Estradiol-dependent Collagenolytic Enzyme Activity in Long-Term Organ Culture of Human Breast Cancer

Jean-Claude Heuson, Jean-Lambert Pasteels, Nicole Legros, Jeanine Heuson-Stiennon, and Guy Leclercq


SUMMARY

An organ culture method suitable for the maintenance of viable human breast cancer for at least 14 days has been described. This method was applied to a total of 94 breast cancer specimens. It allowed good survival of "soft" tumors of various histological types, with loose connective stroma even in hormone-free medium. In contrast, "scirrhous" cancers showed poor survival in hormone-free medium; viable cells were maintained only at the very periphery of the explants. Supplementation of the medium with insulin (10 µg/ml), ovine prolactin (5 µg/ml), and hydrocortisone (1 µg/ml) in various combinations seemed to induce enlargement of viable cancer cells and moderate loosening of the stroma in some cases. However, it did not improve the survival of central tumor cords in scirrhous explants. Further supplementation of the medium with 17β-estradiol (minimum effective dose, 0.1 to 10 ng/ml), although it did not affect soft tumors, markedly improved survival of the cancer cells of scirrhous tumors throughout the whole explants, with evidence of collagen digestion around the neoplastic cells. This was observed in 18 of 20 scirrhous cancers subjected to this treatment. Estradiol need not be present during the whole culture period; the results at 14 days were identical in explants treated with estradiol for the first 7 days only or for the entire period. Addition of purified collagenase during the first 24 or 48 hr of culture resulted in complete dissolution of the collagen. After such treatment, culture under the usual conditions resulted in excellent survival of the explants without improvement from hormone supplementation; thus, while estradiol was necessary when collagen was present, it was no longer required after collagen digestion. It can be concluded that breast cancer cells in organ culture are only slightly, or not at all, hormone dependent for survival, provided that they are not restrained by a dense collagen barrier. The estrogen-induced changes allowing survival inside the scirrhous explants strongly suggest the presence of an estrogen-dependent collagenolytic enzyme system in the collagen-rich breast cancers. This system could represent an important component of the hormone dependency of human breast cancer growth.

INTRODUCTION

The mechanisms of hormone dependence of human breast cancer that form the basis of its endocrine treatments are far from being understood. Their study can be approached by in vitro experiments that may serve the additionally useful goal of selecting patients for hormonal therapies. Organ culture is likely to provide an appropriate method for that purpose because it preserves the normal relationships of epithelial tumor cells and surrounding connective tissue. In contrast to cell or tissue culture methods, it avoids growth competition between tumor cells and fibroblasts and allows a clear recognition of the histological nature of the cells. The technique should be able to sustain living tumor tissue for several weeks, ensuring that hormone assays, even if performed early in the culture experiment, are not carried out on dying cells (17).

Organ culture methods were successfully applied to mammary tumors of laboratory animals (7, 10, 14-16, 27, 35). Unfortunately, long-term organ culture of human breast cancer appears to be especially difficult, particularly in the case of scirrhous tumors (1, 21, 32, 34, 36). This is probably the reason why several authors have proposed the use of cell or tissue cultures (2, 3, 11, 25) or very short-term organ cultures (4, 5, 18, 21, 29, 30, 32).

The present paper describes experiments of organ culture where explants from human breast cancer, including the scirrhous type, were maintained for periods up to 14 days. It describes unexpected findings on the influence of estrogen on survival of the scirrhous tumors.

MATERIALS AND METHODS

Ninety-four breast cancer tissue samples were obtained from 88 female patients and 1 male patient. These samples were from primary breast carcinomas in 75 cases, from both the primary and an invaded axillary lymph node in 5 cases, and from metastatic lesions (nodes, skin) in 9 cases. The patients' ages ranged from 29 to 94 years, with a mean value of 60 years. Twenty-three patients were premenopausal, 8 were within 1 year of their last menstrual period, 56 were postmenopausal, and 1 was of undetermined status.

1 This work was supported by grants from the "Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite de Belgique" and performed in part under contract of the Ministère de la Politique Scientifique within the framework of the Association Euratom, University of Brussels, University of Pisa.

Received January 8, 1975; accepted April 24, 1975.

AUGUST 1975

2039
Immediately after excision, the sterile tissue samples were maintained in Earle’s base at 0°C and transferred to the laboratory within 15 min. In the 1st 23 experiments, explants were prepared for culture by mincing the specimen with opposed surgical scalpel blades operated on a porcelain surface. However, histological examinations revealed that this procedure produced a considerable amount of crushing when applied to scirrhous carcinomas. From then on, in order to avoid this harmful effect, the samples were prepared on a solidified paraffin surface by means of opposed razor blades (Schick Ultra Platinum; Eversharp Nederland B.V., Amsterdam, The Netherlands) selected for the sharpness and hardness of their cutting edge. Adhering fat, connective tissue strands, and necrotic areas were dissected out and the tumors were cut into 1- to 2-mm explants. Five explants were placed on a Millipore filter, pore size 0.45 μm (Millipore, Malakoff, France), or on a small block of agar gel (8 x 8 x 4 mm) (Difco Laboratories, Inc., Detroit, Mich.) and transferred on stainless steel grids in Falcon disposable Petri dishes (Falcon Plastics, Los Angeles, Calif.) containing 2.5 ml of liquid culture medium. The height of the grids was so calculated that the explants were at the liquid-gas interphase. Agar gel was prepared by the method of Willcox and Thomas (36). A 1% solution in Earle’s base was autoclaved and then poured into sterile Petri dishes to form a 4-mm thick layer that was solidified at room temperature. Agar-gel blocks of appropriate size were then cut. The cultures were carried out at 37°C in a water-saturated atmosphere of 95% O₂:5% CO₂. Medium 199 Earle’s base (Flow, Irvine, Scotland, or Gibco Biocult, Glasgow, Scotland), supplemented with glucose to reach a concentration of 250 mg/100 ml, was used as basic medium. It was changed every 2 days. Hormone or enzyme supplementation was carried out as follows.

**Insulin.** A stock solution of insulin (Sigma Chemical Co., St. Louis, Mo.) was prepared by dissolving hormone in 0.005 N HCl; it was added to the medium at a final concentration of 10 μg/ml.

**Prolactin.** Prolactin (ovine, NIH-P-S-10, Bethesda, Md) was dissolved at a concentration of 0.33 mg/ml in 0.9% NaCl, pH 8.4; the final concentration in the culture medium was 5 μg/ml.

**Hydrocortisone and 17β-Estradiol.** The stock solutions (Sigma) were prepared by dissolving the hormones at a concentration of 1 mg/ml in absolute ethanol. Hydrocortisone was added to the culture medium at a final concentration of 1 μg/ml; estradiol was added at final concentrations of 0.1 and 1 ng/ml. Some experiments involved additional concentrations from 0.01 ng to 10 μg/ml. The final ethanol concentration never exceeded 0.1%.

**Bacterial Collagenase.** A preparation (Calbiochem, San Diego, Calif.) titrating at 100,000 C units/g was added to the culture medium at a final concentration of 1 mg/ml.

The cultures were maintained for 4, 6, 7, or 14 days. At the end of each culture period, all explants from 2 or 3 Millipore discs or agar-gel blocks for each experimental condition were fixed in Bouin’s fluid for histological examination. They were cut serially, and 8 regularly spaced sections were placed on a slide. They were stained with hematoxylin and eosin. In selected experiments, specific staining of the collagen was carried out by use of Masson trichrome, Van Gieson staining, and periodic acid-Schiff reaction (24).

Histological typing of the tumors was performed on samples collected at the start of the experiment according to Stewart (31) and Kuzma (20). The tumors were further subdivided into 2 categories: (a) “soft” tumors with scanty or loose stroma, and (b) “collagen-rich” (scirrhous) tumors.

Before culture, a tissue specimen of each tumor was saved for estrogen receptor determinations. The method used has been described (23) and is based on the preparation of the tissue cytosol fraction and measurement of its specific binding affinity for 17β-[³H]estradiol.

### RESULTS

#### Histological Typing

The histological types of the 94 human breast cancers studied are listed in Table 1. Thirty-two were soft tumors and 62 were collagen-rich tumors. The mean age of patients bearing tumors of these categories was 61 and 59 years old, respectively, with little variation among histological types. The proportion of postmenopausal patients in each group was 58 and 68%, respectively, the remainder being premenopausal or rarely menopausal. The groups and various histological types were thus homogeneous with respect to age and menopausal status.

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>No. of Cases</th>
<th>Central Survival</th>
<th>Peripheral Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma (carcinoma simplex)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Colloid carcinoma</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Comedocarcinoma</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Papillary carcinoma (infiltrating)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Paget’s disease (infiltrating)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Infiltrating lobular carcinoma</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collagen-rich tumors</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrating ductal carcinoma (scirrhous)</td>
<td>60</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Comedocarcinoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Paget’s disease (infiltrating)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Central or peripheral survival, presence of viable cancer cells at the end of the culture period in central or in peripheral regions of the explants, respectively.

* Comparison of central survival; soft versus collagen-rich tumors: χ² = 61.55, p < 0.001.

* Comparison of peripheral survival; soft versus collagen-rich tumors: χ² = 3.35, not significant.
Effect of Hormones in Culture

Insulin, Prolactin, and Hydrocortisone. Explants cultured in hormone-free medium or in medium supplemented with insulin, prolactin, or hydrocortisone, either alone or in various combinations, failed to display obvious differences. It is possible that, in occasional cases, insulin alone or in combination with prolactin induced some enlargement of viable cancer cells and moderate loosening of the stroma. Actually, survival during culture depended on the type of tumors, whether soft or collagen rich (Table 1).

In the soft tumors, survival was very satisfactory. The architecture of the explants was essentially the same as before culture, and viable cells were present in central as well as in peripheral areas, provided the diameter did not exceed 1 mm (Fig. 1). Only the 5 medullary carcinomas, which are tumors characterized by heavy lymphocyte infiltration, showed poor survival.

In striking contrast, survival of the collagen-rich tumors was very poor. The only viable cells were located at the extreme periphery of the explants; in deeper parts, the tumor cells were completely pyknotic. The appearance of a typical scirrhous infiltrating ductal carcinoma before and after 6 days of culture is shown in Fig. 2, A and B. A few bundles of cancer cells survived only at the very periphery of the explants. In their depth, cell death usually spared connective tissue cells and, when present, normal and dysplastic mammary tissues.

17β-Estradiol. Supplementation of the medium with 17β-estradiol in combination with insulin or with insulin and prolactin was tested on 23 breast cancers.

Three of the tumors were soft tumors: 1 carcinoma simplex, 1 comedocarcinoma, and 1 papillary carcinoma. In none did estradiol improve survival of the cultures that was already excellent under control conditions; neither did steroids induce stromal changes.

Dramatic changes occurred, however, in most collagen-rich tumors. Twenty such tumors were studied: 1 comedocarcinoma and 19 scirrhous infiltrating ductal carcinomas. In 18 of them, culture in the presence of estradiol resulted in marked loosening and dissolution of the collagen, as well as in significant improvement of cancer cell survival in the depth of the explants. Fig. 2C illustrates this effect of estradiol after 6 days of culture. Collagen had vanished and cords of very healthy tumor cells were present throughout the whole explant, interspersed with connective tissue cells. Fig. 3A is a higher-magnification micrograph of the same explant. Occasional mitoses were seen in the tumor cords. Complete dissolution of the collagen, as observed in Figs. 2C and 3A, did not occur in all cases. In some experiments, estradiol-induced lytic effects on the collagen were limited to the area surrounding strands of viable cancer cells. This is illustrated by the experiment of Fig. 4. In this experiment, after 7 days of culture in the absence of estradiol, viable cancer cells located at the periphery of explants were surrounded by intact collagen, whereas cancer cells located more centrally were pyknotic (Fig. 4A). Culturing with added estradiol for 4 or 7 days resulted in a progressive digestion of the collagen around cords of living cells (Fig. 4, B and C). Special staining of the material indicated that the lytic effects described here were indeed exerted on the collagen itself. Lytic effects on the collagen were not observed far from epithelial cancer cells or in explants that, by random occurrence during preparation, did not contain such cells. The minimum concentration of estrogen effecting these changes was usually 1 ng/ml but ranged from 0.1 to 10 ng/ml. Concentrations as high as 10 μg/ml did not appear distinctly noxious, but the tests were too few to reach valid conclusions. Failure to respond to estrogen at any concentration occurred in only 2 cases.

Estradiol need not be present for the whole culture period to insure cell survival in the depth of scirrhous explants. This was demonstrated in 5 experiments where explants from scirrhous carcinomas were cultured for 7 days in a medium containing insulin, prolactin, and 17β-estradiol, 1 ng/ml, and then cultured for an additional 7 days in the same medium without estradiol. At the end of each 7-day period, examination of the explants revealed an excellent survival of cancer cells in cords surrounded by a loosened stroma. Figure 4D represents a culture at the end of the 2nd, estradiol-free period.

Effect of Collagenase

Explants from 25 tumors were cultured for an initial period of 24 to 48 hr in the presence of added collagenase. After washing out collagenase, they were then further cultured under the usual conditions. Hormones, when present, were added at the start of culture. Of these 35 tumors, 8 were soft: 4 cases of carcinoma simplex, 2 papillary carcinomas, 1 comedocarcinoma, and 1 colloid carcinoma. The 27 others were collagen-rich tumors: 25 scirrhous infiltrating ductal carcinomas, 1 infiltrating Paget's disease, and 1 comedocarcinoma. At the end of the 1- or 2-day period of culture with collagenase, histological examination disclosed that complete dissolution of collagen had occurred. Tumor cells and connective tissue cells appeared intact and settled on the culture support, whether Millipore filter or agar gel, forming flattened explants of very high cellular density. When further cultured for periods ranging from 5 to 13 days in fresh medium with or without added hormones, the cells maintained essentially the same pattern. With respect to the soft tumors, cell survival was not improved over that in explants not initially treated with collagenase, where survival was already excellent. In sharp contrast, scirrhous tumors cultured after an initial treatment with collagenase were considerably improved over their controls. In each case the entire explant was composed of viable tumor cells and connective tissue cells (Figs. 2D and 3B). Pyknosis was very infrequent. The tumor cells arranged themselves as epithelial cords, allowing clear distinction between tumor and connective tissue. Maintenance of these explants did not seem grossly influenced by added hormones. As already described, control explants without initial culture with collagenase showed only poor peripheral survival of tumor cells (Fig. 2B).

Tissue Estrogen Receptors

Samples of tumor tissue were saved before culture for
estrogen receptors assay in all cases but 1. Specific receptors were found present in 26 of 32 soft tumors (81%) and in 47 of 61 collagen-rich tumors (79%). The proportion of receptor-positive tumors and the mean concentration of receptors were essentially the same in all histological types of Table I. In an experiment described above, 17β-estradiol was found to improve survival of cultured scirrhous carcinomas in 18 of 20 cases. Seventy-two % of these 18 tumors contained receptors. Both tumors found unaffected by estradiol in culture were receptor positive.

DISCUSSION

Whereas successful organ cultures of soft, collagen-poor types of breast cancer were reported by various laboratories (34, 36), this paper describes the 1st fully successful attempt at maintaining scirrhous ductal carcinomas in long-term organ cultures. This success may be ascribed partly to technical improvements and partly to the use of proper hormonal supplementation.

One difficulty encountered in culturing scirrhous breast cancer lies in the preparation of the explants. Extreme care is required during the cutting procedure in order to avoid shearing and crushing of the cells engulfed in the dense collagen matrix. The best results were obtained in our hands by the use of crossed razor blades selected for the sharpness and hardness of their cutting edge and operated on a solidified paraffin surface. Explants prepared by this procedure even from very hard collagen-rich tumors showed excellent survival abilities under appropriate conditions.

Survival of the soft tumors of whatever histological type was satisfactory in un-supplemented synthetic culture medium. Cords of cells at the very periphery of scirrhous explants also survived, but those located in their depth died. Supplementation of the medium with various combinations of insulin, prolactin, and hydrocortisone seemed to effect in some cases minor changes that could not be accurately defined by histological methods. It would thus appear that mere survival of breast cancer cells in soft tumors or at the periphery of the scirrhous explants is only slightly, if at all, hormone dependent in vitro. It is quite unlikely that explants would contain enough endogenously produced hormones at the time of explantation to assure cell survival for 14 days. Survival in excess of this duration was not investigated.

In scirrhous carcinomas, normal or benign hyperplastic mammary epithelial cells were able to survive in the depth of the explants, while cancer cells survived only at their periphery. This may indicate that the cancer cells have more stringent requirements for nutrients or oxygen than does the normal or hyperplastic epithelium and that the dense collagen is the factor responsible for the shortage of nutrients or oxygen available to the cancer cells inside these scirrhous explants.

The experiments reported here seem to suggest a new effect of estradiol on breast cancer. Other authors (4, 30) have reported that estrogen could improve the survival of short-term organ cultures of human breast cancers in a small proportion of cases. In the present study, estradiol in combination with insulin or with insulin and prolactin was found to permit the survival of breast cancer cells in the depth of scirrhous explants during long-term culture in most cases. Furthermore, this estradiol-induced survival was accompanied by lysis of the collagen around the cancer cell cords and, in some cases, total dissolution of the collagen in the whole explant. One possible explanation for these effects is that estradiol stimulates the connective tissue components to greater activity in a putative role of supporting life of the cancer cells. The importance of stromal-epithelial interactions has been well documented (12), in particular during embryonic development of the mammary gland (19). Another explanation is that estradiol would be required at the tumor cell level to insure survival. In both hypotheses assuming inherent collagenolytic properties of the tumor cells, their survival would be the cause of the observed lytic effects on the surrounding collagen. A 3rd explanation is that estradiol would stimulate elaboration or activation in breast cancer cells of collagenolytic enzymes. The partial or total destruction of the surrounding collagen would then suppress the shortage of nutrients or oxygen and thereby allow cell survival. This 3rd explanation is the most plausible for 1 major reason: breast cancer cells do not seem to be estrogen dependent for survival in culture, provided they are not surrounded by dense collagen. Thus, good survival was obtained without added estradiol under the following circumstances having in common the absence of a collagen barrier: (a) when the cells were located at the periphery of the explant; (b) when centrally located cells had already digested the surrounding collagen during an initial culture with added estradiol; (c) when the collagen had been eliminated by added exogenous collagenase during the 1st 2 or 2 days of culture.

From these experiments, one can perhaps deduce a tentative hypothesis stating that survival of breast cancer cells in scirrhous ductal carcinoma depends in organ culture upon elaboration or activation by these cells, under the influence of estradiol, of enzymes dissolving the collagen matrix, thus allowing diffusion of the necessary nutrients. In all experiments, estradiol was added in combination with insulin or insulin and prolactin. It cannot therefore be answered whether estradiol alone would produce the same effect. Nor can it be said that estradiol is specific in triggering this enzymatic process. In rare instances, it was suspected that insulin or insulin plus prolactin induced some loosening of the stroma adjacent to peripherally located cords of cancer cells. Yet, this effect was insufficient to permit survival of centrally located cancer cells. Progesterone and other steroids were not tested in the present experiments. Additional studies are required, therefore, to draw a complete picture of the hormonal requirements of human breast cancer in organ culture.

Since specific tissue estrogen receptors are the supposed mediators of the metabolic effects of estrogens in their target tissues, they were assayed in all breast cancer samples subjected to culture and found present in about 80%. In the estradiol culture experiments, 5 of the 18 estradiol-responsive tumors were devoid of receptors while the 2 unrespon-
The present data support this view and further suggest that conclusions of Elliot and Turner (8) on the development of estrogen-sensitive metabolic reactions. Most if not all breast cancers, at least of the scirrhous type, are receptor positive, because, as reported elsewhere (22), negative receptor assays might be due to methodological artifacts. The possibility was suggested that it could act on substrates like collagen. The authors' opinion was "that the spreading factor is either elaborated or activated in the growing mammary gland cells by hormones (especially estrogens) which initiate both duct and lobule-alveolar growth of the mammary glands. The spreading factor may then break down the connective tissue in some manner allowing the rapid forward growth of the mammary ducts and lobes." The question arises whether this hypothesis may apply to breast cancer in vivo. In the present culture experiments, the minimum effective concentrations of estradiol were quite low, ranging from 100 to 10,000 pg/ml. The lower concentrations are not far above physiological levels (15 pg/ml in the serum of postmenopausal women). In earlier in vivo studies, Emerson et al. (9) described the loosening of connective tissue stroma around foci of active cancer in breast cancer patients subjected to estrogen therapy. The estrogen stilbestrol was administered at high doses in order to try to inhibit tumor growth. In their careful description, the authors observed that "the immediate cause of the softening of the tumor was in each case a loosening of the connective tissue surrounding the tumor cells." Such loosening appeared to result from partial dissolution of the collagen, for there was an overall depletion of collagen. It mainly occurred in the connective tissue immediately adjacent to the epithelial elements. Several authors reported collagenolytic enzyme activity in various neoplasms, as demonstrated in homogenates of a tumor (13) or in organ culture experiments (6, 26, 28, 33), suggesting that membrane-bound collagenase could confer upon tumor cells a distinct degree of mobility and invasiveness. Such studies were conducted on a few cultures of breast cancer with negative results (6, 26). No hormone supplementation was tried in these works and, as stated by Riley and Peacock (26), "cell death results in instantaneous cessation of the enzyme activity, and the failure of many tissue fragments to produce collagenolytic enzyme could be failure of tissue culture methods to sustain life for 5 days." Our own data, bearing on viable long-term cultures, strongly suggest that human breast cancer, at least of the collagen-rich types, is able to produce collagenolytic enzymes, but that this production is estrogen dependent. Moreover, this estrogen-dependent collagenolytic activity could well be an important part of the mechanism underlying hormone dependency of human breast cancer growth.

ACKNOWLEDGMENTS

We are grateful to the surgeons and to the pathologists of the Institut J. Bordet for kindly providing the specimens. We are indebted to S. Vermette and M. C. Deboel for their skilful technical assistance. Our thanks are due to Dr. P. G. Condiff for the National Institutes of Health, who generously provided ovine prolactin.

REFERENCES


Fig. 1. Culture of a soft infiltrating ductal carcinoma. H & E. A, initial condition. The tumor (large cells) invades nontumoral mammary tissue (small cells). Connective tissue is sparse, with loose collagen fibrils. B, central region of a 5-day organ culture of the same tumor. Culture in chemically defined Medium 199 supplemented with insulin (10 μg/ml) and hydrocortisone (1 μg/ml). As a general rule, tumor cells are well preserved. Collagen liquefaction is not necessary for the maintenance of viable tumor within the whole explant.

Fig. 2. Culture of a scirrhous infiltrating ductal carcinoma. H & E. A, initial condition. Tumor is characterized by dense collagen stroma. The explants were prepared without apparent loss of tumor cells. B, peripheral area of 6-day organ culture in Medium 199 + insulin (10 μg/ml) + prolactin (5 μg/ml). A cluster of viable tumor cells is present (arrow). Well-preserved fibroblasts and intact collagen bundles can be found even in deeper locations (*). C, same conditions as for B, but with further supplementation of the medium with 17β-estradiol (0.3 ng/ml). Nearly complete disappearance of collagen resulting in increased cellular density throughout the whole explant is observed. Under such conditions, viable tumor cords are present in central parts of the explant (arrows). D, same conditions as for B, except that the initial 48-hr period of culture was carried out in the presence of collagenase (4 mg/ml). The collagen is totally dissolved. The micrograph shows the whole explant with its very high cellular density, probably explained in part by the disappearance of the collagen bulk. Most viable cells are epithelial tumor cords (for higher magnification, see Fig. 2B). Collagenase treatment was sufficient for the maintenance of viable tumor cells in the absence of estrogen.

Fig. 3. Higher-magnification micrographs showing the effects of estradiol and collagenase. H & E. A, same conditions as for Fig. 2C. Detail of the central area of an explant exposed to 17β-estradiol (0.3 ng/ml). A cluster of viable, healthy tumor cells is observed. It is surrounded by connective tissue with increased cellular density and a very loose network of thin collagen fibrils. B, same conditions as for Fig. 2D. As a result of collagenase treatment, the collagen is totally dissolved. Viable tumor cells were maintained in the absence of estrogen. They are easily recognized from fibroblasts as a cluster of densely packed epithelial cells.

Fig. 4. Details of culture of a scirrhous infiltrating ductal carcinoma. H & E. A, 7-day culture in medium containing insulin (10 μg/ml) and ovine prolactin (5 μg/ml). Peripheral area of an explant; viable tumor cells are still present. No liquefaction of collagen around them is observed. In deeper locations, the tumor is pyknotic (arrows). Such deeper locations are to be compared to B, C, and D for survival of tumor in the central area of the explants. B, 4-day culture in the same medium but further supplemented with 17β-estradiol (0.1 ng/ml). Central area of an explant; survival of tumor is observed, with dissolution of the collagen in close contact with tumor cells. Survival of this tumor cord should be compared to the necrotic condition in the deeper locations indicated with arrows on A. C, 7-day culture in the same medium as for B. Central area of an explant; extensive digestion of collagen is visible. The picture was focused on stroma. Only tumor cells can be clearly recognized in the lower part of the central cluster. They look healthy, with prominent nucleoli. D, 14-day culture in the same medium as for B and C, except that 17β-estradiol (0.1 ng/ml) was added only during the initial 7-day period of culture. Central area of an explant. Once sufficient loosening of stroma is obtained in such a central area, estradiol is no longer necessary for the maintenance of very healthy tumor cells. The presence of prominent nucleoli, suggesting ribosomal synthesis, is proof of their good condition.
Estradiol-dependent Collagenolytic Enzyme Activity in Long-Term Organ Culture of Human Breast Cancer

Jean-Claude Heuson, Jean-Lambert Pasteels, Nicole Legros, et al.