EFFECT OF CYCLOHEXIMIDE ON PROTEIN, RNA, AND DNA SYNTHESIS IN CHO AND DIPLOID HUMAN FIBROBLAST CELL CULTURES

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Responses of cells to extremal factors have not yet been adequately studied, although the dynamics of protein, RNA, and DNA synthesis during irradiation and after partial hepatectomy has been well investigated [4, 9]. Similar changes have been found in the liver of rats receiving a sublethal dose of cycloheximide (CHI), namely 0.3 mg/100 g [8]. CHI is an antibiotic causing inhibition of protein synthesis in eukaryotic cells at the elongation stage [10]. The similarity of the changes observed in the cases of irradiation, hepatectomy, and exposure to CHI led to the thought that this inhibitor may be used to study the response of cells to extremal factors [8]. Protein, RNA, and DNA synthesis under conditions of exposure to CHI has been studied previously on cells in culture [5, 6, 11], although in this case no attention was paid to several factors as the change of medium and the addition of serum, both of which could modify the pattern of the cell response to CHI.

The aim of the present investigation was to study changes in incorporation of precursors of protein, RNA, and DNA synthesis in Chinese hamster ovarian (CHO) cells and in human diploid fibroblasts (HDF) in culture, exposed to different doses of CHI for different periods, on different phases of the cell cycle and during repair processes in the cell, while possible influences of additional external factors on the test system were reduced to a minimum.

EXPERIMENTAL METHOD

A culture of HDF was obtained from abortions undertaken on medical grounds (6-9 weeks) by the standard method [2], and cultured in Eagle's medium with the addition of 10% bovine serum and 5% human umbilical serum. To equalize the experimental conditions, the CHO cells were cultured under the same conditions. The level of protein, RNA, and DNA synthesis was estimated by measuring incorporation of ^{3}H-leucine, ^{3}H-uridine, and ^{3}H-thymidine, respectively. The label, in a concentration of 5 μCi/ml, was added for 0.5-3 h. The cells were fixed with 5-7% TCA for 5-10 min in the cold, and then lyzed with 0.1 N NaOH (40 min) at 37°C. Radioactivity of the lysate was measured in Bray's scintillator on a "Mark" (sic) counter. To cause the cells to accumulate in G_0, they were cultured for 3 days in medium with a normal content of serum, then washed with Hanks' solution and kept in serum-free medium for 48 h [1]. The cells were synchronized by seeding, on the assumption that after 20 h the main mass of the cells is in the S-phase [1].

EXPERIMENTAL RESULTS

Dependence of incorporation of precursors of protein, RNA, and DNA synthesis on the concentration of CHI and the duration of its action on HDF is illustrated in Fig. 1. The label was introduced all the time that CHI was present in the culture medium. The graphs show that as the CHI concentration rose to 1 μg/ml there was a sharp decline in incorporation

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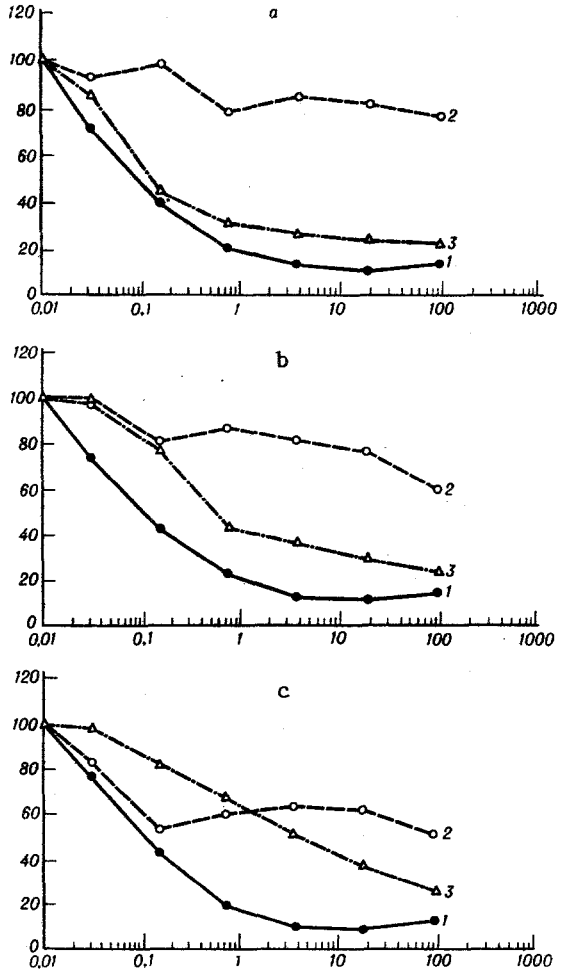

Fig. 1. Dependence of incorporation of ^{3}H-leucine, ^{3}H-uridine, and ^{3}H-thymidine in S-phase of HDF on CHI concentration and duration of exposure to it; a) 30 min; b) 60 min, c) 180 min. Here and in Figs. 2 and 3: 1) incorporation of ^{3}H-leucine, 2) of ^{3}H-uridine, 3) of ^{3}H-thymidine; here and in Fig. 2, abscissa — CHI-concentration (in μg/ml); ordinate — incorporation of label (in %).

of ^{3}H-thymidine and ^{3}H-leucine, but with a further increase of concentration up to 100 μg/ml there was no change. Maximal inhibition of protein synthesis amounted to 90%. Incorporation of ^{3}H-uridine also declined, but less steeply. Similar relationships also were observed in a culture of CHO cells (data not given).

To study the effect of serum factors on manifestation of the action of CHI and to compare effects of different doses of the inhibitor on macromolecular synthesis CHI was added in the S-phase for 1 h together with the label (Fig. 2a, b). When the medium was changed for serum-free medium, during addition of CHI to the CHO culture, against the background of inhibition of protein and DNA synthesis an increase in incorporation of ^{3}H-uridine was observed, which reached its highest level of 160-165% when the concentration of the antibiotic exceeded 1 μg/ml. Similar data also were obtained for HDF, but the increase in RNA synthesis was small (130-135%), and was reached when the CHI concentration was 0.016-0.073 μg/ml. The increase in ^{3}H-uridine incorporation was observed in a culture of HDF treated with CHI at the G_0 stage (Fig. 2c). Here also, against the background of inhibition of ^{3}H-thymidine incorporation, the incorporation of ^{3}H-uridine was reduced in the presence of small doses of the antibiotic (0.01-0.25 μg/ml), but in a dose of 10 μg/ml RNA synthesis was increased up to 200%.

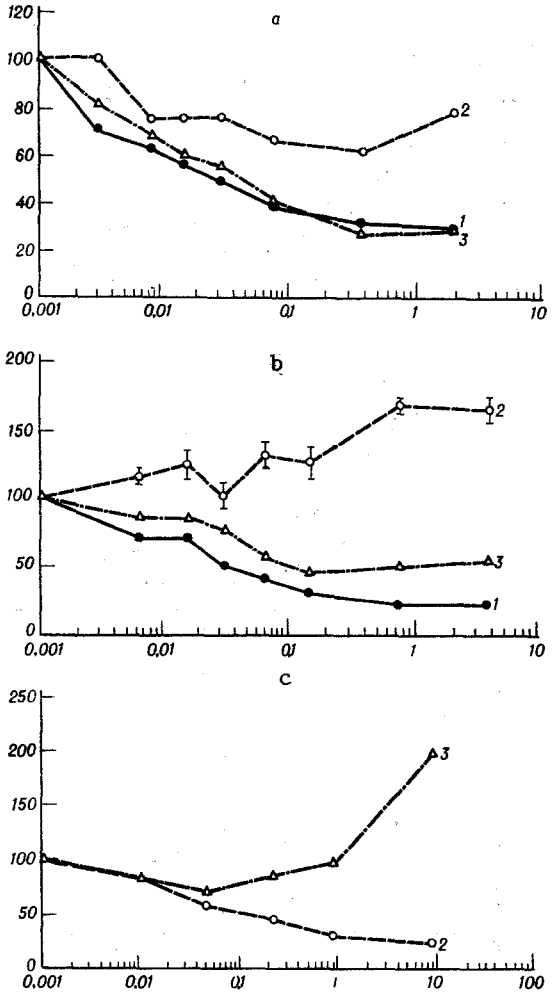

Fig. 2. Dependence of incorporation of ^{3}H-leucine, ^{3}H-uridine, and ^{3}H-thymidine in cells of CHO and HDF cultures on CHI concentration (CHI was injected for 1 h along with the label); a) CHO culture, S-phase, medium with normal serum concentration, b) CHO culture, S-phase, medium without serum; c) HDF, G_0 phase, medium without serum.

To study recovery of biosynthesis during the constant presence of the inhibitor in the culture medium, a dose of 1 μg/ml was used (if a higher dose was used, inhibition of protein synthesis remained virtually unchanged). The culture of diploid fibroblasts was in the G_0 stage in serum-free medium, and on the addition of CHI the medium was not changed. The label was added 2 h before fixation. In the first 6 h of the experiment a sharp decrease was observed in protein and DNA synthesis (by 80%), whereas RNA synthesis was increased, to reach a maximum (160%) after 10 h. This was followed by gradual recovery of incorporation of ^{3}H-leucine and ^{3}H-thymidine (16 h). The level of synthesis of all three types of macromolecules was close to the control level after 24 h, and it remained at that level until the end of the experiment (Fig. 3).

Previous investigations most frequently examined changes in intracellular macromolecular synthesis after short-term exposure to CHI and they observed restoration of synthesis up to and even above the control level [5, 6, 11]. However, the increase in synthetic activity of the cells can be connected with the change of medium, which was carried out in order to remove the CHI. Serum growth factors are known to stimulate macromolecular synthesis, and for that reason, in order to detect the cellular response proper to the action of CHI, it seemed advantageous to use serum-free medium.

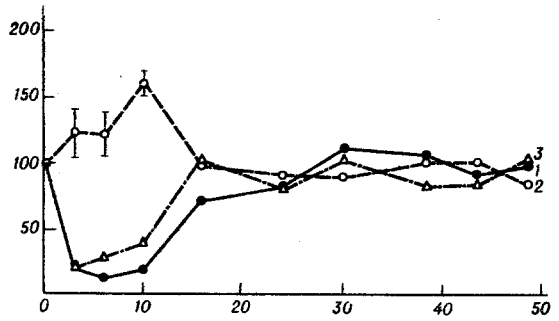

Fig. 3. Time course of incorporation of ^{3}H-leucine, ^{3}H-uridine, and ^{3}H-thymidine into HDF (G_0 phase) when CHI was constantly present (1 μg/ml) in the culture medium (serum-free medium). The label was added 2 h before fixation. Abscissa, time (in h); ordinate, incorporation of label (in %).

A certain analogy can be drawn between this experiment (Fig. 3) and the investigation of hepatocytes from rats receiving a sublethal dose of CHI [8]. In both cases the cells were in the G_0-phase and the inhibitor was present throughout the experiment. A common feature was inhibition of protein and DNA synthesis but enhancement of RNA synthesis during the first hours of the experiment. However, whereas the control levels were restored in the fibroblasts after 10-16 h, and thereafter they remained unchanged, in the hepatocytes subsequent activation of protein synthesis (24-36 h) and DNA synthesis (54-66 h), and a second burst of RNA synthesis (18-48 h) were observed.

These differences can probably be explained by active excretion of CHI from the organs. For instance, the half-elimination time of ^{3}H-CHI from rat liver is 28.8 h [3].

During the first few hours after treatment of cells with CHI in the G_0-phase, a response at the cellular level is observed. This is expressed as inhibition of protein and DNA synthesis accompanied by enhancement of RNA synthesis; subsequently, recovery processes begin to develop. Restoration of the control level is synthesis during long-term incubation with CHI can be explained by the development of compensatory processes in the cell, aimed at restoring the protein-synthesizing apparatus [7], although the possibility of a decrease in the concentration of the inhibitor in the medium as a result of its sorption on large subunits of ribosomes cannot be ruled out [10].

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