

# Reduction in Cellular Adhesiveness upon Contact with a Carcinogen\*

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## SUMMARY

In the experiments reported below, the inner aspects of the pinnae of rabbits' ears were treated for 1 hour with the carcinogen 7,12-dimethylbenzanthracene, 1 per cent in acetone. Other ears were treated with the noncarcinogen anthracene in acetone, and still other ears were left untreated. Discs of skin were removed from these ears, and the relative adhesiveness of the epidermal cells was determined by mechanical shaking and cell counts. It was shown that exposure to the carcinogen resulted in a decreased adhesiveness of the epidermal cells, suggesting that an alteration of the cell surface had occurred within 1 hour after contact with the carcinogen. The findings are discussed and further studies suggested.

Reduced mutual adhesiveness has been shown to be characteristic of neoplastic cells (2, 5). Decreased adhesiveness has been correlated with impairment of the capacity of the cancer cell to bind calcium and, coupled with the ameboid motility of the resulting free-living individual cells, has been proposed as the biological basis for the invasiveness of cancer (3). The facts that adhesiveness is reduced and that the cancer cell is incapable of binding adequate amounts of calcium suggest that there is an alteration in the structure of the external membrane of neoplastic cells, and studies with replicas of cell surfaces with the electron microscope have, indeed, revealed differences in the ultrastructure between normal and neoplastic cells (4). From these combined studies, it seems apparent that during the transformation of a normal to a neoplastic cell an alteration of the external membrane takes place. The question arises as to when, in the process of carcinogenesis, this change occurs. Is it a late phenomenon, associated only with the development of the full-blown neoplastic cell? If, on the contrary, such alteration occurs in an early stage of carcinogenesis, the possibility arises that a change in the cell surface or external cell mem-

brane may be of importance in the initial stages of the process of carcinogenesis.

The experiments here reported were designed to determine how soon, after the application of a chemical carcinogen, there occurs an alteration in the cell membrane or cell surface, as reflected in a reduction of mutual adhesiveness of the cells.

## MATERIALS AND METHODS

The inner aspects of the pinnae of anesthetized domestic rabbits were depilated with a commercial cosmetic preparation. In some instances, the skin surfaces were then treated with the carcinogen 7,12-dimethylbenzanthracene, 1 per cent in acetone, or with anthracene in acetone, as a noncarcinogenic control. Some ears were left untreated. One hour after a single application of the carcinogen or its noncarcinogenic analog, the rabbits were sacrificed and their pinnae removed. A rectangle of skin about 5 × 2 cm. was removed by outlining its area with a scalpel and dissecting one edge free from the underlying cartilage. The free edge could then be grasped in a hemostat and the rectangle of skin stripped off. (If difficulty is encountered in establishing the cleavage plane between dermis and cartilage, subcutaneous injection of physiological saline with a 30-gauge needle will reveal it quickly.) The skin was tacked to a cork board, and discs  $\frac{1}{4}$  inch in diameter were cut from it with a sharp stainless steel cork borer. Two such discs were

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placed in a small glass weighing bottle, together with fifteen glass beads 3 mm. in diameter and 0.5 ml. of physiological salt solution (Earle's). The bottle was then placed in a holder mounted on a clinical blood pipette shaker and agitated for 10 minutes. A drop of metachromatic dye was added to the bottle and mixed with the fluid for a minute, after which a drop of the fluid was withdrawn from the bottle in a blood pipette and run into a hemocytometer chamber where the dislodged cells in all nine squares were counted. In this way, normal untreated rabbit epiderm was examined. In addition, rabbit epiderm, 1 hour after exposure to a single application of 7,12-dimethylbenzanthracene, was studied. As a control, the noncarcinogen, anthracene, also in acetone, was used.

### RESULTS

Chart 1 presents the results in the most graphically impressive form, showing the frequency distribution of the three families of figures. Thirty-two cell counts were made in each category of the samples, and these were taken from eight different animals for any one test substance and for the untreated controls.

Table 1 presents a statistical analysis of the data, showing the difference between the means, the standard error of the difference, the value of "t," and the probability determinations.

As shown in Chart 1, far more cells were dislodged by shaking discs of skin removed from ears that had been treated with the carcinogen than from untreated ears, or ears treated with the noncarcinogen, the statistical difference being highly significant (Table 1). The difference in cell counts between untreated ears and ears treated with the noncarcinogen was not statistically significant. In other words, within an hour after application of the carcinogen, an alteration in the cell surface had occurred which was reflected in a reduced adhesiveness of the cells.

It is apparent that contact with the carcinogen resulted in a highly significant elevation of the cell counts, indicating a reduction in the adhesiveness of the treated cells. Treatment with the noncarcinogenic analog in the same medium (acetone) did not alter adhesiveness as compared with the normal untreated cells.

### DISCUSSION

The demonstration in these experiments of reduction in cellular adhesiveness within an hour

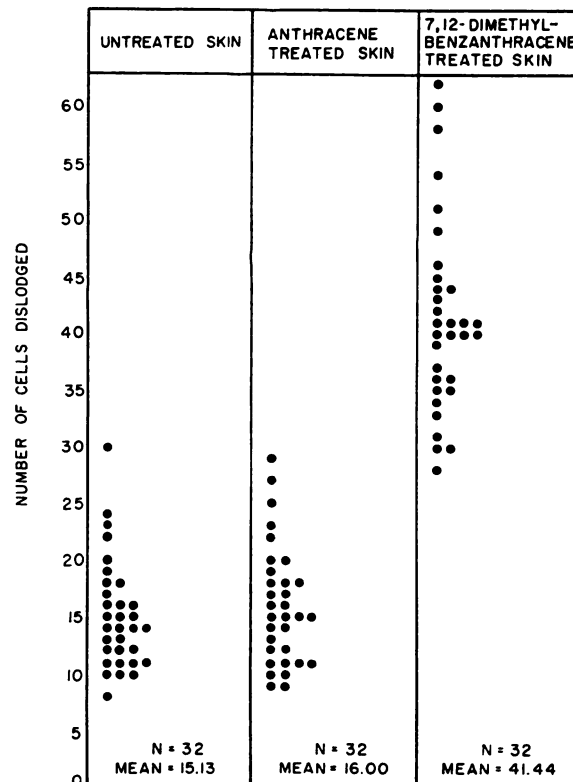


CHART 1.—Frequency distributions of numbers of cells dislodged by shaking treated and untreated skin samples.

TABLE 1  
COMPARISON BY MEANS OF NUMBERS OF CELLS DISLODGED BY SHAKING  
AFTER VARIOUS TREATMENTS OF SKIN

Conditions compared	Difference between means	Standard error of difference	"t," (d.f. = 62)	P
Anthracene-treated with untreated	.87	1.25	.70	> .50
7,12-Dimethylbenzanthracene-treated with untreated	26.31	1.73	15.21	< .001
7,12-Dimethylbenzanthracene-treated with anthracene-treated	25.44	1.76	14.45	< .001

after exposure to a carcinogen suggests that an alteration of the cell surface or external cell membrane occurs early in the process of carcinogenesis. The possibility arises that the surface alteration may be an initial and necessary step in the transformation of a normal to a neoplastic cell. It would be pertinent to study the effects of other carcinogens, such as viruses and physical radiations, to determine whether or not this is, indeed, a constant feature of carcinogenesis. Also, the comparative effects of fat solvents, chelating agents, and nonspecific irritants should be examined in this regard.

It deserves emphasis that the alteration in adhesiveness took place in the present experiments long before any other discernible effects of carcinogenic action were apparent, such as hyperplasia and anaplasia. There would no doubt be a much greater loss in adhesiveness when the tissues had become morphologically neoplastic, but the method employed would yield erroneous counts on such tissues because of the increased number of cells present in relation to the non-neoplastic controls. Whether this initial reduction in adhesiveness is correlated with a reduced calcium content in the cells should be determined, since Carruthers and Suntzeff (1) reported a two-step drop in the calcium content of mouse epiderm during carcinogenesis with methylcholanthrene, the first occurring soon after the carcinogen was applied and the

second when the tissues became frankly neoplastic. Zeidman (6), in this laboratory, found that, when a suspension of epidermal cells was allowed to settle on a glass slide coated with crystals of methylcholanthrene, the adhesiveness of attached pairs of epithelial cells was reduced in comparison with that of untreated cells.

It must be emphasized that the results reported in this paper were obtained with a single chemical carcinogen and that, before generalizations can be made about initial changes of the cell surface as a constant and perhaps essential stage in carcinogenesis, the problem must be more rigorously investigated.

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