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"In vivo Analysis of UV-B Induced Photooxidations"
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Period: 8/28/78 - 4/28/80

The basic objective of this project is an attempt to elucidate the mechanism of UV-B induced photooxidations occuring in vivo, utilizing cholesterol synthesized in vivo as a substrate for photooxidative reactions. By analyzing the cholesterol oxidation products formed when mice are irradiated with UV-B, it is our intention to be able to describe the types of photooxidations initiated by UV-B in vivo.

During the first phase of this project (8/28/78 - 8/27/79) we concentrated on the development of a suitable HPLC system for the rapid and quantitative resolution of cholesterol and of some of the expected oxidation products. We have used an Altex 310/50 HPLC with a variable wavelength detector coupled to a reverse-phase column for the separation and analysis. The following compounds have been prepared from cholestrol to serve as chromatographic standards:

	Retention time (min)
cholesterol	11.5
cholesterol-5α, 6 α-epoxide	
cholesterol-5β, 6 β-epoxide	
3β-hydroxycholest-6-ene-5α-hydroperoxide	7.0
7-α-hydroperoxy cholesterol	6.0
7 α-hydroxy cholesterol	
7 β-hydroxy cholesterol	
7-β-hydroperoxy cholesterol	9.5
3β, 5α, 6β-trihydroxycholesterol	
7-keto cholesterol	

In addition, we have also prepared several of these compounds from ^{14}C-cholesterol to serve as internal standards and, in particular, to locate some of these steroid derivatives that do not have an adequate absorption at 205 nm to permit detection in our variable wave length detector. The ^{14}C-steroids synthesized in our laboratory from ^{14}C-cholesterol include: ^{14}C-cholesterol-5α, 6α-epoxide, ^{14}C-cholesterol-5β, 6β-epoxide and 3β, 5α, 6β-trihydroxy cholesterol

The retention times for several of these standards are listed above, using acetonitrile as the developing solvent.

For the purpose of providing a radioactive substrate for the UV-B photooxidations, we have utilized RS-(5-^{3}H)-mevalonic and (*MVA) injected intraperitoneally into mice. Although there are no reports in mice, *MVA has been utilized in rats and found to give significant incorporation into skin steroids within 30 min (J. Lip. Res. 2:344, 1961). We have now been routinely injecting 0.25 μ curies of *MVA into each experimental animal in order to obtain an adequate quantity of *cholesterol in the skin steroids. Our present protocol consists of injecting *MVA and incubating in vivo for 60 min, either in darkness or exposed to 2 GTE Sylvania fluorescent lamps, F40T12/2021 at a distance of 15 cm.

The experimental animals are nude mice, Skh :hr-1, obtained from Temple University Skin and Cancer Animal Hospital, Philadelphia, PA. Following in vivo incubation, the skin is removed and the lipids are extracted into chloroform/methanol using a Brinkmann Polytron Homogenizer. The entire

process is carried out in a nitrogen-filled chamber under dim light to avoid spurious oxidation of the *cholesterol. After suitable preparation, the steroids are separated by preparative thin-layer chromatography to yield a "steroid" fraction. To remove polar lipids the TLC plate is irrigated 3 times. This material is then subjected to HPLC analysis, with the effluent collected directly in scintillation vials via a Gilson FL-100 fractionator.

Our results are still preliminary, but do indicate that the major radioactive skin steroid observed 1 hr after IP administration of *MVA is cholesterol. There are also smaller amounts of two other radioactive compounds, one more polar and the other less polar than cholesterol. The more polar compound has been tentatively identified as the 3β,5α,6β triol. As yet, we have not been able to identify the less polar compound, but we may be dealing with a precursor of cholesterol such as 7-dehydro cholesterol.

In the extracts of animals exposed to UV-B, we find increased amounts and types of more polar radioactive peaks. The precise characterization of these peaks is difficult, due to the very low levels of radioactivity observed and a problem in reproducibility. We are quite concerned that some of the changes we are observing may be due to the UV-B catalyzed formation of vitamin D_3, via pre-vitamin D_3, from 7-dehydrocholesterol, as recently described by Holick et al. (Biochem. 18, 1003-1979). We are in the process of obtaining some radioactive standards of the compounds described by Holick et al. to compare to our UV-B induced radioactive peaks.

Our remaining effort in this project period (8/28/79 - 8/27/80) will be to characterize these products of UV-B photooxidation of *cholesterol.

Dr. Cameron, NCI: I think this question has been raised before, but how much work has been done with skin painting of these varoius strains? I also have another naive question. What is a sencar mouse? Is that a hairless, too?

Dr. Orme, NCI: No. The sencar mouse is one that was developed by Tom Slaga at Oak Ridge. What they did over a number of generations was select mice that developed tumors after treatment with DMBA-TPA. Since the tumors were not lethal, they could breed tumorigen bearing animals, and, in that manner, developed a population with high susceptibility to DMBA-TPA. They also developed another line, which was supposedly the resistant counterpart.

I may not have that story completely straight because I have obtained it verbally and have never seen any publications on the sencar mice, but Tom Slaga has described it as a very useful system, not only because of its high susceptibility for screening of skin carcinogens and looking at the promotion phenomenon, but also because he has explanted skin cells from the sencar mouse and has an in vitro transformation system which he says is remarkably similar to the in vivo system as far as its response to a spectrum of chemicals is concerned.

It has always been puzzling to me why hairless mice were not picked up for skin painting studies, but to my knowledge they have never been used extensively for that.

Dr. Cameron, NCI: Would you propose that that be an avenue for further research?

Dr. Orme, NCI: Yes. Dr. Fred Urbach at Temple has proposed to do some screening studies with hairless mice. He wants very badly to start screening for skin carcinogens using the HRA strain. That is a possible use.

Dr. Cameron, NCI: That would seem to me to have an occupational smattering, would it not? There would be different exposures for different occupations.

Dr. Gass, OSHA: I have one question - a kind of fun question. Do juvenile hair coats in susceptible strains offer any protection against the UV range?

Dr. Orme, NCI: I do not know if anybody has done that experiment.

Dr. Gass, OSHA: It would be a neat control.

Dr. Orme, NCI: It would be a hard one to do, because the juvenile hair coat is gone so fast.

Dr. Gass, OSHA: It lasts about six days?

Dr. Cameron, NCI: Their juvenile hair coat lasts about ten days, doesn't it?

Dr. Orme, NCI: I am not really sure.

Dr. Gass, OSHA: They do not get a hair coat; that is the problem. If hair does protect against UV, would the juvenile hair coat protect? That is the question.

Dr. Orme, NCI: I think the protection with the hair is just a neutral density protection. It is a neutral density filter protection.

Dr. Gass, OSHA: That is why I would use it for a control.

Dr. Orme, NCI: There is an interesting phenomenon which occurs in the C3H mouse, which is being used by Margaret Kripke in her immunology studies, where she has shown that there is a UV-B induced antigen on skin tumors which develop after UVB irradiation. These are all fibrosarcomas. I do not know, and I do not think anybody knows, why irradiation of some mice leads to fibrosarcomas and irradiaiton of others leads uniformly to squamous cell carcinomas. Hairless mice, for the most part, respond to UV irradiation with squamous cell carcinomas. Haired mice yield a variety of tumors.

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