were preincubated at 37 °C for 1 min. WGA (0.01 mg/0.1 ml of isotonic NaCl-Tris) was added to agglutinate the cells and agglutination was observed within 5 min. To study the effectiveness of agglutination in vivo, L_{1210} mouse leukemic cells (1×10^5 cells/mouse) were inoculated i.p. into BDF_1 mice. After 24 h the mice received the 1st of 5 daily injections of the daunomycin entrapped erythrocytes and WGA at intervals of 10 min.

Results and discussion. 1 ml of resealed erythrocytes entrapped about 4 mg of daunomycin. Resealed erythrocytes (0.5 ml) were suspended in 5 ml of isotonic NaCl-Tris at 37 °C and efflux of daunomycin was estimated at 1, 3, 6, 12 and 24 h and 23, 42, 78 and 84% of entrapped daunomycin was leaked out respectively.

WGA agglutinated the L_{1210} leukemic cells and resealed erythrocytes in vitro (figure 2). The greatest increase in survival time was obtained in vivo when the daunomycin entrapped erythrocytes and WGA were given (table).

WGA mediates attachment of resealed erythrocytes to tumor cells. The effectiveness of daunomycin entrapped erythrocytes against leukemic cells especially demands targeting of the erythrocytes to leukemic cells, and it may be possible through the use of the lectins which do not have agglutinability of normal erythrocytes and mitogenic activity to lymphoyctes but have agglutinability of leukemic cells and surface modified erythrocytes.

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Evidence for the presence of viable endothelial cells in cultures derived from dissociated rat brain[1]

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Summary. The morphology and histochemistry of dissociated newborn rat brain was studied in tissue culture. Direct microscopy of developing cells, electron microscopy and the alkaline phosphatase activity were used to identify the capillary endothelial cells.

Tissue cultures obtained from mechanically dissociated brain tissue have been used for many years[3]. As a consequence there is a cumulative information on the distinctive properties of neuronal and glial cells, and on the extent and nature of glial-neuronal interactions. However, none of the reports published so far have dealt seriously with the possibility that in dissociated cultures, the cells deriving from the brain capillaries can also grow under the same conditions.

In the present paper, we show how the capillary fragments obtained by mechanical disruption of brain tissue as inevitable contamination can undergo morphological changes in vitro, and result in growing cells of capillary origin. Furthermore, these cells show some histochemical characteristics typical for the endothelial cells of brain capillaries.

Cerebral hemispheres from 3-day-old rats were dissociated in sterile conditions by pushing the minced brain tissue through nylon sieves of 250 and 125 mesh pore sizes. Fragments being attached to the sieve were suspended in tissue culture medium and plated out immediately on the cover-slip, according to the culture method described in detail previously[4]. The homogenate obtained was centrifuged and processed further as described by Joó and Karnushina[5] for the isolation of capillaries from brains of adult animals. After differential and density gradient centrifugations, the pellet was resuspended and in part seeded. The ultrastructure of the pellet was checked in the electron microscope (figure 1). From the fragments of dissociated brain tissue, as expected, several different types of cells started to grow. Among the neuronal and glial cells, large (about 25 µm in diameter) occasionally elongated, but as a rule round and flat cells were growing (figure 2). The same type of cell of unknown nature was also present in those cultures, which were derived from the pellet of centrifugations. It was clearly seen that when the short segments or longer networks of capillaries settled in vitro, the large and flat cells originated from the smaller, elongated cells of the capillary tubes themselves (figure 3). The cells of capillary origin, possibly due to their stronger viability, have grown faster than other cells and within some days (varying from 2 to 5 days) formed a continuous monolayer (figure 2). Several neuronal and glial cells were observed to grow later on the surface of the monolayer, establishing contacts either with each other or with the capillary cells. To characterize the enzyme pattern in the cells of capillary origin, histochemical reactions were performed. Alkaline phosphatase[6] and dopa-decarboxylase activities confined to the capillary wall have been regarded as characteristic marker enzymes for the endothelial cells of brain capillaries. Many large and flat cells exhibited strong alkaline phosphatase activity (figure 4), which could easily be observed among the nonreactive neuronal and glial cells. There were, however, cells which did not show alkaline phosphatase activity, although they had the characteristics of capillary origin. L-dopa was taken up to varying extent by almost every cell regardless to its nature and origin (figure 5). Dopa-decarboxylase, though believed to indicate an

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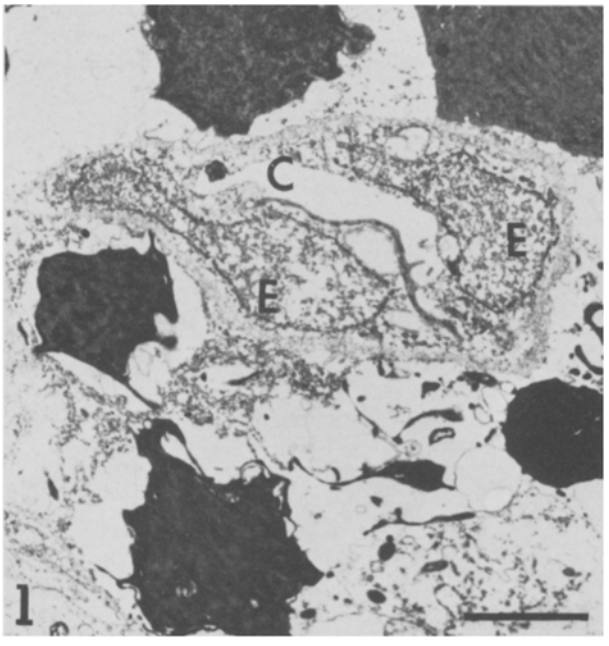
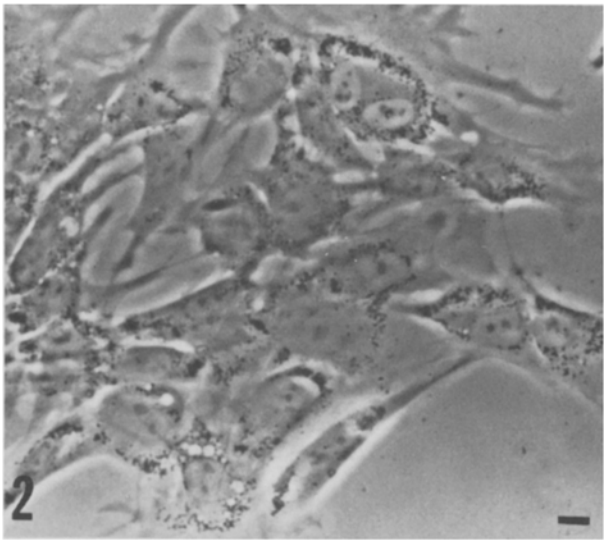




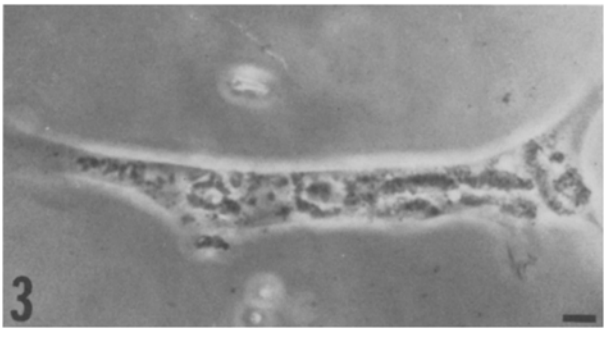


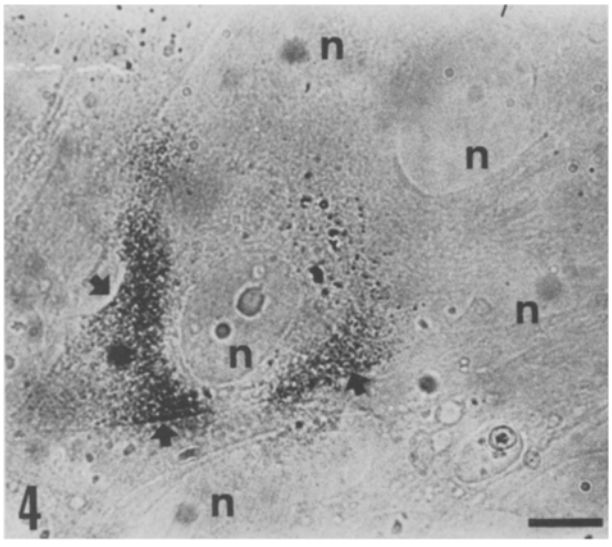
Fig. 1. Ultrastructural view of the capillary fraction of the centrifugate. A transverse section of a capillary wall (C) is seen in the upper middle part. 2 nuclei of the endothelial cells (E) are seen. Scale bar 5 μm.

Fig. 2. A monolayer of flat, rounded or elongated cells has formed after 12 days' culture. The cells were cultivated from the fragments harvested from the nylon mesh. Phase contrast microscopy. Scale bar 10 μm.

Fig. 3. A piece of capillary tube is observed after 2 days' culture by the phase contrast microscopy. Scale bar 10 μm.

important function closely related to the blood brain barrier of brain capillaries, could not be used in the identification of cells. Routine electron microscopic investigation of the cultures revealed that, among other cells, there were large cells showing features characteristic of endothelial origin. Detailed description of the ultrastructural histochemical localization of the enzymes will be given elsewhere.

It is conceivable that, in dissociated brain cultures prepared with the conventional use of 45 μm nylon sieve, segments of capillary tubes were fragmented into smaller units which caused some difficulties in the identification of the cells of capillary origin. It may have been misleading that, in immunohistochemical studies, cells with morphological features similar to the endothelial cells have been found to react with antibody raised against glial



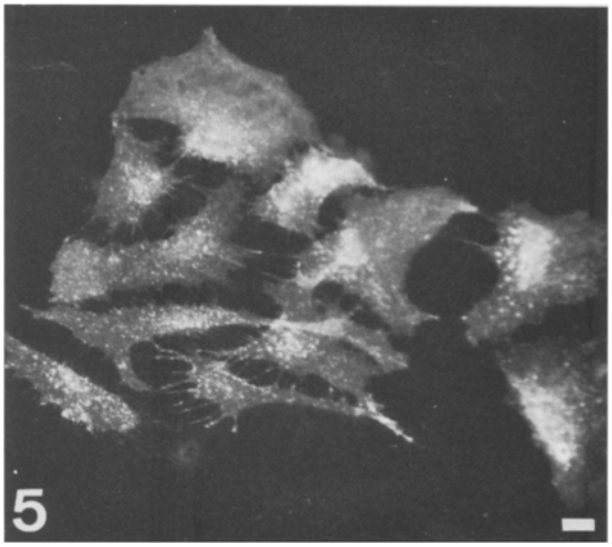


Fig. 4. Light microscopic alkaline phosphatase activity is localized in the cytoplasm of a large, flat, most probably endothelial cell (indicated by arrows). The surrounding cells show no enzyme activity in their cytoplasm (n = nucleus). Scale bar 10 μm.

Fig. 5. Glyoxylic acid induced fluorescence after L-dopa treatment of 14-day-old cultures. The cells originate from the fragments, which were attached to the nylon mesh. Granular fluorescence is noticed in all cultured cells. 2% glyoxylic acid treatment for 5 min, drying and heating thereafter for 5 min at 100 °C. Scale bar 10 μm.

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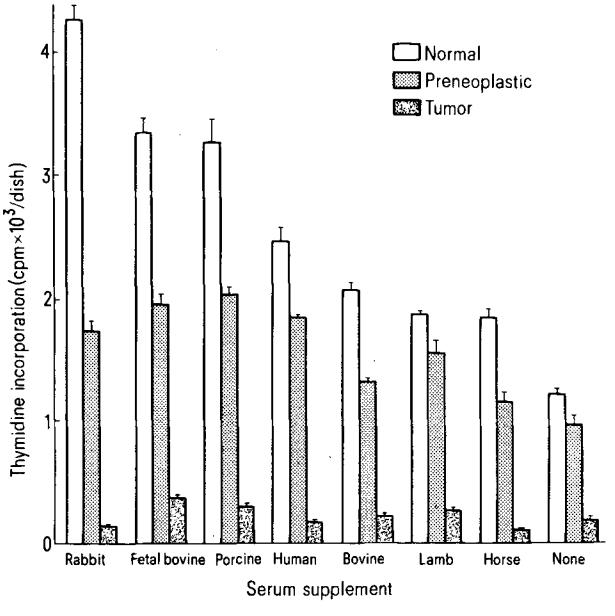
Summary. Monolayer cultures of normal, preneoplastic and neoplastic murine mammary epithelial cells were exposed to various types of mammalian serum. A progressive decline in levels of thymidine incorporation together with a change in the ordering of sera which stimulates optimal incorporation was observed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