PROPERTIES OF RNA ISOLATED FROM LEUKEMIC TISSUE OF RATS IN TISSUE CULTURES

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The study of the nature of malignant changes and the role of viruses in producing them is closely linked to an understanding of the biological properties of the nucleic acids of neoplastic tissues, among which are leukemic tissues. Data obtained in recent years [3, 10, 13-16, 18] confirm that it is possible to induce leukemic and certain neoplasms in mice with the assistance of high-molecular compounds.

While studying the biological action of the nucleic acids of leukemic tissue, we pointed out [4] that it is possible to reproduce leukemia and to develop certain neoplasms in rats 3-8 months after the injection of newborn rats with RNA isolated from the leukemic tissue of rats with erythromyelosis. In addition, these facts confirm that disturbances in the metabolic processes play an exclusive role in establishing malignancy and are considered to be additional evidence for the viral nature of leukemia [1, 2, 6-9].

However, as in similar research work including ours, because the RNA was isolated not from the virus but from the leukemic tissue, it was impossible to exclude the participation of transformed cell RNA.

With the aim of making a further study of the properties of the RNA of leukemic tissue and of finding a possible cause of the disease in the present work we have carried out an investigation of the effects of this RNA on tissue cultures.

A number of reports have been published about the effects on tissue cultures of nucleic acids secreted by leukemic tissue [11, 12, 13] RNA, isolated from cells of human leukemia and placed on a culture of human amnion, leads to the development of a cytopathogenic effect which takes the form of an early degeneration of the single layer culture. These changes, together with the results of other investigations on the RNA in question, are considered to be indications of the infectiousness of nucleic acid of viral origin.

EXPERIMENTAL

The original leukemic material used for the separation of the RNA in our experiments was transplanted from the strain of rats with erythromyelosis obtained in 1957 [19] and the transplanted cell-free filtrate. In the control experiments the source for the material used for the separation of RNA was the spleen tissue and bone marrow of healthy rats.

Kirby's phenol method [17] and the method of G. P. Georgiev [5], with certain modifications, formed the basis of the method employed for obtaining bulk preparations of RNA. These preparations of RNA have the following characteristics: concentration 1-4 mg/ml, protein content up to 0.02%, ratio of nitrogen to phosphorus 1.68, temperature effect on viscosity 1-4.1, DNA impurities not detected.

The experiments were carried out on single layer cultures of mouse and rat embryonic tissue. The cells were grown on No. 199 medium with the addition of 10% calf serum. For the experiments three-day-old cultures were usually taken. To each of them was added 0.2-0.4 ml RNA in flasks, the cells being in contact with the RNA for 60 min at 4°C. Subsequently, the cultures with the nutrient solution were kept at 37°.

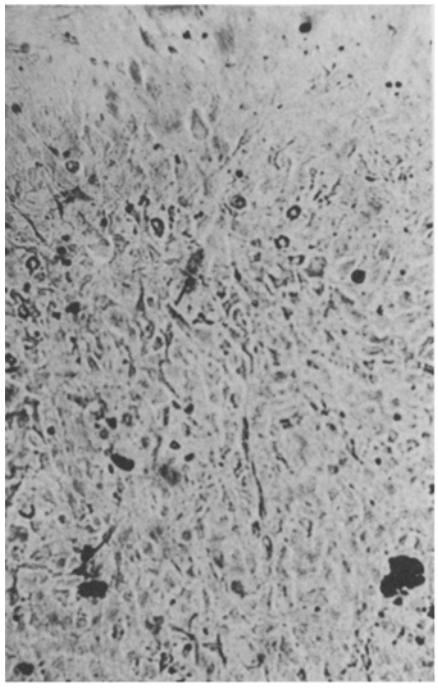

Fig. 1. Three-day old culture of mouse embryonic tissue. Magnification x 150.

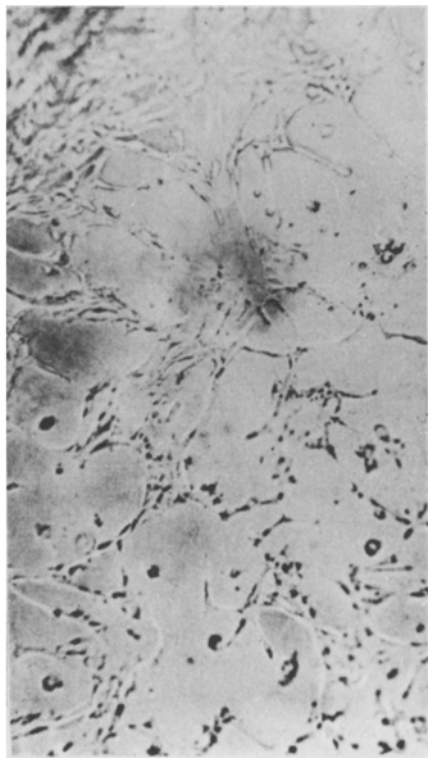

Fig. 2. Culture of mouse embryonic tissue 5-7 days after the action of leukemic RNA. Magnification, x 150.

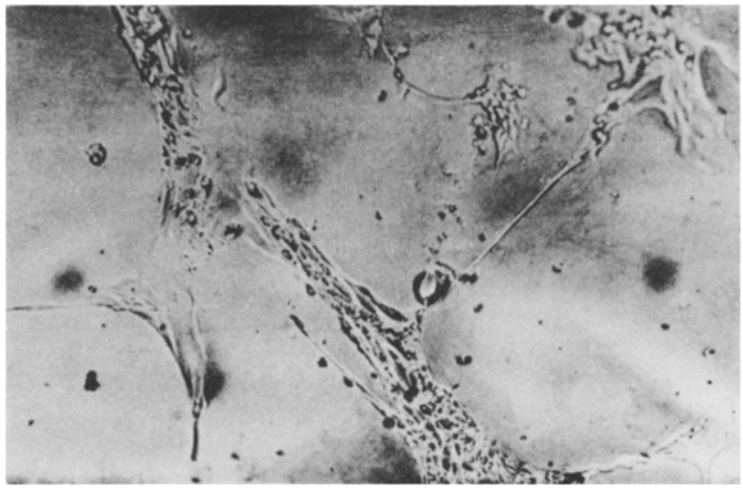

Fig. 3. Culture of mouse embryonic tissue 10-12 days after the action of leukemic RNA. Magnification x 150.

In the control experiments, the cultures were maintained under similar conditions with RNA from normal tissue and with RNA from leukemic tissue but treated with RNase and physiological solution. In all eleven experiments were conducted.

RESULTS

At the beginning of the experiments the explants consisted of a monolayer formed basically of cells resembling fibroblasts and differing in size and shape (Fig. 1). Three to five days later, after the cultures had been "inoculated" with RNA isolated from leukemic tissue, cytopathogenic effects were observed which were exhibited as a gradual elongation and attenuation of the cells or as a rounding off and swelling of the cells with an enlargement of the nucleus leading to the appearance of "giant" cells. In the long run, tissue defects were recorded up to the total disappearance of cells (Fig. 2). Complete lysis of cells set in on the 10th-12th day (Fig. 3). A fairly rapid mass dying of cells was observed in a number of instances. In one of the experiments, cytopathogenic effects were absent and, this was possibly due to a loss of the infective property of RNA since, in this experiment, "inoculation" with RNA was made two days after its isolation. The changes in the cell elements which were observed in the experimental cultures were not recorded in the control flasks; normal cell growth continued in the controls even up to the time when the cells in the experimental cultures were all dead.

The changes in the tissue culture preparations treated with leukemic RNA and stained with azure-eosin were exhibited as a loss of the syncytial connections between the cells, pycnosis or a swelling of the nucleus, the presence of coarse grains of chromatin and a shriveling of the cytoplasm. We were also able to observe the above-described changes in the cells during the transference from flasks of culturing liquid with a definite cytopathogenic action (two experiments were carried out; two transfers in the first and three in the second) and this cytopathogenic action was recorded by the 3-7th day. Such changes were not noticed when the culturing liquid was treated with RNase and the culture inoculated with it.

At the present time observations are being carried out on animals injected with the culturing liquid of cultures.

From the results obtained we may conclude that RNA, isolated from leukemic cells of mice with erythromyelosis displaying leukemic and oncologenic action in the animal organism, has the property of viral nucleic acid, that is, the capacity for infection leading to a fairly rapid appearance of cytopathogenic effects in normal cultures of mouse and rat embryonic tissue. The dependence of the cytopathogenic action on the presence of RNA is confirmed by the complete loss of the capacity for infection by RNA treated with ribonuclease.

The possibility of transmitting the agent responsible for the cytopathogenic action in a few transfers of cultures proves that, in the interaction of leukemic RNA with the cell, the virus-like agent is in all probability reproduced, but further diverse and special researches are required before a final answer can be given to this question.

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