Macromolecular Synthesis following a Single Application of Alkylating Agents Used as Initiators of Mouse Skin Tumorigenesis

T. J. Slaga, G. T. Bowden, B. G. Shapas, and R. K. Boutwell

McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, Wisconsin 53706

SUMMARY

After the application of initiating dosages of certain alkylating agents, the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively, in mouse skin epidermal preparations was determined. Histological changes were compared with the metabolic activities. β-Propiolactone, γ-propane sultone, and bis(chloromethyl) ether inhibited DNA synthesis for periods up to 24 hr; this was followed by stimulation and, in turn, by epidermal hyperplasia. The maximal rate of RNA synthesis with both β-propiolactone and bis(chloromethyl) ether was attained at 12 hr, whereas the major peak with γ-propane sultone was observed at 2 days. Urethan depressed DNA synthesis for 24 hr without a subsequent increase and without detectable epidermal hyperplasia. Chloromethyl methyl ether, a weak or inactive initiating agent, produced no change in macromolecular synthesis, and the skin sections appeared normal. When either β-propiolactone or urethan was applied to mouse skin in which DNA synthesis had been prestimulated with a noncarcinogenic irritant, these agents blocked DNA synthesis.

INTRODUCTION

Chemical carcinogenesis in mouse skin can be divided into at least 2 steps, initiation and promotion (4, 5). A single application of an agent such as β-propiolactone, urethan, or 7,12-dimethylbenz(a)anthracene initiates the process of tumor formation. Subsequent repeated applications of a promoting agent such as croton oil or its active components to skin in which carcinogenesis has been initiated will elicit papillomas and carcinomas. However, at the dosages used, neither the initiator nor the promoter will by itself necessarily cause the formation of tumors in this system.

All of the known carcinogens for the skin are effective initiators at certain dose levels. However, urethan (18) and possibly 1,2,3,4-dibenzanthracene (24) and chloromethyl methyl ether (23) appear to be unique in that they act as initiators but are not complete carcinogens for the skin. There have been several studies of the effects of carcinogens (particularly the polycyclic aromatic hydrocarbons) on DNA and/or RNA synthesis in whole mouse skin, in attempts to determine what changes are required for carcinogenesis (1, 3, 11–13, 15, 16, 21). Hennings and Boutwell (13) reported that 7,12-dimethylbenz(a)anthracene, β-propiolactone, 4-nitroquinoline-N-oxide, and urethan inhibited DNA synthesis in whole mouse skin, but there appeared to be no direct relationship between the degree of inhibition of DNA synthesis and the number of tumors initiated. Other studies have shown an inhibition of the synthesis of whole mouse skin DNA (3, 11, 15, 16) and, in some cases, this was followed by stimulation (3, 16).

We felt that if we studied the biochemical effects of low initiating doses of carcinogens, we might diminish or eliminate toxic effect not directly related to the initial carcinogenic change. After a single application of an initiating alkylating agent, we determined the following: (a) the histological consequences; (b) tumorigenesis after croton oil treatment; and (c) the effect of the agent on incorporation of thymidine, cytidine, and leucine into the DNA, RNA, and protein, respectively, of the epidermal fraction of mouse skin. The effect of β-propiolactone or urethan on DNA synthesis in epidermis in which DNA synthesis had been prestimulated was also studied. The alkylating agents investigated were β-propiolactone, γ-propane sultone, urethan, bis(chloromethyl) ether, and chloromethyl methyl ether. The results are compared with the effects of the hyperplastic agent, acetic acid, on macromolecular synthesis. Alkylating agents were chosen for the initial study because they are ultimate carcinogens, i.e., they require no metabolic activation, so that the metabolic consequences of treatment may be detected within a few min.

MATERIALS AND METHODS

Animals. Female Charles River CD1 mice were purchased from Charles River Mouse Farms, North Wilmington, Mass. Mice (7 to 9 weeks old) were carefully shaved with surgical clippers 2 days before treatment. Only those mice in the resting phase of the hair cycle were used for biochemical experiments.

Tumor Experiments. Two days after being shaved, groups of 20 to 30 mice each received a single topical application of an alkylating agent. Two levels of each initiator were tested. This
treatment was followed, beginning 1 wk later, by 2 applications of 0.25% croton oil weekly for the duration of the experiment.

Chemicals. β-Propiolactone was purchased from Fellows Medical Manufacturing Co., Oak Park, Mich.; urethan (ethyl carbamate) was from Merck and Co., Inc., Rahway, N. J.; and bis(chloromethyl) ether, chloromethyl methyl ether, and γ-propane sultone (1-propanesulfonic acid-3-hydroxy-sultone) were from Eastman Organic Chemicals, Rochester, N. Y. Croton oil was purchased from S. B. Penick and Co., New York, N. Y. The radiochemicals (thymidine-methyl-3H, 3.0 Ci/mmole; cytidine-5-3H, 6.0 Ci/mmole; and L-leucine-4,5-3H, 6.0 Ci/mmole) were obtained from Schwarz/Mann, Orangeburg, N. Y.

All compounds were used as purchased except γ-propane sultone, which was purified by distillation at 114°/1.4 mm.

Application of Compounds Used. All compounds were applied to the shaved backs of mice in a measured volume of reagent grade of solvent, by means of a semiautomatic pipetting device. Urethan, γ-propane sultone, and croton oil were applied in 0.2 ml of acetone; (3-propiolactone was applied in 0.2 ml of acetone; and bis(chloromethyl) ether and chloromethyl methyl ether were applied in 0.2 ml of benzene. The i.p. injections of urethan were made in 0.1 ml of 0.85% NaCl solution.

Extraction of DNA, RNA, and Protein from Mouse Epidermis. Each time point on the charts represents an average of 2 to 3 groups of 4 mice each. Mice were always killed between 8:00 a.m. and 1:00 p.m. to minimize the effects of diurnal variation. Mice were killed by cervical dislocation 30 min after the i.p. injection of 30 µCi of thymidine-3H, or 1 hr after the injection of 20 µCi of cytidine-3H or 100 µCi of leucine-3H.

The method used to obtain epidermis was a modification of the procedure of Cowdry (9). The skins were removed, stretched dermis side down against a piece of filing card, and placed in a beaker containing 200 ml of 1% acetic acid at 5° for 24 hr. The skins were then placed on a porcelain plate on ice, and the epidermis was peeled off with tweezers.

A modification of the Schmidt-Thannhauser procedure (19) was used to obtain the nucleic acids and proteins, as described in detail by Baird et al. (2). Epidermis sheets from each group of 4 mice were placed in a tube containing 20 ml of ice-cold water and were homogenized with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.) for 30 sec at 70 V. Then we added 0.90 ml of concentrated HClO₄ to adjust the concentration of the homogenate to 0.4 M HClO₄. The precipitate was collected by centrifugation, and the pellet was washed once with 10 ml of cold 0.2 M HClO₄ and twice with 10 ml of 100% ethanol. For mice given injections of cytidine-3H, the RNA was hydrolyzed from the precipitate with 10 ml of 0.3 M KOH for 3 hr at 37°. Aliquots (0.2 ml) of the hydrolysate were used for measurement of the radioactivity and for determination of the amount of RNA by the orcinol reaction (20).

The epidermis sheets from mice given injections of leucine-3H or thymidine-3H were hydrolyzed in 10 ml of 0.5 M HClO₄ at 90° for 20 min. The DNA-containing hydrolysates from the mice given thymidine-3H were decanted and filtered, and the radioactivity was measured in 0.2-ml aliquots. The amount of DNA was determined by the diphenylamine reaction (7). After hydrolysis in HClO₄, the precipitate from the mice given leucine-3H was washed twice with 10 ml of cold 0.5 M HClO₄ and once with 100% ethanol. The protein was hydrolyzed in 10 ml of 0.5 M NaOH at 80° for 30 min and centrifuged, and the supernatant was decanted and filtered. Aliquots (0.1 ml each) were used to measure the radioactivity and to determine the amount of protein by absorbance at 280 nm, with serum albumin as the standard.

Radioactivity Measurements. A Packard Tri-Carb liquid scintillation spectrometer was used to measure the radioactivity of the samples. Duplicate 0.1- or 0.2-ml aliquots of each sample were counted in 10 ml of counting solution (composed of 295.2 g of naphthalene, 18.4 g of PPO, 0.1839 g of α-naphthylphenyloxazole, 1400 ml of xylene, 1400 ml of dioxane, and 840 ml of ethanol). To correct for quenching, automatic external standard ratios were used.

The specific activity for each group was calculated by the formula (dpm/µg DNA, RNA, or protein)/(µCi injected/g body weight). The specific activities for each group are

**Chart 1.** The effect of a single application of either 240 or 480 µmoles of β-propiolactone on the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively. Incorporation times for the tritiated precursors were 30 min for thymidine-3H and 1 hr for cytidine-3H and leucine-3H. The specific activity for each group is expressed as percentage of the acetone-treated controls. Time points, average of 2 to 3 groups containing 4 mice each; standard deviation of the control group. The average specific activity for the controls was 32 for DNA, 44 for RNA, and 28 for protein.
expressed as a percentage of the values of the control group.

**Histology.** Skin samples from the treated area of the backs of mice were taken at Days 1, 2, 4, and 6 after the single application of each dose of every test compound. Duplicate mice were killed at each time point for this study. The skin samples were fixed in neutral formalin and stained with hematoxylin and eosin.

**RESULTS**

The effect of a single application of each test compound to the skin of the back of the mice was determined by measurement of the incorporation of precursors into epidermal macromolecules. At least 2 levels of each compound were used; the times ranged from 3 hr to 6 days after treatment. An acetone control was included at each time point. The specific activity is expressed as a percentage of the control values. The average percentage standard deviation for the control group for each experiment is plotted on each chart to indicate the extent of variation. In most cases, a point on a chart represents an average of 2 to 3 groups.

The effects of either 240 or 480 μmoles of β-propiolactone on the incorporation of thymidine-3H into DNA, or cytidine-3H into RNA, and leucine-3H into protein are depicted in Chart 1. Treatment with 240 μmoles of β-propiolactone inhibited the incorporation of thymidine-3H into DNA for 12 hr; this was followed by a stimulation, which reached a peak by 18 hr and decreased to the control value by Day 2. Treatment with 480 μmoles of β-propiolactone brought about a greater and longer inhibition in thymidine-3H incorporation, followed by a gradual increase to twice the control level at 6 days. Both doses of β-propiolactone caused a slight depression in cytidine-3H incorporation into RNA, followed by stimulation that reached a peak more than twice the control values. Incorporation of leucine-3H into protein was stimulated after the application of 240 μmoles of β-propiolactone, reaching a peak at 3 hr, whereas the application of 480 μmoles of β-propiolactone caused a delayed stimulation that peaked at Day 1 and was still at 160% of control at Day 2.

Histological studies revealed that the skins of mice that received the lower dose of β-propiolactone developed a moderate hyperplasia, characterized by 4 to 5 layers of basal cells at Day 2, but by Day 4 the skin sections again appeared normal. Treatment with 480 μmoles of β-propiolactone caused a gradual increase in (to 6 or 7) basal cell layers by Day 6. Some grossly visible wounds were observed between Days 4 and 6 after treatment with the higher dose.

The tumor incidence resulting from initiating doses of β-propiolactone, followed by croton oil promotion, is shown in Table 1. A single dose of 240 or 480 μmoles of β-propiolactone, followed by twice weekly applications of 0.2 ml of 0.25% croton oil, caused 1.3 and 2.8 papillomas/mouse, respectively, at 15 weeks.

The effects of either 9 or 18 μmoles of bis(chloromethyl) ether (CMME) were applied topically in acetone; the rest of the compounds were applied topically in benzene.

### Table 1

<table>
<thead>
<tr>
<th>Initiator</th>
<th>Dose a (μmoles)</th>
<th>Promotion</th>
<th>No. of mice</th>
<th>Week 15</th>
<th>Week 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPL</td>
<td>240</td>
<td>Yes</td>
<td>28</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>BPL</td>
<td>480</td>
<td>Yes</td>
<td>30</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>BPL</td>
<td>480</td>
<td>No</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BCME</td>
<td>9</td>
<td>Yes</td>
<td>27</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>BCME</td>
<td>18</td>
<td>Yes</td>
<td>28</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>BCME</td>
<td>18</td>
<td>No</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CMME</td>
<td>12.5</td>
<td>Yes</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CMME</td>
<td>25</td>
<td>Yes</td>
<td>30</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>CMME</td>
<td>50</td>
<td>Yes</td>
<td>30</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>CMME</td>
<td>125</td>
<td>Yes</td>
<td>26</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>CMME</td>
<td>25</td>
<td>No</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GPS</td>
<td>50</td>
<td>Yes</td>
<td>28</td>
<td>2.3</td>
<td>3.2</td>
</tr>
<tr>
<td>GPS</td>
<td>100</td>
<td>Yes</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GPS</td>
<td>100</td>
<td>No</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urethan</td>
<td>340</td>
<td>Yes</td>
<td>29</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Urethan</td>
<td>680</td>
<td>Yes</td>
<td>28</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Urethan</td>
<td>680</td>
<td>No</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urethan</td>
<td>d</td>
<td>Yes</td>
<td>30</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Urethan</td>
<td>d</td>
<td>No</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a BPL, β-propiolactone; BCME, bis(chloromethyl) ether; CMME, chloromethyl methyl ether; GPS, γ-propane sulfone.
b BCME and CMME were applied topically in benzene; the rest of the compounds were applied topically in acetone.
c In those groups not subjected to promotion, 0.2 ml of acetone was applied to the skin of the back twice weekly.
d In these 2 groups only, urethan was administered by i.p. injection in 0.85% NaCl solution at a dose of 11.2 μmoles/g body weight.
ether on the incorporation of the tritiated precursors are shown in Chart 2. Both doses of bis(chloromethyl) ether caused an inhibition in thymidine-3H incorporation for at least 1 day, followed by a stimulation that peaked at Day 4. The incorporation of cytidine-3H into RNA was stimulated early, reaching a peak at 12 and 24 hr for both doses of bis(chloromethyl) ether. The incorporation of leucine-3H was stimulated only slightly by either dose. The skins of mice treated with 9 or 18 μmoles of bis(chloromethyl) ether developed a moderate hyperplasia by Day 6.

The tumor response after both doses of bis(chloromethyl) ether is shown in Table 1. Van Duuren et al. (23) stated that bis(chloromethyl) ether produced a moderate tumor response as an initiator; in our laboratory it has had an initiating capacity that was less than that of β-propiolactone. A single application of 9 or 18 μmoles followed by croton oil resulted in 0.4 and 0.9 papilloma respectively, per mouse, at 15 weeks.

In contrast to bis(chloromethyl) ether, chloromethyl methyl ether had no effect on the incorporation of tritiated precursors into macromolecules (Chart 3). Furthermore, the skins of mice treated with 12.5 and 25 μmoles of chloromethyl methyl ether appeared normal histologically and, at these dose levels, chloromethyl methyl ether was ineffective as an initiator of skin tumors (Table 1). However, when either 50 or 125 μmoles were administered once, followed by croton oil, a marginal initiating effect was observed. Van Duuren et al. (23) found that, as a result of treatment with either 1.25 or 12.5 μmoles of chloromethyl methyl ether, followed by phorbol ester as a promoter, 7 of 20 and 5 of 20 animals, respectively, developed papillomas; 4 of 20 and 1 of 20, respectively, developed squamous carcinomas (fewer tumors at the higher dose). These investigators also reported that multiple applications of 25 μmoles each were noncarcinogenic.

The effects of γ-propane sulfone on the incorporation of the precursors is shown in Chart 4. The effect of both doses of γ-propane sulfone is similar to that of β-propiolactone and bis(chloromethyl) ether, i.e., an early depression in incorporation of thymidine-3H into DNA, followed by stimulation. The maximal rate of RNA synthesis was attained at 12 hr with β-propiolactone and bis(chloromethyl) ether, whereas the major peak with γ-propane sulfone was observed at Day 2. Leucine-3H incorporation was stimulated at 18 hr and at 4

Chart 2. The effect of a single application of either 9 or 18 μmoles of bis(chloromethyl) ether on the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively. The incorporation times were as stated in the legend to Chart 1. The specific activity for each group is expressed as percentage of the benzene-treated controls. *Time points*, average of 2 to 3 groups containing 4 mice each; ····, standard deviation of the control group. The average specific activity for the controls was 34 for DNA, 43 for RNA, and 30 for protein.

Chart 3. The effect of a single application of either 12.5 or 25 μmoles of chloromethyl methyl ether on the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively. Incorporation times for the tritiated precursors were as stated in the legend to Chart 1. The specific activity for each group is expressed as percentage of the benzene-treated controls. *Time points*, average of 2 to 3 groups containing 4 mice each; ····, standard deviation of the control group. The average specific activity for the controls was 31 for DNA, 47 for RNA, and 25 for protein.
Macromolecular Synthesis after Alkylating Agents

Chart 4. The effect of a single application of either 50 or 100 μmoles of γ-propane sultone on the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively. Incorporation times for the tritiated precursors were as stated in the legend to Chart 1. The specific activity results for each group are expressed as percentage of the acetone-treated controls. Time points, average of 2 to 3 groups containing 4 mice each; ——, standard deviation of the control group. The average specific activity for the controls was 32 for DNA, 41 for RNA, and 24 for protein.

days after treatment with 50 μmoles, whereas a 100-μmole application of γ-propane sultone caused a peak at Day 2. The peak of epidermal hyperplasia after administration of 50 μmoles γ-propane sultone was between Days 2 and 4, whereas the peak after 100 μmoles was between Days 4 and 6. There was some visible wounding by the 5th and 6th day after treatment with 100 μmoles.

Druckrey et al. (10) reported that γ-propane sultone is a potent carcinogen and, as one might predict, we found it to be an effective initiator of skin tumors. To attain the same tumor incidence required about one-fifth to one-fourth as much γ-propane sultone as β-propiolactone on a molar basis. Groups of mice that received a single application of 50 or 100 μmoles of γ-propane sultone per mouse, followed by croton oil, developed 1.2 and 2.3 papillomas/mouse, respectively, at Week 15 (Table 1). Urethan given i.p. at a dose of 11.2 μmoles/g of body weight, followed by croton oil, resulted in 0.6 papilloma/mouse at Week 15.

The effect of 480 μmoles of β-propiolactone on the incorporation of thymidine-3H into DNA, after DNA synthesis had been prestimulated by multiple applications of pressure-sensitive tape, is depicted in Chart 6. The application and removal of pressure-sensitive tape 15 times across the preshaved back of a mouse stimulated the incorporation of thymidine-3H into the DNA of that area of skin by Hr 6, reached a peak of 440% of the control levels at Day 2, and

Chart 5. The effect of a single topical application of 340 or 680 μmoles or of an i.p. injection of 11.2 μmoles of urethan per g of body weight on the incorporation of thymidine-3H, cytidine-3H, and leucine-3H into DNA, RNA, and protein, respectively. Incorporation times for the tritiated precursors were as described in the legend to Chart 1. The specific activity results for each group are expressed as percentage of the acetone or 0.85% NaCl solution controls. Time points, average of 2 to 3 groups containing 4 mice each; ——, standard deviation of the control group. The average specific activity for the controls was 38 for DNA, 44 for RNA, and 27 for protein.
A number of investigators (1, 11–13, 15, 16, 21) have studied the effects of various carcinogens on the synthesis of DNA or of DNA and RNA in whole mouse skin in an attempt to determine any metabolic changes in the macromolecules that may be required for carcinogenesis. Our work represents an extension of these studies to include the measurement of the incorporation of appropriate precursors into protein as well as both DNA and RNA. Emphasis was placed on the initiation component of the carcinogenic process, and therefore the effects of small initiating doses of carcinogens were studied. This also served to minimize extraneous side effects of larger, carcinogenic applications. Epidermal tissue was used instead of whole skin for 3 reasons: (a) skin tumors are known to develop from epidermal cells; (b) incorporation of radioactive precursors into the macromolecules of the dermal cells was eliminated from the study; and (c) the infiltration of leukocytes into the dermis, which accompanies the inflammation caused by some carcinogens, would alter the specific activity and thereby invalidate the results of the isotope incorporation measurements.

An effective strategy to ascertain which biochemical observation(s) may be critical to initiation and promotion and therefore to carcinogenesis is to vary either dose or structure and observe possible correlations of these alterations to the tumor incidence, to other morphological effects, and to biochemical effects. In the studies presented here, structure was the more significant variable under study. Although at least 2 dose levels of each agent were tested, serial dilutions ranging from a dose that causes a maximal effect to one that has no effect are more useful for correlative purposes.

The compounds used varied greatly in their biological effect. Acetic acid is not a carcinogen, nor have we found that it initiates or promotes tumor formation. It is an irritant, capable of causing inflammation, hyperplasia, and wounds; and it was used as a hyperplasiogenic, nonpromoting control. Urethan was included because it is capable only of initiating skin tumors in mice; it is without carcinogenic or promoting activity in mouse skin. There were no morphological changes detectable in the skin of mice given initiating doses of urethan either topically or i.p. Of the alkylating agents, 3 are initiating agents (β-propiolactone, bis(chloromethyl) ether, and γ-pro-
exert initiating action; their half-life in tissue is short, resulting in pulse-like action, and the metabolic consequences of treatment may be detected within a few min after application. 

The effect of these agents on the incorporation of appropriate precursors into RNA, protein, and DNA is not susceptible to simple correlation with the morphological consequences of treatment. One might suppose that, in those cases in which RNA, protein, and DNA synthesis were stimulated, the agent was capable of causing gene activation; β-propiolactone, bis(chloromethyl) ether, γ-propane sulfone, acetic acid, and pressure-sensitive tape are examples. Cell division is the culminating event of gene activation in those systems that are well defined (references in Ref. 17); all of the agents listed caused hyperplasia in the treated area. It is not yet known whether the agents were effective directly as gene activators or indirectly by virtue of cell damage and death (or removal, in the case of the pressure-sensitive tape). It is unlikely that DNA repair could account for the stimulation of incorporation of thymidine-3H into DNA that occurred after treatment with any of the agents, since the magnitude of the synthesis is much too large. Furthermore, repair synthesis usually occurs soon after exposure to the damaging agent, whereas the increased thymidine incorporation occurred from 24 hr to several days after insult (14). 

Preceding the increase in DNA synthesis, an inhibition of DNA synthesis followed immediately after treatment with β-propiolactone, bis(chloromethyl) ether, γ-propane sulfone, urethan, and acetic acid. Even when β-propiolactone or urethan was applied to skin in which thymidine-3H was 3 or 4 times greater than the control level, these agents had turned off the increased DNA synthesis at the earliest time measured. The depression of apparent DNA synthesis caused by these agents might occur by a number of mechanisms, such as (a) interference with strand separation and/or DNA polymerase activity because of the presence of a covalently bound foreign molecule in DNA, (b) reduction in the number of cells synthesizing DNA because of cell death or other toxic reactions, (c) breakdown of (labeled) DNA after treatment, and (d) defective enzymes resulting in failure to utilize nucleoside precursors.

Obviously, β-propiolactone, γ-propane sulfone, bis(chloromethyl) ether, and acetic acid are toxic to cells and cause cell death, as shown by wounding and by histological observation, thereby reducing thymidine-3H incorporation. However, after smaller, less toxic initiating doses of urethan, β-propiolactone, γ-propane sulfone, and bis(chloromethyl) ether, there may be less chance of binding to sites in DNA or to other cellular targets vital to the maintenance of cell life. Thus, at these lower doses of alkylating agents, cell death becomes a less important factor in the apparent inhibition of DNA synthesis. However, the small amounts of DNA-bound carcinogen that have been reported (6, 8, 22) might account for the observed inhibition of DNA synthesis by interference with DNA polymerase activity. On the basis of this rationale, it is likely that all initiators will be found to inhibit DNA synthesis, since interaction of an initiating agent with DNA of the target cells is probably the primary event of initiation. Urethan is a case in point; it is an initiator that showed evidence neither of toxicity nor of gene activation at initiating doses, yet DNA synthesis was inhibited.

REFERENCES


Macromolecular Synthesis following a Single Application of Alkylation Agents Used as Initiators of Mouse Skin Tumorigenesis

T. J. Slaga, G. T. Bowden, B. G. Shapas, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/4/769

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.