

# Formation of a Carcinogen of Natural Origin in the Etiology of Ultraviolet Light-induced Carcinogenesis<sup>1</sup>

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## SUMMARY

The time course of cholesterol  $\alpha$ -oxide formation in the skin of hairless mice receiving chronic suberythemic levels of ultraviolet radiation was determined. The concentration of this carcinogen increased and reached a peak level at 10 weeks, after which squamous cell carcinomas appeared. The facts that an increase in formation of this carcinogen precedes the appearance of tumors and that this increase is apparently related to the dose of ultraviolet received suggest that this photoproduct, of natural origin, is involved in the etiology of ultraviolet-induced carcinogenesis.

## INTRODUCTION

The photochemical conversion of sterols to carcinogenic substances has been proposed as a potential explanation for the cancer causing effects of light upon skin (5, 8). It has recently been demonstrated that one such compound, cholesterol  $\alpha$ -oxide, which possesses carcinogenic properties, is formed *in vitro* in human skin exposed to UV radiation (3, 7). This compound has also been reported in skin of hairless mice exposed to UV (2).

The presence of a compound with known carcinogenic properties does not, in itself, constitute sufficient evidence to implicate the compound in the etiology of the disease. Thus, this report seeks to provide further evidence that the photochemical conversion of cholesterol to a compound with carcinogenic properties is involved in the etiology of UV-induced carcinogenesis.

## MATERIALS AND METHODS

One hundred thirty-five albino hairless mice (Laboratory for Animal Science and Toxicology, Riverside, Conn.) were divided into 4 experimental groups. Two groups (one of 50 and one of 24 animals) received daily (5 days/week) suberythemic levels of UV radiation from a mercury arc lamp (GE-UA3; G.E. Lamp Division, Cleveland, Ohio). The minimal erythema dose of UV was determined prior to

these experiments, and no reddening or acute effects were observed during the course of these studies. One of the groups was maintained for the duration of the experiment to determine percentage of tumor formation during any selected time interval. Animals from the other group were sacrificed at designated times, usually at 2-week intervals, to determine cholesterol  $\alpha$ -oxide concentrations. The 2 control groups of animals were maintained on the same regimen except for radiation. Three animals constituted the sample size of each control and radiated group from which comparisons were made at each selected time interval. Samples were taken at approximately the same time each day for each selected time interval to minimize the effects of diurnal fluctuations on sterol levels.

A flap of dorsal skin from the shoulder to the hip and extending ventrally to the midquadrant of each side was removed from the sacrificed animals. The skin was trimmed of s.c. tissue, minced, and homogenized. Total lipids were extracted as described before (4). The total lipid extract was subjected to preparative thin-layer chromatography to separate the polar photoproducts from other lipid constituents. They were eluted and cholesterol  $\alpha$ -oxide separated by an additional thin-layer chromatography. Experimental details and chromatographic characteristics of skin photoproducts in multiple solvent systems have been described previously (7).

After isolation, the cholesterol  $\alpha$ -oxide was esterified by reaction with acetic anhydride-<sup>14</sup>C (New England Nuclear, Boston, Mass.) at alkaline pH. The ester was extracted from the reaction mixture and prepared for gas-liquid radiochromatography. A stream splitter was placed in line with the flame ionization detector of the chromatograph. Nonlabeled reference cholesterol  $\alpha$ -oxide ester was added to the skin cholesterol  $\alpha$ -oxide ester fraction and the effluent from the split stream collected from the chromatograph at 2-min intervals. Procedures and chromatographic conditions have been described previously (2).

Levels of skin cholesterol  $\alpha$ -oxide were based upon recoveries of <sup>14</sup>C eluted from the gas chromatograph and the specific radioactivity of the acetic anhydride used in esterification.

## RESULTS AND DISCUSSION

Previous studies have demonstrated that the techniques used in this investigation were adequate to separate and

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quantitate many of the polar photooxidation products of cholesterol (2, 3, 7). The compatibility of the chromatographic detector response of reference compound, in this study, to the radioactivity profile of the skin cholesterol  $\alpha$ -oxide ester fraction can be seen in Chart 1.

The pattern of cholesterol  $\alpha$ -oxide formation *in vivo* can be immediately seen in Chart 2 when levels of the compound formed in radiated animals are plotted as a ratio to that found in the control sample at the specific sampling period. No effect on cholesterol  $\alpha$ -oxide formation is seen until after 6 weeks of chronic exposure to UV. At that time an increase occurs until Week 10 when the level of the compound begins to decrease whereupon tumors first appear. Tumors were assessed when the lesions were approximately 1 mm in diameter.

Minimal criteria have been proposed as providing the basis for evidence of causal involvement of a naturally occurring compound in the etiology of UV-induced carcinogenesis (2). These can be stated as: (a) the primary incitant (UV) and the UV-induced compound must both be capable of causing tumor formation; (b) UV-induced compound

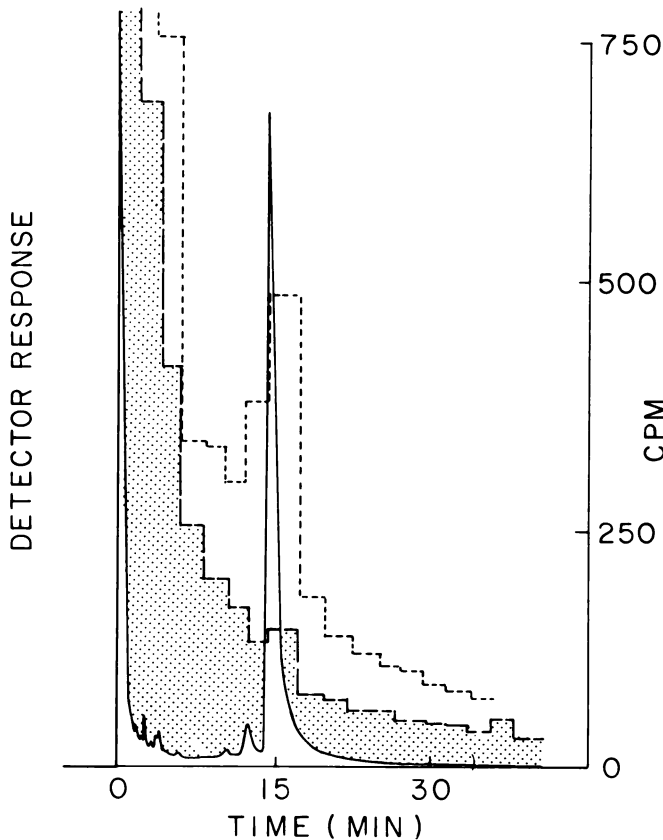


Chart 1. Gas chromatographic trace of reference cholesterol  $\alpha$ -oxide acetate ester. The histogram represents cpm of  $^{14}\text{C}$  in sequential fractions of the gas chromatographic effluent. The radioactivity is that of the mouse skin fraction separated by thin-layer chromatography and esterified with acetic anhydride- $^{14}\text{C}$ . *Open bars*, fraction obtained from mice that had received 10 weeks chronic UV exposure; *stippled bars*, nonradiated controls. Injection size was the same for both control and radiated samples and are comparable on a unit weight basis of skin. Levels of the compound in control animals ranged from approximately 0.3 to 1.0  $\mu\text{g/g}$  skin.

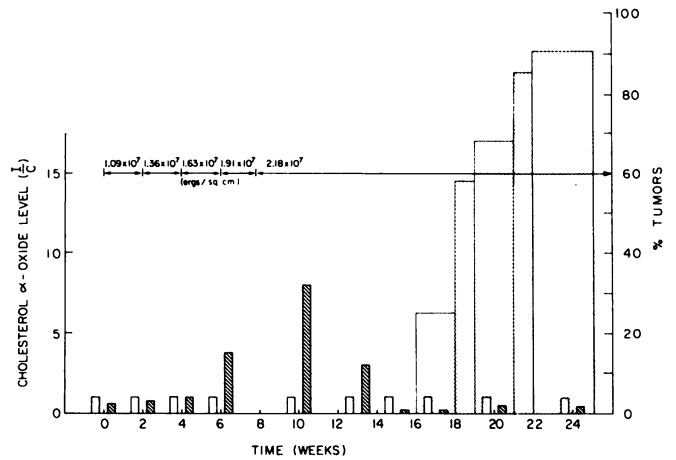


Chart 2. Relationship of cholesterol  $\alpha$ -oxide formation to onset of UV-induced tumors. The levels of cholesterol  $\alpha$ -oxide are expressed as the ratio of levels from radiated samples to those of controls. *Open bars*, controls; *hatched bars*, radiated samples; *top abscissa*, regimen of UV radiation. Exposure levels were increased at 2-week intervals to compensate for epidermal thickening. *Stippled bars* and *right ordinate*, percentage of tumors. The tumors were histopathologically diagnosed as squamous cell carcinomas. No spontaneous tumors occurred in controls.

must be formed prior to the appearance of UV-induced tumors; and (c) the ability of UV to cause tumor formation is related to its ability to induce formation of the compound under question.

The data presented here complete fulfillment of those criteria. One should be cognizant, however, that the criteria are only logical consequences that support the hypothesis that photochemical conversion of cholesterol to a carcinogenic substance occurs and that the substance plays a role in the etiology of disease development. At best, only a positive correlation can be obtained by satisfying these criteria.

There still remain questions which may have serious implications to the validity of this hypothesis. First, comparisons of the total cutaneous load of cholesterol  $\alpha$ -oxide achieved at peak levels are equal to about 16  $\mu\text{g}/\text{animal}$ . Bischoff (1) obtained 20 to 50% tumor formation in mice after s.c. injection of 20 mg of cholesterol  $\alpha$ -oxide. It could be argued that cholesterol  $\alpha$ -oxide would have to be much more carcinogenic than reported by Bischoff and that the increase from 2  $\mu\text{g}$  in the control animals to 16  $\mu\text{g}$  in the radiated ones represents an unusually steep dose-response curve. Further, Bischoff reports the formation of sarcomas after injection of cholesterol  $\alpha$ -oxide. No evidence is available for the compound causing carcinomas either after s.c. injection or topical application.

Indeed, it would seem that comparisons between dose of compound applied either s.c. or topically to that which may occur from natural origin within the target tissue are invalid. The formation of the compound within the target tissue would allow participation in the disease development in a highly specific fashion that could not be duplicated by introduction of the carcinogen through other routes. The same argument applies to type of tumor formed in response to cholesterol  $\alpha$ -oxide. When cholesterol  $\alpha$ -oxide is introduced s.c. it promotes tumors of dermal origin. Thus, with its carcinogenicity established, it would follow that this

property should be exhibited in the epidermis, the target tissue of UV and the most probable site of the formation of the compound. It is conceivable, if cholesterol  $\alpha$ -oxide acted in a specific manner at its site of formation, that topical application of the compound would fail to induce tumor formation. If the increase in formation of cholesterol  $\alpha$ -oxide were simply a manifestation of epidermal hyperplasia one might expect the levels to remain high or continue to increase in relation to epidermal thickening.

In summary, the role of UV in the etiology of certain types of skin cancers has been firmly established (6). The carcinogenicity of cholesterol  $\alpha$ -oxide has also been demonstrated (1). The formation of this compound in skin upon exposure to UV has been reported (2, 3, 7). The present demonstration that a relationship between the formation of cholesterol  $\alpha$ -oxide and the onset of UV-induced tumors exists and that the formation precedes the appearance of tumors completes the fulfillment of the suggested criteria. These data indicate that cholesterol  $\alpha$ -oxide plays a role in the etiology of UV-induced carcinogenesis. Further lines of evidence, however, are currently being sought to test this thesis.

## ACKNOWLEDGMENTS

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