CHANGES IN THE MITOTIC ACTIVITY AND NUMBER OF PATHOLOGICAL MITOSES IN THE EPIDERMIS OF THE MOUSE'S EAR IN THE INITIAL PERIOD OF CARCINOGENESIS

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The intensity of the mitotic activity is a valuable indicator of the rate of cell proliferation in carcinogenesis. The changes in mitotic activity in carcinogenesis have often been investigated.

Authors differ in their descriptions of the changes in mitotic activity in the course of carcinogenesis, depending on the test object, the number of applications of the carcinogen, its dose, and the time intervals after which the number of mitoses was counted. Some authors [9] have found an increase in mitotic activity with an increase in the number of local applications of a 0.6% solution of methylcholanthrene in benzene to the epidermis. Others [5] found that after application of the carcinogen the mitotic activity in the epidermis of the mouse's ear at first fell slightly, and then rose considerably for 23 days. It has been reported that the administration of o-aminoazotoluene to mice depressed the proliferative power of the liver cells [2].

An increase in the mitotic activity has been observed in the epidermis of the mouse 2 h after a single application of a 1% solution of 3-methylcholanthrene in benzene to the skin in the dorsal region. The mitotic activity fell to normal after 6 h [7]. It was later found that the period of intensification of the cell proliferation in the epidermis under the influence of carcinogenic substances follows a temporary depression of the mitotic division [8].

Growth of a malignant tumor is characterized not only by intensification of cell proliferation, but also by the appearance of various forms of pathological mitoses. An increased percentage of pathological mitoses has been observed in malignant papillomas of the human larynx [3, 4, 6].

It has not yet been established whether the pathology of mitosis is the result of malignant transformation of the cell or whether, on the contrary, the disturbances of mitosis cause the transformation of normal cells into malignant [1]. Although there are reports of a disturbance of mitosis in the early stages of carcinogenesis [5], further study of this problem is required.

To study the special features of cell division in the early stages of carcinogenesis, an investigation was made of the changes in mitotic activity and of the number of pathological mitoses in relation to the number of applications and an increase in the length of the time intervals after stopping application of the carcinogen.

EXPERIMENTAL METHOD

Experiments were conducted on male mice of the high-cancer C57BL line, weighing 20-30 g. As carcinogen a 0.05% solution of 9, 10-dimethyl-1,2-benzanthracene (DMBA) in acetone was used. The DMBA solution was applied 3 times a week to the inner and outer surfaces of the ear in a dose of 0.02 ml to each surface. The material was fixed in Carnoy's solution. Total preparations were made of the epidermis of the inner surface of the ear, and stained with Hansen's trioxyhematein. The intensity of mitotic activity was taken as the number of mitoses in an area of 0.78 mm^2. In some experiments the fixed material was embedded in paraffin wax and the mitotic activity was counted in sections 7 μ thick in the stratum basale of the epidermis for a distance of 2 cm. The percentage of pathological mitoses in relation to the total number of mitoses was calculated and the nature of the pathology of mitosis determined.

In the experiments of series I the changes in mitotic activity and the number of pathological mitoses were studied as the number of applications of carcinogen to the epidermis of the mouse's ear increased. The animals

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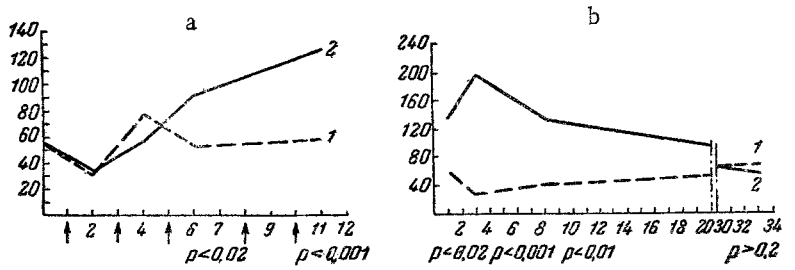

Fig. 1. Changes in mitotic activity depending on number of applications of 9,10-dimethyl-1,2-benzanthracene (a) and time elapsing after 5 applications of DMBA (b). 1) Application of acetone (control); 2) application of DMBA. Here and in Fig. 2 the arrows point to the times of repeated applications of the carcinogen. Along the axis of ordinates—mitotic activity, along the axis of abscissas—time (in days).

were divided into six groups (4-8 mice in each group). In five groups the left ear of each animal was treated with DMBA solution and the right (control) with acetone. One group consisted of intact animals. The animals were sacrificed 24 h after the end of the applications.

In the experiments of series II the changes in mitotic activity and the number of pathological mitoses were studied as the time interval after the fifth application of the carcinogen increased. The animals were divided into eight groups (4-7 animals in each group). The ears of the mice of four groups were treated with carcinogen and the ears of the animals of the other four groups with acetone (controls). The animals were sacrificed 1, 3, 8, and 33 days after the end of the applications. A statistical analysis of the results was carried out by the Fisher—Student method.

EXPERIMENTAL RESULTS

The results of the experiments of series I showed (Fig. 1a) that with an increase in the number of applications there was a clear tendency for the mitotic activity to increase in intensity. After 5 applications of DMBA the mitotic activity rose sharply by comparison with the control animals — by 100-150% ($P < 0.001$). The intensity of the mitotic activity 24 h after 5 applications of DMBA was measured four times. In every case a considerable increase in mitotic activity was observed. The results also showed that repeated applications of acetone, used as solvent for the DMBA, had no significant effect on the intensity of the mitotic activity, which differed only very slightly from the intensity of the mitotic activity in the intact epidermis (see Fig. 1a). After 7 and 10 applications of DMBA to the epidermis the mitotic activity in the sections was 100% higher than in the controls ($P < 0.01$). After 10 applications of the carcinogen, changes of a proliferative character were seen in the sections: the number of layers of the epidermis increased, and tongue-shaped zones of proliferation appeared, sometimes branching in character.

In the experiments of series II the intensity of mitotic activity was determined 1, 3, 8, and 33 days after 5 applications of DMBA to the epidermis of the mouse's ear. The results of these experiments (Fig. 1b) showed that after 24 h the mitotic activity was increased by 150% over the control level. After 3 days a further increase in mitotic activity was observed, and it was now 600% above the control level. After 8 days the mitotic activity showed a decrease, but it was still 200% above the control level. After 1 month (33 days) the mitotic activity was close to its value in the control animals.

Hence, 24 h after 5 applications of DMBA solution to the epidermis of the mouse's ear a marked increase in mitotic activity ($P < 0.02$) was observed, and this increase was still more pronounced 3 days after the end of the applications ($P < 0.001$); later the mitotic activity fell slightly, although it remained high for several days, and not until 1 month after the end of the applications had it returned to normal.

The appearance of the following types of pathological mitoses was observed in the epidermis under the influence of DMBA: delay in the movement of the chromosomes during metakinesis, and delay in their divergence toward the poles and in the formation of bridges.

In the experiments of series I the number of pathological mitoses was determined 24 h after 1, 2, 3, 4, 5, 7, and 10 applications of the carcinogen to the epidermis of the mouse's ear (Fig. 2a). Whereas the number of pathological mitoses in the epidermis treated with acetone was 0.3%, after three applications of DMBA it was 0.9% ($P < 0.05$). After 7 applications the number of pathological mitoses was much greater, namely 2.5% ($P < 0.001$), and it remained at the same level after 10 applications (2.6%).

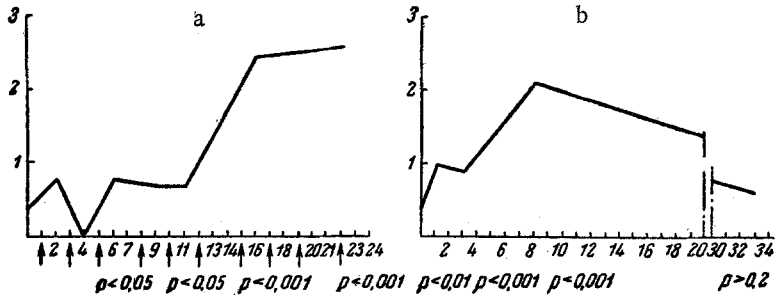

Fig. 2. Changes in the number of pathological mitoses in relation to number of applications of 9,10-dimethyl-1,2-benzanthracene (a) and at different times after 5 applications of DMBA (b). Along the axis of ordinates—pathological mitoses (in %), along the axis of abscissas—time (in days).

In the experiments of series II the changes in the number of pathological mitoses were studied at various periods after 5 applications of DMBA to the epidermis (Fig. 2b). The number rose to 1.1% after 24 h. After 8 days a further increase was observed in the number of pathological mitoses — to 2.1% ($P < 0.001$). The number showed a decrease to 0.7% 33 days after the end of the applications.

Hence, after the end of the applications of DMBA, the number of pathological mitoses in the epidermis rise for a short time, and then (after 3-4 weeks) fell slightly. After many applications the sharp increase in the number of pathological mitoses developed sooner. For instance, 24 h after 5 applications of DMBA the percentage of pathological mitoses was 0.9, and it rose to 2.1 only after 8 days, whereas it was 2.6 only 24 h after 10 applications.

The results of these experiments showed that in the process of carcinogenesis regular changes take place, while still in the early stages, in the intensity of cell division and pathological changes in cell division appear. With an increase in the number of applications of the carcinogen the mitotic activity rises and the percentage of pathological mitoses increases. For a short time after the end of the applications of carcinogen the mitotic activity and the number of pathological mitoses continue to increase.

SUMMARY

After the application of 9,10-dimethyl-1,2-benzanthracene to the epidermis of the ear in a mouse (strain C57BL) it was found that in the early period of carcinogenesis there occur regular changes in the intensity of cell division, manifested by the initial inhibition of mitotic activity and its further increase with an increase in the number of carcinogen applications to the epidermis. After the termination of carcinogen applications, the mitotic activity in the epidermis continues to increase during several days. In addition to this, in the early period of carcinogenesis pathology of cell division also develops. The number of pathologic mitoses in the epidermis grows with an increase in the number of DMBA applications. During a certain period after the termination of applications the number of pathologic mitoses continues to increase.

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