Variations in Cell Overlapping and Growth Depending on the Substratum (36366)

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The inhibition of growth under conditions of cell crowding has been studied in cultures of several types of normal and virus transformed cells in many laboratories (1). In some cases the data suggested that the transformed lines had a decreased sensitivity to the inhibition of division but that the ultimate expression of the phenomenon depended on the affinity of the cells with the substratum (2, 3). To verify this hypothesis one would need a surface for the growth of cell monolayers where cell attachment could be easily modified. The present work describes the growth of Earle's L mouse cell line (4) on such a surface made of a film of a water insoluble protein polymer (5) which could have broader applications in tissue culture.

Materials and Methods. Bovine serum albumin (BSA) was polymerized with glutaraldehyde by adding with constant stirring 1.2 ml of a 2.5% aqueous solution of glutaraldehyde to 1 ml of a sterile 30% solution of BSA in 0.1 *M* phosphate buffer pH 6.8 (5). A volume of this solution adequate to make a layer 2–3 mm thick was quickly poured on the surface of 30 ml plastic Falcon bottles and the protein was allowed to polymerize for 3 hr at room temperature. Following polymerization the protein was covered for 24 hr with borate buffer 0.1 *M* pH 8.4 or with borate buffer containing 1 mg/ml of either polyglutamic acid (molecular weight 45,000), polylysine (molecular weight 175,000) or calf thymus histones. The polymer was then washed twice with culture medium and the cells were seeded. Control cultures were grown on noncoated plastic surfaces. The cells were maintained in Eagle's minimal essential medium supplemented with 10% calf serum and 50 μg/ml aureomycin. Before seeding the cells were pooled and then they were distributed evenly into the bottles. Each day thereafter cells from duplicate cultures

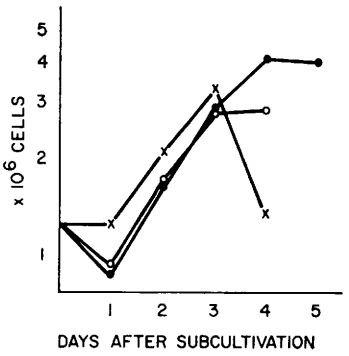

FIG. 1. Mean cell counts found in cultures growing on a plastic surface (x—x) and on the BSA polymer treated with borate buffer (○—○) or with polyglutamic acid (●—●).

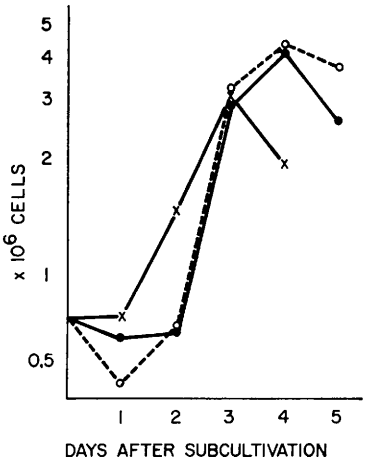

FIG. 2. Mean cell counts found in cultures growing on a plastic surface (x—x) and on the BSA polymer treated with polylysine (○- - -○) or histones (●- - -●).

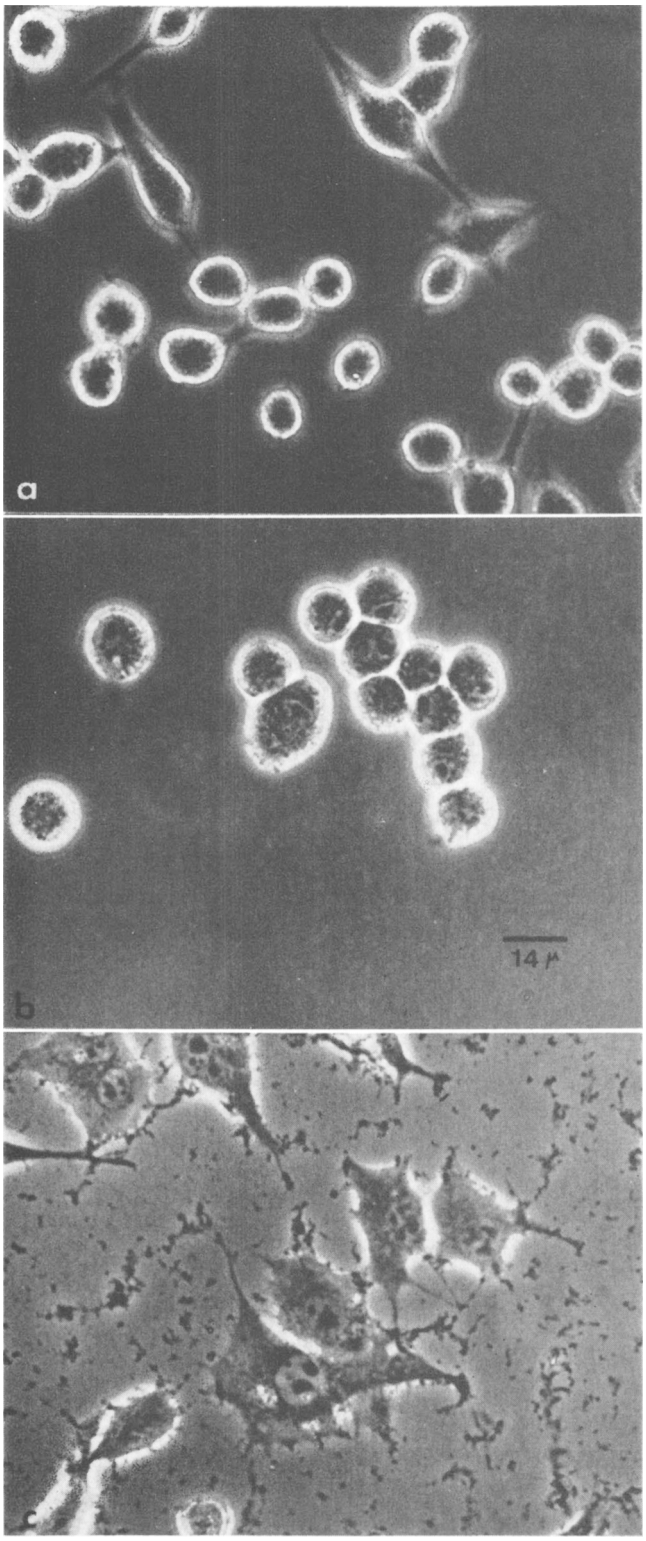

FIG. 3. L cells photographed by phase contrast microscopy after the first day of subcultivation on the BSA polymer treated with buffer (a), with polyglutamic acid (b) or with histones (c). The particles seen around the cells in (c) are due to a histone precipitate. Cells in the control group had the same morphology as cells in (c). The scale is the same for (a), (b) and (c).

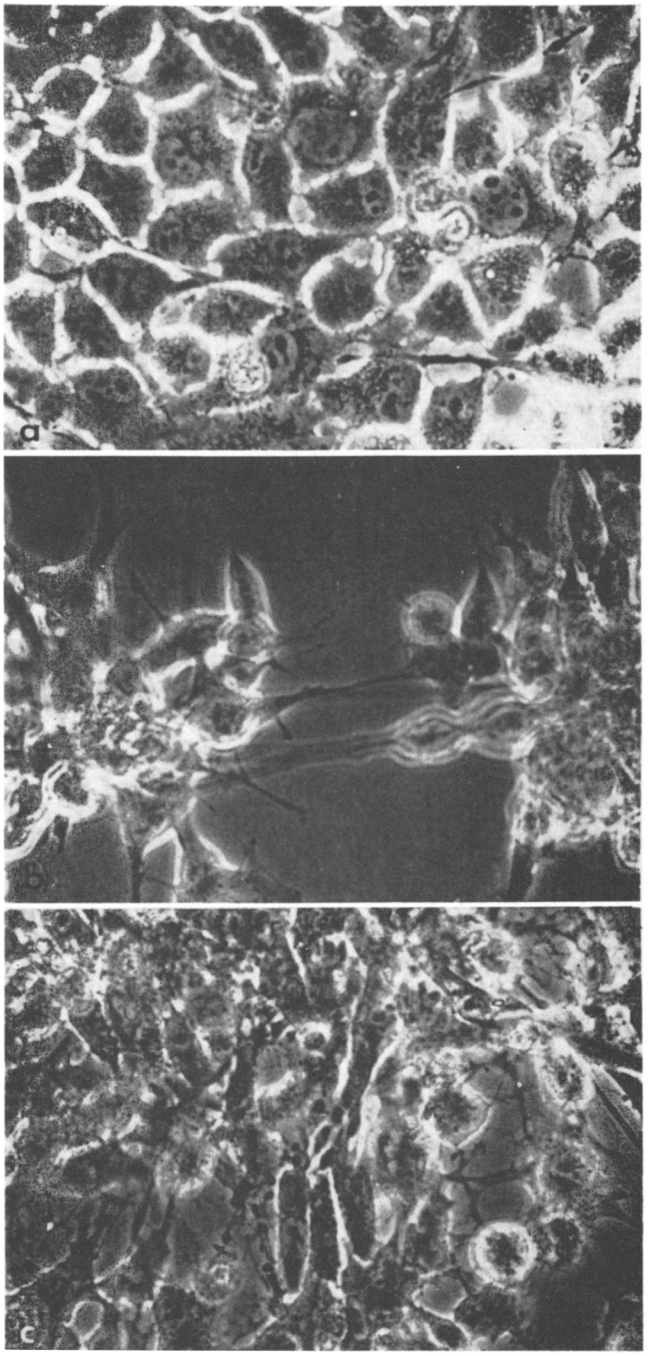

FIG. 4. L cells photographed with phase contrast when growth stopped after proliferation on the plastic surface (a) and on the BSA polymer treated with buffer (b) or with polylysine (c). Cells on the polymer treated with the basic proteins had the same morphology. The scale is the same as in Fig. 3.

in each group were suspended in a phosphate buffer salt solution (PBS) without calcium and magnesium which contained trypsin (0.25 mg%) and EDTA (0.2 mg%), and they

were counted in an electronic Coulter counter. Cell viability was checked by doing simultaneous counts with trypan blue in a haemocytometer. Since cell death was negligible the counts obtained whith both methods did not differ significantly. The difference found within samples was never greater than 5%.

Results. The counts showed that the growth of the cells varied with the treatment of the substratum. During the first 24 hr there was an initial diminution in the number of viable cells in all cultures growing on the BSA polymers as compared with those placed directly on the plastic surface (Figs. 1 and 2). During the second and third day of cultivation, the cells on the BSA polymers treated with borate buffer or with polyglutamic acid (Fig. 1) exhibited a growth rate identical with that of the control cultures after which the cell number decreased in the control cultures, remained stationary in the borate buffer treated BSA cultures and increased in polyglutamic acid treated BSA cell culture. Cells growing on BSA treated with histones, exhibited a longer (2 days) lag period before growth occurred (Fig. 2) but then the rate of growth and the maximum number of cells that were found were greater on the polymer treated with the basic proteins than in the controls. Cells adhering to BSA treated with polylysine and histones took a longer time to detach during trypsinization.

Morphologic analysis revealed different patterns of cell attachment. Isolated cells examined 24 hr after plating presented a fusiform or spherical shape on the BSA polymer treated with buffer or polyglutamic acid (Fig. 3a and b). There was slightly more spreading on the latter. Spreading was much better after treatment with the basic proteins (Fig. 3c) and resembled that of control cells. At the time that growth had ceased, *i.e.*, the third day in the controls and the fourth day in the cultures on the treated BSA polymers, control cells (Fig. 4a) had reached the stage of a confluent monolayer of flattened cells. Cells grown on the buffer treated polymer (Fig. 4b) were not confluent; they formed isolated aggregates or clusters, more than one cell layer thick, in which some of the cells, particularly those at the periphery of the cell islands, had a fusiform shape. Cell arrangement on the substratum treated with polyglutamic acid was of the intermediate type, *i.e.*, formation of aggregates mixed with areas of monolayering. Treatment with polylysine or histones resulted in a monolayer which was distinguishable from the cultures attached to the plastic surface by the fibroblastic shape with disorderly arrangement (criss-cross pattern) of the former (Fig. 4c).

Discussion. It has been previously shown by Carter (6) that overlapping of cells depended on the affinity of the cells with the substratum, but he did not study their growth. The changes in morphology that we observed could be explained on the basis of Carter's experiments (6). Cells would tend to form aggregates on the polymer due to little affinity with the substratum. Covering the BSA surface with a basic protein would increase cell attachment since the cell membrane has mainly negative charges. The variations observed in the maximal number of cells attained on the various surfaces could be due to a selection of cells with different sensitivities to the inhibition of division under conditions of cell crowding. A selection of cells could also explain the initial reduction in the number of cells, if those that are unable to grow on the substratum are eliminated. The results favor the previous suggestions (3, 6) that the expression of contact inhibiton of movement and of division depends to a certain extent on the substratum available to the cells.

To the best of our knowledge this is the only surface usable in tissue culture that can be easily prepared, can be covered with substances with various physicochemical properties, and on which cells grow and migrate differently. This surface could find many applications in cell and tissue culture, such as the study of growth control mechanisms, cell differentiation and the selection of different cell types.

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