EFFECT OF TUBERCULIN ON MITOTIC ACTIVITY OF CELL CULTURES

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The effect of tuberculin on unsensitized cell cultures was studied. After contact for 30 min between human amnion cells and tuberculin in a concentration of 1.5, 1.0, and 0.5 mg/ml, the number of pathological mitoses was sharply increased (up to 90-95%), and pathological forms not present in the control were found (adhesion of the chromosomes in metakinesis). The authors consider that these changes are evidence of the toxic action of tuberculin on the cells. The action of tuberculin is reversible. The mitotic activity 3 h after exposure was identical with the control.

The sensitizing action of tuberculin on cell cultures has frequently been investigated [11, 12, 14]. However, little attention has been paid to the study of its toxic effect. Degenerative changes in internal organs, metabolic disturbances, and also death of animals under the influences of large doses of tuberculin have been described [9]. Only one investigation of the effect of tuberculin on cell cultures is known to the authors [13]. In that study no degeneratively changed cells were found.

The object of the present investigation was to study the effect of tuberculin on mitotic activity of human cell cultures. Mitotic activity provides an adequately sensitive index of the physiological state of the cells and it is changed by the action of many different factors [2-6, 10].

EXPERIMENTAL METHOD

Transplantable human amnion cells (strain Fe) were used as the test object. The cells were grown on watch glasses in penicillin flasks in medium No. 199 with 10% bovine serum and antibiotics; the seeding density was 220,000 cells per ml. A 48-h culture with mitotic activity of 19-25‰ and with 10-20% of pathological mitoses was used.

Dry purified tuberculin, prepared in the authors' institute by the method of Lazovskaya and Golubeva [8], was used in concentrations of 1.5, 1.0, 0.5, 0.2, and 0.02 mg/ml.

Before contact, and after contact for 30 min with tuberculin diluted in medium No. 199 without serum, the cells were washed twice with physiological saline warmed to 37°C and investigated immediately or 20 min and 1, 2, 3 and 24 h after contact.

Specimens fixed with ethyl alcohol were stained by Carazzi's method. Altogether 5000-8000 cells were counted. Mitotic activity was expressed in promille; pathological forms, determined by Alov's method [1], and the number of pathological mitoses are given in percentages of mitotic activity.

EXPERIMENTAL RESULTS

Mitotic activity showed no significant change under the influence of tuberculin during the first 3 h of observation under different experimental conditions. A slight decrease in mitotic activity (down to 13-18‰)

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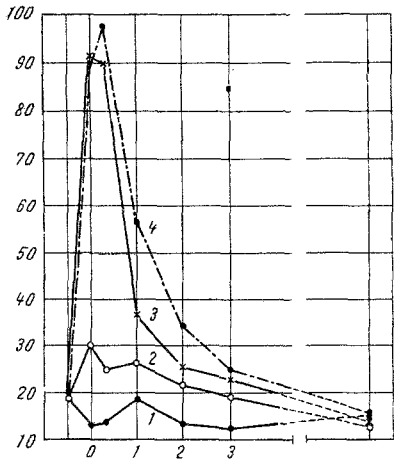

Fig. 1

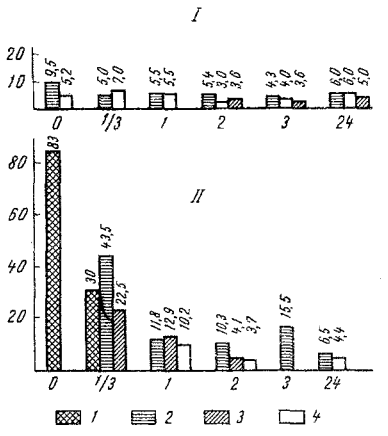

Fig. 2

Fig. 1. Change in number of pathological mitoses in cell culture during the action of tuberculin: 1) control; 2-4) tuberculin in concentrations of 0.1, 1.0, and 1.5 mg/ml respectively; here and in Fig. 2: abscissa, time of cultivation (in h); ordinate, pathological mitoses (in percent of total number of mitoses).

Fig. 2. Predominant forms of pathology of mitosis under normal conditions (I) and after treatment with tuberculin in concentrations of 1.5 and 1.0 mg/ml (II). 1) Adhesion of chromosomes; 2) scattering of chromosomes in metakinesis; 3) three groups at metaphase; 4) deletion of chromosomes in metaphase.

was observed as the result of changing the nutrient medium. Characteristically, metaphases were predominant at all stages of study of the culture (50-70% of the total number of mitoses).

Treatment with tuberculin in a concentration of 1.5 mg/ml led to a sharp change in the phases of mitosis. In some experiments during the first 20 min the number of prophases was sharply increased, to reach 60-70% (two to three times more than in the control), and the number of telophases rose to 40-100%. Metaphases were absent. Tuberculin in a concentration of 1.0 mg/ml in some cases also caused a sharp decrease in the number of metaphases and an increase in the number of telophases to 80%. Neither low concentrations of tuberculin nor a change of medium had any effect on the relative numbers of the phases of mitosis.

Delay of mitosis in prophase is evidence of damage to the chromosome by tuberculin, which is presumably connected with a disturbance of DNA synthesis [1].

Tuberculin had a particularly marked effect in increasing the number of pathological mitoses. The dynamics of the number of aberrant mitoses followed a characteristic curve with an increase in the early periods of observation and a more gradual decrease thereafter (Fig. 1). The action of tuberculin depended on its concentration in the medium. In concentrations of 1.5 and 1.0 mg/ml tuberculin caused on the average a sixfold increase in the number of pathological mitoses (up to 90-95%), while in a concentration of 0.5 mg/ml the increase was more than twice (25-30%) the control level. Lower concentrations of tuberculin, the medium used to dilute it, and a change of nutrient medium had no effect on the dynamics of the number of pathological mitoses. After transfer of cells which had been in contract with tuberculin in a concentration of 1.5 mg/ml into medium not containing tuberculin the number of pathological mitoses continued to increase for a further 20 min.

The differences between the dynamics of the number of pathological mitoses after exposure to tuberculin in concentrations of 1.5 and 1.0 mg/ml were statistically significant at the 95% level of probability 20 min and 1 h after contact, while between concentrations of 0.5 and 1.0 mg/ml they were significant at once and after 20 min and 1 h. Starting from 2 h, differences in the number of pathological mitoses depending on the tuberculin concentration were no longer statistically significantly different from the control level throughout the first 3 h of observation.

Under the influence of tuberculin not only the number of pathological mitoses, but also their form changed. In the control culture scattering and deletion of chromosomes in metaphase were the predominant forms, with the formation of three-group metaphases (Fig. 2). Immediately after treatment with tuberculin most of the dividing cells had adherent chromosomes. After the lapse of 20 min, besides adhesion of the chromosomes there were many cases of scattering of chromosomes and three-group metaphases. Later, the predominant forms of pathology were mainly the same as in the control culture.

The change in the forms of pathological mitoses, especially in the first two tests, can probably be explained by transition from one form of pathological mitosis to another. There are reports in the literature [7] of interconnection between pathological forms such as deletion of chromosomes in metaphase, three-group metaphase, and complete scatter of chromosomes.

Changes in mitotic activity after treatment with tuberculin (delay of mitosis in prophase, predominance of adhesion of chromosomes) thus differ significantly from the changes in mitotic activity in neoplastic conditions [1] (delay in metaphase, broad spectrum of pathological mitoses). The transient character of the tuberculin effect and the predominance of pathological mitosis associated with adhesion of chromosomes suggest that these changes are due to the toxic action of the substance. A study of changes in mitotic activity can be used to assess the side-effects of a tuberculin preparation.

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