TIME-LAPSE PHOTOGRAPHY AND CYTOCHEMICAL INVESTIGATION OF TISSUE CULTURES TREATED WITH RNA FROM LEUKEMIC TISSUE

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UDC 612.014.1.014.46 : 616-006.446-008.939.633.2

Morphological changes, preceded by cytochemical changes, develop in the cells of primary monolayer cultures of chick embryonic tissue under the influence of RNA isolated from leukemic tissue. The intensity of the reactions for RNA and ribonuclease is reduced, while activity of DNA, desoxyribonuclease, and acid phosphatase is increased. The localization of acid phosphatase in the cultures is changed. No such changes are produced by DNA isolated from leukemic tissue.

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In reports of our previous investigations [2, 3] we described changes in cells due to the cytopathogenic action of RNA isolated from leukemic tissue of a transplanted rat erythromyelosis on primary monolayer cultures of rat embryonic tissue, testifying to its virus origin.

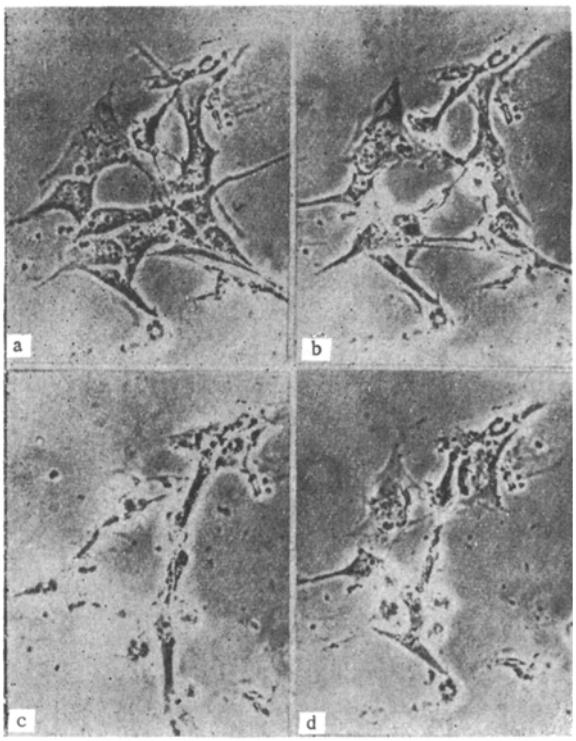

Fig. 1. Single frames from a motion picture film reflecting the dynamics of changes in cells of primary cultures after treatment with RNA isolated from leukemic tissue. Time-lapse photography, phase-contrast, 210×.

In the present investigation a more detailed morphological analysis was made by time-lapse photography and a cytochemical study was made of primary cultures of rat embryonic cells treated with RNA isolated from leukemic tissue.

EXPERIMENTAL METHOD

RNA was obtained from a subcutaneous nodule of transplanted rat erythromyelosis and also from the spleen and bone marrow of healthy rats by the phenol deproteinization method suggested by G. P. Georgiev [4].

Monolayer cultures of rat embryonic tissue were prepared in the usual manner [9, 11]. The cultures were inoculated on the 3rd day of growth. Three groups of tissue cultures were investigated: treated with leukemic RNA, treated with normal RNA, and controls. RNA was added in a dose of 0.2-0.4 ml per flask, in a concentration of 3-6 mg/ml. Slides with cultures were removed daily starting from the first day after addition of RNA and in the corresponding controls.

The cells were fixed with absolute ethyl alcohol or neutral formalin. To detect RNA, DNA, and acid alkaline phosphatase the cultures were stained by the methods of Brachet, Feulgen, and Gomori respectively, while to detect ribonuclease (RNase) and desoxyribonuclease (DNase) Pearse's method, modified in our laboratory, was used. For photography the cultures were kept in a perfusion chamber designed by ourselves.

Laboratory of Etiology and Pathogenesis of Leukemia, Kiev Research Institute of Experimental and Clinical Oncology, Ministry of Health of the Ukrainian SSR (Presented by Active Member of the Academy of Medical Sciences of the USSR A. D. Timofeevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 2, pp. 87-90, February, 1968. Original article submitted April 20, 1966.

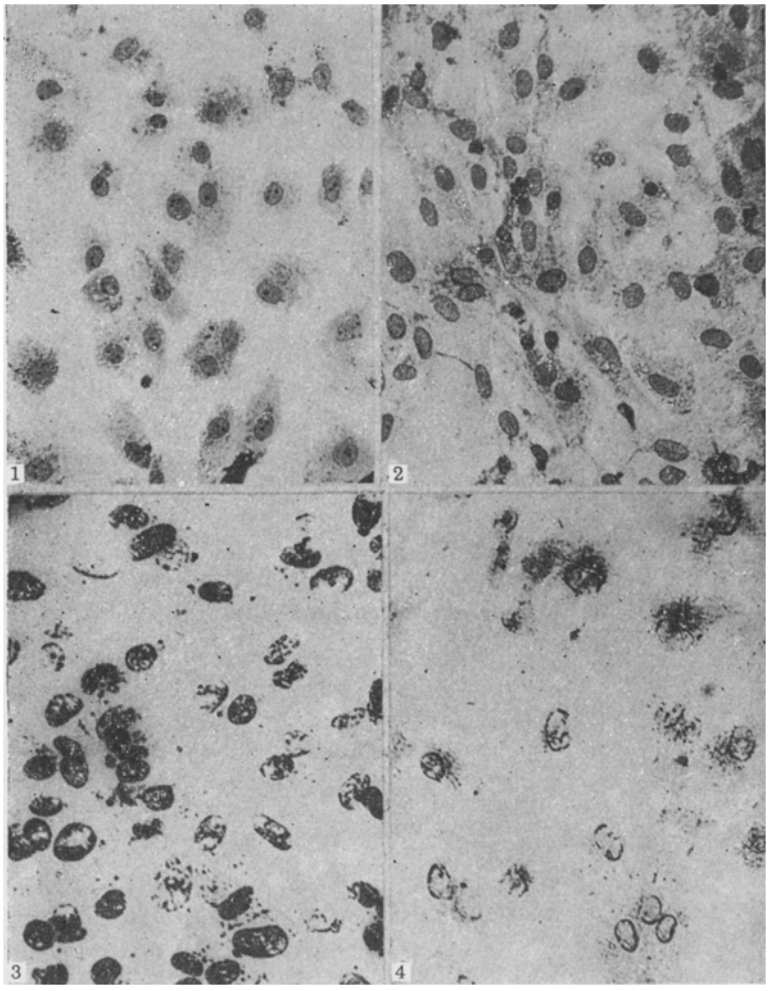

Fig. 2. Acid phosphates and DNase activity in cells of control tissue cultures and of cultures treated with leukemic RNA. 1) Acid phosphatase (control); 2) acid phosphatase (experiment); 3) DNase (control); 4) DNase (experiment).

Photography was carried out by means of the MKU-1 miniature camera fitted with a thermostatically controlled chamber. The speed of filming was one frame per min. Time-lapse miniature photography with a phase-contrast microscope was used [6, 7].

EXPERIMENTAL RESULTS

The onset of changes in the cells brought about by leukemic RNA was discovered by time-lapse photography on the 2nd day after inoculation, whereas by ordinary microscopic examination of cultures growing in flasks they cannot be seen until the 3rd-5th day. The cells at first shrank, the outlines of their cytoplasm because sharper, after which they became thinner and very elongated in shape; in some cells the nuclei became swollen, while in others they underwent pycnosis. After strong contractions of the cells the very thin bands of cytoplasm joining them ruptured and the syncytial connections between the cells were broken. The intensive movements of the nuclei in time ceased, indicating death of the cells. Ultimately debris was formed. Extracts taken from the film, demonstrating changes in the cells under the influence of leukemic RNA, are shown in Fig. 1. The speed of development of the degenerative changes depended on the dose of RNA.

The cytochemical changes in the cells of the experimental cultures took the form of a decrease in intensity of the reaction for RNA by comparison with the controls and an increase in intensity of the reaction for DNA. These changes appeared 24 h after incubation of the cells with leukemic RNA and were very stable. Only if the cytopathogenic action was very strong was there an increase in the number of cells possessing increased pyroninophilia. The DNA granules varied in size; most of the nucleus in the control preparation was filled with a mass of tiny granules, while in the experimental there were many more large granules.

Parallel with the change in the content of nucleic acids, changes were observed in the reactions for the corresponding enzymes (nucleases). The very small RNase content in the control cultures diminished still further in the experimental; the DNase content, on the other hand, increased. In the control cultures DNase was found in the nuclear membrane the chromatin of the nuclei, and the cytoplasm (as a few tiny granules); in the cultures inoculated with leukemic RNA, DNase activity in the cell nuclei showed a marked increase (Fig. 2).

Judging by the reaction for acid phosphatase, the cells of rat embryonic tissue contain a large quantity of it, but the localization of this enzyme in the experimental cultures differed essentially from that in the control, where acid phosphatase was found in the chromatin of the nucleus, the nuclear membrane, and nucleoli, with smaller amounts in the cytoplasm; in the experimental cultures the acid phosphatase content in the nucleus was relatively diminished, while the cytoplasm contained large numbers of granules (Fig. 2). In both experimental and control cultures only small amounts of alkaline phosphatase were present in the rat embryonic tissue cells.

Cytochemical differences between the cells began to appear on the 1st or 2nd day after inoculation, somewhat earlier than the first morphological signs. In cells treated with normal RNA no significant difference was found between the changes in or localization of the studied cell components compared with the controls.

DNA isolated from the same leukemic tissue did not produce any of the changes described above.

At this stage it is difficult to give a definite interpretation of the significance of the observed changes in relative content of nucleic acids and of their specific enzymes in cells treated with RNA from leukemic tissue. They may be considered in conjunction with other published observations [5, 8, 12], and recent developments in the study of biosynthesis of virus nucleic acids in cells [1, 10].

The results obtained are evidence of considerable disturbances of intracellular metabolism under the influence of RNA from leukemic tissue.

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