LIBERATION OF RIBONUCLEIC ACID FROM CELLS OF EHRLICH'S CARCINOMA

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Cells of Ehrlich's carcinoma during incubation in nutrient medium secrete acid-insoluble RNA. Accumulation of RNA in the medium is unrelated to damage to or disintegration of the cells.

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A special feature of cancer cells is the modification of their external and internal membranes [2, 11]. The study of compounds secreted by tumor cells into the surrounding medium is of considerable interest. Published data show that intact tumor cells can secrete enzymes [4, 9, 12, 17, 18]. As a rule the liberation of nucleic acids is attributed to cell damage [1, 7, 13].

The object of the present investigation was to study liberation of RNA from intact tumor cells and from the same cells irradiated with UV light.

EXPERIMENTAL METHOD

Experiments were carried out with the ascites form of Ehrlich's carcinoma. Cells were separated from the ascites fluid by centrifugation, washed with Hanks' solution, and resuspended in Hanks' solution containing 10% calf serum, in a density of three million cells/ml. The suspension was poured into penicillin flasks in a volume of 3 ml and some flasks were incubated at 37°, others at 4°.

A portion of the cells was irradiated with UV light from a type BUV-60p bactericidal lamp. Exposures of 1, 10, and 30 min were given. Immediately after irradiation of the tumor cells, 10% calf serum was added to the Hanks' solution, the cell suspension was poured into flasks, and incubation carried out at 4°.

At various time intervals the presence of nucleic acids in the nutrient medium was examined and the state of the cells studied. The ascites fluid was separated from the cells by centrifugation followed by filtration through several layers of filter paper. Nucleic acids were precipitated with 3% perchloric acid. The residue was washed twice with 3% perchloric acid, alcohol, and ether and dried. Nucleic acids were determined in the residue by the method of Tsanov and Markov [5], and acid-soluble products of nucleic acids were determined in the supernatant. The quantity of these products was determined from the difference: $E_{260} - E_{286}$.

The state of the cells was determined by vital staining with neutral red as described by Nasonov and Aleksandrov [3].

The ribonuclease (RNase) activity of the culture fluid was determined from the increase in content of acid-soluble products of RNA hydrolysis in the following incubation mixture: 0.5 ml RNA solution (6 mg/ml) in tris-HCl buffer, pH 7.6; 0.5 ml cell-free nutrient medium. The mixture was incubated for 20 min (exactly) at 37°. The reaction was stopped by addition of a precipitating agent: 0.75% uranyl acetate in 2.5% perchloric acid solution. A parallel control was set up with substrate and nutrient medium only. Yeast RNA, twice reprecipitated in 96° alcohol, was used for the reaction. Activity was expressed in units of increase in density per hour.

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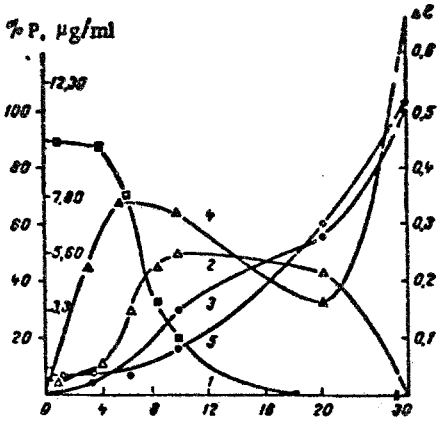

Fig. 1

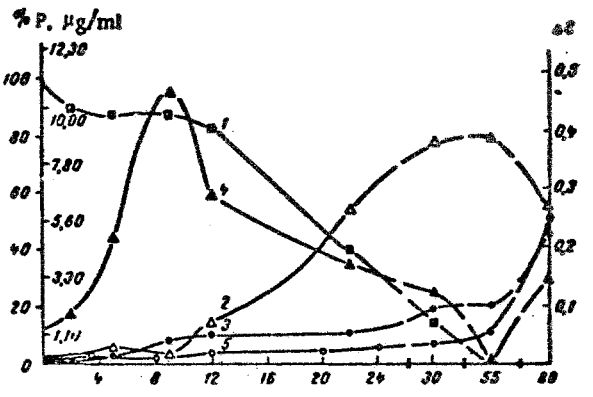

Fig. 2

Fig. 1. Incubation of cells at 37°. Abscissa, time of incubation (in hours); ordinate, on the left: number of cells (in percent) with granular staining (1); with diffuse staining (2), and number of unstained cells (3); on the right: RNA phosphosur (4, in µg/ml). Supplementary ordinate: difference $E_{260} - E_{285}$ of acid-soluble products of nucleic acids (5).

Fig. 2. Incubation of cells at 4°. Legend as in Fig. 1.

TABLE 1. Change in RNase Activity of Nutrient Medium and Its RNA Concentration during Incubation of Tumor Cells

Time of incubation (in h)	% of cells			RNA phosphorus (µg/ml)	RNase activity of medium (in specific units/ml/h)
	intact	in state of paranecrosis	dead		
0	98	1.6	0.4	—	5
2	94	3.6	0.4	3.3	5
4	94	3.6	0.4	7.8	5
6	88	11.4	0.6	7.0	15
8	78	21.4	0.6	6.5	20
12	70	28.0	2.0	5.8	20
24	52	43.0	5.0	1.0	25
36	20	77.0	13.0	—	50
48	15	45.0	40.0	4.2	25
72	0	40.0	60.0	11.5	20

EXPERIMENTAL RESULTS

Vital staining with neutral red enabled the following states of the cells to be distinguished: 1) intact cells: bright red granules of dye were present in the cytopla , the cytoplasm and nucleus themselves were unstained; 2) damaged cells in a state of paranecrosis [3]: no granules of dye were present, the cytoplasm and nucleus were diffusely stained; 3) dead cells: no staining whatever, nuclei visible.

During incubation of the cells at 37°, accumulation of RNA in the nutrient medium was observed in the first 4 h, and most cells showed no signs of injury (Fig. 1). As the state of the cells changed to paranecrosis and the number of dead cells increased, the RNA content in the medium fell sharply. Intensive death and destruction of the cells after incubation for 20 h caused a further increase in the RNA concentration in the medium.

Incubation of the cells at 4° (Fig. 2) enabled them to survive longer. Accumulation of RNA in the medium took place comparatively slowly. With an increase in the number of cells in a state of paranecrosis the RNA concentration in the medium fell.

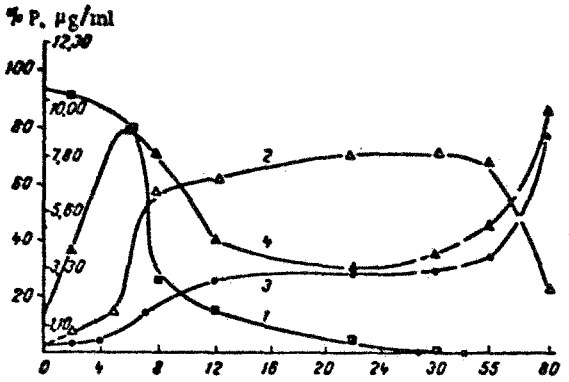

Fig. 3. UV irradiation of cells for 1 min followed by incubation at 4°. Legend as in Fig. 1.

In the experiments with exposure to UV light for 1 min (Fig. 3), within 8 h most cells were in a state of paranecrosis. Accumulation of RNA in the medium took place most intensively in the first few hours, while most cells still remained capable of forming granules of dye. An increase in the length of exposure to UV light caused more rapid death of the cells. However, an increase in RNA concentration in the medium was also observed at a time when most cells showed no signs of injury. In all the experiments DNA appeared only after disintegration of the cells.

The increase in concentration of acid-insoluble RNA in the nutrient medium in the first hours of incubation, when most cells still showed no signs of injury, evidently took place as a result of liberation of RNA from intact cells.

The decrease in RNA concentration in the medium when the state of the cells changed to one of paranecrosis was due in all probability to the appearance of RNase activity. We studied the presence of RNase activity in the nutrient medium in additional experiments.

As Table 1 shows, as the cells changed into a state of paranecrosis their RNase activity increased, resulting in a decrease in concentration of acid-insoluble RNA in the medium. After death of the cells the RNase activity fell and the RNA concentration again rose. The decrease in RNase activity can be attributed to liberation of enzyme inhibitors during lysis of the cells. The presence of active RNase inhibitors in tumor cells has frequently been demonstrated experimentally [8, 10, 15].

The liberation of RNA polymer by cancer cells which we observed may be the result of increased permeability of the cell membrane [4]. Its liberation can cause a disturbance of intracellular regulatory mechanisms, which must be reflected in continuing RNA synthesis, because one of the mechanisms of regulation of RNA synthesis is inhibition of the RNA-polymerase reaction by soluble RNA [16]. The possibility of such a disturbance has been confirmed by other investigations [6, 14]. In cells of Ehrlich's carcinoma, in contrast to normal cells, RNA synthesis remains at a constant and high level at all stages of growth and cell division.

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