fibrillary acidic protein and interpreted as astrocytes[8]. However, the demonstration of capillary origin and the presence of marker enzyme, alkaline phosphatase, being characteristic of the endothelial cells, render it possible that the original tissue used as antigen was in parti contaminated by capillaries. Another possible interpretation could be that the cells growing in cultures with very similar morphological characteristics may be composed of 2 types: one reacting with the antibodies of glial fibrillary acidic protein, and another showing alkaline phosphatase positivity. Before performing biochemical experiments, one has to be certain of the cell population of cultures. The demonstration of alkaline phosphatase in cells of endothelial origin may be of help in determining the ratios between cells growing in vitro.

The current state of knowledge of neural dissociation has been claimed[3] to be still far from satisfactory. Our results showed that, after mechanical dissociation of brain tissue, viable cells of capillary origin, whose nature was evidenced as endothelial cells, were present in the cultures. Keeping in mind the important transport processes underlying the complex regulatory function of the blood brain barrier, attempts are made to obtain cultures consisting mainly of endothelial cells in the hope of using them as a novel approach in further studies.

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Decreased serum responsiveness by primary monolayer cultures of preneoplastic and neoplastic mammary epithelial cells

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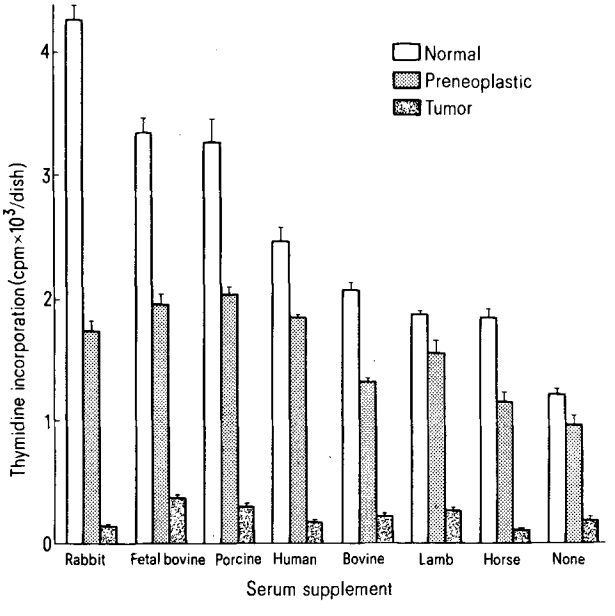
Summary. Monolayer cultures of normal, preneoplastic and neoplastic murine mammary epithelial cells were exposed to various types of mammalian serum. A progressive decline in levels of thymidine incorporation together with a change in the ordering of sera which stimulates optimal incorporation was observed in the transformed cells.

Monolayer cultures of normal mouse mammary epithelial cells (MMEC) respond to the presence of serum by increasing a) their levels of DNA synthesis[2–4], b) mitotic rates[5] and c) final cell densities[6]. Tumor cells obtained from spontaneously arising (MTV-induced) mammary adenocarcinomas respond in a similar manner[7–8], However, cultures originating from cells of the preneoplastic, hyperplastic alveolar nodule (probably NIV-induced) have not yet been examined. Although it is generally known that both normal and abnormal mammary cells synthesize greater amounts of DNA in the presence of serum, we sought here to compare the degrees of responsiveness to various types of mammalian sera under otherwise identical cell culture conditions. The results of these experiments form the subject of this report.



Histogram showing levels of incorporation of ^{3}H-thymidine in the presence of various types of mammalian serum on normal, preneoplastic and neoplastic mammary epithelial cells. Each value represents the average of 4 determinations. SEM for each value is indicated by the bars.

Material and methods. Normal MMEC were obtained from the glands of 16–17-day pregnant BALB/cfC3H mice (Cancer Research Laboratory, Berkeley, California). Preneoplastic cells were collected from primary hyperplastic outgrowth (HOG) of a nodule arising spontaneously in an 8–10-month-old mouse, whereas tumor cells were obtained from a mammary adenocarcinoma arising from a similar HOG, both from BALB/cfC3H mice. Normal and preneoplastic tissues were enzymatically dissociated as previously described[9]. Neoplastic tissue was minced and dissociated

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with 0.25% trypsin in Puck's saline A[10] for 30 min at 37 °C in a gyratory water bath shaker.
In all cases, the cells were initially plated in a Dulbecco's MEM medium[11] containing 4500 mg glucose/l and supplemented with 20% fetal bovine serum (FBS) and gentamycin (0.05 mg/ml, Shering) at 37 °C in an atmosphere of 5% CO_2:95% air. After 3 days the amount of FBS was reduced to 5% and a day later the medium was changed to one containing 5% of the mammalian serum to be tested. The final cell density of each culture well at this point was $5.0–6.0 \times 10^4$ cells/cm^2 of surface area as determined by standard hemocytometric methods. Sera selected for testing included the following: bovine (Grand Island, Santa Clara, Calif.), fetal bovine (Grand Island), horse (Grand Island), human (Microbiological Associates, Los Angeles, Calif.), lamb (Grand Island), porcine (Grand Island), and rabbit (Microbiological Associates).
Thymidine incorporation was measured as follows: After the cells had been exposed to the selected serum for 20 h, ^{3}H-thymidine (45 Ci/mmole, Research Products Corp., Elk Grove Village, Ill.) was added to the medium at a concentration of 1 μCi/ml media for the final 2 h of incubation. The cells were washed once in unlabeled media, twice with cold 5% trichloroacetic acid (TCA) and then placed at 4 °C for the next 2 days with fresh TCA to remove any unincorporated ^{3}H-thymidine. Cells were finally rinsed with distilled water and dissolved in 88% formic acid. An aliquot of this solution was assayed for radioactivity and the results were expressed as cpm incorporated into TCA-insoluble material over the 2-h labelling period.
Results and discussion. Normal MMEC responded markedly to selected sera by incorporating more ^{3}H-thymidine than serum-free controls. Rabbit serum was the most potent (3.5 times the control), followed by porcine (2.8 times), calf (2.7 times), and human sera (2.1 times) (figure). Preneoplastic cells, on the other hand, incorporated somewhat less ^{3}H-thymidine in response to serum (only 2fold above serum-free controls in the presence of porcine serum) (figure). Regardless of the type of serum used, the HOG cultures always incorporated less ^{3}H-thymidine than similarly supplemented normal cultures. A further observation is that both normal and preneoplastic cells respond almost identically in serum-free medium. Thus, it appears that cultures of preneoplastic cells, while displaying a decreased sensitivity to serum, retain as high a rate of incorporation in the absence of serum as do identically cultured normal cells.
Tumor cells incorporated ^{3}H-thymidine at a rate markedly lower than cells from normal glands and hyperplastic outgrowths (figure). FBS yielded the maximum stimulus of 1.8fold above serum-free controls. However, unlike normal and preneoplastic cells, tumor cells exposed to serum-free medium incorporated comparatively low levels of ^{3}H-thymidine. Thus, we may conclude that these cultured tumor cells have a decreased sensitivity to serum and possess a substantially reduced ability to incorporate thymidine in medium free of serum.
The significance of these data lies in the area of cellular recognition. Morphologically and biochemically, monolayer cultures of normal, preneoplastic and neoplastic MMEC often closely resemble one another. Thus, the differential responsiveness of normal and abnormal cells observed in these studies may prove important as an additional means of identifying these cells in vitro.

Les cellules mammotropes et somatotropes de l'antehypophyse chez la souris C3H porteuse de tumeur mammaire spontanée. Etude quantitative au microscope électronique[1, 2]

Mammotropic and somatotropic pituitary cells in spontaneous mammary tumor bearing C3H female mice. A quantitative electron microscope study

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Summary. Pituitaries of mammary tumor-bearing mice (C3H) were examined by quantitative electron microscopy. The results indicate that considerable modifications occur in mammotropic and somatotropic cells. Both types show an increase of the surface of the endoplasmic reticulum and a decrease of the volume of secretory granules (in percent of cytoplasmic volume), suggesting a heightened secretory activity of these cells during mammary carcinogenesis.

Il existe de nombreux arguments en faveur du rôle de l'hypophyse dans la génèse et la croissance des tumeurs mammaires tant chez l'animal[3–9] que chez l'homme[10–13]. Parmi les hormones hypophysaires, la prolactine a une action majeure et sa production semble augmentée au cours de la carcinogénèse mammaire. Ainsi la concentration en prolactine de l'hypophyse est plus élevée chez la chienne atteinte de cancer mammaire[14] et chez la femme atteinte de cancer du sein à un stade avancé[15–17] que chez les sujets témoins. De même la concentration dans l'hypophyse est généralement plus élevée chez la souris de souche à haute incidence cancéreuse[18], et le taux plasmatique de prolactine semble augmenté chez les femmes à haut risque[19].
A l'opposé les dérivés de l'ergot de seigle, qui inhibent la sécrétion de prolactine, réduisent l'incidence et la croissance des tumeurs mammaires chez le rat et chez la souris[20]. L'influence de l'hormone de croissance est moins bien précisée mais semble également importante: chez la souris hypophysectomisée, ovariectomisée et surrénalectomisée l'injection d'hormone de croissance est nécessaire à l'induction de lésions prénéoplasiques et à leur transformation cancéreuse, au moins dans certaines souches[4, 5].
L'objet de ce travail est d'étudier les modifications morphologiques des cellules mammotropes et somatotropes chez la souris porteuse de tumeur mammaire spontanée et d'apprécier le degré d'activité de ces 2 types cellulaires de façon aussi précise que possible par l'application de méthodes morphométriques au microscope électronique.
Matériel et méthodes. Le matériel provient de 8 souris C3H ♀ (MTV +) porteuses de tumeur mammaire spontanée et de 4 souris C3H ♀ (MTV +) témoins non