the regulation of certain pituitary trophic functions and, in particular, corticotrophic, lactotrophic, somatotrophic, and thyrotrophic [1]. Accordingly, one of the mechanisms lying at the basis of the stimulating action of β-endorphin on secretion of ACTH, prolactin, and STH is evidently a fall in the concentration of catecholamines and, in particular, DA and NA, in the hypothalamus. This leads to activation of specific hypothalamic factors (and somatotrophin releasing factors) and to release of the pituitary trophic hormones.

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ACTION OF CYTOCHALASIN D ON DNA SYNTHESIS IN CELLS IN CULTURE

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KEY WORDS: culture of normal cells; cytochalasin D; DNA synthesis.

To explain the role of changes in the cytoskeleton and, in particular, cortical microfilaments in the regulation of cell multiplication, the effect of cytochalasin B (CCB), which destroys the system of actin microfilaments, on proliferation of cells in culture has been studied. It has been shown that CCB inhibits DNA synthesis in normal cells [2, 4, 6]. However, it has not been explained whether this effect is the result of changes in the cytoskeleton, for two other possible explanations have not been ruled out. We know that when cells are incubated in the presence of CCB, the last stage of mitosis (cytokinesis) is disturbed [1], and as a result of this part of the culture becomes binuclear. Some workers explain the inhibition of DNA synthesis during culture of cells in CCB by the direct inhibitory effect of the two nuclei on each other, the so-called "effect of restriction of polynuclearity of normal cells" [5]. Another problem not yet solved is whether inhibition of DNA synthesis is the result of a side effect of CCB — blocking of glucose transport into the cell [3].

In the present investigation, to solve the problem of the effect of changes in the actin cytoskeleton on DNA replication during the action of cytochalasins, the effect of long-term incubation of normal cells with cytochalasin D (CCD), which selectively destroys the microfilament system but does not affect transport of sugars, when investigated. Incorporation of labeled thymidine into mononuclear and binuclear cells in the presence of CCD and after its removal by rinsing also was studied separately.

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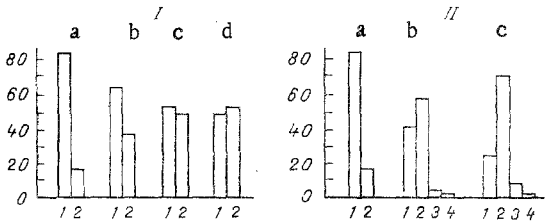

Fig. 1. Histogram of distribution of number of nuclei per cell after different times of incubation of MEF (I) and 3T3 cells (II) in medium with CCD. Abscissa, number of nuclei per cell; ordinate, % of total number of cells. I: a-d) 1st, 2nd, 3rd, and 8th days of incubation respectively; II: a-c) 1st, 3rd, and 8th days respectively.

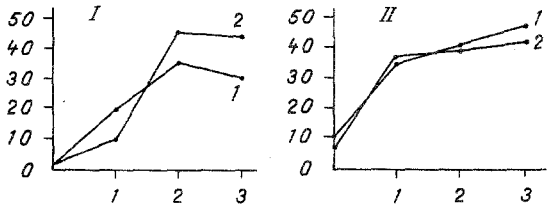

Fig. 2. Effect of CCD on incorporation of ^{3}H-thymidine (1 μ Ci/ml, 1 h) into nuclei of MEF (I) and 3T3 (II) cells. Abscissa, duration of incubation of cells in medium with CCD (in days); ordinate, ratio of index of labeled cells in medium with CCD to index of labeled cells of control culture (in %). 1) Mononuclear, 2) binuclear cells.

EXPERIMENTAL METHODS

A culture of mouse fibroblasts of the BALB/3T3 line and a secondary culture of fibroblasts obtained by trypsinization of mouse embryos (MEF) were used. The cells were seeded with a density of 10-20/cm^2 on coverslips, placed in plastic dishes, and cultured at 36°C in Eagle's medium with 45% lactalbumin hydrolysate and 10% bovine serum, in the case of MEF, or 10% embryonic calf serum in the case of 3T3. The medium was changed 24 h after seeding of the cells for fresh medium to which 0.2-0.3 μg/ml of CCD (from Sigma, USA) was added. The cells were incubated in medium with CCD for 7-8 days and the medium was changed every 3 days. To investigate DNA synthesis the method of autoradiography with ^{3}H-thymidine was used; for this purpose 1 μCi/ml of ^{3}H-thymidine was added to the culture medium at 1 h, or 0.1 μCi/ml at 24 h, was added to the culture medium (specific activity of the ^{3}H-thymidine was 15.6 Ci/mmole). The cells were fixed with a mixture of 96% ethanol and glacial acetic acid (3:1) for 20 min, the coverslips were transferred for 20 min into 96% ethanol, after which they were glued to slides. The preparations were rinsed 3-4 h in running water, dried in air, and coated with type M nuclear emulsion. Autoradiographs were exposed for 72 h at 4°C, fixed, and counterstained with Ehrlich's hematoxylin. To determine the index of labeled cells and the percentage of cells with different numbers of nuclei, 400-500 cells were counted.

RESULTS

A few hours after addition of 0.2-0.3 μg/ml of CCD binuclear cells began to appear in the culture of normal (MEF and 3T3) cells, the number of which increased progressively with lengthening of the incubation time (Fig. 1). By the 8th day the largest number of nuclei in cells in the MEF culture did not exceed two, whereas in the 3T3 culture 6-7% of cells with 3-4 nuclei were observed.

In the first hours of incubation with small doses of CCD, the index of labeled cells remained close to that of the control, but 16 h after addition of CCD to the culture medium the index comprised 5% compared to 98% in the control. The change occurred not only for

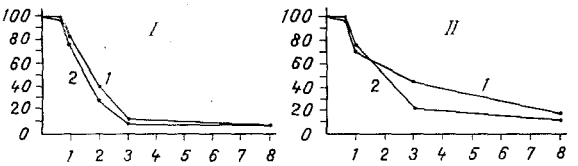

Fig. 3. Recovery of incorporation of ^{3}H-thymidine in MEF (I) and 3T3 cells (II) in fresh medium after incubation for each day in medium with CCD. Abscissa, time after rinsing culture to remove CCD (in days); ordinate, index of labeled cells (in %). 1) Mononuclear, 2) binuclear cells. ^{3}H-thymidine (0.1 μCi/ml) was added to the medium 24 h before fixation.

mononuclear, but also for binuclear cells (the indexes of labeled cells in the mononuclear and binuclear populations were compared). Furthermore, with the lengthening of incubation time, the percentage of cells in the medium with CCD gradually decreased (Fig. 2): Toward the 8th day it reached 1% and 6% in the MEF and 3T3 cultures, respectively (pulsed labeling).

During the first days in medium with small doses of CCD the shape of the cells changed very little: They were merely compressed a little, but preserved their fibroblast-like shape with a distinct lamella. With lengthening of the incubation time marked changes could be observed in the cell morphology, and after the 4th-5th day cells of various shapes and sizes began to appear in the culture: polygonal cells with long processes, large round cells with thickened edges, sickle-shaped cells with long, frequently branching processes. In the center of the large round cells the cytoplasm sometimes was reduced in thickness and broke up, and as a result of separation of the edges, fan-shaped cells with wide, curved processes were formed.

Inhibition of DNA synthesis and the morphological changes described above after incubation of MEF cells and 3T3 cells for 8 days in media containing small doses of CCD were reversible. When the cells were transferred to medium without CCD, synthesis of DNA recommenced after 16-20 h in both mononuclear and binuclear cells (Fig. 3). On the first days after rinsing, the cells were still larger than in the control, but their morphology returned to fibroblast-like. After a few days the number of binuclear cells in the culture decreased.

Thus on incubation of MEF and 3T3 cells gradual inhibition of DNA synthesis is observed. By contrast with observations made by other workers [2, 6], who found that DNA synthesis was inhibited in a culture of normal cells only 6-12 h after addition of CCB to the medium, in the present experiments with CCD no such early block was observed. Cells in the presence of CCD passed through their whole cycle of development during the first 2 days and DNA replication took place not only in mononuclear, but also in binuclear cells, but starting with the 2nd-3rd day DNA synthesis showed a sharp decline. This gradual inhibition can most probably be explained by the specific action of CCD on the active cytoskeleton of the cells as they pass through the cell cycle, for unlike CCB, CCD does not reduce glucose transport into the cell. Inhibition of DNA synthesis cannot be explained by the so-called effect of limitation of polynuclearity of normal cells, for in the early stages in medium with CCD and, in particular, in culture after removal of the CCD by rinsing, binuclear cells synthesize DNA.

The results indicate that structural changes in the active cytoskeleton can abruptly and reversibly disturb passage of the normal cell through the cycle.

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