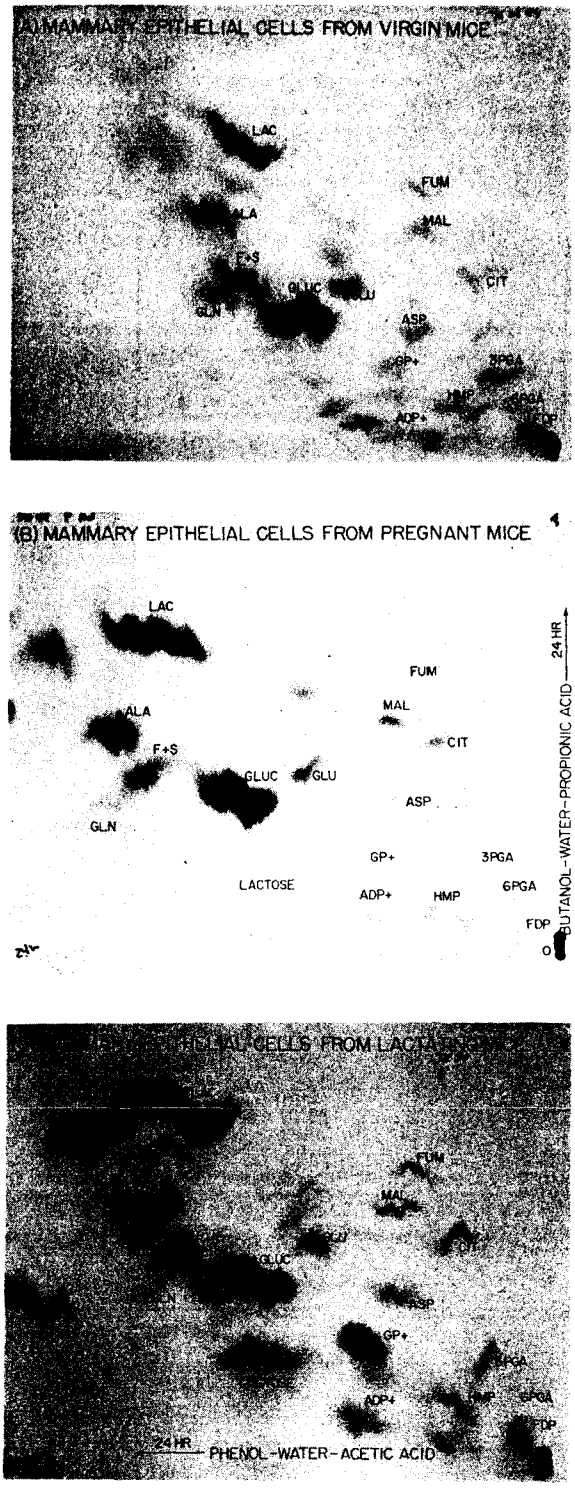
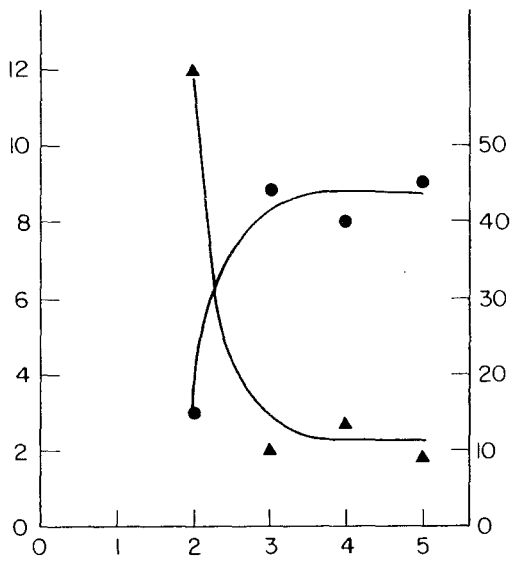


Fig. 1 XBB805 6663



XBL 803-4066A

Fig. 2