CHANGES IN MITOTIC BEHAVIOR OF A CELL CULTURE CAUSED BY SENSITINS

L. N. Yakimenko

UDC 612.014.3:612.6 [.014.46:615.281.221.1

A study of the effect of three types of lyophilized preparations of sensitins on human amnion cell cultures showed an increase in the number of pathological mitoses, arrest of division in metaphase, and agglutination of chromosomes which were not present in the control. These changes indicate a harmful action of these preparations on chromosomes and on the achromatic spindle and, in the author's opinion, they characterize the toxic properties of sensitins.

KEY WORDS: sensitins; pathological mitoses; chromosomes; achromatic spindle.

Sensitins – preparations of the tuberculin series, by means of which diseases resembling tuberculosis and caused by a typical mycobacteria can be diagnosed – have now been prepared and are beginning to be used. Sensitins are being tested in accordance with the scheme used in tuberculin production, where the question of the toxicity of the preparations issued remains open. Model' [6] rightly considers that there are insufficient experimental data to prove that tuberculin is nontoxic for healthy animals.

The need for testing new preparations of the tuberculin series in a biological system of trials has been raised in a paper by Yablokova et al. [7]. In the investigation described below, sensitins also were tested for toxicity.

EXPERIMENTAL METHOD

Changes in the indices of mitosis of a 2-day transplantable culture of human amnion cells, strain FL, were used as criteria of toxicity. Sensitins were studied from three species of mycobacteria: M. fortuitum, M. kansasii, and M. battey. Lyophilically dried preparations of sensitins, diluted with nutrient medium to a concentration of 2 mg/ml, were kept in contact with a cell monolayer for 30 min. Indices of mitosis were studied immediately after contact with the preparations, and 20 min and 1, 3, and 24 h later. Details of the method were described previously [4].

EXPERIMENTAL RESULTS

Contact between the cells and the preparations of sensitins for 30 min led to a sharp increase in the number of pathological mitoses – up to 90-100% (Fig. 1). This number continued for an hour, but then fell during the next 24 h to reach the control level. Until 3 h of observation the curves for the number of pathological mitoses during exposure to all the sensitins studied differed significantly from the control (level of significance 0.95), but they did not differ from each other. Since this state of affairs also applied to other indices of mitosis examined below, data for only one of the sensitins are given in Figs. 2 and 3.

The preparations tested changed the forms of abnormal mitosis. Immediately after contact with the agents, swollen and adherent chromosomes began to appear in large numbers (up to 77-98%) in metaphase, although they were completely absent in the control culture. According to data in the literature [2, 5] this pathology of mitosis is evidence of disturbances of the surface structure of the chromosomes; it appears

Gor'kii Research Institute of Epidemiology and Microbiology. Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 237-239, February, 1976. Original article submitted July 11, 1975.

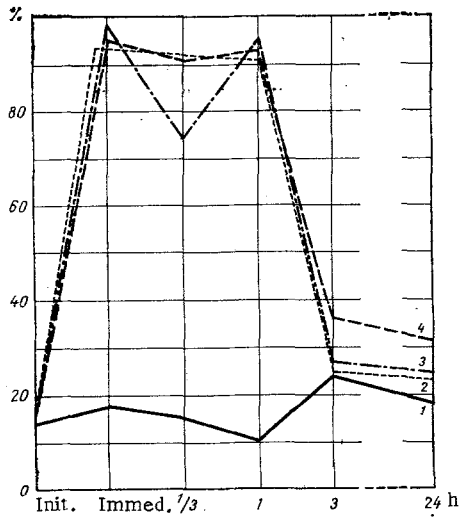

Fig. 1

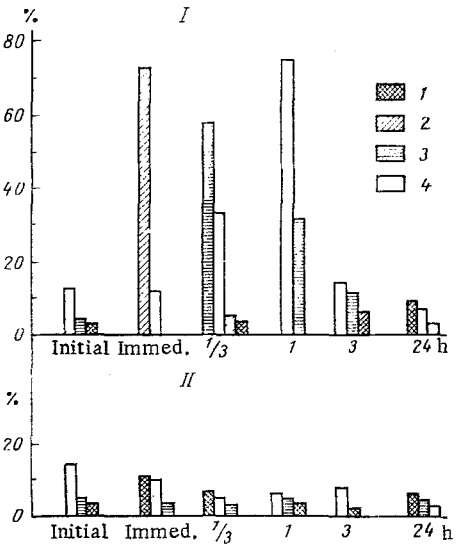

Fig. 2

Fig. 1. Changes in number of pathological mitoses in culture of FL cells during the action of sensitins. Here and in Fig. 2: abscissa, time after beginning of exposure, (in h); ordinate, pathological mitoses (in % of total number of mitoses). 1) Control, 2-4) sensitins from M. kansasii, M. fortuitum, and M. battey respectively.

Fig. 2. Predominant forms of pathological mitoses in culture of FL cells during the action of sensitins from M. kansasii (I) and under normal conditions (II). 1) Deletion of chromosomes; 2) agglutination of chromosomes; 3) dispersion of chromosomes; 4) triplet metaphase.

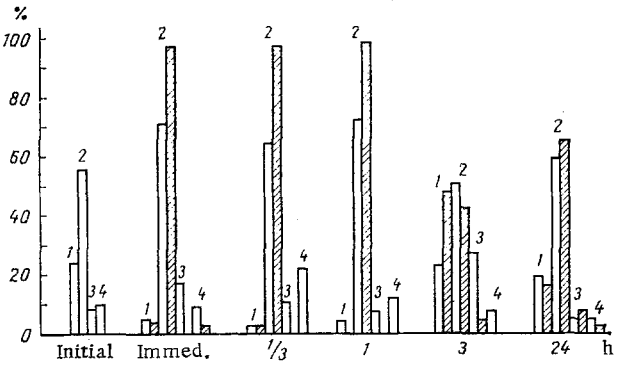

Fig. 3. Ratio between phases of mitosis in culture of FL cells acted upon by sensitin from M. kansasii: 1) prophase, 2) metaphase, 3) anaphase, 4) telophase. Unshaded columns – control; shaded columns – experiments. Abscissa, time of cultivation (in h); ordinate, phases of mitosis (in %).

frequently when mitotic poisons act on the cell and, to some extent, it may evidently reflect the toxicity of the preparation. In two successive tests, dispersion of chromosomes in metaphase and triplet metaphase appeared in numbers many times greater than the control level. These pathological forms are associated with separate disturbances in the structure of the division spindle [5]. Consequently, sensitins have a harmful action both on chromosomes and on the achromatic spindle. Simultaneously with the changes described, in the material tested after 1 h delay of mitosis at the metaphase stage was observed, and occurring simultaneously with a high percentage of pathological mitoses (Fig. 1, curve 3), this indicates that

the sensitins act upon the thiol mechanism of mitosis, responsible for assembly of the mitotic apparatus [3].

The sensitins tested did not alter the mitotic activity of the cells in the same way as tuberculin, as the writer has shown previously [4]. These results can be compared with the observations of Pearmain et al. [8], who found that tuberculin has no action on mitotic activity of peripheral blood leukocyte cultures from healthy donors.

Lyophilically dried preparations of sensitins, like tuberculin, thus have no effect on the degree of mitotic activity of unsensitized cells in culture, but they considerably increase the number of pathological mitoses, disturb the structure of the division spindle, and cause adhesion of chromosomes, thereby enabling their toxic properties to be detected.

LITERATURE CITED

1. L. A. Alov, Vestn. Akad. Med. Nauk SSSR, No. 11, 58 (1965).
2. I. A. Alov, The Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
3. I. A. Alov and M. E. Aspiz, Dokl. Akad. Nauk SSSR, 166, 965 (1966).
4. I. S. Golubchik and L. N. Yakimenko, Pyull. Éksp. Biol. Med., No. 5, 105 (1972).
5. L. F. Kurilo, "A study of the morphology and mechanisms of origin of triplet metaphases," Author's Abstract of Candidate's Dissertation, Moscow (1970).
6. L. M. Model', The Biology and Biochemistry of Mycobacterium tuberculosis [in Russian], Moscow (1952).
7. T. B. Yablokova, D. T. Levi, A. L. Lazovskaya, et al., Zh. Mikrobiol., No. 2, 99 (1971).
8. G. Pearmain, R. R. Lycette, and P. H. Fitzgerald, Lancet, 1, 637 (1963).