BENZPYRENE METABOLISM IN CULTURES OF NORMAL AND MALIGNANT FIBROBLASTS

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Normal and malignant rodent fibroblasts differ in their sensitivity to the toxic action of polycyclic carcinogenic hydrocarbons [4, 10, 11, 13]: the cells of sarcomas induced by chemical carcinogens, by cellophane discs, by polyoma and SV40 viruses are far less sensitive to these agents than the homologous normal cells. The increase in resistance to carcinogens is evidently connected with the acquisition of malignant properties by the cell [1, 2].

The object of the present investigation was to study the rate of metabolism of the carcinogenic hydrocarbon benzpyrene (BP) in cultures of normal and malignant fibroblasts of mice and hamsters. Similar investigations were also carried out with cultures of normal human fibroblasts, which are resistant to the toxic action of carcinogenic hydrocarbons [13].

EXPERIMENTAL METHOD

Experiments were carried out on embryonic mouse, hamster, and human fibroblasts in the second subculture, and also on a number of strains of malignant hamster and mouse fibroblasts cultivated for a long time (Table 1).

From 24-48 h after the cells were seeded in Carrel flasks, the medium was changed with the addition of 10 ml medium No. 199 with 10% serum to each flask. In some cases the carcinogen was added in 0.1 ml acetone so that its concentration in the medium was 0.1 μg/ml. In other cases the carcinogen was dissolved beforehand in serum, in which case its concentration in the medium was 0.05-2.4 μg/ml (Table 2). The flasks were incubated for between 30 min and three days (Table 2). At the end of this time the medium was poured from each flask into a separate tube, the cells were removed with trypsin and counted, and then transferred into the same tube. In the course of the experiment the number of cells in the culture varied considerably. At the time of addition of BP it varied from 500,000 to 1 million per flask, and later in the sensitive tissue it fell to 150,000 -200,000, while in the resistant tissue it rose to 2-3 million. The cells of some strains (APO, No. 866) became detached from the glass towards the end of the experiment as the result of monolayer formation. For this reason the quantity of destroyed carcinogen was determined per flask and not in relation to a certain number of cells.

BP was extracted from the culture medium and the cell mass with normal octane at 80° with continuous shaking. BP in the extract was determined quantitatively by a fluorescence spectrometric method in solutions in n-octane frozen to -196° [5, 6, 8, 9].

In experiments in which the intensity of fluorescence was determined by a microfluorometric method, the cells were cultivated in chambers made from optically polished glass with parallel walls.

The apparatus for recording the intensity of fluorescence of the BP penetrating into the cells consisted of three main parts: an ML-2 luminescence microscope; a type UM-2 monochromator, and a recording system consisting of a type FÉU-17 photomultiplier, on the photocathode of which fell light from the exit slit of the monochromator, a dc amplifier, and a self-writing ÉPP-0.9 potentiometer.

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TABLE 1

Strain	Animal from which tissue obtained	Carcinogenic agent	Origin of strain
BHK-21	Hamster	Spontaneous malignant change	[15]
L	Mouse	Methylcholanthrene or spontaneous malignant change	[14]
No. 866	Hamster	Polyoma virus	I
No. 874	Hamster	" "	I
SA-1	Hamster	Virus SV40	[3]
PKh-128	Hamster	" SV40	II
APO	Mouse	Spontaneous malignant change	[7]

Legend: I) Strain obtained from I. S. Irlin at the N. F. Gamaleya Institute of Microbiology and Immunology; II) strain obtained from A. D. Al'tshtein at the L. A. Tarasevich Control Institute. The authors are grateful to both.

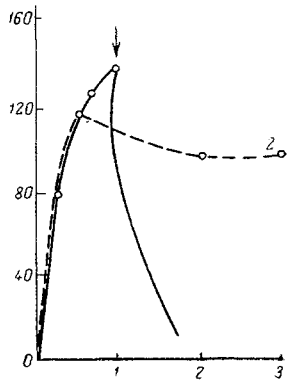

Fig. 1. Changes in intensity of fluorescence of benzpyrene (BP) (4030 Å) in a monolayer of mouse fibroblasts. 1) Chamber in which medium containing BP in a concentration of 1 μg/ml was replaced after 1 h by medium without carcinogen; 2) chamber with medium containing BP in concentration of 1 μg/ml. Abscissa—time (in hours) after addition of carcinogen; ordinate—conventional units of fluorescence (intensity of fluorescence of BP solution containing $1 \cdot 10^{-7}$ μg/ml in benzene taken as 50).

Excitation was carried out with the ultraviolet part of the spectrum from a type DRSh-250 lamp, selected with a type UFS-2 filter.

EXPERIMENTAL RESULTS

Within a few minutes after its addition to the medium, BP began to accumulate in the cells. After 30 min this accumulation in the cells ceased (see Fig. 1). In the course of the first two hours after its addition to the medium, all the added BP (95-100 %) could be extracted from the muscle fibroblasts and the culture medium. Later the quantity of BP extractable from the cultures gradually diminished. The rate of decrease of the BP concentration in cultures of mouse fibroblasts in experiments when 0.1 μg/ml was added remained approximately constant throughout the experiment: the quantity of BP in the culture was halved in the course of about 12 h. In experiments in which the intensity of fluorescence of BP in the cells was determined, this intensity also diminished gradually; if the medium with BP was replaced by a medium not containing this hydrocarbon, the intensity of BP fluorescence fell very rapidly (in the course of 1 h) to zero (see Fig. 1). The amount of BP extractable from the culture of mouse fibroblasts 2-3 days after its addition was only 3-10% of the initial quantity of hydrocarbon added (0.1 μg/ml). In some experiments the residue after extraction of the cultures with octane was hydrolyzed at 80° in a 4.5 N KOH for 20 h. The amount of BP obtained by a second extraction of the hydrolysate was less than 10% of the initial BP content in the culture.

Similar results were obtained in experiments with normal hamster fibroblasts as in the experiments with mouse fibroblasts (Table 2). In cultures of human embryonic fibroblasts the decrease in the BP content took place much more slowly than in the experiments with rodent fibroblasts. In cultures of some malignant lines of mouse and hamster fibroblasts which were tested,the BP content hardly changed at all during the three days of the experiment (lines L and BHK), while in cultures of other tumors it fell, although much more slowly than in cultures of the homologous normal fibroblasts (Table 2).

The BP added to cultures of normal rodent fibroblasts was gradually converted into a compound not possessing the characteristic maximum of fluorescence at 4030 Å. Oxidative systems metabolizing BP are found in the liver cells of various animals and also in cells of the intestine and certain other tissues [12].

TABLE 2. BP Metabolism in Cultures of Normal and Malignant Cells

Animals from which tissue obtained	Tissue or strain	BP concentration (in μg/ml)	Duration of experiment (in hours)			
			10—12	24	48	72
			residue of BP (in % of control)			
Mouse	Normal embryonic fibroblasts	0,1	49±5	21,6±2,6	8±2,2	3±1,3
		0,09—0,12	—	11,7±1	<1	—
		0,3—0,5	—	22±6	5±6,5	<1
		0,6—0,8	—	41±3,3	—	12,1±4
		1,6	—	—	—	32±3
	L	0,1	—	93±3	80±7	98±13
	APO	0,1	—	—	90±0	76±16
		0,6	—	—	—	89±10
Hamster	Normal embryonic fibroblasts	0,1	—	25±3	9,6±1	3±1
	BHK	0,1	—	—	99±1	105±1
		0,6	—	—	—	88±15
		2,4	—	—	—	99±11
	PKh-128	0,085	—	—	45±6	—
		0,7	—	—	—	53±7
		1,0	—	—	61±10	—
	№ 866	0,1	—	—	—	38±9
	№ 874	0,05	—	—	74±6	—
		0,15	—	—	85±8	—
	SA-1	0,075	—	—	65±13	—
Man	Normal embryonic fibroblasts	0,09	72±6	110±7	—	59±6
		0,09	—	84±0	—	47±6

BP in these experiments was probably the result of activity of these same enzyme systems present in the cells of tissue cultures of rodent embryos. These cells, actively metabolizing BP, were highly sensitive to the toxic action of this hydrocarbon. Human embryonic cells, and also the cells of certain tumors of mice and hamsters, with much lower sensitivity to carcinogenic hydrocarbons, hardly metabolized BP at all, or did so much more slowly than normal rodent cells. This correlation between the rate of BP metabolism and its toxic action shows that during metabolism this hydrocarbon may be converted into a highly toxic derivative. This hypothesis requires verification. The difference in sensitivity of various types of cells in vitro to carcinogens is partly due to differences in the rate of metabolism of these compounds. Possibly, however, sensitive and resistant cells differ not only in the rate of metabolism of carcinogenic hydrocarbons, but also in the character of the metabolites formed and also in the character of the cell components with which these metabolites interact.

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