A Putative Role for Cell-Cell Epithelial Contacts in Lactose Secretion

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Abstract Rates of synthesis and secretory release and the maximal requisite intracellular transit time ($T_{sec}$) for lactose were measured in vitro for three preparations of lactating guinea pig mammary tissue: tissue slices, mammary epithelial cell (MEC) acini, and mono-dispersed MEC. The $T_{sec}$ values for tissue slice and acini preparations were similar, lactose required approximately 16 min to pass from its site of synthesis (Golgi) to the extracellular medium. Dispersal of mammary tissue into single cells by collagenase disruption of all cell-cell junctional complexes increased the $T_{sec}$ value to approximately 25 min but did not alter kinetics of lactose synthesis and secretory release. These data suggest a possible involvement of cell-cell junctional contacts in intracellular transport of lactose.

Adjacent constituent cells of the mammary epithelium characteristically possess several morphologically distinct classes of intercellular junctions (Pitelka et al., 1973). These regions of cell contact are believed to play important primary or secondary roles in directing and stabilizing the three-dimensional morphology of ductal and acinar (alveolar) mammary epithelia, influencing the shape of individual milk secretory cells and possibly providing avenues for intercellular solute transfers (Kater and Gavin, 1978; Loewenstein et al., 1978; Pitelka et al., 1973; Staehelein, 1974).

The nature and distribution of most breast epithelial junctional complexes appear to change substantially just prior to the commencement of active milk secretion. As the acinar epithelium becomes actively secretory, all desmosomes and most intermediate junctions between cells disappear whereas gap junctions remain and the network of tight junctions becomes compact and highly ordered (Hollmann, 1974; Pitelka et al., 1973). This persistence and reorganization of the tight, or occluding, junctions between acinar cells, which results in a relatively impermeable barrier for paracellular solute movement, has been viewed as responsible for the sharp decline in serum-derived solutes in milk at the onset of

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lactation (LINZELL and PEAKER, 1971a, b, 1974; PEAKER and TAYLOR, 1975; LINZELL et al., 1975).

The present paper reports the effect of mild disruption of junctional contacts between milk secretory acinar cells in vitro on rates of lactose synthesis, intracellular transport, and secretion. Evidence is presented showing that disruption of the normal three-dimensional architecture of acini significantly increases the pre-secretory intracellular transit time but not the synthesis or secretory release of lactose.

MATERIALS AND METHODS

Animals. Multiparous, 1–3 day lactating guinea pigs (Cavia porcellus) nursing 1–2 pups each were purchased from Simonsen Laboratories, Inc., Gilroy, CA.

Tissue Preparations. Guinea pigs were killed by carbon dioxide asphyxiation and the mammary glands removed under aseptic conditions. Mammary tissue was immediately sliced by hand into approximately 3–4 mm thick strips and briefly incubated with gentle agitation at 37°C in Krebs-Henseleit bicarbonate buffer (gassed with 95% O₂ : 5% CO₂) modified by the addition of 5 mm glucose, 1 μg/ml bovine insulin (24 IU/ml, Sigma) and fortified with 0.1 × Dulbecco’s Minimum Essential Medium essential and non-essential amino acids (GIBCO, Grand Island, N.Y.).

Tissue slices of approximately 1 mm thickness, 2 mm width and 4 mm length, free of grossly obvious areas of adipose, connective and vascular tissues, were prepared by gently compressing larger tissue samples between two sterile, 3 cm × 3 cm, plastic boards and hand trimming the tissue edge with a surgical scalp blade. Tissue slices were then incubated at 37°C under 95% O₂ : 5% CO₂ for 30–60 min with aeration and gentle agitation in order to wash out residual milk.

Mammary epithelial cell (MEC) acini and mono-dispersed MEC were prepared from minced mammary tissue by a collagenase dispersal technique detailed elsewhere (FOSTER, 1977, 1979; FOSTER and FELDMAN, 1975). Collagenase-dispersed mammary tissue was gently passed through nylon filters of progressively smaller pore diameters (FOSTER and FELDMAN, 1975). Tissue excluded by 25 μm pore diameter mesh but passing through 105 μm pore diameter mesh formed the acini preparation. Mono-dispersed MEC represented tissue which passed freely through the 25 μm pore diameter filter. Included in this population were variable numbers of non-MEC, e.g., fibrocytes, possible adipocytes, and erythrocytes, as well as occasional cell clusters composed of 2–3 MEC. Cell counts were performed on acini and mono-dispersed MEC preparations by hemocytometry, the secretory MEC were readily distinguished from other cell types by their substantially greater diameter and characteristic content of secretory milk lipids (FOSTER, 1977). Prior to cell counting, an aliquot of the acini preparation was mono-dispersed with 0.1% (w/v) trypsin (1: 250, Difco Labs., Detroit, MI).

Incubation procedures. Tissue slices were incubated at 37°C in the modified

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Krebs-Henseleit incubation medium at approximately 50–75 mg wet tissue per ml medium and gassed with a small stream of 95% O₂: 5% CO₂ bubbled through the medium. Acini and mono-dispersed MEC were suspended in incubation medium at a density of 2–3 × 10⁶ cells per ml at 37°C. A slow stream of 95% O₂: 5% CO₂ continuously bubbled through the medium maintained a constant suspension of cells. ¹⁴C-lactose synthesis was initiated for all groups with the addition of 2.5 μCi (in 2.5 μl 20% ethanol) D-[U-¹⁴C]glucose (ICN, Irvine, CA) per ml medium. Media samples were collected after allowing the tissue slices to settle to the bottom of the culture vessel. At the end of each study, the incubation medium was aspirated, tissue slices briefly blotted on absorbent paper and then frozen on dry ice. Later, the tissues were ground in distilled water with sea sand using a mortar and pestle, centrifuged and the supernate assayed for ¹⁴C-lactose. Acini and dispersed MEC were separated from their incubation medium by centrifugation and then assayed for ¹⁴C-lactose as reported elsewhere (FOSTER, 1978).

¹⁴C-lactose assay. ¹⁴C-activity in lactose was measured in deionized tissue extracts, cell and media samples by thin-layer chromatography as described previously (FOSTER, 1978).

Statistics. Data are reported as means and standard errors of the mean. Statistical significance was determined using the Student’s t-test for unpaired samples.

RESULTS

Photomicrographs of representative samples of each of the three mammary tissue preparations are presented in Fig. 1. Both tissue slice and MEC acini preparations showed numerous sites at which secretory cells lining the acinar lumen were in immediate contact with the incubation medium (arrows, Fig. 1A, D). The secretion of milk products by these acinar cells therefore proceeded directly into the incubation medium. The appearance in the incubation medium of milk products from the other secretory cells located more deeply in tissue slice preparations was expected to be less immediate, requiring initial passage through primary or secondary mammary ducts to points where these pathways open at the cut edges of the tissue. The acini preparations were largely deficient in ducts and appeared composed of single acini or clusters of 2–6 acini, each acinus containing approximately 20–40 cells (Fig. 1C, D). The pathway for milk product release into the incubation medium in such preparations appeared either immediate or required only a minimal diffusion distance. Junctional complexes between adjacent acinar cells being intact, stabilized the characteristic, three-dimensional appearance of each acinus even though mesenchyme and possibly myoepithelium normally surrounding each acinus in situ had been stripped away during the preparative collagenase treatment.
Fig. 1. Photomicrographs of several *in vitro* preparations of lactating guinea pig mammary epithelium. 

A. Tissue slice, hematoxylin and eosin stained, 1 μm section. B. Monodispersed MEC, several of which have loosely reaggregated. Note secretory lipid droplets within cells and sites of apparent lipid extrusion at cell periphery. Phase optics. 

C. Isolated acini, phase optics. D. Isolated acini, hematoxylin and eosin stained, 1 μm section. Arrows in A and D indicate areas of immediate contact between incubation medium and acinar lumen. Note differences in scale.

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Fig. 2. Time course of $^{14}$C-lactose synthesis and secretion by tissue slices of lactating guinea pig mammary gland. Incubation medium (▲) was sampled periodically whereas tissue $^{14}$C-lactose, (■) was assayed only at the end of the study. Overall $^{14}$C-lactose production (○) represented the sum total of secreted $^{14}$C-lactose plus final tissue $^{14}$C-lactose content. Assuming a constant rate of lactose synthesis, the time course of tissue $^{14}$C-lactose build-up was estimated as the difference between $^{14}$C-lactose production and medium $^{14}$C-lactose content. Arrow indicates moment of first appearance of $^{14}$C-lactose in the incubation medium.

Mono-dispersed MEC appeared spherical, showed areas of active milk fat secretion and contained numerous light refractile (lipid) droplets in their cytoplasm. Very few of the cells were still attached by junctional complexes although many of the mono-dispersed cells had a tendency to weakly reassociate into clusters of 2–6 cells.

A representative time course of synthesis, tissue build-up and release into the incubation medium of $^{14}$C-lactose by mammary tissue slices is illustrated in Fig. 2. In this study the medium alone was sampled periodically, the tissue mass remaining constant throughout the time course. Rate of $^{14}$C-lactose synthesis was assumed to be linear throughout the duration of this study. In other mammary tissue slice experiments (data not presented) rates of lactose and secretory protein synthesis were found to be constant for at least 5 hr under similar incubation conditions. $^{14}$C-lactose first appeared in the medium approximately 15 min after the addition of $^{14}$C-glucose to the culture system and progressively accumulated thereafter in the medium in a linear or slightly curvilinear manner. The commencement of $^{14}$C-lactose secretion into the medium represents the minimal interval for synthesis, intracellular transport and secretory release of lactose. Previously it was found that $^{14}$C-lactose is both synthesized and maximally labeled by $^{14}$C-glucose in less than 1 min after exposure to $^{14}$C-glucose (FOSTER, 1979). The first appearance of $^{14}$C-lactose in the medium, therefore, provides a maximal value for the intracellular transport time ($T_{sec}$) of lactose (FOSTER, 1979).
Tissue $^{14}$C-lactose content, estimated as the difference between total $^{14}$C-lactose synthesis and $^{14}$C-lactose in the medium, progressively increased with continued incubation in $^{14}$C-glucose medium, approaching a maximal level somewhat later than the time at which $^{14}$C-lactose first appeared in the medium. Both the kinetics of tissue $^{14}$C-lactose build-up and the curvilinear trend of $^{14}$C-lactose accumulation in the medium with time but not the time required for the first appearance of $^{14}$C-lactose in the medium varied considerably between tissue batches (data not presented). This probably reflected gross differences in ratios of secretory acini at the cut edge to more deeply situated acini and variable differences in the rate at which various tissue compartments such as the deeply situated acinar lumens and primary and secondary ducts reached steady-state conditions in the influx and efflux of $^{14}$C-lactose.

Rates of $^{14}$C-lactose synthesis and secretory release into the incubation medium also were linear with time for MEC acini preparations (Fig. 3). In these preparations $^{14}$C-lactose accumulated exclusively within the acinar cells for approximately 15–17 min before the commencement of secretory release of $^{14}$C-lactose into the medium and the establishment of a constant acinar level of $^{14}$C-lactose (small arrows, Fig. 3).

Mono-dispersed MEC, likewise synthesized and secreted $^{14}$C-lactose at a constant rate throughout the cultivation period (Fig. 4). However, the interval for the first appearance of $^{14}$C-lactose in the medium for mono-dispersed MEC preparations was nearly 10 min greater than for tissue slice and acini preparations (small arrows, Fig. 4). Table 1 summarizes estimates of these times, termed the intracellular secretory transit time ($T_{sec}$) of lactose (Foster, 1979), by tissue slice, acini and mono-dispersed cell preparations derived from studies similar to those presented in Figs. 2, 3 and 4. $T_{sec}$ values for tissue slice and acini preparations

![Fig. 3. Time course of $^{14}$C-lactose synthesis and secretion by MEC acini. $^{14}$C-lactose content of acinar cells (○) and the incubation medium (△) were determined periodically and total $^{14}$C-lactose synthesis (□) estimated as the sum of these values. Arrows indicate interpolated time at which $^{14}$C-lactose content of acinar cells first reaches its maximum value and at which $^{14}$C-lactose first appears in the incubation medium.

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Fig. 4. Time course of $^{14}$C-lactose synthesis and secretion by fully mono-dispersed MEC. Data presented as in Fig. 3.

Table 1. Rate of Synthesis and Intracellular Secretory Transit Times ($T_{sec}$) of Lactose by Various in vitro Preparations of Mammary Tissue from Lactating Guinea Pig.

<table>
<thead>
<tr>
<th>Tissue preparation</th>
<th>Synthesis (nmols/10^6 cells/hr)</th>
<th>Significance</th>
<th>$T_{sec}$ (min)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Tissue slice</td>
<td>N.D.</td>
<td>—</td>
<td>15.4±1.24</td>
<td>—</td>
</tr>
<tr>
<td>(B) Acini</td>
<td>36.1±4.0</td>
<td>—</td>
<td>15.9±0.95</td>
<td>N.S. (A vs. B)</td>
</tr>
<tr>
<td></td>
<td>(n=3)</td>
<td></td>
<td>(n=3)</td>
<td></td>
</tr>
<tr>
<td>(C) Mono-dispersed cells</td>
<td>34.4±4.3</td>
<td>N.S. (B vs. C)</td>
<td>25.0±1.00</td>
<td>$p&lt;0.001$ (B vs. C)</td>
</tr>
<tr>
<td></td>
<td>(n=4)</td>
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<td>(n=4)</td>
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N.D.= not determined. N.S.= not significant. Values are means±S.E., $n=$ number of separate mammary tissue preparations.

were similar. $T_{sec}$ values for mono-dispersed MEC were significantly different from both tissue slice and acini preparations. Overall rates of $^{14}$C-lactose synthesis and secretion by acini and mono-dispersed cell preparations are similar (Table 1, Figs. 3, 4), ruling out possible damage to cellular transport and other metabolic processes supporting the synthesis and release of lactose. Both preparations also maintained similar intracellular levels of $^{14}$C-lactose (Figs. 3, 4), suggesting that intracellular compartments involved in secretory transport of lactose were functioning comparably.

**DISCUSSION**

In another report (FOSTER, 1979), it was suggested that the interval between the first appearance of $^{14}$C-lactose in the cell and its appearance in the incubation medium, i.e., the $T_{sec}$ value, represents the maximal requisite time for the intracellular transport of lactose between its sites of synthesis and secretion. The linear accumulation of cellular $^{14}$C-lactose was found to reflect the progressive increase...
in cell lactose specific activity and the overall kinetics of $^{14}$C-lactose flux provided evidence of a vectorial transport of lactose in a linear, non-mixing, assembly-line manner from its site of synthesis, the Golgi apparatus, to its site of secretory release at the apical cell membrane (Foster, 1979).

The results presented here suggest that junctional contacts between adjacent MEC are supportive in the secretion of lactose by the mammary epithelium. Disruption of all epithelial cell-cell junctional complexes by collagenase results in destruction of the three-dimensional acinar morphology and subsequent dispersal of constituent MEC. This disruption is accompanied by a distinct increase in the interval ($T_{sec}$) for the intracellular transport of lactose.

This change is not explained by gross damage to the cell membranes or by disturbances in key metabolic processes since the rate of lactose synthesis and the integrity of the exocytotic release mechanisms at the cell periphery appear similar between acini and mono-dispersed MEC. It is unlikely that an increased $T_{sec}$ value originated from direct, albeit unknown, detrimental actions of collagenase on mono-dispersed MEC since acini preparations also received identical collagenase exposures. Additionally, $T_{sec}$ values for tissue slices, preparations which were not exposed to collagenase or subsequent preparative filtrations, were not significantly different from $T_{sec}$ values for collagenase-treated acini.

The manner in which junctional complexes between secretory MEC support the intracellular transport of lactose is not clear. The present study does not indicate whether an increase in the $T_{sec}$ value reflects a decreased rate of intracellular transport or lengthened or perturbed routes of transport. It is clear, however, that junctional contacts between MEC must play a secondary role in lactose synthesis and secretion since both processes continue in disjuncted, mono-dispersed MEC.

It is generally considered that lactose is transported to the cell periphery in Golgi apparatus-derived secretory vesicles, along with certain milk proteins, notably caseins and $\alpha$-lactalbumin, water and several inorganic electrolytes (Linzell and Peaker, 1971a). The cellular mechanisms governing secretory vesicle formation and transport are little understood. Ollivier-Bousquet and Denamur (1973) report an acceleration of intracellular casein secretion, i.e., increased $T_{sec}$ value, following treatment of rabbit and ewe mammary tissue in vitro with theophylline, an inhibitor of cyclic AMP phosphodiesterase, and dibutyryl cyclic AMP. This finding, however, appears not to be the case for the intracellular transport of lactose (Foster, unpublished observations), although lactose synthesis and hence the ultimate rate of appearance via secretion of lactose in the incubation medium or milk is markedly inhibited by theophylline and/or cyclic AMP (Loizzi et al., 1975; Foster, unpublished observations).

Microtubules are prevalent structures in MEC, notably in the apical regions (Franke et al., 1976; Pitelka et al., 1969). Franke et al. (1976) report that casein-containing vesicles, which presumably co-transport lactose, are closely associated
with microtubules. They propose (FRANKE et al., 1976) that secretory vesicles are both structurally and functionally associated with these elements in such a manner as to provide mobility and/or guide elements for vectorial translocation of the vesicles. This relationship appears supported by the uncoupling of secretion from synthesis of casein (OLLIVIER-BOUSQUET and DENAMUR, 1973), lactose (GUERIN and LOIZZI, 1978) and overall milk secretion (PATTON, 1974, 1976) by colchicine, an inhibitor of tubulin polymerization into microtubules (BORISY and TAYLOR, 1967) and by vincristine, which induces formation of highly ordered tubulin paracrystals (BRYAN, 1971).

It is possible that the network of microtubules which is presumed to be involved in facilitating milk secretory vesicle transport are either directly or secondarily associated with specific junctional complexes such that disruption of these junctions disorganizes the microtubular network. Likewise, secretory vesicle formation and vectorial transport or the microtubular system itself may rely upon a particular arrangement or order of other cytoplasmic structures which is lost when coupled cells are dispersed.

It remains to be determined whether loss of the acinar morphology through disruption of cell-cell junctional contacts also modifies the kinetics of intracellular transport of other milk constituents. Of particular interest here would be the transport of caseins and α-lactalbumin, milk proteins presumably co-transported with lactose (LINZELL and PEAKER, 1971 a) and of milk triglycerides, whose routes of intracellular transport and mechanism of release from the cell are qualitatively distinct from lactose (LINZELL and PEAKER, 1971 a).

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