Skin Carcinogenesis: Cholesterol-5α,6α-Epoxide Hydrase Activity in Mouse Skin Irradiated with Ultraviolet Light

Abstract. *Cholesterol-5α,6α-epoxide hydrase activity was 96 percent greater in skin of hairless mice that were receiving suberythemic ultraviolet light irradiation for 15 weeks than in nonirradiated controls. This enzyme system, which metabolizes cholesterol-5α,6α-epoxide (a known carcinogen), appears to be substrate-inducible and is apparently responsible for the concomitant reduction of the sterol carcinogen that occurs prior to tumor induction.*

The photochemical conversion of sterols to carcinogenic substances has been suggested as a possible mechanism for the cancer-causing effects of sunlight and ultraviolet light on skin (*1*). Cholesterol-5α,6α-epoxide (CAE), a known carcinogen, is formed in both human and hairless mouse skin upon exposure to ultraviolet light (*2*). When the time course of CAE formation in skin of hairless mice receiving long-term, suberythemic levels of ultraviolet light was determined, it was found that the carcinogen increased, reached a peak at about 10 weeks, and after this squamous cell carcinomas appeared at week 15 (*3*). These data suggested a causal relation of this compound to carcinogenesis induced by ultraviolet light.

Since epoxides are thought to be the metabolically activated form of polycyclic hydrocarbons, both the mixed-function oxidases leading to epoxide formation and aryl hydrocarbon hydroxylases responsible for their detoxification have received much attention (*4*). Epoxide hydrases transform epoxides to much less reactive dihydrodiols (*5*). Activities of these enzymes, therefore, could be of vital importance with respect to accumulation of epoxides and thus to the mutagenicity and carcinogenicity of the respective hydrocarbon. A sterol epoxide hydrase has been found in mouse skin and catalyzes the reaction of CAE to cholestane-3β,5α,-6β-triol (triol) (*6*). Here we report the influence of long-term irradiation with ultraviolet light on CAE hydrase activity and its relation to CAE levels in the skin of mice.

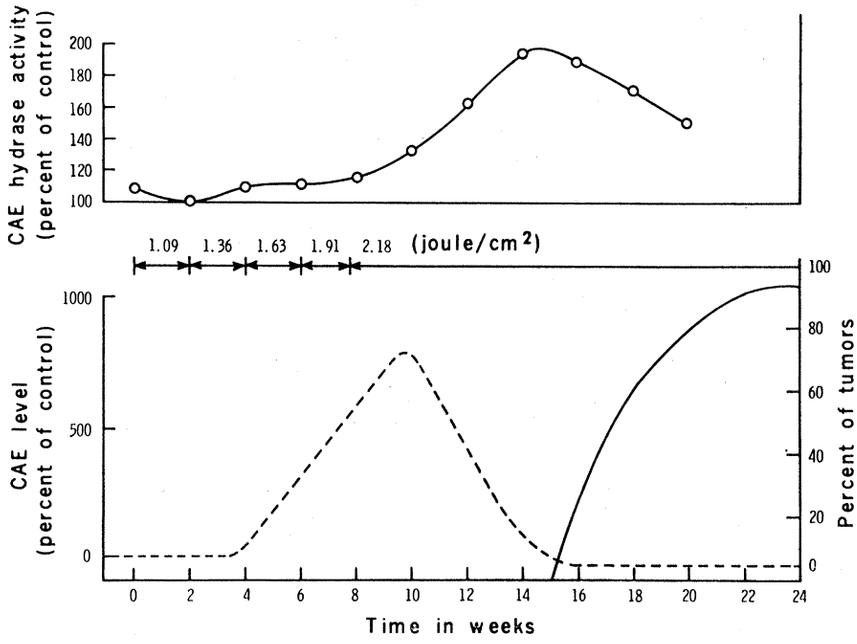

Fig. 1. Relation between CAE hydrase activity and CAE levels to onset of tumors induced by ultraviolet light. The upper curve illustrates CAE hydrase activity in skin of mice irradiated with ultraviolet light as the percentage of nonirradiated controls. Enzyme activity was calculated from the conversion rate of CAE to triol per milligram of protein. Each point on the curve represents the mean value from triplicate incubations. The standard deviation of the percentage of conversion within each triplicate set of incubations did not exceed ± 0.63. Average control activity was 0.16 nmole of CAE per milligram of protein per 2 hours. In the lower curves, the dashed line represents levels of CAE in the skin of irradiated mice as the percentage of controls; the solid line and right ordinate represent the percentage of animals bearing tumors. The middle abscissa shows the regimen of ultraviolet radiation. Exposure levels were increased at 2-week intervals to compensate for epidermal thickening. [Data from (*3*)]

Hairless mice (hrhr) were maintained on a regimen of long-term, suberythemic ultraviolet light as previously described (*3*). Mice were killed at 2-week intervals, a flap of dorsal skin removed, subcutaneous tissue scraped away, and the skin was minced and homogenized in four volumes of Krebs-Ringer phosphate buffer (*p*H 7.4). The homogenate was centrifuged at 500*g* for 10 minutes, and the supernatant (enzyme) was removed for assay. The reaction mixture contained 33 nc (4.6 mc/mmole) of [4-^{14}C]cholesterol-5α,6α-epoxide, 50 μl of Tween 80 solution (86.6 mg of Tween 80 in 1 percent ethanol), and 1 ml of enzyme. Incubation was carried out at 37°C for 2 hours. At the end of 2 hours the reaction was halted by the addition of 5 ml of a mixture of chloroform and methanol (2 : 1, by volume) and the total lipids were extracted. The total lipid extract was chromatographed on thin-layer chromatographic (TLC) plates in a mixture of chloroform and acetone (9 : 1, by volume). The areas of the TLC plate corresponding to the respective standards (CAE and triol) were scraped off and radioactivity was determined. The triol was identified by recrystallization to a constant specific radioactivity and radio-gas-liquid chromatography. Cholesterol-5α,6α-epoxide hydrase activity was calculated from the percentage of conversion of ^{14}C-labeled CAE to [^{14}C]triol per milligram of protein.

The results in Fig. 1 demonstrate that there is a significant increase of CAE hydrase activity beginning after 8 weeks of ultraviolet light radiation. The increased activity reaches a maximum at 15 weeks and remains at a substantially higher level than that of controls through 20 weeks. The initiation of higher activities of CAE hydrase corresponds to an increased level of CAE and suggests that the enzyme is substrate-inducible. Further, the higher activity of enzyme occurring after 12 weeks explains the subsequent decrease in CAE levels.

If CAE is indeed involved in the etiology of carcinogenesis induced by ultraviolet light, then an enzyme that detoxifies this compound is of paramount importance. In the case of hydrocarbon arene oxides, the impor-

tance of the hydrase reactions lies in (i) detoxification of carcinogenic compounds to less harmful ones, (ii) possible alteration of this detoxification potential by environmental factors thus leading to the malignant state, and (iii) potential manipulation of this metabolism as a therapeutic tool. Generally, the hydrase enzymes have been associated with the mixed-function oxidases —the latter being responsible for the activation of certain hydrocarbons to their more carcinogenic oxides. Efforts to manipulate the enzymes responsible for detoxification of the oxides are complicated by unwanted effects upon the enzymes responsible for formation of the oxides. In the case of CAE hydrase, the substrate is of natural origin and is formed photochemically; the fact that CAE levels appear to be directly related only to irradiation and CAE hydrase activity suggests that mixed-function oxidases are not involved. Thus the paradox faced by others in the manipulation of enzyme systems related to both carcinogen activation and detoxification is not pertinent to the CAE hydrase system. This might allow alteration of those enzymes involved in detoxification of CAE in a way that could be of preventive value.

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Human Vaginal Secretions: Volatile Fatty Acid Content

Abstract. *Vaginal samples (682) were collected by a tampon method from 50 healthy young women. Samples were analyzed by gas chromatography. The volatile aliphatic acids increased during the late follicular phase of the menstrual cycle and declined progressively during the luteal phase. Women on oral contraceptives had lower amounts of volatile acids and did not show any rhythmic changes in acid content during the menstrual cycle. These same substances possess sex-attractant properties in other primate species.*

Volatile aliphatic acids (acetic, propanoic, methylpropanoic, butanoic, methylbutanoic, methylpentanoic) have been demonstrated in the vaginal secretions of the rhesus monkey (*Macaca mulata*), anubis baboon (*Papio anubis*), patas monkey (*Erythrocebus patas*), pigtail monkey (*M. nemestrina*), crab-eating monkey (*M. fascicularis*), and squirrel monkey (*Saimiri sciureus*); thus, they are present in a wide range of primate species (*1, 2*). These acids possess sex-attractant properties in the rhesus monkey and stimulate the sexual activity of males via olfactory pathways (*3*). Preliminary studies have also demonstrated their occurrence in women (cooperative wives of colleagues) (*4*). In an effort to obtain quantitative data on a larger human sample, we collected secretions from 28 women attending an infertility clinic at the Samaritan Hospital for Women, London (*5*). Samples were collected by lavage, and only 2 of the 28 contained acids in concentrations similar to those observed in the earlier study. Women presenting themselves at a clinic for gynecological examination sometimes take such extreme steps to prepare themselves by washing and douching that it is impossible even to collect vaginal smears for cytology and, because the negative results from the clinic population contrasted with our preliminary findings, it was considered essential to investigate a population of normal, healthy women.

The subjects were recruited for study as follows. University women were simply informed in a leaflet that a study on the physiology of women was being undertaken that involved measures on the biochemical composition of body fluids, and that this would require participants to wear vaginal tampons. Those women indicating their willingness to cooperate were then contacted individually by a mature female psychologist who helped them complete a questionnaire designed to exclude those with gynecological problems. After participants signed a consent form protecting their human rights and welfare, they were (i) offered a gynecological examination, (ii) provided with a box containing tampons and bottles, and (iii) given detailed instructions on their use (see below). Identities were protected by code numbers. A month after their distribution, boxes were collected under conditions preserving confidentiality, and a second questionnaire was completed. Efforts were made to recheck menstruation dates, but attention was also given to the use of oral or vaginal contraceptives and feminine hygiene products. Subjects were not aware of the specific purpose of the study or of the type of assays to be conducted. There was no direct contact between laboratory and subjects, the only link being the psychologist who recruited women to the project and who distributed and collected the boxes. The biochemists conducting assays were aware only of code numbers. When assay of all 682 samples had been completed, data were grouped in relation to the stage of the menstrual cycle, and subjects were separated into oral contraceptive users and nonusers.

Self-collection of vaginal secretions by aspiration with a pipette containing distilled water (pipettes were used successfully in infrahuman primates) proved quite distasteful to the majority of women. Accordingly, the following acceptable method was developed by which women could conveniently and reliably take a sample of their own vaginal secretions. A commercial tampon was reduced to about 1 cm in length, washed with hot methanol in a Soxhlet extractor for 2 hours, dried at 110°C, and hermetically sealed in a polyethylene bag. This procedure removed waxes and other extractable matter that would later interfere with gas chromatography. Each subject was provided with a convenient box containing 16 tampons (1, a control blank) and 15 numbered, snap-cap bottles each containing 20-ml of methanol. Subjects were requested to wear each tampon in the usual way for 6 to 8 hours and, on removal, to drop it immediately in the bottle provided. Tampons could be worn by day or by night, whichever was most convenient,