ELECTROPHORETIC MOTILITY OF MOUSE HEPATOMA 22 CELLS AND NORMAL MOUSE LIVER CELLS SUBSEQUENT TO THE ACTION OF DISPERSING AGENTS

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In recent years, data has appeared in the literature on disruption of cell adhesion in tumor tissues. Abercrombie and Heaysman observed a difference in the behavior of normal fibroblasts and sarcoma cells in tissue cultures. The meeting of two normal fibroblasts causes a "contact inhibition" of their movement [2,3], while a meeting of sarcoma cells with one another, or with a normal fibroblast, does not depress movement [4,5]. It was shown that this difference in behavior of the cells is related to a decrease in the adhesiveness of the surface of tumor cells. The latter is probably caused by an increase in the surface electrical charge as compared with normal cells [7]. In the works of Klein, Purdom, and Ambrose, a correlation was established between the development of sarcoma and the progressive increase in electrophoretic motility of the cells obtained from the sarcoma at different stages in its development [13].

An analogous increase in electrical charge was observed in comparing the electrophoretic motility of regenerating liver cells with homologous nonregenerating cells [10,11], and of embryonal fibroblasts with adult ones [12]. The increase in electrophoretic motility of cells in all cases of intensified morphogenetic movements impels postulating that the increase in density of electrical charge is not a specific characteristic of malignant change, but a manifestation of growth.

It was of interest to elucidate the reason for the increased charge in the indicated cases. There were two possible routes for increasing the charge of the cells: 1) a change in the number of charged groups on the surface, 2) a change in the capacity to adsorb positive ions. We carried out a comparative study of the electrophoretic motility of mouse hepatoma 22 cells and normal mouse liver cells subsequent to the action of dispersing agents.

EXPERIMENTAL METHOD

The cells were separated according to the method of Anderson [9], by perfusing with dispersing solutions and a 5% solution of saccharose (1:1). The liver was perfused through the portal vein, and the hepatoma through the heart. Perfusion of the hepatoma 22 was performed chiefly for removal of erythrocytes, since it easily dissociates into cells without special treatment. As the dispersing agents, we used a 0.02% solution of EDTA (ethylenediamine tetraacetate), phosphate buffer pH 8.0, and a solution of trypsin (1:50). In those cases where the perfusion was carried out with the solutions of trypsin or EDTA, after passing 80 ml of these solutions through the liver (hepatoma) it was washed out with a buffer, pH 7.3, and a 5% solution of saccharose (1:3). When the perfusion employed the phosphate buffer pH 8.0, all subsequent operations were carried out in the same solution.

The perfused liver (hepatoma) was shredded with scissors. The resultant cell suspension was allowed to settle for 15 min. Then, the supernatant fluid, together with the cell fragments which it contained, was drawn off, and the sediment was mixed with the solution in which the electrophoretic measurements were performed. We obtained a cell suspension that did not contain erythrocytes. We rejected centrifugation, since it destroyed the cells and did not lead to improvement of the cell suspension.

Electrophoretic motility was measured in an Abramson plane quartz chamber [6], with $Cu/CuSO_4$ electrodes and agar bridges, containing 10% KCl solution in a 3% agar gel. All measurements were made in the phosphate buffer + 10% saccharose solution (1:3). The saccharose was added in order to retard the settling of the cells. When complete

Electrophoretic Motility of Normal Liver Cells and Hepatoma 22 Cells Subsequent to the Action of Dispersing Agents

Method of separation	pH of the medium during measurement	Motility of the liver cells (in micra/sec/v/cm)	Motility of the hepatoma 22 cells*
Phosphate buffer 8.0 + 5% saccharose	7.3	0.99 ± 0.024	1.17 ± 0.03
Phosphate buffer 8.0 + 5% saccharose	8.0	1.18 ± 0.02	1.13 ± 0.02
0.02 M EDTA + 5% saccharose	7.3	1.24 ± 0.065	1.16 ± 0.04
0.02 M EDTA + 5% saccharose	8.0	1.23 ± 0.065	
Trypsin 1/50 + 5% saccharose	7.3	1.22 ± 0.045	1.01 ± 0.03

*Motility of the erythrocytes at pH 7.3 was equal to 1.31 micra/sec/v/cm; at pH 8.0–1.34 micra/sec/v/cm.

settling had occurred, the chamber was turned on the other side, and the measurements were continued. In all the measurements, the internal control was represented by human erythrocytes, that had previously been twice washed in the phosphate buffer pH 7.3

EXPERIMENTAL RESULTS

The results of the measurements (see the table) indicate not only an absolute increase in the ζ-potential subsequent to malignant change, but also a change in the reactivity of the surface of the tumor cells. While the ζ-potential of the normal liver cells rose by approximately 20% after treatment with EDTA, trypsin, or after raising the pH from 7.3 to 8.0, the hepatoma cells did not react to these influences. This points to a connection between increased negative charge of the hepatoma 22 cells and change in the capacity to adsorb positive ions.

At this time, we cannot draw any definite conclusions as to what causes this change. We do not have sufficient knowledge of the physico-chemical properties of the membrane, or of the mechanism of action of the indicated agents on the chemical components of the membrane (for example, there are no data on the adsorption of cations by phospholipids). This information can be obtained from experiments on model systems. One of the possible changes in the membrane, leading to these effects, may be related to the partial loss of proteins by the external membranes, and "denudation" of the lipoid containing membrane. This hypothesis is in good accord with the data of authors showing that tumor cells of the kidney can be verified by the action of lipase, while cells of the original, normal tissue do not react to it [8].

It should be noted that hepatoma 22 is one of the most malignant tumors. It is still not known whether the observed change in the capacity to adsorb positive ions is a general property of all tumors, and is more pronounced in the cells of regenerating or embryonal tissues.

SUMMARY

Electrophoretic motility of hepatic cells and of hepatoma 22 cells was studied after treating these cells with trypsin and disodium ethylene diamine tetra-acetic acid and after changing pH of the medium. As demonstrated, at pH of the medium 7.3 electrophoretic motility of the hepatoma 22 cells is 20% higher than of hepatic cells. Electrophoretic motility of hepatic cells increases in treating them with trypsin and disodium ethylene diamine tetra-acetic acid, as well as with the rise of pH of the medium from 7.3 to 8.0. There was no increase of hepatoma cells motility after the same treatment.

A conclusion was drawn that the rise of the negative electric charge of the hepatoma 22 cells (as compared with hepatic cells) was connected with changed capacity to absorb positive ions.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.