THE EFFECT OF COLCHICINE ON MOSAIC PATTERNS IN CULTURED CELLS

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Bizarre geometric figures have been identified recently within cells in vitro (1, 2). Visible under phase-contrast illumination, these complexes are called mosaic patterns. They have been observed in cultured cells derived from a variety of sources, including human oral epithelium, melanoma, and carcinoma of the lip (2), but have not been reported in fixed tissues. In a careful study of the morphologic characteristics of mosaic pattern cells, Rose and Cattoni (2) concluded that the figures were expressions of cell differentiation. The structural alterations which account for the patterns in the cells are unknown. One theory suggests that the designs reflect changes in the physiochemical state of the cytoplasm, the phase-white areas indicating a sol state and the phase-gray bars indicating a gel (2).

Recently mosaic patterns were observed in cells of a primary culture derived from a human basal cell carcinoma. To investigate the basis for the patterning, particularly regarding the physiochemical state of the cytoplasm, I recorded alterations in the patterns with time, observed the movements of phase-dense particles within the cells, and examined the effect on the cells of colchicine, a drug which alters intracellular sol–gel relationships.

METHODS

Primary cell cultures were derived from a large solitary basal cell carcinoma located on the right leg of a 67 yr-old white male, according to a method described previously (3). Fragments of the tumor were incubated in 0.25% trypsin solution for 30 min. The cells were dispersed with paired needles, and the cell suspensions, containing 200,000 cells per milliliter, were introduced into four glass Cruickshank chambers. The nutrient solution consisted of Eagle's minimal essential media containing 10% calf serum to which penicillin (200 units per milliliter) and streptomycin (200 μg/ml) were added. The cultures were incubated at 37°C. Media were changed 1 day after planting and every 2 days thereafter. On day 14, colchicine (5×10^{-4} M) dissolved in media was added to two chambers and allowed to remain for 30 min. All four chambers were then rinsed and filled with fresh media.

Still photographs of selected cells were taken with Kodak Plus X film (Eastman Kodak Co., Rochester, N.Y.). Time-lapse cinemicrographic sequences were filmed at eight frames per minute with Kodak Plus X reversal film.

RESULTS

On day 2, the cells showed a great variability in shape. Both spindle and epitheloid forms were seen. The cells showed no evidence of contact inhibition (Fig. 1). They were motile and multiplied readily.

Mosaic figures began to appear in a few scattered cells about 96 hr after planting. The number of mosaic cells gradually increased, constituting approximately 80% of the total cell population by day 14. The cells in all four chambers were affected equally.

The geometric forms noted during the first week of culture consisted largely of scattered, light-dark alterations arranged in bands. The figures gradually grew more complex during the second week and manifested sharply angular and stellate outlines (Figs. 2 and 3). In some cells, the bands were only in the center; in others they extended to the periphery. In addition to the angular complexes, rounded phase-white clear areas or "holes" were noted (Fig. 5 *a*). Although some bands overlaid nuclear regions, the patterns did not appear to involve nuclei themselves.

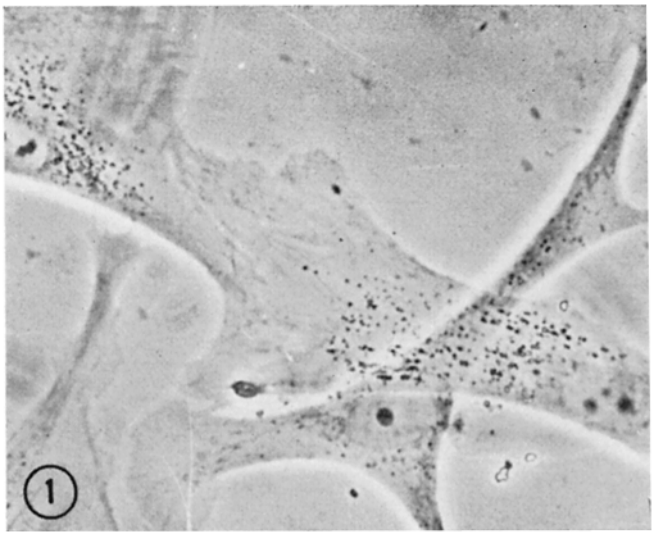

FIGURE 1 Cells in primary culture, derived from a human basal cell carcinoma, day 2. Contact inhibition is absent. × 40 objective, phase contrast.

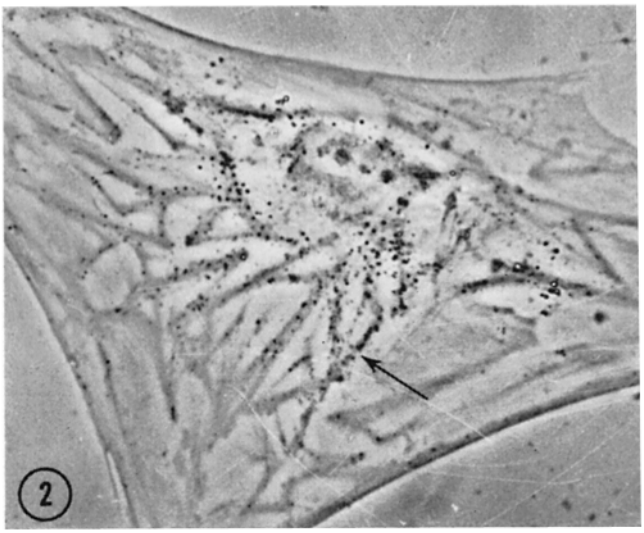

FIGURE 2 Mosaic pattern in cell, day 10. Phase-dense granules, which demonstrate saltatory movements, are concentrated along the gray bars (arrow). × 70 objective, phase contrast.

Time-lapse cinemicrographic studies indicated that the patterns were not fixed but modulated gradually. Phase-dense granules (Fig. 4 *a*) moved irregularly in a linear direction along the dark bands. The displacements were outside the range of Brownian movement.

Approximately 45 min after the cells had been exposed to colchicine, the mosaic patterns began to fade, but did not disappear after 2 hr (Figs. 4, 5). Pattern dissolution was irregular and lysis of the figures was more prominent in some cells than in others. The phase-dense granules which were localized along the dark bands tended to disperse (Fig. 4 *b*). The mosaic patterns did not reappear although traces were evident for 2 wk.

The cells in both the colchicine-treated and control chambers began to disintegrate during the fourth week of culture and were dead 27 days after planting.

DISCUSSION

The characteristics of mosaic patterns in cells examined in this study support the hypothesis that these complexes are related to physiochemical alterations of the cytoplasm. The modulations in the patterns suggest their labile rather than fixed

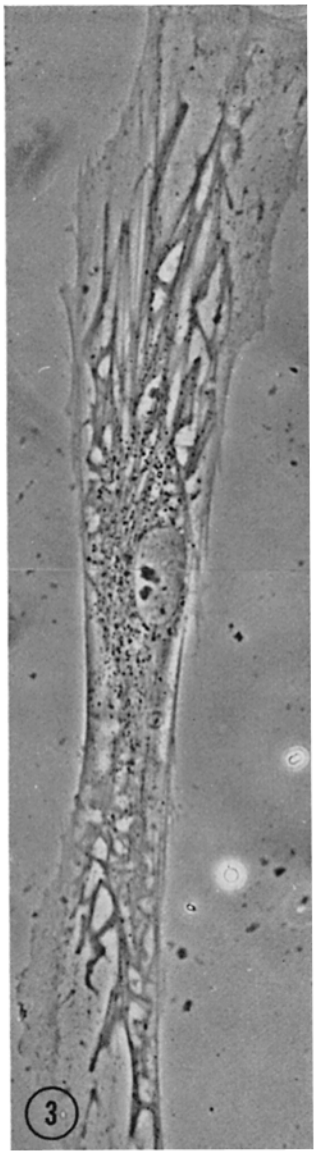

FIGURE 3 Mosaic pattern in cell, day 12. × 70 objective, phase contrast.

nature. The nonBrownian (saltatory) movements of phase-dense granules along the cytoplasmic bars indicate the presence of linear constraining elements (microtubules) (4), which have been related to gel strength in the cells (5). Colchicine-induced fading of the patterns is consistent with the drug's capacity to destory microtubules and to interfere with protoplasmic gelation in relatively low concentrations (6 and 7).

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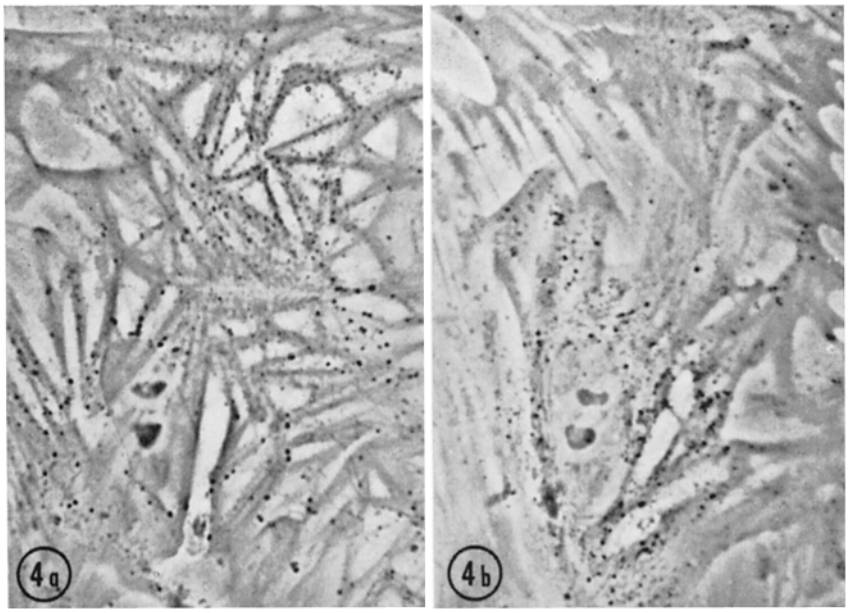

FIGURE 4 *a*, Mosaic pattern in cell, day 14. Phase-dense granules are lined up along the bars. *b*, same cell 2 hr after incubation with colchicine (5×10^{-4} M), showing dissolution of the complexes and dispersion of the phase-dense granules. × 70 objective, phase contrast.

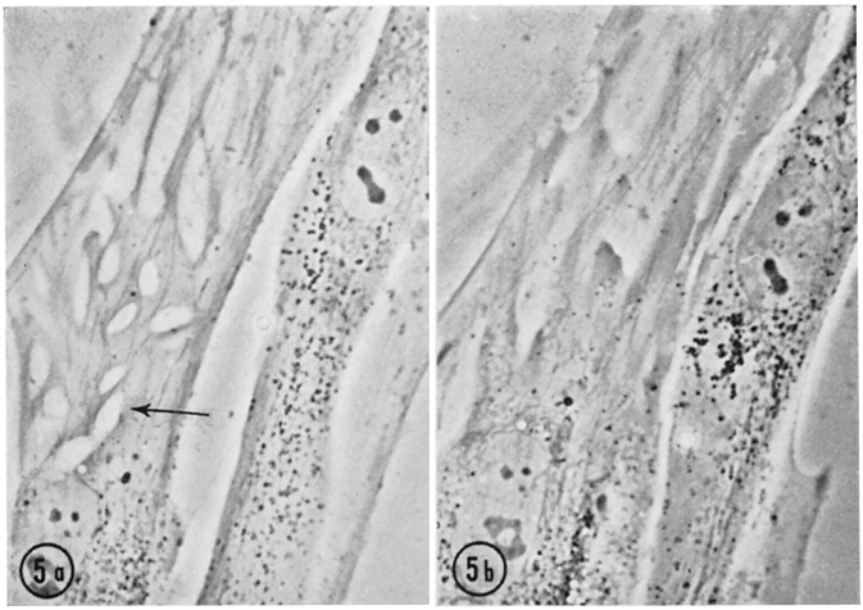

FIGURE 5 *a*, Cell with mosaic pattern showing phase-white holes (arrow) lies adjacent to uninvolved cell, day 14. *b*, Same cell 2 hr and 30 min after incubation with colchicine (5×10^{-4} M), showing fading of the holes. × 70 objective, phase contrast.