BIOCHEMICAL CHANGES IN RAT EPIDERMIS INDUCED BY CHRONIC ULTRAVIOLET-LIGHT EXPOSURE.

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One of the most important functions of the epidermis is to protect underlying tissue from the environment. Only a few biologically active ultraviolet wave length of sunlight penetrates the epidermis into the dermis. However, an exposure to intense ultraviolet light produces a number of biochemical alterations. Most of the effect of ultraviolet light of the skin are harmful instead of beneficial. The exposure of ultraviolet is a major cause of cancer of the skin.

The metabolism of sulfur compound in the epidermis is a challenging subject, because of its fundamental relationship to cellular biochemical activities. This report presents data and discusses the comparatively long term effects of ultraviolet light on the sulfhydryl compounds in living rat epidermis.

MATERIAL AND METHODS

Young albino male rats were used in this experiment. After the hair was clipped from their backs, the rats were irradiated in their cage for two minutes daily at a distance of 50 cm with a hot quartz broad spectrum ultraviolet lamp (G.E., UA-3, 3.3×10^3 erg/cm²/sec.). Non irradiated animals were used as a control.

At selected intervals; 2 and 5 weeks, 3 and 7 months, 5 to 10 animals in each group were killed and their skin was excised. The epidermis was separated by Van Scott's stretch technique in a cold room to prevent degradation of tissue. Separated epidermis was immediately lyophilized and kept in a refrigerator until analysis was performed.

The epidermal sulfhydryl (non protein-bound SH and protein-bound SH), disulfide content and polarographic filtrate wave height were determined as described in a previous paper (1). Epidermal RNA and DNA content were determined by our modification of Scott's ultramicro technique using ultraviolet absorption (2). To determine an epidermal nuclei morphologically (2), skin from the back of each rat was fixed in 10 per cent formalin. Sections were cut 8 μ thick and stained by a routine hematoxylin-eosin technique. All observations and measurements of the sections were made using an ocular micrometer calibrated against a stage micrometer. The number of nuclei in the Malpighian layer was determined by counting ten locations on each sample, and the total number of nuclei was expressed as the number per mm². The

maximum diameter of twenty nuclei was expressed in microns.

To find the effect of ultraviolet exposure, the statistical technique of single variable of classification with repeated samples was applied at the level of 0.05.

RESULTS

The total SH level of normal pre-irradiation epidermis was $93 \pm 3.1 \times 10^{-2}$ mM in 100 g dried epidermis (non protein-bound SH was 38 ± 1.8 , protein-bound SH was 56 ± 2.0) and normal disulfide level was 0.27 ± 0.01 per cent. The RNA content of normal epidermis was 20.2 ± 1.4 μ g per mg of dried epidermis. The DNA content was 12.6 ± 0.70 μ g. The RNA/DNA ratio was 1.61 ± 0.12 . The results of the SH pattern and nucleic acid of epidermis following chronic exposure are summarized in Table 1, 2 and 3.

Figure 1 shows the epidermal SH pattern based on DNA content. This alteration of sulfhydryl is approximately proportional changes within epidermal cell.

TABLE 1
Epidermal Sulfhydryl Pattern

	Sulfhydry group.		Disulfide.	Filtrate wave height.
	Non protein-bound	Protein-bound		
Non-irradiated group.	38 ± 1.8	56 ± 2.0	0.27 ± 0.01	4.35 ± 0.36
2 weeks' exposure	22 ± 1.6	56 ± 1.2	0.29 ± 0.02	3.60 ± 0.36
5 weeks' exposure	18 ± 2.4	56 ± 3.3	0.25 ± 0.02	3.90 ± 0.33
3 months' exposure	26 ± 2.1	27 ± 3.4	0.21 ± 0.03	2.55 ± 0.21
7 months' exposure	29 ± 1.9	44 ± 2.6	0.36 ± 0.00	4.20 ± 0.15

SH is expressed as $\times 10^{-2}$ mM in 100 g dried epidermis.

Disulfide is expressed as per cent.

Filtrate wave height is expressed as μ A.

TABLE 2
Epidermal Nucleic Acid Pattern

	RNA	DNA	RNA/DNA
Non-irradiated group	20.2 ± 1.4	12.6 ± 0.7	1.61 ± 0.12
2 weeks' exposure	16.3 ± 0.9	11.0 ± 1.2	1.65 ± 0.14
5 weeks' exposure	16.3 ± 0.2	9.6 ± 0.9	1.89 ± 0.13
3 months' exposure	20.6 ± 0.3	14.3 ± 0.6	1.45 ± 0.03
7 months' exposure	18.5 ± 0.3	9.5 ± 0.7	1.97 ± 0.12

DNA, RNA content: μ g in 1 mg dried epidermis.

TABLE 3
CORRELATION OF EPIDERMAL NUCLEAR SIZE AND NUMBER
WITH DNA CONTENT.

	Number of nuclei	Diameter of nuclei	DNA
Unexposed group. (control)	10416 $\pm$ 772	8.0 $\pm$ 0.13	12.6 $\pm$ 0.7
7 months' exposed group. (8.5 $\times 10^7$ erg/cm ²)	9025 $\pm$ 485	7.5 $\pm$ 0.15	9.5 $\pm$ 0.7

Number of nuclei is expressed as a number per mm² of section 8 μ thick.

Diameter is expressed as μ unit.

DNA is expressed as μ g per mg dry epidermis.

Fig. 1 Epidermal Sulfhydryl
per 1 μ g DNA in 1 mg epidermis

The chronic ultraviolet exposure caused the epidermal SH pattern to decrease. A further analysis of epidermal SH revealed that chronic exposure caused the non protein-bound SH to decrease, however protein-bound SH remained relatively unaltered. But there was a significant decrease in protein-bound SH level at 3 months' exposure. The disulfide level appeared to be unaffected, but did manifest a significant increase after 7 months' exposure. The polarographic filtrate wave height showed a gradual decrease.

Chronic exposure caused the DNA content and diameter of nuclei to decrease. The number of nuclei appeared to decrease, although a significant difference was not detected at the 0.05 level. A significant difference was not detected in the RNA content.

DISCUSSION

Many investigators studied biochemical changes induced by irradiation focused their attention on bone marrow and internal organs, but neglected the epidermis which is first in line of exposure to absorb the energy of ionizing radiation, because of the tedious procedures to obtain a free hair-stubble sample from small amount of epidermal material.

Some of the most important metabolic processes occurring in the epidermis involves sulfhydryl and disulfide. Sulfhydryl groups are essential for the activity of a number of enzymes and for cellular oxidation-reduction mechanisms. They are also important in keratinization, antibody formation, cell wall permeability, cellular division, growth and carcinogenesis.

In a previous paper (3), the effect of a single exposure of ultraviolet on sulfhydryl pattern in living human skin was reported. Ultraviolet irradiation caused an immediate increase in sulfhydryl concentration, however, this was followed by a return to normal range in twenty-four hours. By three days after exposure, sulfhydryl concentration had become lower than normal. In approximately ten to fifteen days following exposure the sulfhydryl values again were normal.

Chronic exposure of skin to ultraviolet light is not considered to be only an accumulation of single exposure. The primary and secondary photo-biochemical changes are to be overlapped, and also the adaptation to ultraviolet irradiation with repetitive exposure must be occurred.

In the present experiment the repeated exposure of epidermis to ultraviolet light caused a decrease in the sulfhydryl level. The non protein-bound SH level quickly fell to below normal value, but protein-bound SH remained relatively unaltered. In the non protein-bound SH fraction, sulfosalicylic acid (0.2 M of final concentration) was used to prepare a protein-free filtrate, since glutathione does not tend to become auto-oxidized in this reagent. Glutathione is not the only SH containing component of acid extracts of tissue, but is the biologically interesting peptide. It has been known that the thiol enzyme requires the SH group for its activity. In a previous paper (4), succinic dehydrogenase activity (thiol enzyme) was attacked by a single exposure of ultraviolet light, but glutathione could restore the enzyme activity, probably by reducing the oxidized enzyme-SH group. The chronic exposure of ultraviolet light caused the non protein-bound SH to decrease. Therefore the restoring effect of glutathione on the oxidized SH caused by ultraviolet exposure will be eliminated. The

decrease of non protein-bound SH would reflect the enzyme activity in the epidermis.

Ultraviolet caused protein-bound SH remained relatively unaltered. However, it was found that the protein-bound SH decrease significantly at the 3 months' exposure. Apparently a chronic exposure of rat epidermis may cause a structural changes in protein molecule. Additional studies in progress on protein hopefully will provide answers to this and other problems.

Calcutt et. al (5) reported that an elevated level of tissue-sulfhydryl is an essential feature of tumor induction. During chemical carcinogenesis, it has been observed that elevated tissue-SH concentration was followed by subnormal levels that persisted into the resultant tumor. Elevations in SH concentration recorded in all tumor-susceptible tissue usually for several weeks to several months. Since ultraviolet light is a known carcinogen, our findings were not strictly compatible with Calcutt's hypothesis. However, our experiment was considered in the desired period of seven months. The tumor production with ultraviolet light in this dosage takes approximately three more months (total energy is above 10^8 erg/cm²). Therefore the relationship between epidermal SH level and tumor production could not be determined.

In 1961, Soffen et al. (6) reported the quantitative measurements of changes in mouse ear skin following a single dose of ultraviolet light. The incidence of mitosis figure, number of epidermal cells, thickness and nuclear diameter were discussed. All the measured aspects changed quantitatively in similar fashion following the irradiation; increasing rapidly at first, reaching a maximum and then falling gradually toward normal. Ito (7) found in mild irradiation of ultraviolet light a slight increase in RNA content of the cytoplasm when examined 3 to 5 days after irradiation. In more intensity irradiation the RNA content decrease. With more irradiation the nuclear DNA was found to gradually decrease or to coagulate. In the present paper, the number of nuclei, diameter and DNA content following chronic exposure were determined. The chronic exposure caused the diameter of nuclei and DNA content to decrease. Ultraviolet irradiation produced photochemical changes in DNA

TABLE 4
Serum Sulfhydryl Pattern

	Sulfhydryl μ M/dl.	Disulfide mg/dl.	Polarographic filtrate wave height. μ A.
Non-irradiated group	35 ± 2.3	75 ± 1.5	11.4 ± 0.8
2 weeks' exposure (4.0×10^6 erg/cm ²)	25 ± 1.1	85 ± 4.0	14.2 ± 0.6
5 weeks' exposure (1.0×10^7 erg/cm ²)	26 ± 2.7	83 ± 1.2	18.2 ± 1.0
3 months' exposure (4.1×10^7 erg/cm ²)	26 ± 1.2	76 ± 4.9	20.0 ± 1.4
7 months' exposure (8.5×10^7 erg/cm ²)	19 ± 0.3	75 ± 2.4	28.6 ± 2.7

which modified its physical chemical properties. These alterations were functionally harmful and resulted in lowered biological activity.

Based on the possibility that serum SH level reflect tissue level, the serum sulfhydryl level was determined in the same animal (Table 4). The serum SH level in the unexposed control was $35 \pm 2.3 \mu\text{M/dl.}$ and decreased significantly, while the disulfide content remained in the normal range (75–85 mg/dl). The polarographic filtrate wave height showed a gradual increase (from 11.4 μA to 28.6 μA). It is apparent that the lowering of the serum SH content is an indication of a pathological change but is not specific for cancer (8). A greater difference between normal and pathological serum has been found in the polarographic filtrate reaction (8). Cancerous and other pathological sera give a high catalytic double wave than in normal serum. In the present experiment, it was apparent that serum obtained from exposed rat showed a pathological change.

Little is known concerning the actual mechanism in malignant transformation of tissues exposed to ultraviolet light. In all instances this is extensive destruction of tissues and cellular proliferation subsequently occurs. During this process of new cell formation or during mitosis the ultraviolet radiation probably results in alterations in the nucleoproteins.

SUMMARY

The biochemical changes of sulfhydryl compound in rat epidermis were observed after chronic exposure to ultraviolet light for seven months. The total energy received was $8.5 \times 10^7 \text{ erg/cm}^2$.

Chronic exposure caused the non protein-bound SH to decrease, but protein-bound SH, and disulfide content remained relatively unaltered. However, the protein-bound SH did manifest a significant decrease after 3 months' exposure. The polarographic filtrate wave height showed a gradual decrease.

The DNA content decreased significantly, but no significant difference was found on RNA content. The diameter of nuclei showed to decrease, but a significant change in the number of nuclei was not detected.

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