# BENZ(a)PYRENE SENSITIVITY OF EPITHELIAL AND MESENCHYMAL EMBRYONIC LUNG CELLS IN MICE PREDISPOSED (STRAIN A) AND RESISTANT (C57BL) TO PULMONARY CARCINOGENESIS

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It was shown previously that differences in the sensitivity to induced pulmonary carcinogenesis in mice of strains A and C57BL arise during prenatal development and persist in the isolated organ explanted into organ culture [1, 5, 7]. Under these circumstances we have found significant differences in the growth potential of normal embryonic lungs of mice of the above strains, in the relative numbers of epithelial and mesenchymal cells, in their proliferative activity, and certain other properties [1, 3]. On the basis of these data it has been suggested that a combination of the above factors of mouse lung tissue plays a definite role in the realization of genetically determined sensitivity to spontaneous and induced carcinogensis [3], so that the need has arisen for a further differential study of the role of cell—tissue factors in the mechanisms of pulmonary carcinogenesis.

There is information in the literature that the proliferative activity of cells in target organs is one of the important factors affecting sensitivity to carcinogenic influences, and in particular, transplacental [8]. We also know that changes in the proliferative activity of cells in target organs is one of the characteristic effects of chemical carcinogens, and one which correlates sufficiently closely with the sensitivity of the cells to their action.

This paper gives the results of a comparative autoradiographic study of the proliferative activity of the epithelium and mesenchyme of embryonic lungs of mice belonging to strains A and C57BL, under normal conditions and under the influence of the pulmonotropic carcinogen benz(a)pyrene (BP). The method of organ culture, whereby epithelial-mesenchymal relations can be preserved in the target organ and possible modifying influences of systemic factors of the organism on carcinogenesis can be ruled out, was used.

## EXPERIMENTAL METHOD

Experiments were carried out on organ cultures of the lungs of 17-day embryos of A and C57BL mice by the method described in detail previously [3]. Explants were cultured in nutrient medium containing BP in concentrations of 3, 6, and 12 μg/ml. Control explants were grown on medium without the carcinogen. On the 14th day $^3$H-thymidine was added to the nutrient medium of the control and experimental explants in a concentration of 1 μCi/ml. After 1 day the cultures were fixed with 70% ethyl alcohol, treated histologically, and embedded in paraffin wax; serial sections were cut to a thickness of 4 μ. The dewaxed sections were coated with type M photographic emulsion, exposed for 2 weeks at 4°C, developed, and stained with hematoxylin. Proliferative activity of altogether 366 cells of the explants was studied in 10 series of experiments. To determine the labeling index (LI), two to four laminar sections were chosen from each explant at different levels. In each section virtually all cells of the alveolar and bronchial epithelium were counted (about 800-1200 cells), as well as mesenchymal cells (about 400-500 cells) of the explant proper, and cells migrating on the surface of the supporting substrate (filter). The $\chi^2$ test was used for statistical analysis of the results.

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## EXPERIMENTAL RESULTS

The morphology and cellular structure of organ cultures of normal embryonic mouse lungs of A and C57BL strains have been fully described previously [1, 3, 5]. The cell mass of the explants from both strains on the 15th day of culture consisted mainly of epithelial cells of alveolar (EA) and bronchial (EB) structures, and to a lesser degree, of fibroblast-like mesenchymal cells surrounding the epithelium (ME). Mesenchymal cells migrating on to the filter (MS) formed an extensive zone of growth around the explants.

Autoradiographic study of the explants showed that under normal conditions after 15 days of culture LI of the EA cells was about equal in the embryonic lungs of mice of both strains. However, LI of EB cells was significantly higher in strain A. Differences between the strains also were found in the proliferative activity of ME and MF cells (Fig.1). The direct action of different concentrations of BP on the cultures caused an increase or decrease in LI of the cells, or had no significant effect, depending on the type of cells and the dose of BP used. For instance, in experimental cultures of embryonic lungs of A mice exposure to BP in a concentration of 3 µg/ml considerably increased LI of the EA, and in particular, of the EB cells. LI of the ME cells, under these circumstances, was indistinguishable from normal, but LI of MF cells was sharply reduced (Table 1). When a dose of 6 µg/ml was used LI of the EA cells was virtually indistinguishable from normal, whereas LI of the EB cells was the same as when a dose of 3 µg/ml was used. ME rose sharply, but MF in the cells remained just as low as with a dose of 3 µg/ml. Under the influence of BP in a dose of 12 µg/ml, LI of the EA cells was indistinguishable from normal (just as with a dose of 6 µg/ml), but in the EB cells it was higher than in the experiments in which smaller doses of BP were used. LI of ME and MF cells was a little lower than with a dose of 6 µg/ml.

Exposure to BP in a dose of 3 µg/ml sharply increased LI only in the population of EB cells in experimental explants of embryonic lungs of C57BL mice, unlike the mice of strain A. In the MF cells, just as in strain A, it fell sharply compared with normal. LI in the EA cells was reduced a little, whereas in the ME cells it was raised somewhat. An increase in the dose of BP to 6 and 12 µg/ml caused a further slight decrease in LI of the EA and ME cells. LI of the EB cells was lower than with a dose of BP of 3 µg/ml, but significantly above normal. LI of the MF cells was almost the same when a dose of 6 µg/ml was used as with a dose of 3 µg/ml, but after exposure to a dose of 12 µg/ml it fell to zero (Table 1).

The effect of BP thus depended on its concentration, but it was also determined by the type of cells and by the strain of the donor mice. The data given in Table 1 and in the histogram in Fig. 1 show that dependence of the effect on the dose of BP differed for different types of cells for mice of the same strain, and on the other hand, it differed for the same types of cells but of different strains of mice. The writers showed previously that transplacental and direct exposure to BP has a marked growth-stimulating effect in organ cultures of embryonic lungs of A mice, unlike those of C57BL mice, and leads to the development of focal and total adenomatous growths and microadenomas [7]. The results of this investigation showed that the main source of these pretumor proliferative foci is the EB cells, whose proliferative activity was increased most sharply as a result of exposure to all concentrations

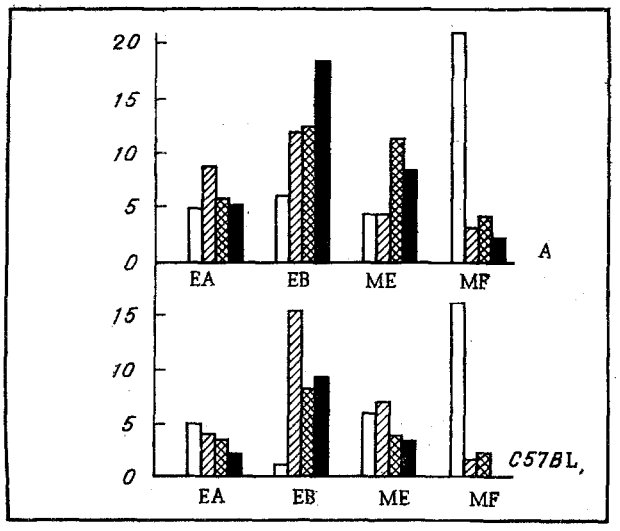


Fig. 1. LI of epithelial and mesenchymal cells of embryonic lung explants from A and C57BL mice under normal conditions and on exposure to different concentrations of BP. Abscissa, type of cells; ordinate, LI (in %). Unshaded columns — normal; oblique shading — 3 µg/ml of BP; cross-hatching — 6 µg/ml of BP; black column — 12 µg/ml of BP.

TABLE 1. Effect of BP on Proliferative Activity of Epithelial and Mesenchymal Cells of Embryonic Lungs of Mice of Strains A and C57BL

| Type of cells | Parameter | Strain of donor mice | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | | | | C57Bl | | |
| | | normal | BP concentration, μg/ml | | | normal | BP concentration, μg/ml | | |
| | | | 3 | 6 | 12 | | 3 | 6 | 12 |
| EA | All cells | 38 101 | 3 364 | 11 146 | 1 701 | 27 610 | 12 663 | 20 914 | 26 967 |
| | Number of which were labeled | 1 894 | 557 | 625 | 91 | 1 267 | 458 | 710 | 881 |
| | LI, % | 4,97 | 8,75 | 5,61 | 5,35 | 4,59 | 3,62 | 3,39 | 3,27 |
| | P | | <0,001 | <0,01 | >0,1 | | <0,001 | <0,001 | <0,001 |
| EB | All cells | 15 083 | 10 344 | 24 452 | 4 440 | 79 777 | 9 961 | 18 027 | 14 666 |
| | Number of which were labeled | 906 | 1 269 | 3 035 | 833 | 89 | 1 139 | 1 262 | 1 164 |
| | LI, % | 6,01 | 12,27 | 11,41 | 18,76 | 1,12 | 11,43 | 7,0 | 7,66 |
| | P | | <0,001 | <0,001 | <0,001 | | <0,001 | <0,001 | <0,001 |
| ME | All cells | 10 613 | 10 884 | 8 281 | 2 082 | 12 157 | 16 544 | 19 551 | 21 859 |
| | Number of which were labeled | 463 | 482 | 941 | 173 | 1 572 | 870 | 700 | 794 |
| | LI, % | 4,36 | 4,43 | 11,36 | 8,31 | 4,71 | 5,26 | 3,58 | 3,63 |
| | P | | >0,1 | <0,001 | <0,001 | | >0,1 | <0,001 | <0,001 |
| MF | All cells | 15 627 | 2 234 | 5 102 | 497 | 1 590 | 1 377 | 2 937 | 1 361 |
| | Number of which were labeled | 3 378 | 68 | 202 | 9 | 258 | 51 | 45 | 124 |
| | LI, % | 21,62 | 3,04 | 3,96 | 1,81 | 16,23 | 3,70 | 1,53 | 9,11 |
| | P | | <0,001 | <0,001 | <0,001 | | <0,001 | <0,001 | <0,001 |

of BP used, and that the effect increased with an increase in dose. Dependence of effect on dose also was observed by the writers previously when the results of experiments with BP and with other carcinogens were estimated morphologically [5, 6]. It is important to emphasize, however, that the increase in proliferative activity of the EB cells in the experimental explants was combined with an increase in proliferative activity in ME cells also (Fig. 1). The essential feature is that during exposure to BP, LI of the EB cells also was increased in lung explants from C57BL mice, but dependence of the effect on dose was not observed, and in contrast to strain A (and this is particularly important) LI of the ME cells was below normal. These results confirm the important role of the mesenchyme and of epithelial-mesenchymal interactions in the realization of the carcinogenic effect by epithelial target cells, which was demonstrated for the first time by the writers in experiments with urethane and a model of epithelial-mesenchymal recombinants [2, 4]. The results of the present investigation also confirm the view that proliferative activity of the cells in target organs is of significant importance as a factor affecting sensitivity to carcinogenesis [8]. Proliferative activity of the cells in lung embryos of mice of strain A, and in particular, of target cells (EB) is higher on the whole than in strain C57BL (Fig. 1). The results of this investigation thus conform the hypothesis that local cell — tissue factors play a definite role in the realization of the genetically determined predisposition of mouse lung tissue to spontaneous and induced carcinogenesis.

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