Hormone-Independent DNA Synthesis by Epithelial Cells of Adult Human Mammary Gland in Organ Culture[1] (37323)

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(Introduced by D. H. Moore)

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The technique of organ culture for studying controls of cell behavior in the normal mammary gland has been applied mainly to nonhuman species. Numerous studies have demonstrated the importance of various hormones or other serum factors in regulation of proliferation and function of the epithelial cells (1–5). Similar studies of normal human mammary gland are few in number (6–8) and considerable work remains in determining control factors operative in this system. The present study demonstrates that maintenance of the epithelium and stimulation of DNA synthesis in the human mammary gland *in vitro* is, to a considerable extent, not dependent on hormonal or serum factors, thus emphasizing the importance of species differences when analyzing control mechanisms.

Materials and Methods. The method of dissecting ducts from human mammary glands has been described in detail elsewhere (8). Ducts were obtained from 6 mammary glands removed surgically because of an associated carcinoma. The region of mammary gland utilized included part of the nipple and underlying tissue which were far removed from the cancerous region and determined grossly to be free of disease. Portions of dissected ducts were routinely examined histologically to verify the gross impression. No patients had any history of hormone therapy.

[1] This work was supported by grants No. 1 PO1 CA 11536 from The National Cancer Institute, and No. 1 PO 1 AM 15515 from The National Institute of Arthritis and Metabolic Diseases. Also supported by grant No. CA-08515 from The National Cancer Institute and contract NIH-NCI-G-72-3868.

Isolated ducts, comprising epithelium and underlying connective tissue were opened longitudinally and cut into fragments 3–4 mm^2. The explants were placed epithelial side up on a fine mesh screen in the well of organ culture dishes (Falcon). All tissue was grown in Eagle's minimal essential medium (MEM) under the folowing conditions (a) MEM alone, (b) MEM with 10% fetal calf serum, (c) MEM with crystalline zinc insulin (Lilly), 5 μg/ml, and (d) MEM with bovine prolactin, 5 μg/ml. The stock solution of bovine prolactin (gift of National Institutes of Health) was made by dissolving 10 mg of powder in 4.2 ml phosphate buffered saline. NaOH (0.1 *N*) was added to aid in solubilizing the powder. Culture fluid was renewed every 4 days. Cultures were incubated at 37° in an atmosphere of 5% CO_2 in air.

To study DNA synthesis, tritiated thymidine (^{3}HTdR) (sp act 6.7 Ci/mmole) was added to 7 day cultures at a concentration of 2 μCi/ml for 4 hr. Cultures were then washed 3 times in Gey's balanced salt solution, fixed in 10% buffered formalin, sectioned at 4 μm for autoradiography, dipped in Kodak NTB-2 emulsion, exposed for 2 wk at 4° and developed. They were then stained with hematoxylin.

Results. The epithelium in large and medium-sized ducts prior to culture generally comprised 2 layers of cuboidal cells (Fig. 1). Many specimens also contained very small ducts lying in the connective tissue wall of the larger duct. The epithelium in these ductules also consisted of 1–2 layers of cuboidal cells. Careful searching failed to

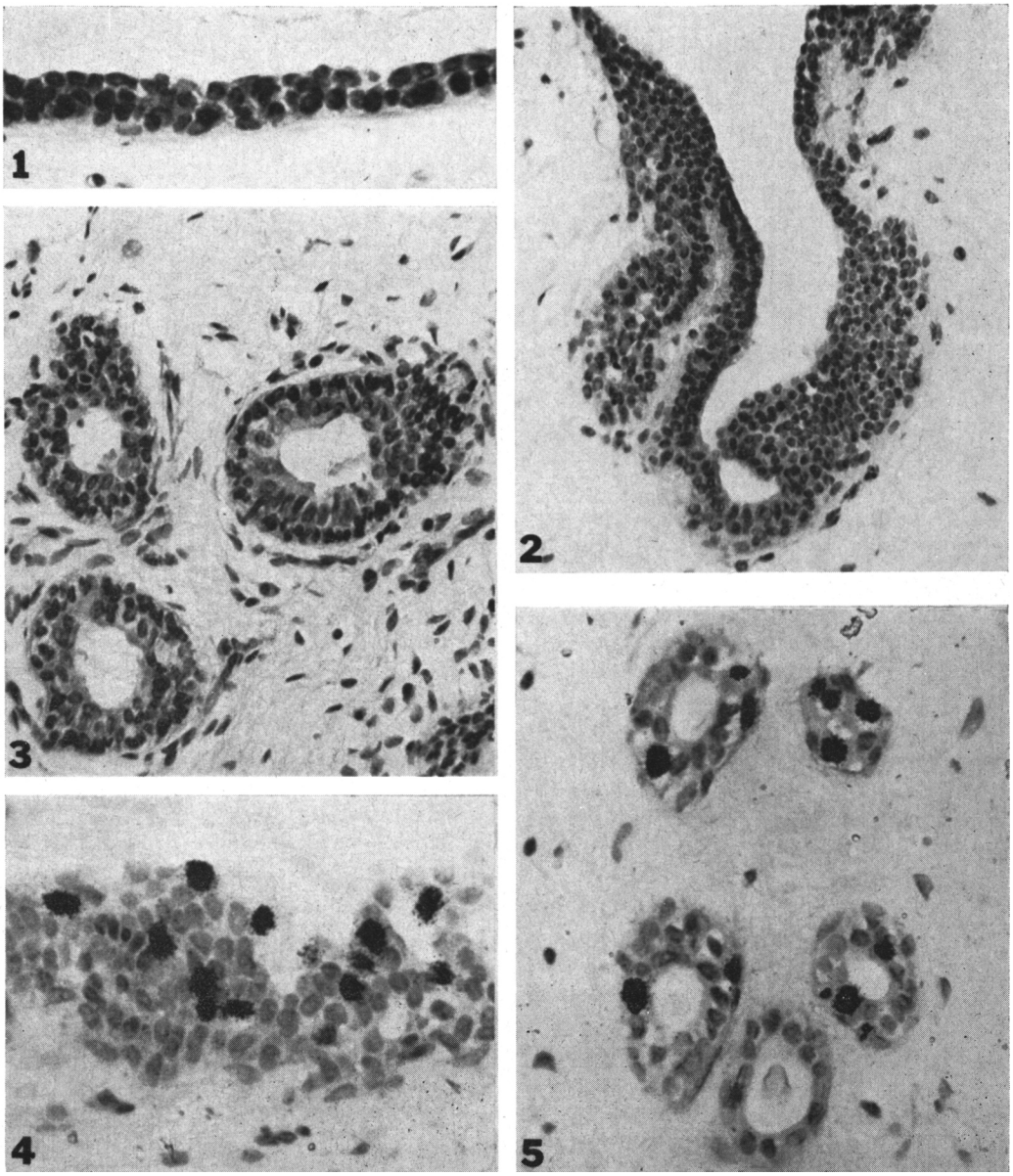

FIG. 1. Human mammary gland epithelium from large duct (uncultured specimen) comprising double layer of cuboidal cells. ×340.

FIG. 2. Organ culture of human mammary gland after 16 days in defined medium. Epithelium of large duct is well maintained and shows increased number of layers when compared with Fig. 1. ×180.

FIG. 3. Organ culture after 16 days in defined medium. Small ducts retain their shape and epithelium is well maintained. Note normal connective tissue cellularity. ×210.

FIG. 4. Epithelium of large duct after 7 days in organ culture in defined medium. Note labeling of numerous nuclei that have incorporated tritiated thymidine during a 4 hr pulse. Autoradiograph. ×500.

FIG. 5. Epithelium of several small ducts after 7 days in defined medium. Labeled nuclei provide evidence of DNA synthesis. Autoradiograph. ×450.

reveal more than a rare mitosis anywhere.

Ducts grown only in chemically defined medium without serum or hormones were sampled at intervals from 4 to 16 days after being placed *in vitro*. Fifty-five pieces were examined and all showed excellent preservation of the entire explant (epithelium and connective tissue), even after 16 days (Figs. 2 and 3). The epithelium of both large and small ducts was intact and continuous along its entire length. It often appeared thicker than in control uncultured material due to an increase in the number of cell layers and height of the individual cells. The underlying connective tissue appeared quite normal in its cellular and collagenous components. Small blood vessels remained intact.

Studies with ^{3}H-TdR were carried out on 7 day cultures in defined medium and showed that during a 4 hr pulse, a significant number of epithelial cells were synthesizing DNA in both large and small ducts (Figs. 4 and 5). Labeled interphase nuclei were found in the cuboidal or columnar cells at all levels, even at the luminal surface. The labeling index of 34/1000 was determined by counting the number of labeled nuclei in a population of 1000 cells or more on each of 6 duct specimens. There was no apparent difference between large and small ducts. Mitoses, although more readily identified than in uncultured material, were not especially numerous and the mitotic index was only 1–3/1000. Some labeled mitoses were found, however, indicating that at least some labeled cells had moved from S phase to mitosis. Labeled nuclei were not found in the connective tissue cells.

Ducts grown in medium containing serum, insulin or prolactin (40–50 explants/group) all showed the same preservation of architecture and epithelial maintenance up to 16 days *in vitro*. There was no readily discernible qualitative difference when compared with ducts grown in defined medium. The labeling index for cultures after 7 days in serum was 45/1000, which was not significantly different from that in defined medium. The labeling index in ducts grown with insulin or prolactin was 69/1000 and 64/1000, respectively, representing a significant increase over that in defined medium. However, a proportional rise in mitotic index was not found, as it remained at 1–3/1000.

Discussion. The dearth of *in vitro* studies of control of proliferation and function in the human mammary gland stands in sharp contrast to innumerable studies on nonhuman species. The main problems that have confronted the investigator studying the human mammary gland are the difficulty in obtaining adequate material and the inability to define the "normal" state of the gland. Thus, experiments become open to the criticism that well-recognized controls do not exist. Such criticism might apply to the present work where so-called "normal" ducts have been dissected from mammary glands with an associated carcinoma. While recognizing the inherent limitations of this system, it still seems important to utilize such material for reference studies.

In the present study, the epithelium of *both* large and small ducts of the adult human mammary gland was maintained in defined medium for up to 16 days. New DNA synthesis and mitosis also occurred in 7 day cultures under these conditions. By contrast, the inability of the epithelium of the adult mouse mammary gland in organ culture to survive and synthesize DNA in defined medium is well known. Many studies have demonstrated that insulin is required for epithelial maintenance and proliferation in the mouse (1–3, 9). In the prelactating gland, addition of insulin results in maintenance of the proliferative state (1, 3, 9, 10, 11). In glands from virgin mice DNA synthesis occurs after an initial lag period (9, 12, 13). Most observations have been made on small ducts and terminal acini. It has been reported, however, that the epithelium of large ducts does survive after several days in defined medium only (2) as does the rudimental ductal tree in the embryonic mouse mammary gland (14). This observation points out the possible importance of both developmental stage and regional differences in epithelial cell response.

Although epithelial maintenance and DNA synthesis by human mammary gland cells was impressive in chemically defined medium

alone, the additional enhancement of DNA synthesis by insulin and prolactin was significant. In 7 day cultures, both hormones caused an elevation in labeling index and thus appear to recruit cells into DNA synthesis in addition to those so stimulated under conditions of growth in defined medium (13). The continued DNA response to insulin after 7 days in culture stands in contrast to the short response in the mouse where, after reaching a peak at 2 days, DNA synthesis rapidly declines and virtually stops by 72 hr (9, 15) even though insulin continues to be present. The effect of prolactin on DNA synthesis seems to vary according to species. Stimulation of DNA synthesis has been reported in the rat (4) but not in the mouse (5). Wellings and Jentoft (7), using ovine prolactin, did not report an effect on survival and proliferation in short-term cultures of human mammary gland. Since bovine prolactin used in the present study did stimulate DNA synthesis, the source of the hormone must also be considered when interpreting data as to its effect or lack thereof.

Failure to find a rise in mitotic index commensurate with the increase in labeling index in the presence of insulin and prolactin might be explained in 2 ways: (a) mitosis is very brief and chances of finding significant numbers of cells without the aid of a metaphase arrest agent are thereby diminished or (b) many cells complete DNA synthesis and enter a prolonged G_2 phase (16). Some cells divide, however, since labeled mitoses were seen.

The relative independence of epithelial maintenance and DNA synthesis by human mammary gland cells demonstrates the importance of species differences and emphasizes that different controls of cell behavior may be operative. Recognition of these differences may be important in elucidating the events of carcinogenesis. While short-term maintenance of the "normal" human mammary gland in defined medium has been reported, no studies of DNA synthesis were performed (6, 7).

Summary. The epithelium of *large and small* ducts from human mammary gland was well maintained for 16 days in organ culture in a chemically defined medium. Significant DNA synthesis and mitosis occurred in the absence of supplemental hormones or serum. Addition of insulin and prolactin, however, did cause an increase in the number of cells synthesizing DNA.

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Received Feb. 1, 1973. P.S.E.B.M., 1973, Vol. 143.