THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 49, No. 6

Printed in U.S.A.

EFFECTS OF ULTRAVIOLET LIGHT ON SKIN CHOLESTEROL*

ELIZABETH W. RAUSCHKOLB, M.D., GORDON FARRELL, M.D.
AND JOHN M. KNOX, M.D.

Surprisingly little is known of the photochemical reactions which occur in human skin upon exposure to sunlight. There are a few noteworthy exceptions, such as the activation of tyrosine, the general assumption of the conversion of 7-dehydrocholesterol to Vitamin D, the transformations in porphyrins which result in skin photosensitization, and the reduction in sulfhydryl linkages (1–4).

Using an *in vitro* technique, a study is underway in our laboratory to explore the biochemical reactions of human skin. One of the first observations was that a striking alteration in the chloroform soluble cholesterol content of skin occurs after exposure to irradiation. This is a report of our findings.

METHODS

1. Extraction and quantitative determination of skin sterols. The skin specimens are frozen and sectioned at 20–50 microns with a freezing microtome. These sections are suspended in 10 volumes (approximately 5 ml) distilled water and shaken with 30 ml chloroform. The solvents are left in contact at 3° C in the dark for 16 hours. The skin constituents are thus separated into two large classes, i.e. lipid-soluble (e.g. sterols, steroids, naturally occurring quinones, terpenes, fatty acids and phenolic carboxylic acids) and aqueous, consisting of (a) water-soluble substance (e.g. amino acids, peptides, soluble mucopolysaccharides and proteins, and inorganic salts) and (b) insoluble material (e.g. proteins, melanin) which remain suspended in the aqueous phase.

In the present study attention was given to the chloroform soluble fraction. The chloroform extract is recovered and the aqueous fraction washed twice with chloroform. The chloroform fractions are combined, reduced to dryness, transferred quantitatively and subjected to thin-layer chromatography. Layers of silica gel G approximately 250 μ thick are prepared on 20 x 20 cm glass plates. The concentrated chloroform fraction is applied quantitatively with a micropipette 1.5 cm from the lower edge of the plate. Reference compounds, cholesterol, cholesterol stearate, triolein, tripalmitin, tristearin, and Vitamin D, are also applied.

The following single phase solvent systems are employed (5):

1. ethylene dichloride(s)
2. ethylene dichloride/methanol (98:2)
3. chloroform/acetic acid (96:4)
4. chloroform/methanol (95:5)
5. n-propanol/12.5% ammonium hydroxide (80:20)

Compounds are located on the developed plates by spraying with 0.5% aqueous potassium permanganate. This sensitive but nonspecific reagent permits the immediate detection of a variety of lipids and sterols as bright yellow spots on a reddish violet background without destruction of the cholesterol nucleus. Presumptive identification of the sterol can be obtained by spraying with concentrated sulfuric acid and acetic acid (1:1) and heating at 90° C for 15 minutes. Cholesterol and cholesterol stearate give red spots on a white background. Visual inspection of the silica gel plates provides a semiquantitative estimate of the amount of sterol present.

For more quantitative data a micromethod was devised. Thin-layer chromatography is performed as described above. For routine use, the ethylene dichloride system proved to be particularly useful. Detection of compounds is by spraying with potassium permanganate. The spot from the skin extract which corresponds to reference cholesterol is quantitatively scraped off the plate, extracted with chloroform and centrifuged at 3° C. The clear supernatant chloroform extract is removed and evaporated under CO_2 or N_2. To the dry sample 1 ml of chloroform and 1 ml of a mixture of acetic anhydride-concentrated sulphuric acid (20 vol:1 vol) are added. An oxidative dehydration transformation occurs to form a specific chromogen (a blue color which soon changes to green; c.f. Liebermann-Burchard reaction). Quantitation is at 620 mμ in a spectrophotometer. The method is sensitive to 2 μg; recovery is on the average 88.6%.

For extraction of any possible sterol fraction which remained in the aqueous phase (water-soluble substances and suspended insoluble material, presumably in a bound form), (v.i.) this fraction is mixed with an equal volume of 15% ethanolic potassium hydroxide containing (4%) pyrogallol. The mixture is refluxed for 30 minutes, cooled for one hour and extracted with iso-octane. The iso-octane fraction is backwashed with water until pH 6 is obtained and is chromatographed as above for isolation of sterols.

2. In vitro irradiation of human skin. An environmental chamber was designed to permit rigid control of temperature, humidity and oxygen ten-

* From the Veterans Administration Hospital and the Departments of Dermatology and Biochemistry, Baylor University College of Medicine, and The Texas Research Institute for Mental Sciences, Houston, Texas.

Presented at the Twenty-eighth Annual Meeting of the Society for Investigative Dermatology, June 18–20, 1967, Atlantic City, New Jersey.

sion. The chamber is a double-walled cylinder of machined aluminum closed at one end. The opposite end is fitted with an optically plane silica lens which transmits wave lengths between 2,200 and 35,000 Å. The space between the inner and outer cylinder walls serves as a water-jacket through which water is circulated by a perfusion apparatus.[1] The temperature is maintained at ±0.04° C. During the experiment the chamber is gassed with water-saturated oxygen. A small receptacle near the rear of the chamber holds wet gauze to maintain saturation with water vapor. The inner surface is painted with black matte finish to reduce reflection.

Human skin, obtained as soon as possible postmortem, is used in the experiments. A specimen approximately 1.5 cm x 3 cm is cut from the lower abdominal wall within 1½ hours after death and kept chilled in the dark until irradiated. The specimen is dissected free of subcutaneous connective tissue and divided into two pieces about 1.5 cm x 1.5 cm, weighing approximately 0.5 grams each, one to be irradiated and the other to serve as control. The skin specimen is suspended by four small stainless steel spring hooks 2 cm behind and parallel to the lens. In operation, the specimen is mounted, the lens is replaced in position and covered with a thin aluminum shutter, the temperature is adjusted to 37° C, and the chamber is gassed with oxygen saturated with water. A mercury arc source, emission spectrum 2,400–4,400 Å,[2] is positioned directly facing the apparatus at a measured distance from the skin surface (40 cm in these experiments). At a signal the shutter is opened and irradiation is carried out for a selected interval of time after which the light source is removed. The skin specimen is promptly frozen for analysis (q.v.). The control specimen is subjected to an identical procedure but not to irradiation.

In a small series of the experiments the irradiation was by sunlight. The environmental chamber with the skin in place was moved outdoors and positioned so that the lens pointed at the sun. Conditions of temperature, humidity and oxygenation were the same as in the experiments performed with the ultraviolet light.

RESULTS AND DISCUSSION

1. Cholesterol in Skin. An interesting observation, which to the best of the authors' knowledge has not been noted previously, is that less than half of the total skin cholesterol can be obtained by direct extraction with chloroform (Table I). This is surprising in the light of the solubility characteristics of the sterol and its fatty acid esters, both of which partition quantitatively into the organic solvents in preference to water. Upon treatment of the aqueous fraction by potassium hydroxide hydrolysis, an additional cholesterol fraction is obtained.

TABLE I

Freely extractable and "bound" cholesterol in Caucasian skin

Experiment	Freely extractable cholesterol (μg/gm whole skin)	"Bound" cholesterol (μg/gm whole skin)
1	350	178
2	330	383
3	340	460
4	208	513
Mean	307	383
Total	690 μg/gm	
% of Total	44.5%	55.5%

The results strongly suggest that skin cholesterol exists in two forms, a freely extractable form which is obtained by extraction with organic solvents, and a bound form associated with the aqueous fraction, possibly with one or more of the skin proteins, which can be released by potassium hydroxide hydrolysis. One wonders if this binding might take the form of esters at position 3, analogous to fatty acid esters, but involving a hydrophilic substance which contains free carboxyl groups available for esterification.

This report covers only the results in experiments dealing with the effects of ultraviolet irradiation on the level of the freely extractable fraction of cholesterol. Studies are underway to examine the effects of irradiation on the bound fraction; the distribution of the sterol between freely extractable and bound forms will be the subject of a later report.

2. Effects of Irradiation In Vitro on the Freely Extractable Cholesterol. In a small series of experiments we found that reduction occurs in the sterol constituents of chloroform extracts of skin biopsies of patients who had been exposed for one hour to the noonday sun (6). This *in vivo* type of experiment posed the question as to the fate of the sterol following irradiation. The skin biopsies had been obtained 24 hours after exposure to sunlight. The typical biological response of the skin, solar erythema and edema, had taken place

[1] Haake, model "F".
[2] *Hanovia* Alpine, Ultraviolet Lamp.

TABLE II

Reduction in the freely extractable cholesterol content of human skin following exposure to sunlight in vitro

Experiment	Control (μg/gm)	Irradiated (μg/gm)	Exposure time (hrs)	Change (%)
1. Negro skin	41.5	13.75	2	−67
2. Negro skin	50.4	32.3	2	−36
3. Caucasian skin	77.5	30.2	1	−61

TABLE III

Reduction in the freely extractable cholesterol of Caucasian skin following exposure to ultraviolet light in vitro

Experiment	Caucasian skin control (μg/gm)	Irradiated (μg/gm)	Exposure time (min)	Change (%)
1	42.5	16.5	2	−61
2	39.5	18.7	2	−53
3	77.5	43.1	1	−45
4	107.0	20.4	2	−81
5	41.0	33.2	2	−17
6	107.0	76.4	2	−28
Mean	69.08	34.66		−47.5 ± 7.6* (S.E.)

* t, by the difference method, = 6.25; $p < .01$.

TABLE IV

Alteration in the freely extractable cholesterol content of Negro skin following exposure to ultraviolet light in vitro

Experiment	Negro skin control (μg/gm)	Irradiated (μg/gm)	Exposure time (min)	Change (%)
1	41.5	33.8	4	−19
2	50.4	41.0	4	−19
3	81.5	19.3	4	−76
4	51.5	56.6	4	+10
Mean	56.2	37.7		−26 ± 18.0*

* This difference fails to be significant because of the large error term.

and was clearly visible. What mechanism was responsible for this decrease in sterol content of the skin? Was it the result of an immediate photoreaction, the metabolism by the skin *in situ* or was it the result of a delayed biological response, an increased circulation and/or production of an inflammatory cell infiltrate which could have removed sterol constituents from the injured skin? To answer these questions, it was necessary to carry out the experiments reported here in such a way that the skin specimen was isolated from the circulatory system and free from the effects of any other physiological system.

In three experiments the isolated skin specimens mounted in the environmental chamber were exposed to the noonday Houston sun (June 1966). The results (Table II) indicate that irradiation induces a highly significant reduction in the sterol extractable from the skin in the absence of any other physiological mechanism.

The quantitative alterations in cholesterol content of Caucasian skin following exposure to ultraviolet light *in vitro* are presented in Table III. The freely extractable cholesterol content in six untreated specimens of Caucasian skin was on the average 69.08 μg/gm. The average decline amounts to 47.5% of the resting level and occurs within 2 minutes of the time that exposure to light began (1 min in one experiment).

By contrast, in three experiments in which the skin was obtained from Negro subjects, the *in vitro* effect of ultraviolet radiation was much less uniform (Table IV). In two experiments the decline amounted to 19%, in a third a much greater decrease (76%) was observed, while in a fourth, an increase (10%) was seen. The reasons for this variability are not clear, but may be related to the effects of variations in skin pigmentation and the consequent variations in light penetration.

Although it seems clearly established that exposure to sunlight or ultraviolet irradiation in Caucasian skin induces striking changes in the amount of cholesterol extractable from the skin, the mechanism for these changes remains unresolved at this time. Cholesterol is synthesized in the skin at a rate almost as high as that of liver, according to one group of investigators (7). Assuming that a reasonably constant level of the sterol is maintained, it is evident that it must be removed by one or more mechanisms at a rate equal to the rate of

synthesis. Since the analyses reported here were specific for free cholesterol, "removal" could occur by a change of species, i.e. to 7-OH or 7-keto cholesterol, esterification, et al.; that is, any change which alters the pool of free cholesterol. In the present series of experiments, "removal" could also be achieved by an increase in the ratio of the bound to freely extractable forms of the sterol.

A decrease in pool size could result also from diminution of the rate of synthesis in the presence of a maintained rate of removal. Experiments using radioactively labelled (C_{14}) cholesterol and its precursor are currently underway, toward elucidating whether a change in the rate of synthesis, or of removal, or both, is responsible for the observed phenomena.

CONCLUSION

Sunlight or ultraviolet light induces a prompt and statistically significant reduction in the amount of freely extractable cholesterol in human skin *in vitro*. It is evident that biochemical alterations in sterol metabolism occur with rapidity when sunlight or ultraviolet light strikes the skin's surface. The implication of this observation in the photobiology of human skin remains to be elucidated.

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DISCUSSION

Thank you, Dr. Urbach, for your pertinent question inquiring for an explanation as to why we observed a wide range of quantitative values of freely extractable cholesterol in human skin. In reviewing our data we discovered that there is a statistically significant seasonal variation in the freely extractable cholesterol content in Caucasian skin (Table appended in discussion). We found that the high values had been obtained in February. By contrast, in experiments performed during the months of June through September the control cholesterol values were all lower. This would suggest that reduction in the freely extractable skin cholesterol seen in our laboratory experiments occurs as a natural phenomenon on exposure to the summer sun in Houston.

TABLE APPENDED IN DISCUSSION

Apparent seasonal change in freely extractable cholesterol in Caucasian skin

Season	Number of specimens	Mean
Winter (February 1967)	4	307 ± 33.2* (S.E.)
Summer (June–September, 1967)	9	82 ± 11.4* (S.E.)
Difference, summer vs. winter	−73%	

* t, by the difference method, = 8.04; $p < .001$.

I would like to take this opportunity to thank you for bringing our attention to this interesting ancillary observation.

In answer to your second question, as to the possibility that ultraviolet irradiation may alter the cholesterol molecule, it is certainly an important consideration to explain the observed alteration found in the freely extractable cholesterol. I should think that it is entirely possible that irradiation alters the cholesterol molecule. This is one of our very real interests in the study and we hope to have additional information soon on this point.

ADDENDUM

It is worthy of comment that another group of workers has reported (in a manuscript appearing after submission of our report) a seas-

onal change in epidermal lipid content. (Nieminen, E., Leikola, E., Koljonen, M., Kiistala, U., and Mustakallio, K. K.: Quantitative Analysis of Epidermal Lipids by Thin-Layer Chromatography with Special Reference to Seasonal and Age Variation. Acta derm.-venereol. *47:* 327–338, 1967). Mono-, di-, and triglycerides, free fatty acids and saturated hydrocarbons were found by these workers to be lower in summer than in winter. The natural phenomenon is presumably related to seasonal variation in the levels of solar radiation.