EFFECT OF COLCEMID ON CELL MIGRATION, MITOSIS, AND DNA SYNTHESIS IN MOUSE EMBRYONIC FIBROBLAST CULTURES

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The action of increasing doses of colcemid on mitosis, cell migration, and DNA synthesis in mouse embryonic fibroblast cultures was compared. Dose-effect curves for the blocking of mitosis and inhibition of cell migration into the wound were similar (minimal effective dose 0.01-0.015 μg/ml). DNA synthesis was stimulated only by doses of colcemid for which inhibition of mitosis was maximal: 0.035 μg/ml and higher.

A study of the action of metaphase inhibitors (colchicine and its derivatives, vinblastin, vincristin) is at present attracting considerable attention because these substances can act selectively on the microtubules of cells [6, 7].

Metaphase inhibitors not only have a characteristic action on mitosis in embryonic fibroblast cultures but also induce marked changes in cells currently in interphase. Stable areas of the cell surface disappear, leading to irregular activation of movements of all parts of the surface, so that the cells are unable to migrate in a fixed direction [4]. Metaphase inhibitors also induce the onset of DNA synthesis in many cells in high-density cultures [5]. However, it has not yet been discovered to what extend the two types of changes are interconnected. One approach to the elucidation of the connection between disturbances of the character of cell migration and stimulation of DNA synthesis is to compare the doses of colcemid inducing these effects.

The object of the present investigation was to compare the dose-effect curves for inhibition of mitosis, disturbance of cell migration into the wound, and stimulation of DNA synthesis under the influence of colcemid.

EXPERIMENTAL METHOD

Primary cultures of mouse embryonic tissue, grown on coverslips in penicillin flasks by the method described previously [3], were used. On the seventh day of cultivation, 24-30 h after a change of medium, part of the monolayer was removed and, at the same time, without changing the medium, 0.02-0.2 ml colcemid (Ciba) solution was added to the culture fluid to give a final concentration of the compound of between 0.0025 and 0.4 μg/ml. Thymidine-H^3 was added to the culture medium in a dose of 1 μCi/ml (specific activity 0.45 Ci/mmole) 24 h after the addition of colcemid and 30 min before fixation. The methods of fixation, staining, and preparation of autoradiographs were described previously [2].

Three experiments were carried out altogether, and at each point in the experiment two to five parallel samples were tested. Numbered preparations were counted by two investigators independently.

To assess the mitostatic action of colcemid the mitotic index was counted in 1000 cells in a preparation and the ratio between the phases of mitosis was determined (in 100 dividing cells per preparation).

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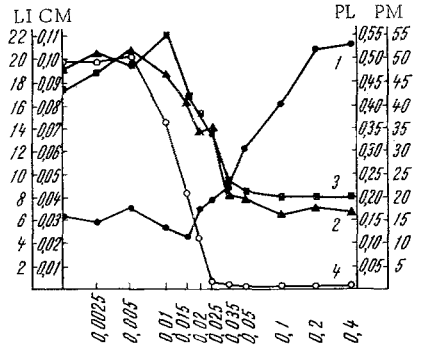

Fig. 1. Action of colcemid on migration of cells into the wound, DNA synthesis, and mitosis. Abscissa, doses of colcemid (in μg/ml), dose intervals plotted on a logarithmic scale; ordinate: 1) LI (in %); 2) CM; 3) PL; 4) percent of late phases of mitosis (PM). Each curve based on mean results of three experiments.

The proportion of cells in the S-phase was determined on autoradiographs and the index of labeled nuclei (LI) was calculated for 500 cells in the specimen.

The intensity of cell migration into the wound was assessed by means of two indices.

1. The coefficient of migration of cells into the wound (CM). The first stage in the work of obtaining this index was to determine the mean density of cells in the monolayer, expressed as the number of cells per mm^2 of culture. The number of cells was counted in at least 60 fields of vision under a magnification of 7 × 90 (area of field of vision 1.2×10^{-3} mm^2) in different parts of the monolayer. The number of cells migrating into the wound was counted above 1-2 mm of the line of division of the monolayer (total length of the line of division 5-7 mm). The ocular grid, consisting of a narrow rectangle (area 6×10^{-3} mm^2), was moved perpendicularly to the edge of the wound, starting from the last cell migrating into the wound. All fields of vision in which the cell density was 75% or less of the mean density of the monolayer were classed as belonging to the wound region. The coefficient of cell migration into the wound was defined as the ratio between the number of cells migrating from 1 mm of the wound edge and the number of cells present in 1 mm^2 of the monolayer.

2. The path length of the migrating cells (PL). Under low power (7 × 10) by means of an ocular grid the distance was determined from the distance of the cell migrating farthest into the wound in a given field of vision as far as the visible ridge formed at the site of division of the monolayer. After 20-30 measurements for each wound the mean index was calculated. At each point in the experiment at least 4 wounds were measured in this way.

EXPERIMENTAL RESULTS

Mitostatic Effect of Colcemid

Blocking of mitosis was observed after treatment with colcemid in a dose of 0.01 μg/ml (Fig. 1). With an increase in concentration of the compound the effect also increased, and doses exceeding 0.035 μg/ml arrested all dividing cells in the c-metaphase. So far as the mitotic index is concerned, a statistically significant increase was observed after doses exceeding 0.02 μg/ml (mitotic index 0.87 ± 0.13% in the control; after colcemid in a dose of 0.02 μg/ml the mitotic index was 2.38 ± 0.46, $P < 0.01$). The results of these experiments agree with those obtained previously [6].

Effect of Colcemid on Cell Migration into the Wound

The value of CM varied with the action of increasing doses of colcemid in the same way as the other index, PL (Fig. 1). These two criteria evidently give equivalent values for the intensity of cell migration.

The indices of intensity of migration began to decrease in cultures treated with colcemid in a dose of 0.015 μg/ml. With an increase in the doses up to 0.035 μg/ml these indices continued to fall. Maximal inhibition of migration of cells into the wound was observed after a dose of 0.05 μg/ml, no further increase in inhibition being observed after a further increase in the dose of colcemid (CM did not exceed 0.04). The cells in the wound were round in shape and were not oriented in any particular direction.

Action of Colcemid on DNA Synthesis

The results show that doses of colcemid which delay only some of the cells in c-metaphase were very close to the doses partly inhibiting cell migration into the wound (0.01 and 0.015 μg/ml respectively). Doses of the compound inducing a total block of mitosis (0.035 μg/ml) and maximal inhibition of cell migration (0.035-0.05 μg/ml) also were similar. The mitostatic effect of colcemid is linked with its action on the microtubules of the division spindle, and the action on cell migration is evidently the result of destruction

of the cytoplasmic microtubules [4]. The results suggest that microtubules of the cytoplasm and of the spindle in the system investigated are destroyed by colcemid in closely similar concentrations.

DNA synthesis in these experiments was stimulated only by doses of colcemid which blocked all cells in c-metaphase and induced maximal inhibition of cell migration into the wound (0.035-0.4 μg/ml). As mentioned previously, inhibition of cell migration into the wound is a reflection of disappearance of stable areas of the cell surface and the appearance of random movements of all borders of the cell. The results thus agree with the hypothesis that induction of DNA synthesis is a result of marked activation of movements of the cell surface. This activation of movements as the result of the action of colcemid may be one type of change in the cell surface induced by a number of different agents and leading to induction of DNA synthesis [1, 3].

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