Concerning the Necessary Coupling of Development to Proliferation of Mouse Mammary Epithelial Cells*

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SUMMARY

The epithelial cells from mammary glands of mature virgin mice synthesize increasing levels of casein and α-lactalbumin when cultured in the presence of insulin, hydrocortisone, and prolactin. When DNA synthesis is blocked by the addition of cytosine arabinoside or 5-fluorodeoxyuridine, augmented milk-protein production does not occur. The effects of cytosine arabinoside are prevented by simultaneous addition of deoxycytidine. Neither cytosine arabinoside nor 5-fluorodeoxyuridine have a postmitotic inhibitory effect on casein or α-lactalbumin synthesis. The insulin-induced increase in the combined activities of the intracellular proteins glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase is not affected by either inhibitor.

Such a necessary coupling of secretory protein production to DNA synthesis or mitosis or both no longer exists in explants from midpregnant mice. Most of the epithelial cells of this tissue have already undergone their "critical mitosis" in vivo.

Freshly isolated mammary gland tissue from mice in the middle of their first pregnancy has the ability to make low levels of the characteristic secretory proteins casein (1, 2) and α-lactalbumin (the B-protein of the lactose synthetase system (3-5)). When explants from this tissue are cultured in the presence of insulin, hydrocortisone, and prolactin, they are stimulated to undergo DNA synthesis (2), develop rough endoplasmic reticulum (6), and make increased amounts of the secretory proteins (2, 3, 7). The peak of synthesis of these agents is reached within 48 hours after explantation (8), and 24 hours after the rate of DNA synthesis has reached its peak (2). This relationship prompted investigators to postulate the necessary coupling of biochemical differentiation in this system to DNA synthesis and cell proliferation (2, 3, 9, 10). This general concept was proposed by Holtzer (11) for the differentiation of chondroblasts and myoblasts. However, the previous studies designed to examine this point in the mammary gland system employed either colchicine (2, 3, 10), hydroxyurea (10), or androgens (9) as inhibitors of cell proliferation, and are subject to closer scrutiny.

Colchicine prevents the increase in casein (2, 10) and α-lactalbumin (3) synthesis which is induced by I, F, and P in midpregnancy explants. However, colchicine arrests cells in metaphase, and some mitotic cells have a limited capacity to make RNA and proteins (12, 13). The inability of colchicine-blocked mammary epithelial cells to make casein and α-lactalbumin in response to the hormones, therefore, may not reflect a requirement for cell proliferation. Androgens, in the presence of I, F, and P, have also been shown to inhibit DNA synthesis and the subsequent increase in casein production by midpregnancy mammary explants (9). However, the present report demonstrates that α-methyltestosterone inhibits casein synthesis even when added to postmitotic cells. Similar effects have been observed with hydroxyurea. Thus, the conclusion that the development of midpregnancy, mouse mammary epithelial cells is necessarily coupled to their proliferation is not justified.

Consequently a critical re-examination of the role of DNA synthesis or mitosis or both in mammary gland differentiation was initiated. For these studies, cytosine arabinoside and 5-fluorodeoxyuridine were employed as inhibitors of DNA synthesis. The concentrations of these agents were carefully titrated to minimize cell death. As a result, the inhibitors have minimal side effects in this system.

The possible role of a "critical mitosis" in the differentiation of mammary epithelial cells from both midpregnant and mature virgin mice was examined. In contrast to glands from pregnant mice, mammary tissue from mature virgin animals is developmentally quiescent. Epithelial cells freshly isolated from this tissue are insulin-insensitive (14), make very little casein (1, 2), and have barely detectable B-protein activity (6). However, when cultured in the presence of I, F, and P these cells acquire insulin-sensitivity (14), make DNA (2, 14), and generate daughter cells competent to synthesize both casein (2, 14), and α-lactalbumin in response to the hormones, therefore, may not reflect a requirement for cell proliferation.
8) and α-lactalbumin (8). This report shows that the development of the capacity to make the secretory proteins is, indeed, necessarily coupled to DNA synthesis or mitosis or both in mammary explants from virgin mice. In contrast, the ability of explants from midpregnant animals to make casein and α-lactalbumin no longer depends on a critical mitotic event in vitro.

**Materials and Methods**

**Chemicals**—Crystalline beef insulin was a gift from the Eli Lilly Co. Ovine prolactin was a gift from the National Institute of Arthritis and Metabolic and Digestive Diseases, National Institutes of Health. Crude collagenase was a product of Worthington Biochemicals Corp. UDP-[14C] galactose (298 mCi per mm) and [14C]-labeled L-amino acid mixture (1 mCi/0.67 mg) were purchased from New England Nuclear. Carrier-free 32P1 orthophosphate and bovine casein (Hammersten) were obtained from Schwarz-Mann. UDP-galactose, N-acetylglucosamine, ATP, bovine serum albumin, 5-fluorodeoxyuridine, glucose 6-phosphate, deoxyctydine, and NADP were purchased from Calbiochem. Cytosine arabinoside was obtained from the Cyclo Chemical Corp. and Upjohn Co.

**Organ Culture Method**—Female mice 10 to 12 days into their first pregnancy, and virgin mice 3 to 4 months old were used. All mice were of the C3H/HeN strain. Mammary glands were removed with aseptic technique and cultured as described previously (7). Explants were cultured in Medium 199 (Microbiological Associates) containing combinations of insulin, hydrocortisone, and prolactin at a final concentration of 5 μg per ml each. The culture medium was changed at least every 48 hours.

**Casein Biosynthesis**—Casein synthesis was measured by incorporation of 32P1 or 14C-amino acids as indicated. After homogenization of the tissue, casein in the 105,000 × g supernatant was precipitated with calcium ions and remin in the presence of carrier casein as described previously (7).

**Lactose Synthetase (EC 2.4.1.28) Activity**—Enzymatic activity in mammary tissue was determined as described previously (8). The lactose synthetase reaction is linear for 30 min. Assays were run at 37° for 15 or 30 min. All data are expressed as picomoles of product (lactose) formed per mg wet weight tissue per 15 or 30 min, and are corrected for production of [14C]galactose by endogenous enzymatic hydrolysis of UDP-[14C] galactose.

**Isolation of Epithelial Cells (DNA Content and Cell Number)**—Epithelial cells were isolated by treatment with collagenase in Medium 199 as described previously (14). Cells to be counted were further treated with 0.5% crude tissue culture trypsin (Gibco) as described previously (8) and counted in a hemocytometer.

DNA content of cells isolated after collagenase treatment was determined by the Burton (15) diphenylamine method.

**Glucose 6-phosphate Dehydrogenase (EC 1.1.1.49) and Glucose 6-phosphate Dehydrogenase (EC 1.1.1.49) Activities**—Epithelial cells collected after collagenase treatment were homogenized in 0.01 M Tris-HCl pH 7.6 containing 0.15 M KCI, and centrifuged at 12,000 × g for 15 min. The supernatant was assayed for the combined dehydrogenase activities spectrophotometrically by determining the rate of reduction of NADP at 340 nm (10). The data are expressed as the change in absorbance at 340 nm per min per mg weight of tissue.

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**Results**

Epithelial cell differentiation in explants from mature virgin mammary glands, represented by an increase in the rate of synthesis of casein and the production of α-lactalbumin, occurs upon addition of insulin, hydrocortisone, and prolactin to the chemically defined medium (2, 8). The peak of casein synthesis occurs after 72 hours of culture, while α-lactalbumin reaches its peak 24 to 48 hours later (8). This increase in secretory protein production is preceded by an increased rate of DNA synthesis which, after an initial 24-hour lag, approaches its maximum after 48 hours in culture (2). When this increase in DNA synthesis is prevented by the presence of the inhibitors cytosine arabinoside and fluorodeoxyuridine, the subsequent development of milk-protein production is blocked.

Table I shows that ara-C at a concentration of 15 μg per ml completely inhibits DNA synthesis for at least 72 hours in the presence of I, F, and P. The increase in the rate of casein synthesis seen after 72 hours in the presence of the three hormones is almost totally blocked by this agent. Delaying the addition of ara-C for 24 hours does not diminish its effect on DNA or casein synthesis, because during this initial 24-hour interval, virgin mammary epithelial cells do not make a significant amount of DNA (14). Delaying the addition of the inhibitor yet another 24 hours, until a significant amount of DNA synthesis has occurred, results in a partial decrease of both total DNA content and casein production. However, when the casein synthesis is expressed in terms of DNA content, it becomes apparent that those cells formed in the 24- to 48-hour interval of culture respond fully to the hormonal stimuli.

The possibility that the effects of ara-C in this system are unique to this agent and result from its general toxicity is rendered improbable by experiments which employ another

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**Table I**

Effect of inhibitors of DNA synthesis on casein synthesis in explants from mature virgin mice

Mammary explants from mature virgin mice were cultured in the presence of insulin, hydrocortisone, and prolactin (IFP) for 72 hours. Cytosine arabinoside (ara-C, 15 μg per ml) or fluorodeoxyuridine (FUdR; 25 μg per ml) was added at the times indicated. Casein synthesis was determined by pulsing with 10 μCi of carrier-free 32P1, from 0 to 4 hours or from 68 to 72 hours in culture. DNA content of epithelial cells was determined as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Casein</th>
<th>Epithelial DNA per mg wet weight tissue</th>
<th>Δ Casein per mg of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr 72 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFP plus 15 μg of ara-C per ml added at 0 hr</td>
<td>350 ± 44</td>
<td>99.6</td>
<td>2.95</td>
</tr>
<tr>
<td>IFP plus 15 μg of ara-C per ml added at 24 hrs</td>
<td>79 ± 3</td>
<td>44.0</td>
<td>0.52</td>
</tr>
<tr>
<td>IFP plus 15 μg of ara-C per ml added at 48 hrs</td>
<td>88 ± 4</td>
<td>43.6</td>
<td>0.73</td>
</tr>
<tr>
<td>0 hr 72 hrs</td>
<td>236 ± 27</td>
<td>65.4</td>
<td>2.75</td>
</tr>
<tr>
<td>IFP</td>
<td>109 ± 5</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>IFP plus 25 μg of FUdR per ml added at 0 hr</td>
<td>439 ± 23</td>
<td>50.8</td>
<td>6.50</td>
</tr>
<tr>
<td>IFP plus 25 μg of FUdR per ml added at 24 hrs</td>
<td>120 ± 6</td>
<td>31.4</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Mammary explants from mature virgin mice were cultured in the presence of insulin and hydrocortisone (IF) or insulin, hydrocortisone, and prolactin (IFP) for 72 hours. Cytosine arabinoside (ara-C) or deoxyctytidine (CdR) or both were added at the time of explantation. Casein synthesis and accumulation were determined by a continuous 72-hour exposure of the explants to a 14C-amino acid mixture at 0.25 μCi per ml. Epithelial cell number was determined as described under "Materials and Methods." 

Inhibitor of DNA synthesis, fluorodeoxyuridine, and by the use of deoxy ytidine, a structural analogue of ara-C. As seen in Table I, FUDR, at 25 μg per ml, also completely suppresses DNA synthesis during 72 hours of exposure to I, F, and P. Moreover, the increased rate of casein synthesis seen after 72 hours in the presence of the hormones is blocked by FUDR. Table II shows that the simultaneous addition of CdR (120 μg per ml) to cultures containing ara-C prevents the effect of this agent on cell proliferation. Similarly, the synthesis and accumulation of casein during 72 hours of exposure to the hormones is no longer suppressed.

Virgin mammary explants also make the secretory protein α-lactalbumin, the B-protein of the lactose synthetase system, when cultured in the presence of I, F, and P. This activity, which is low or undetectable in freshly isolated tissue, begins to emerge after 96 hours in culture and reaches a maximum after 120 hours (8). Table III shows, however, that B-protein activity fails to emerge when DNA synthesis is blocked by the addition of either ara-C or FUDR at the time of explantation.

Table III also shows that, as with casein synthesis, delaying the addition of ara-C until 24 hours after explantation does not affect the ability of this agent to suppress the later emergence of B-protein activity. In all cases the A-protein of the lactose synthetase system is present in sufficient quantities for detection of B-protein (8).

In explants from mature virgin mice, DNA synthesis generally reaches its maximum between 48 and 72 hours of culture in the presence of I, F, and P (2). At this time, B-protein activity is still low or undetectable (8). If the inhibitors are added at 72 hours, however, the activity of B-protein at 120 hours is not affected.

The requirement for DNA synthesis does not relate to the synthesis of all hormonally-induced proteins in the virgin mammary gland. The formation of the nonsecretory, intracellular proteins, glucose 6-phosphate dehydrogenase and glucosone 6-phosphate dehydrogenase is not dependent on DNA synthesis (Table IV). The combined activities of these enzymes have been shown to increase in mature virgin explants in the presence of insulin after an initial lag of 24 hours (14). Table IV shows that when DNA synthesis is completely blocked by the addition of either ara-C or FUDR to explants cultured in the presence of insulin, in combination with F and P, the increase in enzyme activity per cell after 72 and 96 hours is virtually unaffected. Thus, the parent cells in the presence of ara-C or FUDR can develop insulin-sensitivity, and are then capable of producing elevated levels of the combined dehydrogenases in response to insulin.

Taken together, the preceding data support the concept that development of the capacity to manufacture the secretory proteins, casein and α-lactalbumin, is necessarily coupled to DNA synthesis in mature virgin explants.
mitosis in pregnancy mice is largely independent of DNA synthesis and the development of the secretory proteins in explants from mid-pregnant mice, while on a per cell basis, the level of B-protein activity in the presence of I, F, and P' and ara-C is only slightly smaller than inhibition of total B-protein activity after 48 hours in culture, the complete inhibition of DNA synthesis results in a 40 to 50% level of casein synthesis is unaffected (Table VI). Similarly, proliferation is achieved, the synthesis and accumulation of synthesis in vitro. Even when the complete inhibition of cell proliferation during a prior exposure to I and F (18) is added at the time of explantation. Casein synthesis and DNA content were determined as described in the legend to Table I. Lactose synthetase B-protein activity was determined as described under "Materials and Methods." Table IV

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Combined G-6-P-D and Gl-6-P-D activities</th>
<th>Epithelial DNA per mg wet tissue</th>
<th>Enzyme activity per mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>D.O.D. 1.0 X IP/min/mg wet wt tissue</td>
<td>ng</td>
<td>0.010</td>
</tr>
<tr>
<td>72 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFP</td>
<td>1.38</td>
<td>44.3</td>
<td>0.031</td>
</tr>
<tr>
<td>IFP plus 15 µg of ara-C per ml</td>
<td>0.02</td>
<td>31.2</td>
<td>0.020</td>
</tr>
<tr>
<td>0 hr</td>
<td>0.33</td>
<td>52.6</td>
<td>0.0063</td>
</tr>
<tr>
<td>96 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFP</td>
<td>1.20</td>
<td>98.8</td>
<td>0.0121</td>
</tr>
<tr>
<td>IFP plus 25 µg of FUdR per ml</td>
<td>0.55</td>
<td>52.6</td>
<td>0.0105</td>
</tr>
</tbody>
</table>

In contrast to the mature virgin system, epithelial cells in mammary explants from mice in the middle of their first pregnancy make DNA in response to insulin during the first 24 hours of culture (2, 14). Both casein and α-lactalbumin reach their peak after 24 hours of culture in the presence of I, F, and P (8) (24 hours after the peak of insulin-stimulated DNA synthesis (2)). Using ara-C it is possible to show that the synthesis of casein (Table VI) and the increase in B-protein activity (Table VII) in this system are not necessarily coupled to DNA synthesis in vitro. Even when the complete inhibition of cell proliferation is achieved, the synthesis and accumulation of casein during 72 hours of culture in the presence of I, F, and P is only inhibited by 42%. Actually, on a per cell basis, the level of casein synthesis is unaffected (Table VI). Similarly, the complete inhibition of DNA synthesis results in 40 to 60% inhibition of total B-protein activity after 48 hours in culture, but on a per cell basis, the level of B-protein activity in the presence of I, F, and P and ara-C is only slightly smaller than that in the presence of the hormones alone (Table VII). Thus, the development of the secretory proteins in explants from mid-pregnant mice is largely independent of DNA synthesis and mitosis in vitro.

Table V

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Epithelial DNA per mg wet weight tissue</th>
<th>Casein A Casein per mg of DNA</th>
<th>Lactose synthetase B-protein activity</th>
<th>B-protein activity per mg of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>D.O.D. 1.0 X IP/min/mg wet wt tissue</td>
<td>ng</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>72 hrs</td>
<td></td>
<td>33.7</td>
<td>106 ± 9</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>IFP</td>
<td>76.7</td>
<td>333 ± 26</td>
<td>2.96</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>IFP plus 25 µg of FUdR per ml</td>
<td>32.1</td>
<td>90 ± 6</td>
<td>0</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>120 hrs</td>
<td></td>
<td>57.5</td>
<td>51 ± 4</td>
<td>0.904</td>
</tr>
<tr>
<td>IFP</td>
<td></td>
<td>35.6</td>
<td>4 ± 2</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Table VI

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Casein</th>
<th>Epithelial cell number</th>
<th>Casein per 10^9 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>cpn/mg wet wt tissue</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>72 hrs</td>
<td>IFP</td>
<td>193</td>
<td>2.13</td>
</tr>
<tr>
<td>IFP</td>
<td>1584</td>
<td>919</td>
<td>1.10</td>
</tr>
</tbody>
</table>

As shown in Table VIII, ara-C has neither an inhibitory nor super-inductive effect on casein synthesis when it is added with prolactin to cells, most of which already have completed their replication during a prior exposure to I and F (18). Similarly, ara-C has no postmitotic inhibitory effect on B-protein activity. However, α-methyltestosterone, at a concentration used previously (9) to inhibit DNA synthesis during a 24-hour interval, inhibits casein synthesis by 50% even when added postmitotically. Similar postmitotic inhibitory effects were obtained with hydroxyurea at a concentration of 40 µg per ml.
of the nonsecretory proteins glucose 6-phosphate dehydrogenase
addition, the insulin-induced increase in the combined activities
on DNA and casein syntheses can be prevented by the simul-
ous addition of the structural analogue deoxycytidine. In
on the observation that the inhibitory effects of ara-C
hibitors of DNA synthesis. The effects of these agents in this
system do not result from a general toxicity. This conclusion
sensitive to cytosine arabinoside and 5-fluorodeoxyuridine, in-
tivity, casein, and α-lactalbumin, in mammary gland explants
scribed in the legend to Table I. Data shown are representative
several experiments.

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Lactose synthetase B-protein activity</th>
<th>Epithelial cell number</th>
<th>B-protein activity per 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g mol product formed/mg wet wt tissue/15 min</td>
<td>X 10^4 cells/mg wet wt tissue</td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>10</td>
<td>4.06</td>
<td>2.14</td>
</tr>
<tr>
<td>48 hrs</td>
<td>IFP</td>
<td>73</td>
<td>7.18</td>
</tr>
<tr>
<td></td>
<td>IFP plus 45 μg of ara-C per ml</td>
<td>41</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>IFP plus 90 μg of ara-C per ml</td>
<td>30</td>
<td>3.70</td>
</tr>
</tbody>
</table>

**TABLE VIII**

*Effect of ara-C and α-methyl testosterone on casein synthesis in postmitotic cells of midpregnancy explants*

Mammary explants from mice in the middle of their first pregnancy were cultured in the presence of insulin, hydrocortisone, and prolactin (IFP) for 48 hours. Cytosine arabinoside (ara-C) was added at the time of explantation. Lactose synthetase B-protein activity and epithelial cell number were determined as described under "Materials and Methods." Data shown are representative of several experiments.

<table>
<thead>
<tr>
<th>Second incubation conditions</th>
<th>Casein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/mg wet wt tissue/4 hrs</td>
</tr>
<tr>
<td>0 hr, IFP</td>
<td>45</td>
</tr>
<tr>
<td>72 hrs</td>
<td>42</td>
</tr>
<tr>
<td>IF</td>
<td>225</td>
</tr>
<tr>
<td>IFP</td>
<td>210</td>
</tr>
<tr>
<td>IFP plus 30 μg of ara-C per ml</td>
<td></td>
</tr>
<tr>
<td>0 hr, IFP plus α-methyl testosterone</td>
<td>71 ± 1</td>
</tr>
<tr>
<td>(2 x 10^-3 M)</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>24 hrs</td>
<td>IFP</td>
</tr>
<tr>
<td>IFP</td>
<td>271 ± 18</td>
</tr>
<tr>
<td>IFP plus α-methyl testosterone</td>
<td>123 ± 4</td>
</tr>
<tr>
<td>(2 x 10^-3 M)</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The development of the capacity to synthesize the milk-proteins, casein, and α-lactalbumin, in mammary gland explants from mature virgin mice is necessarily coupled to critical events sensitive to cytosine arabinoside and 5-fluorodeoxyuridine, inhibitors of DNA synthesis. The effects of these agents in this system do not result from a general toxicity. This conclusion is based on the observation that the inhibitory effects of ara-C on DNA and casein syntheses can be prevented by the simultaneous addition of the structural analogue deoxycytidine. In addition, the insulin-induced increase in the combined activities of the nonsecretory proteins glucose 6-phosphate dehydrogenase and glucuronate 6-phosphate dehydrogenase, which has been shown to require RNA and protein synthesis (19), is not affected. Also ara-C and FUdR do not affect the emergence of B-protein activity, when added after 72 hours of culture in the presence of I, F, and P, even though transcriptional events are required during this time (8). This also indicates that even though some DNA synthesis occurs in the explants after 72 hours, the cells formed after this time do not contribute to the expression of B-protein activity at 120 hours. In effect, delaying the addition of ara-C or FUdR until 72 hours of culture (before the emergence of B-protein activity) represents a postmitotic control for these B-protein-producing cells. This is similar to the postmitotic test for ara-C in midpregnancy explants (Table VIII).

It was conceivable that ara-C and FUdR inhibit casein and α-lactalbumin production by preventing the virgin epithelial cells from acquiring insulin-sensitivity, as well as by preventing DNA synthesis itself. However, when present from the onset of culture, these agents do not inhibit the increase in the combined dehydrogenase activities even though this increase also requires the acquisition of insulin-sensitivity (14). Also, delaying the addition of the inhibitors for 24 hours (until after insulin-sensitivity is acquired (14)) gives the same results with respect to casein and α-lactalbumin production as does their addition at the time of explantation.

Thus, it appears that critical events occurring between 24 and 72 hours of culture in the presence of I, F, and P are essential to the development of the secretory character of epithelial cells in mature virgin explants. During this 48-hour interval, DNA synthesis and mitosis occur (2). In contrast to mature virgin explants, the development of the secretory proteins in midpregnancy explants appears to be virtually independent of DNA synthesis in vitro. This does not, however, invalidate the concept of a critical mitosis in mammary gland development. It has been shown that by midpregnancy, the epithelial cells in the mouse mammary gland have undergone extensive DNA synthesis and cell division in vitro (17). This may represent the critical mitosis necessary for further development. As a result, most epithelial cells in freshly isolated midpregnancy mammary glands no longer require DNA synthesis in vitro in order to be competent to synthesize casein and α-lactalbumin in response to I, F, and P.

This conclusion is contrary to that of previous studies which postulated a necessary coupling of milk-protein production to DNA synthesis or mitosis or both in midpregnancy explants. This is probably due to the nature of the inhibitors used in these experiments. In the previous studies colchicine (2, 3, 10), hydroxyurea (10), and androgens (9) were used to block cell proliferation. However, since some metaphase cells have a limited capacity to make RNA and proteins (12, 13) it may not have been possible for the colchicine-blocked cells to respond to the hormones by making casein or α-lactalbumin. The conclusion drawn from studies in which androgens or hydroxyurea were added to midpregnancy explants is also unjustified. Both of these agents inhibit casein synthesis even when added to explants postmitotically.

FUdR and ara-C, on the other hand, do not have these side effects in the mammary gland system. Since there is no evidence that either ara-C (20) or FUdR (21) are incorporated into DNA, the possibility that these agents lead to the formation of faulty messenger-RNAs for the secretory proteins seems highly unlikely. Rather, these two agents are thought to act by specifically inhibiting DNA synthesis. While the mechanism
of action of ara-C is not fully understood, it is thought to act via its triphosphate, by inhibiting the DNA polymerase reaction itself (20, 22). FUdR acts by inhibiting thymidylate synthetase (21). In both cases, the majority of cells are caught at the G1 → S interval of the cell cycle. Thus, in mature virgin explants, DNA synthesis and the subsequent production of competent daughter cells via a critical mitosis, is prohibited. In midpregnancy explants, the cells also are trapped at the entrance to the S-phase, but having already undergone their critical mitosis in vivo, they can, nevertheless, synthesize casein and ß-lactalbumin in response to I, F, and P.

Mitoses in the intact animal prior to the onset of pregnancy are “noncritical.” Even though the mammary epithelium in immature mice undergoes extensive proliferation in vivo (23), the resulting daughter cells (i.e. the epithelial cells of mature virgin mammary glands) still must undergo a critical mitosis before they can develop the ability to synthesize casein and ß-lactalbumin. However, critical mitoses by the epithelial cells in mammary tissue from immature mice can be effected in vitro in the presence of I, F, and P (23).

It is not known whether it is DNA synthesis alone or events occurring during mitosis which are necessary for the development of the capacity to synthesize the milk-proteins in mammary epithelial cells. Nor is it understood why this method of control is operative. The elucidation of these points awaits further study.

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Wasser, and ARON A. MOSCONA. Isolation and Charac-
terization of Glutamine Synthetase from Chicken Neural
Retina.

Page 7745, Paragraph 1 under “Molecular Weight of Native
Enzyme,” Line 4 should read:

(0.375, 0.55, and 0.75 mg per ml) in 0.1 M phosphate buffer,

Vol. 248 (1973) 528–533

In YU, C. A., L. YU, and TsOo E. King. Kinetics of Electron
Transfer between Cardiac Cytochrome c1 and c.

Page 531, left-hand column, Lines 9, 11, and 22, \(10^5\)
M\(^{-1}\) s\(^{-1}\) should read:

\(10^6\) M\(^{-1}\) s\(^{-1}\). In all other places, including the Summary, the
data are correctly printed.

Vol. 248 (1973) 472–477

In OWENS, IDA S., BARBARA K. VONDERHAAR, and YALE J.
TOPPER. Concerning the Necessary Coupling of Develop-
ment to Proliferation of Mouse Mammary Epithelial Cells.

Page 475, Table VI, Column 4, the heading should read:

\(\Delta\) Casein per 10^4 cells.

Due to a mishap in printing \(\Delta\) was omitted.

We suggest that subscribers photocopy these corrections and insert the photocopies at the
appropriate places where the article to be corrected originally appeared. Authors are urged to
introduce these corrections into any reprints they distribute. Secondary (abstract) services
are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Concerning the Necessary Coupling of Development to Proliferation of Mouse Mammary Epithelial Cells
Ida S. Owens, Barbara K. Vonderhaar and Yale J. Topper


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