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Glycoprotein Synthesis as a Function of Epithelial Cell Arrangement: Biosynthesis and Release of Glycoproteins by Human Breast and Prostate Cells in Organ Culture

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We demonstrate that a technique is available to investigate glycoprotein synthesis in organ cultures of human breast and prostate surgical specimens where the 3-dimensional epithelial cell arrangement remains intact. Malignant breast and prostate epithelium maintained their capacity to synthesize glycoproteins for at least 3 days as followed by the incorporation of [^{3}H]glucosamine into macromolecules. Over 70% of incorporation was by malignant cells as judged by autoradiography. Labeled glycoproteins were released into glandular lumina and consequently into the culture fluid. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed predominantly one group of macromolecules released with an apparent molecular weight of 48,000 ± 6,000 daltons. This glycoprotein was found in all of the breast specimens studied, which included 1 medullary, 1 infiltrating lobular, and 8 infiltrating duct carcinomas. The pattern was independent of the availability of estrogen receptors. A similar glycoprotein was also observed in the culture media from a Grade I and a Grade II well-differentiated infiltrating prostate carcinoma. Incorporation was below the level of detection in 4 of 6 cases of benign prostatic hyperplasia. A more complex pattern of labeled glycoproteins was found in the media of a Grade II and a Grade III poorly-differentiated prostate carcinoma. The established human mammary carcinoma cell line MCF-7 synthesized and released a similar 48,000 molecular weight glycoprotein but additional components with larger molecular weights were also released. An intriguing interpretation that 3-dimensional tissue integrity restricts some glycoprotein synthesis is discussed. Cells grown in 2-dimensional monolayers could escape from such a topographic restriction and express additional families of glycoproteins.

Key words: breast, prostate, carcinoma, glycoproteins, organ culture

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Human breast and prostate epithelial cells are arranged in vivo in 3-dimensional glandular structures. Glycoproteins are important for organ-specific cell aggregation, for cell-cell recognition (1), for cell surface antigenicity (2), and may play an important role in the arrangement of 3-dimensional cell structures (3). Therefore conditions must be established for studying their synthesis and turnover where the 3-dimensional arrangement of cells is maintained. In organ cultures tissue integrity is largely unaltered, therefore biosynthetic events can be followed in vitro, under conditions which resemble the in vivo environment.

Cancer cells derived from secretory cell types appear to retain secretory capacity, but to date no complete study is available on the molecular nature of glycoproteins secreted by benign hyperplastic prostatic epithelium and by various grades of malignant prostatic or breast epithelium. These glycoproteins are particularly important since they may be markers responsible for immune recognition (4) and in their released form or in combination with their corresponding antibodies (5) could block immune cytotoxicity. Mammary glands from pregnant mice maintained in organ culture have already been shown to be a good model system for studying the hormone-dependent biosynthesis and secretion of proteins (6, 7). Two recent studies, one autoradiographic (8) and the other biochemical (9), used organ cultures of human breast carcinoma to show that the malignant cells retained biosynthetic and secretory capacity. Glycoprotein synthesis by epithelial cells is under hormonal regulation (10) and it is probably influenced by the degree of cell differentiation and the 3-dimensional arrangement of cells as well. Although prostate and breast epithelial cells can be maintained in cell culture using fetal calf serum and various hormones (11, 12), extrapolation to an in vivo glycoprotein-synthesizing activity must be undertaken with caution.

We have investigated the feasibility of using human prostate and breast surgical specimens in short-term organ cultures. The present report describes the biochemical nature of [^{3}H]glucosamine-labeled material released in these organ cultures and from the established human breast carcinoma cell line MCF-7 maintained in a 2-dimensional monolayer culture.

MATERIALS AND METHODS

Organ Cultures

Tumor samples were obtained from 10 mastectomy and 11 prostatectomy specimens under sterile conditions within 15 min after completion of surgery. Adjacent tissue was fixed in formalin for diagnostic purposes. Light microscopy revealed that 8 of the breast tumors were infiltrating duct carcinomas, exhibiting varying degrees of differentiation. The other 2 were a medullary and an infiltrating lobular carcinoma. In 6 prostate specimens, cut surfaces revealed pale hyperplastic nodular areas, portions of which were selected for this study. Microscopic examination revealed nodular and fibroglandular hyperplasia without any evidence of carcinoma. In the other 5 prostate specimens, cut surfaces revealed firm, yellow carcinomatous areas, portions of which were selected. The microscopic diagnosis was Grade I, well-differentiated infiltrating adenocarcinoma in 2 of these cases; Grade II, moderately well-differentiated infiltration adenocarcinoma in 2 cases; and Grade III, poorly-differentiated infiltrating adenocarcinoma in 1 case.

Tissue samples were cut into approximately 1-mm cubes and placed on steel grids in 60 × 15-mm petri dishes (Falcon Plastics, Oxnard, California) containing 5 ml of media

which reached to the upper surface of the explants. Usually 0.5 g of tumor was sliced into more than 50 cubes per incubation. The cultures were carried out at 37°C in a water-saturated atmosphere of 95% air:5% CO_2. Medium 199 (Flow Laboratories, Rockville, Maryland), supplemented with 125 mg/100 ml of glucose, 2 mM glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin, was the basic medium. Twenty-five microcuries of D-[6-^{3}H] glucosamine hydrochloride (specific activity 10.1 Ci/mM, New England Nuclear Corporation, Boston, Massachusetts) was added to the media within 1 h of culture. During the first 24-h labeling period, the cultures were supplemented either with fetal bovine serum (Flow Laboratories) or with patient serum, obtained a day before surgery, to a final concentration of 10%. After 24 h, the labeled explants were removed from isotope-containing media, rinsed with basic media, and added back to petri dishes containing 5 ml of basic media supplemented with serum or bovine serum albumin (BSA) to a final concentration of 1%. The culture fluid containing labeled material was collected at various time intervals and centrifuged to remove cellular debris. Solid urea was added to the supernatant to 10 M final concentration and after 30 min at room temperature the urea-treated media was extensively dialyzed at 4°C for 2 days against 0.01% ammonium bicarbonate and lyophilized.

Estrogen Receptors

The presence of estrogen receptors in the breast tumors was determined commercially by Bioscience Laboratories (Van Nuys, California) using the dextran-coated charcoal assay.

Cell Culture

The established human mammary carcinoma cell line MCF-7 (12), mycoplasma-free, was kindly provided by Dr. John A. Sykes, California Hospital and Medical Center, Los Angeles. These cells were maintained in RPMI-1640 medium (Flow Laboratories) supplemented with 10% fetal bovine serum in monolayer cultures. Six million cells, from passages 189 to 193, in confluent monolayer cultures, were maintained for 24 and 48 h in the presence of 25 μCi of D-[6-^{3}H] glucosamine hydrochloride. Postlabel incubation was carried out for various lengths of time in isotope-free media supplemented with 160 μunit/ml of bovine pancreatic insulin (Sigma Chemical Company, St. Louis, Missouri). The cell-free supernatant was processed in the same manner as the organ culture samples.

Autoradiography

At the end of organ culture, several explants from 3 infiltrating duct carcinomas of the breast, the Grade III prostate carcinoma, and explants from 1 case of benign prostatic hyperplasia were taken for autoradiographic analysis to determine the distribution of radioactivity within the explants. The explants were fixed for 1 h in 2% glutaralydehyde in 0.2 M sodium cacodylate buffer (pH 7.3) and postfixed for 1 h in 1.0% osmium tetroxide in cacodylate buffer. They were then rapidly dehydrated in acetone and embedded in a polyester resin. One-micron-thick unstained sections of the embedded explants were coated with Kodak NTB 2 emulsion (Eastman Kodak Company, Rochester, New York) by dipping the slides on which the sections had been placed into melted emulsion diluted 1:1 with distilled water. The slides were stored at 4°C in lightproof boxes for 1 week and then developed. After development, the sections were stained through the emulsion with toluidine blue. A measure of the amount of label associated with each compartment within the sections was calculated by first estimating the percentage area

over random sections of explants occupied by tumor cells and connective tissue. This was done essentially by the method of Whur (13, 14) and involved placing at random over photographic enlargements of entire sections a stencil containing a large number of randomly-placed small circles of the same diameter. The contents of 50 circles were then analyzed for each section by a point system. The average number of grains over each compartment was then determined on enlargements of micrographs taken at 1,000 X magnification. A measure of the amount of label associated with each compartment was given by multiplying the percentage area of the section occupied by each compartment by the average number of grains over that compartment.

Gel Electrophoresis

Portions of the urea-treated, dialyzed, and lyophilized media samples were subjected to 3 M urea-1% sodium dodecyl sulfate (SDS) electrophoresis, using 7.5% precast polyacrylamide gels (Bio-Rad, Richmond, California) (15). Molecular weight markers were heavy- and light-polypeptide chains from human immunoglobulins. After electrophoresis the gels were cut into 1- or 2-mm slices and the radioactivity within each slice was determined, after treatment with Beckman Tissue Solubilizer, using scintillation counting. The samples were counted for 10 min; the efficiency of counting was 33%.

Enzymatic Treatment

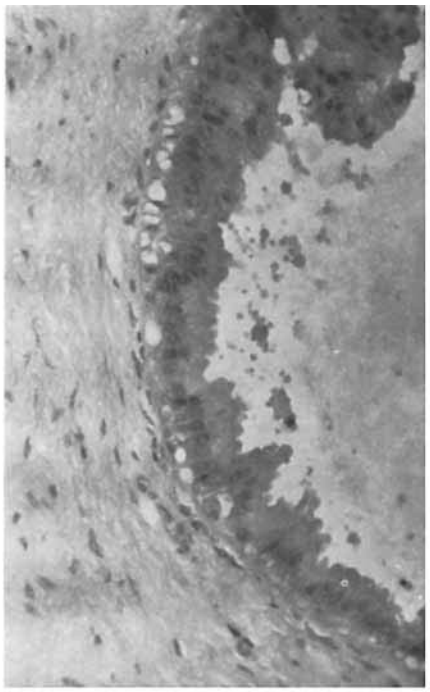
A portion of the cell-free culture medium, 2 mg dry protein equivalent, was incubated in 600 μl of 0.05% NH_4HCO_3 with 0.2 mg of trypsin, TPCK, or with 0.1 mg of chymotrypsin (Sigma Chemical Company) overnight at 37°C. A separate 2-mg sample was treated with 100 μg of pronase (Merck & Co., Inc., Rahway, New Jersey) in 0.1 M phosphate buffer, pH 7.4, and incubated for 1 h at 37°C. The digested samples were dialyzed against water and lyophilized prior to SDS-polyacrylamide gel electrophoresis.

RESULTS

Breast Epithelial Cells

In Fig. 1, the hematoxylin- and eosin-stained paraffin sections of 4 different breast epithelial cell arrangements are illustrated. The normal duct from a nonlactating breast has a single layer of epithelial cells, resting on a basement membrane (Fig. 1A). The epithelium consists of 2 or 3 rows of cells resting on a basement membrane (Fig. 1B) in a hyperplastic duct; however, normal cell morphology and glandular architecture is preserved. In an intraductal carcinoma (Fig. 1C), the duct is filled with many layers of malignant epithelial cells. In this condition the basement membrane remains intact and cells have not invaded the connective tissue stroma. A representative field of an infiltrating duct carcinoma is illustrated in Fig. 1D. The connective tissue stroma is filled with rows of single malignant cells which form small gland-like structures.

Fig. 1. Hematoxylin- and eosin-stained paraffin sections of 4 different breast epithelial cell arrangements (magnification 800 X). A) Normal duct. A single layer of cells rests on a basement membrane. B) Hyperplastic duct. Epithelium consists of 2 or 3 rows of cells resting on a basement membrane. C) Intraductal carcinoma of the breast. Duct is filled with many layers of malignant epithelial cells. Thin line in center of the picture is intact basement membrane; cells have not invaded the connective tissue stroma. D) Infiltrating duct carcinoma. Stroma is filled with malignant cells. They are found in rows of single cells or form small gland-like structures.



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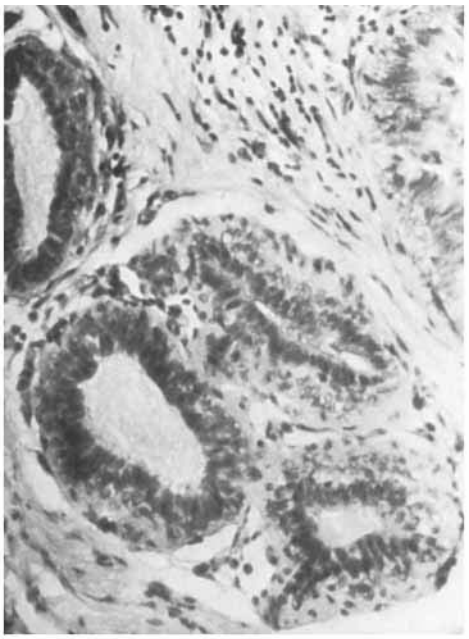

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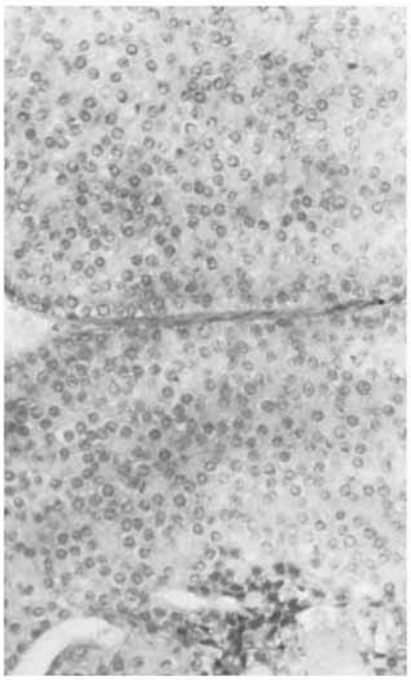

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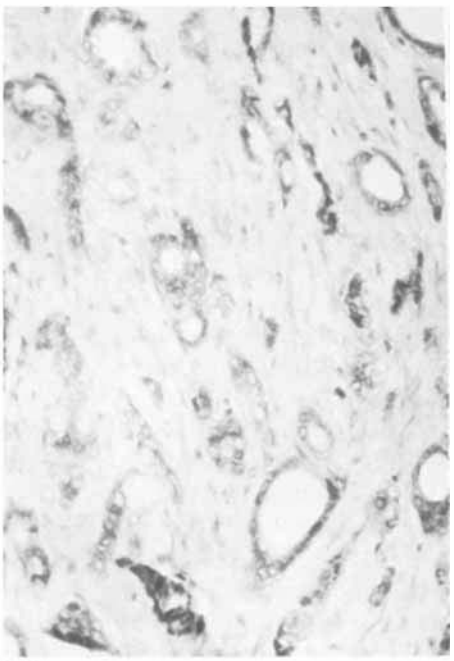

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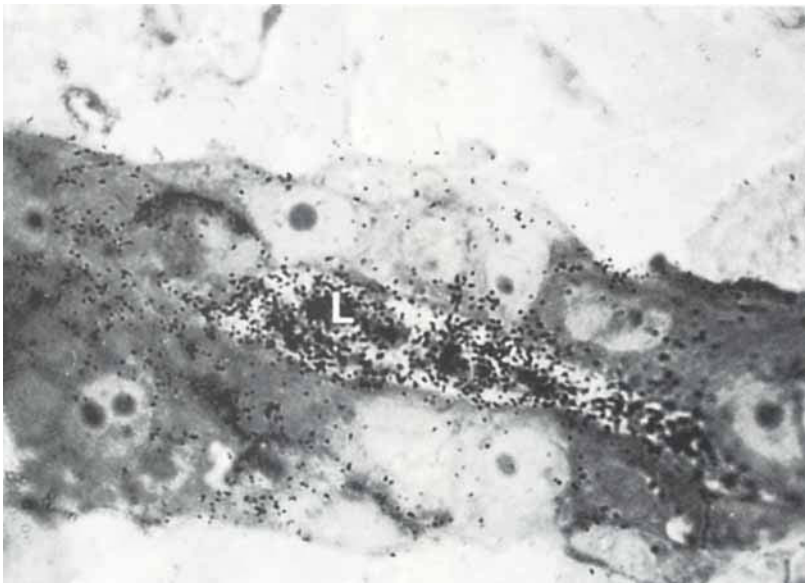


Fig. 2. Autoradiography of an infiltrating duct carcinoma of the breast. Most of the silver grains are found over the cellular elements and lumen (L) of a small malignant gland after 24-h incubation with [^{3}H] glucosamine followed by 48 h in nonisotopic media. Connective tissue stroma around gland contains few grains (enlarged from 1,000 × original magnification).

Explants from the 3 breast tumors chosen for autoradiography were found to contain well-maintained epithelial cells after organ culture for 3 days (24-h incubation with [^{3}H] glucosamine followed by 48 h in nonisotopic media). Most cells contained large nuclei with prominant nucleoli and were often arranged in gland-like structures. Isolated islands of neoplastic cells were also found within the connective tissue stroma. Autoradiography revealed significant amounts of radioactivity within the cellular and luminal components of malignant glands (Fig. 2). In the 3 tumors studied, an average of 25% of the area of sections was occupied by malignant cells and 70% of the total number of silver grains were associated with these cells and their glandular lumina. Thirty percent of the grains were found over noncellular connective tissue. Few fibroblasts and leukocytes were found within the explants of the 3 tumors and less than 3% of the total grains were found over them. These observations indicate that at least 70% of the label was incorporated by neoplastic breast epithelium.

Urea-treated and dialyzed cell-free organ culture media were subjected to SDS-polyacrylamide gel electrophoresis. In Fig. 3, we illustrate representative results from different incubations of surgical specimens from 5 breast cancer patients. These gels were run on standard 7.5% precast acrylamide gels and calibrated with immunoglobulin light and heavy chains. Each sample represents 5% of a culture supernatant. A striking similarity of macromolecular patterns was observed with all the samples studied to date. A predominant component with an apparent molecular weight of 48,000 ± 6,000 was released from all organ cultures. The exact resolution of the major peak is technically not feasible using the 1- or 2-mm slicing techniques. Minor variations in slice thickness may result in artifacts which resemble 2 or more apparent peaks (Graph E in Fig. 3).

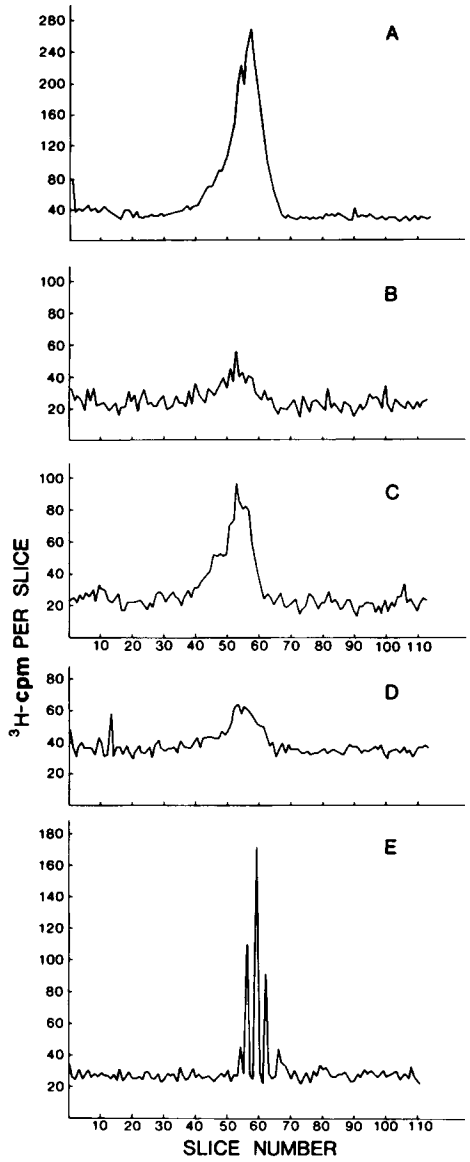


Fig. 3. SDS-polyacrylamide gel electrophoresis of dialyzed breast cancer organ culture media using standard precast 7.5% gels. Slices were 1 mm thick. The dye marker position was between slices 95 and 108. Immunoglobulin light chains migrated to slice positions 68 to 72, and heavy chains to positions 43 to 47. Graph A: Medullary carcinoma. 24 hour post-label incubation. Graph B: Infiltrating duct carcinoma. Second 24-hour post-lable incubation. Estrogen receptor negative. Graph C: Infiltrating duct carcinoma. 48-hour post-label incubation. Graph D: Infiltrating lobular carcinoma. Third 24-hour post-label incubation. Estrogen receptor-positive. Graph E: Infiltrating duct carcinoma. 48-hour post-label incubation. Estrogen receptor-positive.

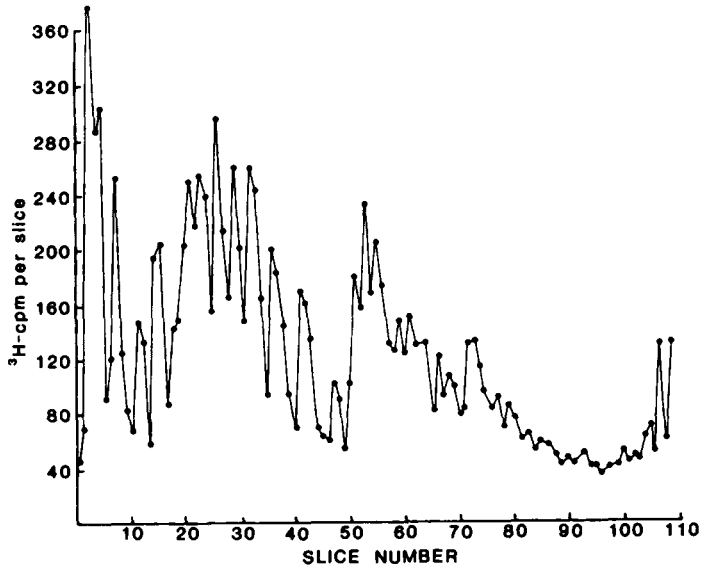


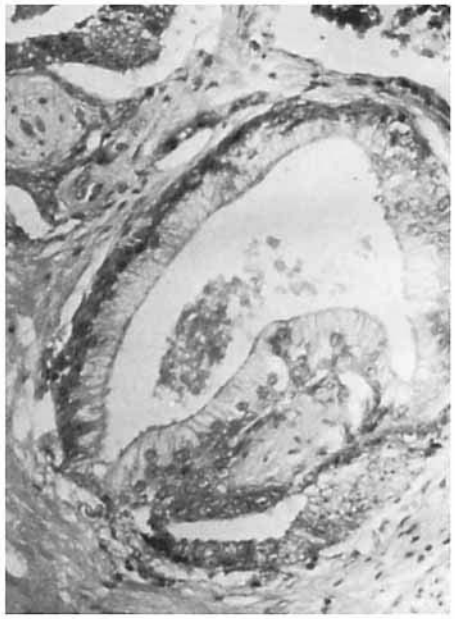
Fig. 4. SDS-polyacrylamide gel electrophoresis of MCF-7 cell culture media from 48-h postincubation. Five to six million cells were grown in a 2-dimensional monolayer culture to confluency. Five percent equivalent of urea-treated, dialyzed, and lyophilized supernatant was applied to the gel. Slices were 1 mm thick. Calibration was the same as in Fig. 3.

An established human mammary carcinoma cell line, MCF-7, was grown in culture to a confluent monolayer in order to have maximum cell-cell contact in this 2-dimensional array of cells. [^{3}H]Glucosamine was introduced for 24 h, and postlabel incubation was carried out in serum-free media. The cell-free culture media from the 48-h postlabel period was treated with urea, dialyzed, and lyophilized. A 5% equivalent was analyzed by SDS-polyacrylamide gel electrophoresis. The results illustrated in Fig. 4 indicate a greater complexity of labeled glycoproteins than observed in any of our organ cultures of human breast carcinoma, even though a family of glycoproteins with apparent molecular weights of 48,000 daltons was also released from this cell line.

Prostate Epithelial Cells

Figure 5 illustrates 3 different prostatic cell arrangements as seen in hematoxylin- and eosin-stained paraffin sections. A single layer of columnar epithelial cells rests on a basement membrane (Fig. 5A) in a normal prostate gland. Normal glandular architecture is preserved in benign hyperplasia (Fig. 5B), but the columnar epithelium is 2 or 3 layers thick. In a Grade III prostatic carcinoma, the stroma is filled with malignant cells which occasionally form small gland-like structures (Fig. 5C).

Fig. 5. Hematoxylin- and eosin-stained paraffin sections of 3 different prostatic cell arrangements (magnification 800 ×). A) Normal prostatic gland. A single layer of columnar epithelial cells rests on a basement membrane. B) Benigh prostatic hyperplasia. Columnar epithelium is 2 or 3 layers thick. Normal architecture of gland is preserved. C) Grade III prostatic carcinoma. Stroma is filled with malignant cells which occasionally form small glands.



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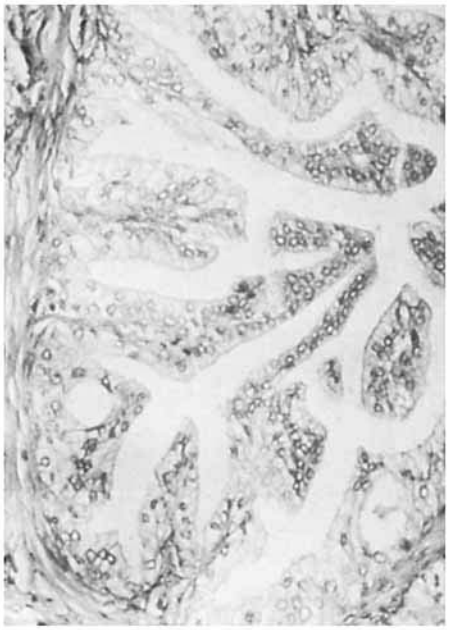

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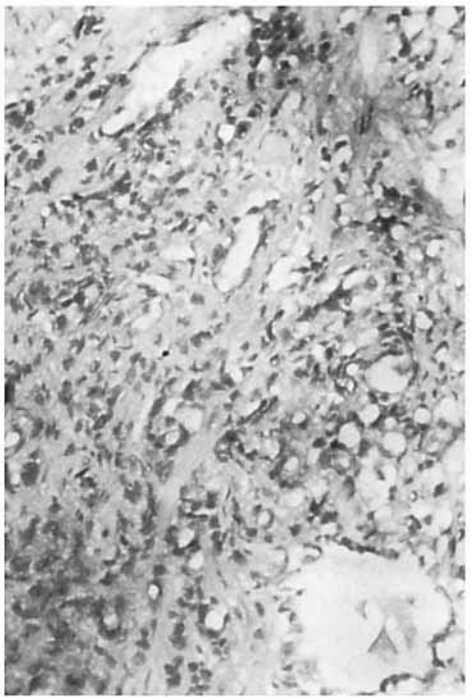

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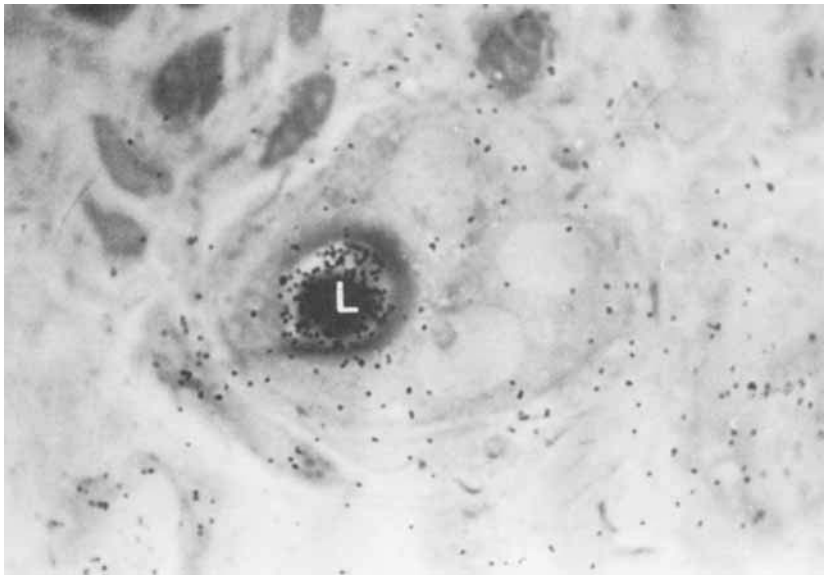

Fig. 6. Autoradiography of a poorly-differentiated Grade III prostate carcinoma. Most of the silver grains are found over the lumen (L) of a small malignant gland. Incubation as in Fig. 2 (enlarged from 1,000 X original magnification).

Well-maintained glandular and stromal structures were found in the hyperplastic explants after organ culture for 3 days, which included 24-h incubation with [^{3}H]glucosamine followed by 48 h in nonisotopic media. Autoradiography revealed significant amounts of radioactivity within the cellular and luminal components of the glands. After organ culture for 3 days, explants from the Grade III, poorly-differentiated carcinoma also contained well-maintained epithelial and stromal components. These explants were characterized by the presence of minute neoplastic acini haphazardly arranged, and neoplastic cells without any distinct glandular formation within the connective tissue stroma. Autoradiography revealed particularly intense reactions over the lumens of the small neoplastic glands (Fig. 6). Quantitation of grains revealed that at least 62% of the incorporation took place by neoplastic epithelium.

Organ culture media from prostate specimens were analyzed by SDS-polyacrylamide gel electrophoresis. No significant release of [^{3}H]glucosamine-labeled macromolecules was found with explants from 4 cases of benign hyperplasia and with one Grade I well-differentiated infiltrating carcinoma. One major family of glycoproteins with an apparent molecular weight of 48,000 ± 6,000 daltons was released by a Grade I well-differentiated and a Grade II moderately well-differentiated infiltrating carcinoma. One fibroglandular and one nodular hyperplasia sample also released similar glycoproteins. Characteristic patterns are illustrated in Fig. 7. A more complicated pattern of labeled glycoproteins was observed in the organ culture media of a Grade II moderately-differentiated and a Grade III poorly-differentiated carcinoma (Fig. 8). In addition to the 48,000 molecular weight glycoprotein, a high-molecular-weight component was observed near the top of the gels and there were additional peaks with an apparent molecular weight range of 30,000–120,000.

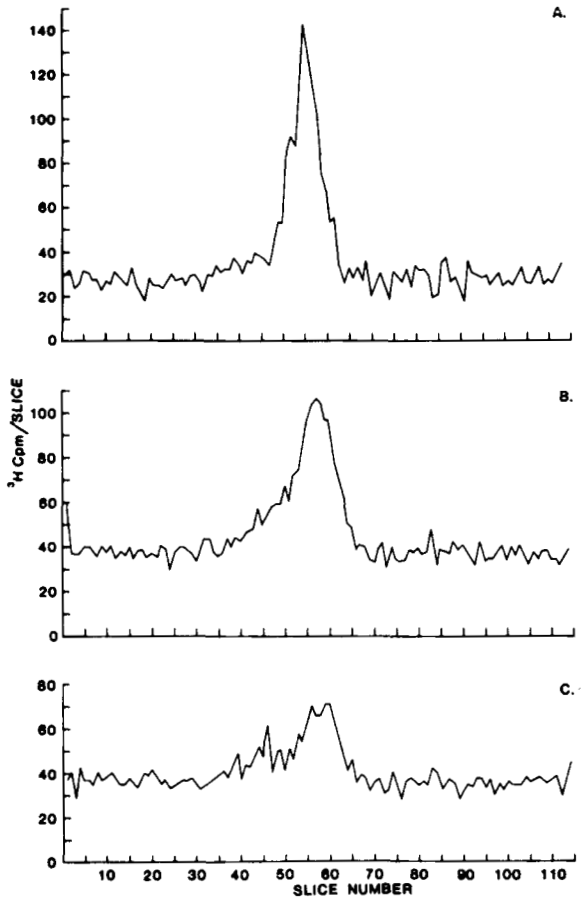

Fig. 7. Distribution of incorporated [^{3}H]glucosamine from prostate organ-culture media in SDS-polyacrylamide gel slices. Slices were 1 mm thick. Conditions and calibrations were the same as in Fig. 3. Graph A: Grade II, moderately well differentiated infiltrating adenocarcinoma. Graph B: Nodular hyperplasia. Graph C: Grade I, well differentiated infiltrating adenocarcinoma.

The dialyzed and lyophilized medium from explants of one case of benign prostatic hyperplasia was treated with trypsin, chymotrypsin, or pronase (Fig. 9). The [^{3}H]glucosamine-labeled glycoproteins were completely digested to fragments smaller than 5,000–10,000 molecular weight, since all of the detectable radioactivity was abolished in SDS gels. Trypsin digestion decreased the apparent molecular weight by approximately 15–20%. All of the SDS-polyacrylamide gel patterns of untreated glycoproteins remained the same when reducing agents were omitted from the sample buffer, indicating that disulfide-linked aggregation was not responsible for the pattern.

DISCUSSION

Breast Epithelial Cells

Active synthesis and release of glycoproteins was observed for at least 48 h in organ cultures of human breast carcinomas. In some samples, for which data is not presented

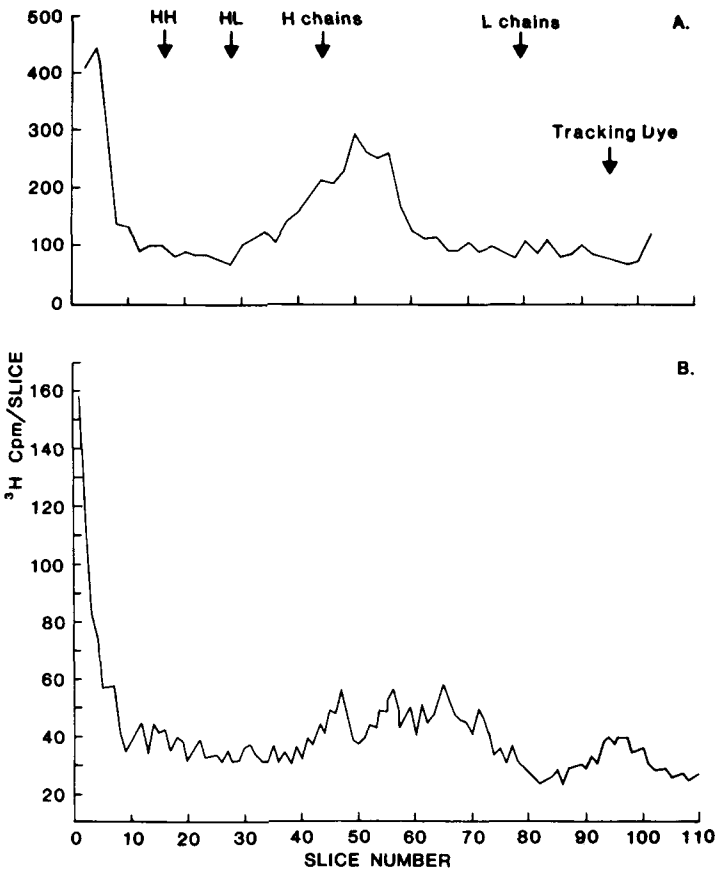

Fig. 8. Distribution of incorporated [^{3}H]glucosamine from prostate organ-culture media in SDS-polyacrylamide gel slices. Slices were 1 mm thick. Graph A) Grade III, poorly-differentiated adenocarcinoma. Graph B) Grade II, moderately-differentiated adenocarcinoma.

here, this activity remained for 5–7 days. Autoradiography performed on explants of 3 infiltrating duct carcinomas of the breast revealed that an average of 70% of the total number of silver grains over random sections were associated with the malignant cells. When glandular structures were present, more intense reactions were found over lumina than epithelial cytoplasm, indicating that the glycoproteins synthesized by malignant epithelium were released into glandular lumina. Similar experiments with early rat mammary carcinomas induced by a carcinogen also indicated the production of an unidentified glycoproteins which has been interpreted as an early detectable sign of malignant transformation (16).

SDS-polyacrylamide gel electrophoresis patterns of the glycoproteins released into the media by 10 different breast tumors, 5 of which are illustrated in Fig. 3, revealed a striking similarity of labeled and released macromolecules. Medullary carcinoma (Graph A), infiltrating lobular carcinoma (Graph D), and 8 infiltrating duct carcinomas, of which only 3 representative samples are illustrated (Graphs B, C, and E), all produced a group of macromolecules with an apparent molecular weight of 48,000 ± 6,000 daltons. Since at this time the percent of carbohydrate is not known, their exact molecular weight cannot

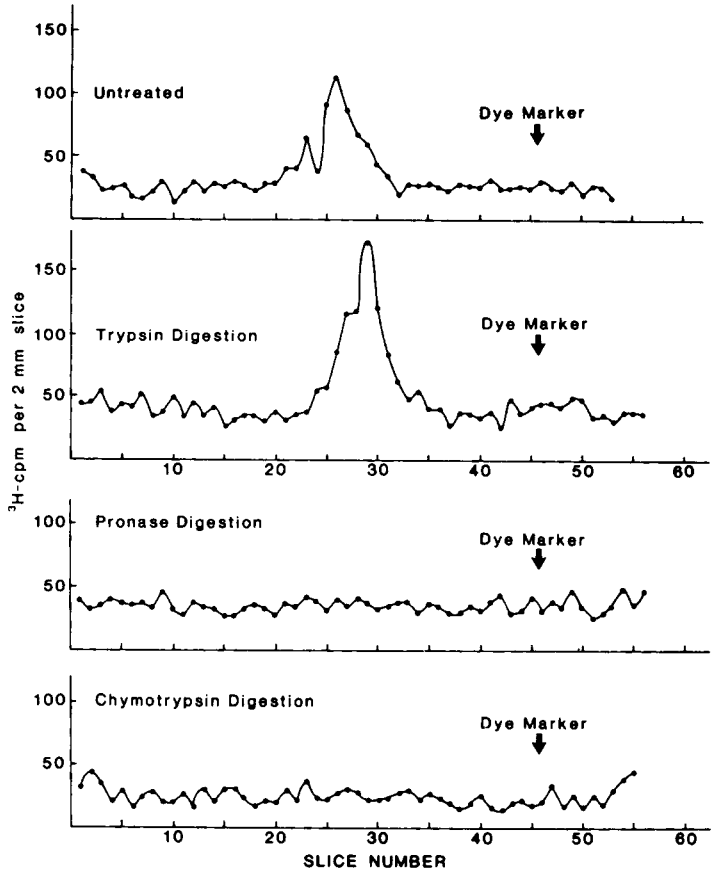

Fig. 9. The effect of proteolytic enzyme treatment on the organ culture supernatant. A media sample from benign prostatic hyperplasia was treated with trypsin, pronase, or chymotrypsin and analyzed by SDS polyacrylamide gel electrophoresis. Slices were 2 mm thick. Conditions and calibrations were the same as in Fig. 3.

be determined. Neuraminidase treatment of these glycoproteins decreased the apparent molecular weight by 3–5% (17) suggesting the presence of sialic acid on these molecules. Since these molecules were also susceptible to pronase digestion, we conclude that the isotope label is incorporated into a glycoprotein (17). The labeled glycoprotein patterns (Fig. 3) appear to be independent of the availability of estrogen receptors since only 50% of the tumors were estrogen receptor positive.

A complex pattern of released glycoproteins, labeled with [^{3}H]glucosamine, was observed in the supernatant of the established human mammary carcinoma cell line MCF-7, grown in 2-dimensional monolayer cultures (Fig. 4). These cells also produced a group of glycoproteins with an apparent molecular weight of 48,000 ± 6,000 daltons. Whether these molecules are antigenically related to the organ culture products is currently under investigation. It is interesting to note, however, that in addition to this group of glycoproteins, the majority of incorporation was into larger macromolecules. This is not due to

a less extensive digestion of glycoproteins after their release in monolayer cultures since these components are not further degraded when introduced in organ cultures for 24 h (Tökés and Lam, unpublished observation).

Prostate Epithelial Cells

Autoradiography performed on explants of a representative case of benign prostatic hyperplasia that had been cultured for 1 day with [^{3}H]glucosamine followed by a chase of 2 days in nonisotopic media revealed that 70% of the total number of silver grains over random sections were associated with glandular-epithelial structures. Usually more intense reactions were found over the lumina of glands than epithelial cytoplasm. Autoradiographic data obtained from explants of the Grade III, poorly-differentiated prostate carcinoma were similar to those obtained from hyperplastic tissue. The major contribution of this data is that the glycoproteins recovered from the media of organ cultures of hyperplastic and carcinomatous human prostate and breast tissue are predominantly products of epithelium.

Labeled glycoproteins were below the level of detection in culture supernatant fractions in 4 of 6 cases of benign prostatic hyperplasia and from 1 well-differentiated Grade I prostate carcinoma. In these samples the number of epithelial cells was similar to or higher than in other biosynthetically-active samples. Therefore the low levels of incorporation are not due to decreased cell numbers but may reflect the generally poor metabolic state of the cells. These cells may also have their biosynthetic activity under more stringent regulation which is inactivated by organ culture conditions. Two of the six hyperplastic prostate cultures synthesized and released a major 48,000 molecular weight glycoprotein fraction, similar to that found in cultures of breast cancer specimens. These glycoproteins were also observed in a Grade I well-differentiated infiltrating prostate carcinoma and in a Grade II, moderatly well-differentiated infiltrating prostate carcinoma (Fig. 7). In 2 of the samples illustrated in Fig. 7, we examined the molecular weight patterns released after 24 and 48 h of postlabel incubation. Even though quantitative differences were observed as expected, both patterns were identical signifying the lack of extensive degradation.

Trypsin treatment of the released glycoproteins from a benign prostatic hyperplasia sample slightly decreased their molecular weight, but both chymotrypsin and pronase treatment resulted in complete digestion (Fig. 9). These observations are identical to the previously reported sensitivity of glycoproteins produced by breast carcinoma specimens to various proteases (17).

The most complex glycoprotein patterns were obtained from one Grade II, moderately-differentiated and a Grade II, poorly-differentiated prostate carcinoma (Fig. 8). The large molecular weight component did not migrate faster in a 5% polyacrylamide gel when introduced with dithiothreitol and urea. Mucins and glycoproteins larger than 500,000 molecular weight behave in this manner. In both of these samples, 48,000 molecular weight components also were released. More samples will be necessary to establish whether the complexity of glycoprotein patterns is related to the undifferentiated state of Grades II and III carcinomas of the prostate.

GENERAL COMMENTS

In organ culture samples of benign hyperplastic prostate where the glandular structure and the basal membrane appeared normal, glycoprotein synthesis and release was

usually below the level of detection. Moderately- or well-differentiated malignant epithelial cells from both prostate and breast released a strikingly similar pattern of glycoproteins. A 48,000 molecular weight glycoprotein was the predominant component in all of the samples where glandular structures and the basal membrane were still recognizable. The most complex pattern was observed with poorly-differentiated prostatic adenocarcinomas or with breast epithelial cells grown in the complete absence of any 3-dimensional glandular structures. These observations suggested to us a hypothesis that the 3-dimensional integrity of glandular structures may restrict synthesis and release of several glycoproteins. With progressive alteration in glandular structure the synthesis and the release of a 48,000 molecular weight glycoprotein would increase. Epithelial cells grown in 2-dimensional monolayer cultures or in vivo without basement-membrane-supported glandular structures would escape from such a topographic restriction and express additional families of glycoproteins. The data presented here is in agreement with this hypothesis; further quantitation of glycoprotein turnover by epithelial cells as a function of progressive disattachment from glandular structures is in progress in our laboratory.

Although the molecular nature of the major 48,000 molecular weight glycoprotein is not yet elucidated, a few observations reveal that it may not be the product of a differentiated cell function, such as the production of a specific milk or prostatic fluid protein. Supplementing organ culture media with hormones, and exchanging the patient's own serum with fetal bovine serum failed to alter the released glycoprotein pattern. Similarly, the availability of estrogen receptors did not alter the pattern. Therefore it is reasonable to hypothesize that these glycoproteins represent an expression of an "epithelial state" of differentiation.

A number of molecular markers have been reported to be associated with prostate and breast cancer (18–23). In addition to these markers, 2 membrane-associated glycoproteins need to be evaluated for their possible relation to the labeled components we have found in organ culture. Human histocompatibility antigens, normal components of most if not all cell types, have approximate molecular weights of 45,000 (24) and cell membrane turnover results in the shedding of these entities. Therefore it is possible that at least a fraction of the labeled glycoproteins belong to this category. A second membrane-associated component, the major envelope glycoprotein of Type C viruses, should also be considered. A polymorphism of these virion-associated and differentiation antigens, encoded by a multigene family, was reported (25) with the murine Type C viruses. This study demonstrated that the tryptic peptides of glycoproteins with molecular weights of 69,000 and 70,000, containing 32% carbohydrate, and the peptides of the 45,000 molecular weight glycoprotein with 6–9% carbohydrate were identical. Therefore gp45 can be considered an incompletely-glycosylated glycoprotein (25). Recent reports (26) also claim that the presence of the gp52 viral glycoprotein associated with mouse mammary tumor can serve in the plasma as a diagnostic indicator of the presence of a solid tumor. Since similar molecular weight glycoproteins, labeled in our experiments, are released, it is reasonable to postulate that they accumulate in the plasma and could therefore act as a diagnostic indicator of neoplasia.

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REFERENCES

1. Cook GMW, Stoddart RW: Surface Carbohydrates of the Eukaryotic Cell. London: Academic Press, 257, 1973.
2. Cook GMW, Stoddart RW: Surface Carbohydrates of the Eukaryotic Cell. London: Academic Press, 257:140, 1973.
3. Beug H, Gerisch G, Kempff S, Riedel V, Cremer G: Exp Cell Res 63:147, 1970.
4. Baldwin RW, Harris JR, Price MR: Int J Cancer 11:385, 1973.
5. Baldwin RW, Price MR, Robins RA: Nature (London) 238:185, 1972.
6. Juergens WG, Stockdale FE, Topper YJ, Elias JJ: Proc Natl Acad Sci USA 54:629, 1965.
7. Turkington RW, Lockwood DH, Topper YJ: Biochim Biophys Acta 148:475, 1975.
8. Dermer GB, Sherwin RP: Cancer Res 35:63, 1975.
9. Hurlimann J, Lichaa M, Ozzello L: Cancer Res 36:1284, 1976.
10. Baulieu EE, LeGoascogne C, Groyer A, Feyel-Cabanes T, Robel P: Vitam Horm (NY) 33:1, 1975.
11. Kaighn ME, Babcock MS: Cancer Chemother Rep 59:59, 1975.
12. Soule HD, Vazquez J, Long A, Albert S, Brennan M: J Natl Cancer Inst 51: 1409, 1973.
13. Whur P, Herscovics A, LeBlond CP: J Cell Biol 43:289, 1969.
14. Dermer GB: J Ultrastruct Res 22:312, 1968.
15. Fairbanks G, Steck TL, Wallach DFH: Biochemistry 10:2606, 1971.
16. Russo I, Saby J, Isenberg W: Proc Am Assoc Cancer Res 16:116, 1976.
17. Dermer GB, Tökés ZA: J Natl Cancer Inst (Manuscript submitted).
18. Chu TM, Bhargava AK, Barnard EA, Ostrowski W, Varkaris MJ, Morrin C, Murphy GP: Cancer Chemother Rep 59:97, 1975.
19. Chu TM, Nemoto T: J Natl Cancer Inst 51:119, 1973.
20. Steward AM, Nixon D, Zamcheck N, Aisenberg A: Cancer 33:1246, 1974.
21. Rosato FE, Seltzer M, Mullen J, Rosato EF: Cancer 28:1575, 1971.
22. Amaral L, Werthamer S: Nature (London) 262:589, 1976.
23. Muller M, Grossman H: Nature (London) New Biol 237:116, 1972.
24. Terhorst C, Parham P, Mann DL, Strominger JL: Proc Natl Acad Sci USA 73:910, 1976.
25. Elder JH, Jensen FC, Bryant ML, Lerner RA: Nature (London) (In press).
26. Ritzi E, Martin DS, Stolfi RL, Spiegelman S: Proc Natl Acad Sci USA 73:4190, 1976.