mv) and the rest potentials for the germanium (−500 to −650 mv) to the point of zero charge (PZC) for germanium. The PZC for germanium (*p* and *n* types) has been reported for 0.1*N* HCl as −900 mv (relative to a SCE), on the basis of capacitance determinations (*9*). Both the rest potential and the potential for enhanced adsorption are several hundreds of millivolts more positive than the PZC.

We conclude that the surface charge does indeed play a strong role in enhancing fibrinogen adsorption from saline solution, with a potential dependence very similar to that observed by Sawyer and his co-workers (*4*). However, this conclusion does not imply that the PZC is the same in our experiments as in those of Sawyer and his co-workers. The technique of infrared internal reflection spectroelectrochemistry should be extremely useful in continued studies of biological adhesion phenomena. The technique is not limited to germanium but can be extended to thin films such as carbon, platinum, copper, iron, and aluminum, by means of vacuum deposition of these materials onto the surfaces of internal reflection elements (*10*).

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Breast Cancer: Induction of Differentiation by Embryonic Tissue

Abstract. *A mouse mammary tumor, adenocarcinoma BW 10232, was maintained in vitro for 14 days, separated from embryonic mammary mesenchyme by a Millipore filter. Tubules developed in the tumor; deoxyibonucleic acid synthesis declined; and a presumptive acid mucopolysaccharide matrix, not evident in the controls, appeared.*

The importance of interactions between epithelium and mesenchyme during embryogenesis has been well documented (*1*). However, there have been few investigations of the effects of inductively active embryonic tissues on the growth of tumors. These studies have demonstrated the following: cell organization in a murine ascites tumor, murine mammary tumor, and some human tumors exposed to chick notochord, neural tube, and mesonephros; emergence of cartilagenous tissue from chick and human sarcomas cultured with chick notochord; one instance of differentiation in 28 cultures of rat renal tumor incubated with rat or mouse neural tube; and generation of nephron elements in a primitive human renal tumor exposed in vitro to fetal mouse neural tissue (*2*). Our studies demonstrate that embryonic murine mammary mesenchyme produced histological evidence of differentiation in a murine breast cancer.

A syngeneic mouse mammary tumor, adenocarcinoma BW 10232 (Jackson Laboratory), carried in C57BL/6J mice, was maintained in organ culture in direct combination with, or on the opposite side of a filter to, embryonic murine mammary mesenchyme tissue. Pieces of tumor no larger than 0.3 mm were cultured for 14 days on a membrane filter (Millipore, type TH, 25 ± 1 μm thick, 0.45-μm pore diameter) in plastic organ tissue culture dishes (Falcon) (*3*). Cultures were immersed in a defined medium composed of Eagle's basal medium with Earle's salt solution (87 percent); horse serum (10 percent); penicillin (100 unit/ml), streptomycin (100 μg/ml), mycostatin (100 unit/ml) (GIBCO), and, either 3 percent or 20 percent of 9-day chick embryo extract (EE) that had been centrifuged at 800*g* to remove debris. Cultures were maintained in a 5 percent CO_2, high-humidity incubator at 37°C.

Mammary mesenchyme was obtained from embryos of CF_1 Swiss white mice which were derived from a breeding colony. On the day 12 of gestation the skin bearing the mammary gland rudiments was peeled from underlying mesenchyme so that the mammary mesenchyme could be freed from the rib anlagen. Small pieces of viable mesenchyme were placed adjacent to the tumor on the filter or held in place by clot (ratio of EE to chicken plasma, 1 : 1) across the filter from the tumor.

The tumor in vivo is characterized histologically by a stable morphology of compact cords of tumor cells with frequent mitoses (Fig. 1A). During an extended organ culture of 144 controls (71 in three percent EE and 73 in 20 percent EE) without embryonic inductive tissue, the tumor cells became randomly oriented (Fig. 1B). Only 2 of 144 control cultures presented any evidence for tubule formation, the presence of which was regarded as the minimum criterion for differentiation. Both of these controls were maintained in medium supplemented with 20 percent EE, which itself has been shown to promote DNA synthesis and support differentiation in vitro of pancreatic epithelium (*4*).

Histological evidence for differentiation was obtained after exposure of the tumor to embryonic mammary mesenchyme tissue either by direct combination or in a transfilter culture. Direct combination with mammary mesenchyme resulted in tubule formation in 8 of 24 tumors (33 percent)

grown in medium containing 3 percent EE, and in all 21 tumors grown in a medium supplemented with 20 percent EE. Exposure to mammary mesenchyme across the filter was associated with differentiation in 2 of 6 tumors (33 percent) in 3 percent EE and in 29 of 48 tumors (60 percent) in 20 percent EE (Fig. 1C).

In contrast to our results with mesenchyme tissue, differentiation did not occur after direct combination of 11-day embryonic brain with 10 tumors in 3 percent EE or with 21 tumors in 20 percent EE. Exposure to embryonic brain across the filter was not associated with differentiation in 6 tumors in 3 percent EE, but some tubule formation was present in 2 of 21 tumors cultured in 20 percent EE.

In the controls, the most active growth was along the filter where the nuclei were largest, mitotic activity most evident, and nucleoli greatest in number and size. Exposing the tumor in vitro to inductively active mammary mesenchyme produced a smaller cell size, a greater population density of cells, less mitotic activity, fewer nucleoli, and coarser chromatin patterns. Tubule formation, ranging from simple to complex branching patterns, was observed on the area of the tumor which was opposite to the filter.

As further evidence of differentiation, we looked for acid mucopolysaccharides in the cultures. Acid mucopolysaccharides have been associated with the epithelial mesenchymal interface during embryonic induction (*5*) and have recently been credited with an important role during cell aggregation (*6*). We stained the sections of the tumor with Alcian blue in order to examine the matrix of the tumor for evidence of acid mucopolysaccharides (*7*). The control cultures of tumor failed to stain positively with Alcian blue after 14 days of culture in vitro. In the embryonic tissue–treated tumors, however, Alcian blue–positive material was present between the epithelial cells in areas where tubule formation was evident.

In order to quantify DNA synthesis in the cultures, we exposed them to [methyl-^{3}H]thymidine ([^{3}H]dT) (Schwarz/Mann), specific activity, 1.9 c/mmole, diluted in the medium to a concentration of 1 μc/ml, for 24 hours on day 6 and day 13 of culture. There were significant differences in labeling rates (Table 1). Reduced or absent labeling was correlated with and preceded the appearance of differentiation in tumor cultured across the filter to embryonic mammary mesenchyme (Fig 1, B and C).

Table 1. Cell kinetic studies of in vitro mammary tumor. In each culture, 1000 interphase cells were counted. There were significantly fewer labeled interphase cells in tumors cultured with mammary mesenchyme. Correlations were calculated at 7 days ($P < .005$) and at 14 days of culture ($P < .001$) (Student's t-test).

Tumor	Culture (No.)	Labeled cells (% ± S.D.)
	7 days	
Control	10	72.0 ± 5.67
Across filter	14	60.1 ± 11.4
	14 days	
Control	13	62.1 ± 9.8
Across filter	13	47.4 ± 4.1

From initial studies it appears that heat-killed mammary mesenchyme can maintain an inductive effect (three of five tumors in 3 percent EE) and that formalin-fixed mesenchyme is not inductive (zero to six tumors in 3 percent EE).

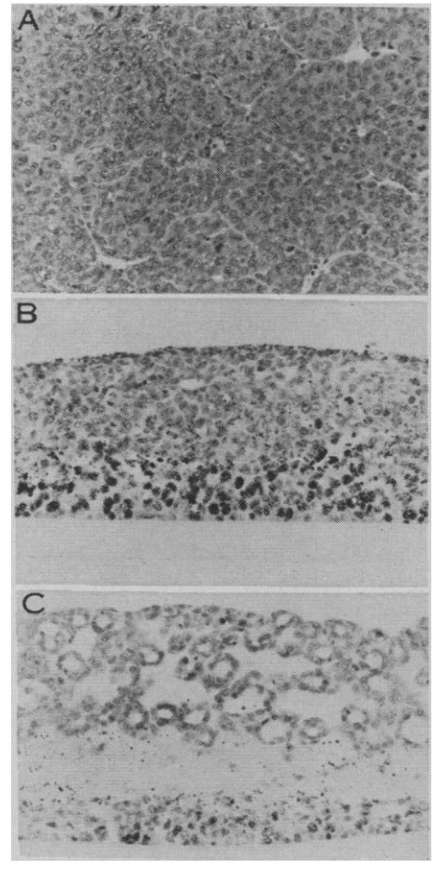

Fig. 1. (A) Mammary tumor cells after 7 days of growth in vivo; (B) after 14 days of culture in vitro and 24 hours of exposure to [^{3}H]dT; and (C) after 14 days of culture in vitro separated from embryonic mammary mesenchyme by a Millipore filter, and 24 hours of exposure to [^{3}H]dT. Each section was stained with hematoxylin and eosin (× 304).

We conclude that an agent or agents which was inactivated by formalin, probably stable to heat, and capable of traversing a 0.45-μm Millipore filter initiated several morphologic and functional changes in the mammary tumor compatible with differentiation: namely, development of tubules; interruption of DNA synthesis; changes in nuclear and cytoplasmic morphology; and appearance of a matrix tentatively identified as containing acid mucopolysaccharides.

These investigations indicate that histological evidence for differentiation of a murine mammary tumor can be obtained by exposing the tumor to inductively active embryonic tissues in an in vitro model. They demonstrate that modulation in cell cycle activity is associated with morphological differentiation and suggest that a matrix containing an acid mucopolysaccharide is a significant concomitant for the emergence of a differentiating population of tumor cells.

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