CHANGES IN ELECTROPHORETIC MOBILITY OF FIBROBLASTS IN TISSUE CULTURE CAUSED BY 3,4-BENZPYRENE

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Under the influence of 3,4-benzpyrene the electrophoretic mobility of fibroblasts in tissue culture is increased, and this is accompanied by the appearance of hyaluronic acid or chondroitin sulfate on their surface.

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The conversion of normal cells is accompanied by considerable changes on the cell surface. One of the manifestations of this conversion, as several authors have shown experimentally, is an increase in the negative electrical charge of cell surfaces during malignant change and a corresponding increase in electrophoretic mobility of malignant cells compared with homologous normal cells [4]. The suggestion is made that the increase in electrophoretic mobility of tumor cells compared with homologous normal cells is due to differences in the adhesive properties of these two cell types [9].

Because it has been shown that under the influence of polycyclic carcinogenic hydrocarbons changes take place in the shape, the mutual arrangement [3], and the character of contacts and movement of the surface membranes of fibroblasts, the investigation of the surface of tumor cells and cells treated with carcinogenic agents is of considerable interest.

In the present investigation the effect of 3,4-benzpyrene on the electrophoretic mobility of fibroblasts in tissue culture was studied.

EXPERIMENTAL METHOD

Primary monolayer cultures of mouse embryonic fibroblasts and cells of strain L were used in the investigation. Strain L was obtained by Earle and co-workers as a result of malignant transformation of fibroblast cultures treated with methylcholanthrene [6]; its malignancy was verified by animal inoculation. The cells of strain L used in our experiments possess all the characteristic properties of malignant cells in culture (rapid growth in the absence of contact inhibition, resistance to the toxic action of carcinogenic substances, and so on).

TABLE 1. Effect of 3,4-benzpyrene on Electrophoretic Mobility (EM) of Fibroblasts

Expt. No.	EM (in μ/sec/V/cm)		P	EM expt.
	in control cells	of cells treated with 3,4-benzpyrene		EM control (in %)
1	1,20±0,03	1,35±0,03	0,005	112
2	1,20±0,03	1,28±0,03	0,05	107
3	1,32±0,03	1,38±0,03	0,10	104
4	1,35±0,04	1,62±0,08	0,005	120
5	1,24±0,08	1,31±0,04	0,50	106
6	1,13±0,04	1,24±0,03	2,2	110

The cells were grown for 8-10 days in flasks in medium No. 199 with 10% serum. 3,4-benzpyrene was added on the 3rd-4th day of cultivation in a suspension in a concentration of 1 μg/ml. The fibroblasts and strain L cells were taken from the glass slide by means of a rubber eraser without preliminary chemical treatment, then washed twice with phosphate buffer (pH 7.3) and centrifuged for 10 min at 1000 rpm.

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The electrophoretic mobility of the cells was measured using the apparatus described previously by A. G. Malenkov and E. A. Modyanova [2].

In each experiment the mobility of 15-20 cells was measured, the mobility of each cell being determined in 2 directions of the field. Concurrently with measurements of the mobility of each fibroblast, the mobility of the erythrocytes was determined, by adding to the cell suspension human erythrocytes twice washed in phosphate buffer. The mobility of the erythrocyte was taken to be 1.31 μ/sec/cm, the absolute mobility of erythrocytes determined previously. In that case, the mobility of the fibroblasts was given by the formula:

$$U_{fibr} = 1.31 \cdot \frac{t_e}{t_f}.$$

where t_f and t_e represent the mean times during which the fibroblasts or erythrocyte, respectively, traverse one division of the ocular micrometer.

EXPERIMENTAL RESULTS

Results showing changes in electrophoretic mobility of fibroblasts under the influence of 3,4-benzpyrene are given in Table 1. As Table 1 shows, the electrophoretic mobility of cells treated with the carcinogen was 7-15% higher than that of the control cells. These changes are statistically significant and were regularly observed in all experiments, although the absolute values of the mobility of the fibroblasts varied slightly. These variations were probably attributable to the fact that embryonic fibroblasts were used in the experiments, and their mobility varied slightly depending on the age of the embryo.

Since the increase in electrophoretic mobility took place on the 5th-7th day of treatment with 3,4-benzpyrene, and it was previously shown that polycyclic carcinogenic hydrocarbons stimulate liberation of acid mucopolysaccharides by fibroblasts during the same period [1], the question arose whether this increase in electrophoretic mobility of the cells was connected with the appearance of acid mucopolysaccharides on their surface. To answer this question, an experiment was performed in which the cells were treated with testicular hyaluronidase. The cells were washed in phosphate buffer and then incubated for 1 h with testicular hyaluronidase in a concentration of 1 mg/ml at 37°. In parallel experiments control cells were incubated in physiological saline. After treatment with the enzyme the cells were again thoroughly washed with phosphate buffer.

Treatment of fibroblasts exposed to the action of 3,4-benzpyrene with testicular hyaluronidase reduced their electrophoretic mobility to the control level (before treatment 1.27±0.03, after treatment 1.15±0.04, control 1.13±0.04). At the same time, this enzyme did not affect the electrophoretic mobility of the control cells. Treatment with hyaluronidase likewise had no action on the magnitude of the negative charge of the strain L cells.

The absolute electrophoretic mobility of the strain L cells was slightly lower than that of the fibroblasts. However, this observation does not conflict with the many observations of increased electrophoretic mobility of connective-tissue cells during malignant transformation, because we used embryonic fibroblasts in the experiments. Comparison of the electrophoretic mobility of mouse embryonic fibroblasts, fibroblasts from an adult mouse heart, and cells of strain L showed that the electrophoretic mobility of the embryonic fibroblasts was much higher than that of the adult mouse fibroblasts, and the strain L cells occupied an intermediate position in this respect [8]. Furthermore, it has been shown in liver cells that the density of the surface-negative charge of proliferating cells of growing tissues is higher than that of resting tissue cells [5]. It may accordingly be concluded that the increase in density of the surface-negative charge is not a specific feature of tumor cells.

The increase in electrophoretic mobility of the cells may take place as a result of various changes in chemical composition of the cell surface. It has been found, for instance, that the increases in surface-negative charge of hamster embryonic cells infected with polymer virus is due to the appearance of sialic acid on their surface [7].

The increase in electrophoretic mobility of the cells under the influence of 3,4-benzpyrene takes place as a result of the appearance of hyaluronic acid or chondroitin sulfate on the cell surface. It cannot be stated more definitely which of these two polysaccharides is liberated by the cells on the basis of the technique used, because both are destroyed by testicular hyaluronidase.

Hence, the ability of cells to form contacts may be modified concurrently with an increase of their charge, as occurs during the action of a carcinogen on fibroblasts. However, simultaneous changes such as these are not a rule to which there are no exceptions. In fact, normal embryonic cells possessing a high surface charge form stable contacts with each other while cells of strain L, possessing a smaller charge, do not form such contacts. Evidently there may be other causes of the changes affecting contacts in tumor cells than an increase in density of the negative charge on the cell surface.

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