STIMULATION OF DNA SYNTHESIS IN THE MOUSE EPIDERMIS AT THE STAGE OF INITIATION OF CARCINOGENESIS

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UDC 576.353:591.871:616.006

The kinetics of the passage of cells in the mouse epidermis into the phase of DNA synthesis during the 24 h after stimulation with proton oil was studied. Application of 7,12-dimethylbenzanthracene, 3,4-benzpyrene, or urethane 5 weeks before croton oil stimulation was found to accelerate the passage of the cells into the S-phase. In the stage of initiation of carcinogenesis the sensitivity of cells of the epidermis to the action of factors stimulating DNA synthesis is increased.

The kinetics of stimulation of DNA synthesis by activators of carcinogenesis (Tween-60, croton oil) provides a method of distinguishing initiated from normal epidermis [1, 2].

In this investigation the kinetics of stimulation of DNA synthesis in initiated epidermis was studied in more detail: several initiating agents were used, and the relationship between effect and concentration of activator and the kinetics of passage of the cells into the phase of DNA synthesis were studied.

EXPERIMENTAL METHOD

Male C3HA mice aged about 3 months were used. A state of initiation was induced by the application of two drops of a 1.5% solution of 7,12-dimethylbenzanthracene (DMBA) in mineral oil (about 600 μg DMBA), 150 μg 3,4-benzpyrene in 0.1 ml acetone, or 120 mg urethane in acetone to the shaved skin of the intrascapular region. The urethane was applied 18 h after application of the 0.5% solution of croton oil in acetone, because preliminary application of croton oil considerably intensifies the inducing action of urethane [4]. Mice to whose shaved skin the solvents (mineral oil or acetone) were applied acted as the control. The skin of some mice was painted with an acetone solution of the noncarcinogenic hydrocarbon anthracene.

Five days after these preliminary procedures the same area of skin was again shaved and treated with 0.02, 0.1, or 0.5% solutions of croton oil in acetone. Thymidine-H^3 (specific activity for Ci/mmole) was injected intraperitoneally in a dose of 1 μCi/g body weight every 4 h 6 or 7 times (the first injection was given at the same time as the application of croton oil). The skin was fixed 1 h after each injection (except the 1st and 2nd) in 4 mice. Mice receiving the preliminary treatment but not the subsequent application of croton oil received 5 injections of thymidine-H^3 every 6 h. The technique of preparing and counting the autoradiographs of the epidermis was described previously [1, 2].

EXPERIMENTAL RESULTS

The passage of the cells into the phase of DNA synthesis in epidermis initiated by DMBA, 3,4-benzpyrene, and urethane and in normal epidermis (application of the solvents) was studied 5 weeks after these procedures. With the lapse of this time all possible changes of proliferative activity in response to the harmful action of the initiators should have disappeared and only the stable changes connected with initiation

Laboratory of Experimental Oncology, Sector of the Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulletein Eksperimental'noi Meditsiny, Vol. 77, No. 4, pp. 93-96, April, 1974. Original article submitted March 30, 1973.

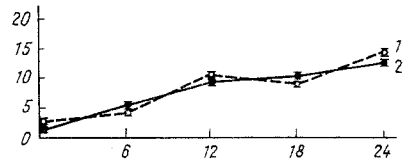

Fig. 1. Labeling index of basal cells of the epidermis 5 weeks after application of 1.5% DMBA in mineral oil (1) or of mineral oil alone (2). Abscissa, time (in h) after first injection of thymidine-H^3; ordinate, percentage of labeled cells after injection of thymidine-H^3 every 6 h.

should still have been present in the tissue. In fact, 5 weeks after initiation with DMBA the passage of the cells into the S-phase in the course of 24 h was identical in the normal and induced epidermis (Fig. 1). Consequently, proliferative activity in the initiated epidermis was unchanged at that time.

A sharp difference between the normal and initiated epidermis was observed on determination of passage into the S-phase after stimulation with croton oil. With three different concentrations of croton oil the labelling index of the basal cells increased much faster in the epidermis initiated by DMBA than in the control (Fig. 2). At each time studied for 20-24 h after stimulation with croton oil, many more cells in the initiated epidermis passed into the S-phase. The greatest differences between the initiated and normal epidermis were observed after application of 0.1% croton oil.

The mean time between the action of the stimulating factor (croton oil) and transition into the S-phase was thus much shorter after initiation. The effect of initiation was most clearly marked in the first 12-20 h after stimulation. Later, as was shown previously, all the cells of the basal layer in the initiated and normal epidermis enter the S-phase identically [1, 2].

The increase in labeling index of the differentiated cells also took place much faster in epidermis initiated by DMBA than in the normal epidermis (Fig. 3). In the initiated epidermis, after application of 0.02 and 0.1% croton oil, labeled differentiated cells were found at times when none had yet appeared in the normal epidermis.

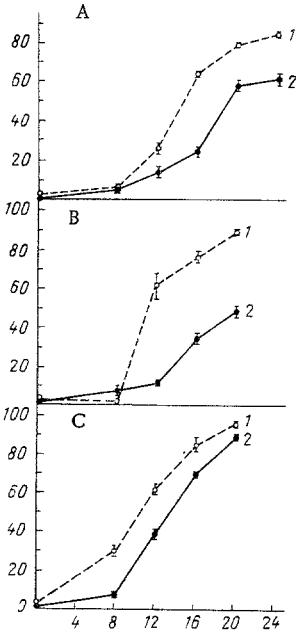

Fig. 2

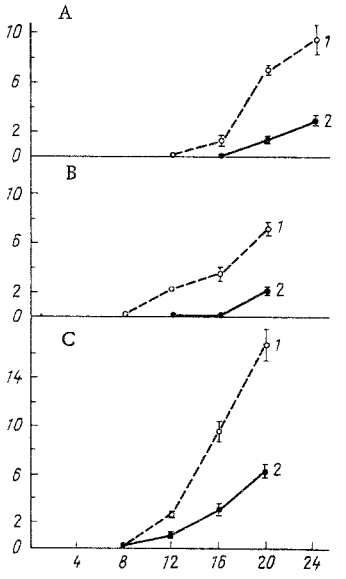

Fig. 3

Fig. 2. Effect of application of various concentrations of croton oil on labeling index of basal cells of the epidermis 5 weeks after application of 1.5% DMBA in mineral oil (1) or of mineral oil alone (2). Abscissa, time (in h) after application of croton oil: 0.02% (A), 0.1% (B), 0.5% (C); ordinate, percentage of labeled basal cells after injection of thymidine-H^3 every 4 h.

Fig. 3. Effect of application of various concentrations of croton oil on labeling index of differentiated cells of the epidermis 5 weeks after application of 1.5% DMBA in mineral oil (1) and of mineral oil alone (2). Abscissa, time (in h) after application of croton oil: 0.02% (A), 0.1% (B), 0.5% (C); ordinate, percentage of labeled differentiated cells after injection of thymidine-H^3 every 4 h.

TABLE 1. Labeling Index in Epidermis at Various Times after Application of 0.1% Croton Oil

Preliminary treatment*	Labeling index of basal cells (in %) †				Labeling index of differentiated cells (in %)
	0 h	12 h	16 h	20 h	20 h
Acetone	3,6±0,1	17,0±1,0	33,5±2,2	46,3±2,4	1,3±0,2
Anthracene 150 μg	4,3±0,6	14,4±4,7	27,8±2,0	57,9±4,3	2,4±0,6
Croton oil 0,5%	4,2±0,4	15,5±3,9	35,5±0,8	62,2±3,4‡	2,3±0,5
Croton oil 0,5%; urethane 120 mg 18 h later	5,8±0,4‡	25,1±2,3‡	48,7±2,7‡	75,9±2,0‡	4,4±0,2‡
3,4-benzpyrene 150 μg	4,8±0,8‡	29,9±1,8‡	47,2±1,5‡	79,2±5,8‡	5,9±1,5‡

*Application to interscapular skin of mice 5 weeks before application of 0.1% croton oil: of anthracene, 3,4-benzpyrene, and croton oil – in 0.1 ml acetone, of urethane twice in doses of 60 mg in 0.2 ml acetone at an interval of 15 min.
† Thymidine-H^3 injected simultaneously with application of 0.1% croton oil and then every 4 h. Fixation 1 h after last injection. Time between application of 0.1% croton oil and subsequent injection of thymidine-H^3 indicated in Table. In the group "0 h" fixation carried out 1 h after a single injection of thymidine-H^3 without application of croton oil.
‡ Difference from control (acetone) statistically significant ($P < 0.05$).

The effect of different initiators and other agents was studied in conjunction with stimulation by 0.1% croton oil for the action of DMBA was exhibited most clearly with this concentration. Of the preliminary treatments listed in Table 1 only 3,4-benzpyrene and urethane, as initiators, accelerated the passage of the basal cells into the S-phase in the early periods and led to an increase in the labeling index of the differentiated cells compared with the control (acetone).

The acceleration of the passage of cells into the S-phase during stimulation was thus observed after the various initiators, both polycyclic hydrocarbons that are complete carcinogens, and of urethane which has only inducing activity for mouse epidermis.

Consequently, this effect correlates with the state of initiation and it can probably be used as an indicator of this state and as a test for screening initiators. However, for a final conclusion it will be necessary to study many initiators and use mice of lines suspersensitive to two-stage carcinogenesis, of the STS type.

Various factors stimulating DNA synthesis, including croton oil, evidently act by activating genes [3, 5]. Since the state of initiation in carcinogenesis is an inherited change in the cells this change most probably involves a gene [6]. Acceleration of the passage of cells into the phase of DNA synthesis in initiated epidermis may perhaps reflect a change in the genetic apparatus of the cell increasing the sensitivity of the genome to the action of stimulating factors.

The authors are grateful to Academician N. M. Emanuel' and Professor L. P. Lipchina for their interest in the investigation and for discussing the results.

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