THE EFFECT OF U. V. IRRADIATION ON ENZYME SYSTEMS IN THE EPIDERMIS

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THE PHYSIOLOGICAL effect of ultraviolet light irradiation upon the epidermis and dermis has been extensively studied from many points of view. Thus the effect of U. V. light on erythema production, melanin formation, long-term changes in dermal collagen, induction of cancer and histological changes in the epidermis have been, and continue to be, important areas of research (1–6).

It is interesting to note, however, that attempts to identify the initial biochemical alterations induced by U. V. light have been in general unsuccessful. Indeed most of the changes brought about by exposure to U. V. light do not manifest themselves until at least several hours after exposure. Thus, for example, erythema begins to develop two to three hours after irradiation (1) and histopathological changes, twenty-four hours later (7).

Recently, Daniels *et al.* (8) using histochemical procedures have examined the histochemical, enzymatic and cellular changes occurring in the epidermis after U. V. exposure. While histological and some histochemical changes were observed as soon as four hours after irradiation, no histologically demonstrable enzymatic changes were noted until extensive cellular damage was observed.

It was the purpose of this investigation to see if any signs of damage resulting from U. V. irradiation could be detected at fairly short periods of time after exposure. Since one might expect enzymatic changes to precede any marked histological changes, our investigation has centered around the effect of U. V. irradiation on some of the enzyme systems present in the skin. The enzymes we chose to investigate were those involved in glucose metabolism and two transaminase enzyme systems.

EXPERIMENTAL

Preparation of Tissue Homogenates—Male rats (200 ± 25 gm.) of the Sprague-Dawley strain were used in all experiments. Four days prior to

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the day of the experiment, the animals were anesthetized with ether and the hair plucked from the dorsal area. The plucking was done four days before the experiment to allow the skin flare reaction caused by the hair removal to subside. Both control and treated animals were sacrificed by cervical fracture and their pelts quickly removed. After scraping away the subcutaneous fat, the pelt was stretched over a chilled drum. The epidermis was then scraped from the dermis with a scalpel, and after weighing on a Roller-Smith precision balance, placed into a Potter-Elvehjem homogenizing tube. A small pair of scissors was next used to mince the tissue, and then a calculated amount of KCl-$KHCO_3$ homogenizing fluid (sufficient fluid to prepare a 5 per cent homogenate) was added and the contents homogenized for three to five minutes in the cold. For spectrophotometric studies, the homogenate was centrifuged at 5000 r.p.m. in a Precision Vari-Hi Speed Centricone centrifuge for fifteen minutes and the resultant supernatant liquid was used.

Injury Inducing Procedure—Four days after plucking, the animals were exposed to U. V. light (Westinghouse R. S. 275W Sun Lamp) for twenty-four minutes at a distance of $10^1/_2$ in. This exposure was sufficient to produce a reaction comparable to a first-degree burn. The animals were then sacrificed at varying time periods after exposure. Control animals were plucked four days prior to the experiment, but were not exposed to U. V. light.

Glucose Oxidation—Glucose oxidation was determined manometrically in a Warburg apparatus by measuring oxygen uptake. The complete system contained homogenate, buffer (phosphate pH 7.4), Mg^{++}, ATP, TPN and glucose with KOH in the center well.

TPN Reduction—Glucose oxidation *via* the hexose monophosphate shunt was determined spectrophotometrically by following the rate of TPN·H formation at 340 mμ. The system contained epidermal extract, buffer (phosphate pH 8.0), Mg^{++}, ATP, TPN and glucose.

Transaminase Activity—Transaminase activity was followed spectrophotometrically *via* the oxidation of DPN·H. Both glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase were studied. The complete system contained: epidermal extract, buffer (phosphate pH 7.4), aspartic acid or alanine, DPN·H, lactic dehydrogenase and alpha keto glutaric acid.

Results

Initial experiments in this investigation were aimed at studying the effect of U. V. light irradiation on glucose metabolism by rat skin. Rats were irradiated in the manner described and sacrificed at one-half hour, one hour, one and one-half hours and two hours after exposure. Epidermal homogenates were prepared from the exposed sites, and glucose metabolism

was evaluated *via* oxygen consumption and compared to homogenates prepared from nonexposed animals. The results indicated that at the one-half hour interval, the rate of O_2 consumption was often comparable or slightly elevated from that of unexposed skin. However, at all other time intervals, the O_2 consumption of the homogenates prepared from the exposed animals was markedly depressed. In some cases this depression in oxygen consumption was as great as 75 per cent.

Since previous work in our laboratories and others (9–11) indicated the presence of the hexose monophosphate shunt in glucose metabolism in skin, it was decided to investigate further the effect of U. V. irradiation on glucose oxidation by following the rate of TPN reduction in epidermal extracts prepared from exposed and nonexposed animals.

Animals were irradiated and sacrificed at intervals of one-half hour and one hour after exposure. Epidermal extracts were prepared from their skins as well as from the skin of a nonexposed animal and the early stages of glucose oxidation followed spectrophotometrically *via* TPN reduction. The results are presented in Table 1.

TABLE 1—THE EFFECT OF U. V. IRRADIATION ON GLUCOSE METABOLISM*

Time, min.	Control Nonirradiated	Irradiated Sacrificed	
		½ hr. later	1 hr. later
1	0.258	0.295	0.090
2	0.300	0.350	0.150

* Glucose metabolism measured as rate of TPN reduction (increase in O.D. at 340 mμ).

TABLE 2—GOT AND GPT TRANSAMINASE ACTIVITY OF IRRADIATED *vs.* NONIRRADIATED RAT SKIN

	GOT Activity	GPT Activity
Nonirradiated	15×10^3 units*	3×10^3 units*
Irradiated (sacrificed one hr. after irradiation)	0	0

* One unit equals a decrease in optical density of 0.001 units per minute per gram wet weight of tissue.

These results suggest an increase in glucose metabolism at the one-half hour interval and a depression at the one hour interval.

Since a marked depression in glucose oxidation was consistently noted at time intervals from one hour to four hours after U. V. irradiation, further attempts were made to find the cause for this inhibition.

Rats were irradiated in the usual manner and sacrificed one hour after exposure. Epidermal extracts were prepared and glucose oxidation studied *via* TPN reduction. As usual, a marked reduction in glucose oxidation by irradiated skin was noted (see Fig. 1). When, however, glucose-6-phosphate was used as a substrate in place of glucose, no dif-

ference was observed between the rate of glucose metabolism in exposed *vs.* nonexposed animals (Fig. 2).

These results suggested that the cause for the decreased rate of glucose metabolism in the irradiated animals' skin was related to some inactivation or inhibition of the glucose phosphorylating mechanism.

The supposition was further confirmed by showing that when exogenous hexokinase (the enzyme which converts glucose to glucose-6-phosphate) was added to the epidermal homogenate system, no difference in the rate of glucose metabolism could be observed between irradiated and nonirradiated skin (Fig. 3).

The effect of U. V. irradiation on one other enzyme system was investigated. In earlier work in this laboratory it was shown that the rat epidermis contains at least two transaminase systems. These are glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT). The activities of these two transaminase systems were compared in epidermal extracts of normal rats *vs.* epidermal extracts of U. V. irradiated animals. The results (Table 2) indicated a complete inhibition of transaminase activity in the extract from the irradiated animals.

Discussion

The time delay between exposure to U. V. irradiation, and the onset of clinical or histological evidence of damage has been explained by a number

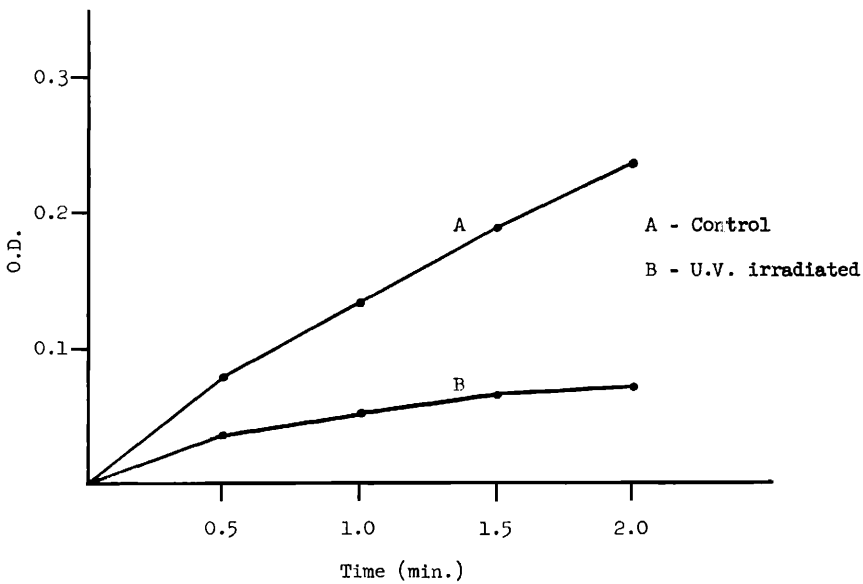

Figure 1.—Effect of U. V. irradiation on glucose metabolism by rat epidermis. Rate of TPN reduction (increase in O.D. at 340 mμ) *vs.* Time (min.).

of different theories (12). Perhaps the most widely accepted theory speculates that sunlight sets up some type of free radical reaction which in turn results in the release of some noxious agent thus setting the stage for the classical symptoms of sunburn. While the nature of these initial reactions is unknown, it appears that it takes several hours for the body to respond *via* gross clinical or histologic changes.

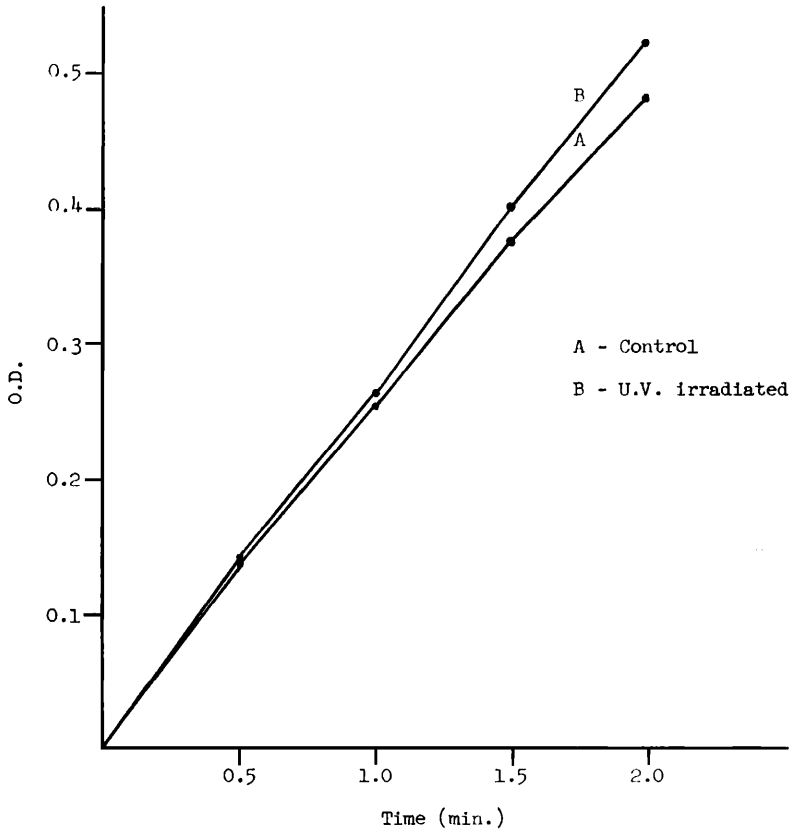

Figure 2.—Effect of U. V. irradiation on metabolism of glucose-6-phosphate by rat epidermis. Rate of TPN reduction (increase in O.D. at 340 mμ) *vs.* Time (min.).

It is only reasonable to expect that during this time lapse, enzymatic changes are occurring which initiate some of the cellular responses seen. While the work reported in this paper does not clarify the mechanism of the initial damaging effects of U. V. irradiation, it does point out some of the early enzymatic changes occurring.

The damaging effects of U. V. irradiation on many enzyme systems is well known (13). Usually these effects have been studied with *in vitro* systems

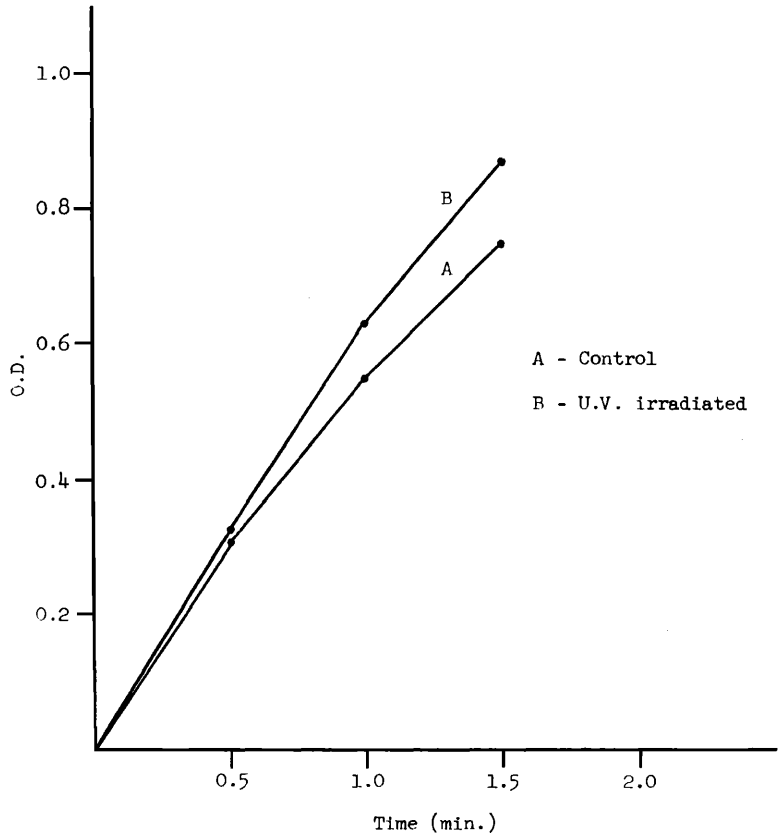

Figure 3.—Effect of U. V. irradiation on epidermal metabolism of glucose with added exogenous hexokinase. Rate of TPN reduction (increase in O.D. at 340 mμ) *vs.* Time (min.).

and are often related to denaturation of the enzyme protein itself or inactivation of some co-factor needed for enzyme activity. Thus, for example, it has been reported that U. V. light can inactivate transaminase systems *in vitro* (14). Exposure of the under side of mouse skin to U. V. irradiation has been reported to cause a decrease in succinic dehydrogenase activity as estimated histologically (15). Exposure of skin or skin extracts to U. V. causes an increase in phosphorylase activity (16, 17).

In our studies, the most marked effects observed as a result of U. V. irradiation of rat skin *in vivo* were a decrease in the rate of glucose oxidation and a complete inhibition of two transaminase systems present in the epidermis. Both of these enzymatic effects manifested themselves within one hour after irradiation and continued up to four hours after irradiation. At time intervals of one-half hour after irradiation, this inhibition of glucose oxidation was not noted, and indeed in several experiments a slight

increase in rate was noted. Measurements of glucose oxidation and transaminase activity were not measured at periods of time longer than four hours after irradiation.

While the mechanism for the inactivation of transaminase activity is unknown, it appears that the mechanism by which the decrease in glucose oxidation occurs is related to some malfunction in the glucose phosphorylating system. Thus it has been shown that in the presence of glucose-6-phosphate as substrate or with the addition of exogenous hexokinase, no difference can be observed between the rate of glucose oxidation of extracts prepared from normal animal skin and those from irradiated animal skin.

It is interesting to note that one of the early histological changes seen in irradiated skin is a build-up in glycogen (8). This glycogen build-up has also been observed after X-irradiation, and it is speculated to result from an enzymatic disbalance between glucose and glycogen metabolism (18). Perhaps the enzymatic changes in glucose metabolism reported herein are related to this imbalance.

Summary

The effect of U. V. light on enzyme systems present in the epidermis of the rat has been investigated. Exposure of rats to U. V. light has resulted in a decreased rate of glucose metabolism in homogenates prepared from the epidermis of the irradiated animals. This marked decrease in glucose oxidation can be observed within one hour after irradiation. Inactivation of the glucose phosphorylating mechanism is believed to be the cause for this decreased glucose metabolism. Complete inhibition of two transaminase enzyme systems was also noted in the epidermis of rats, one hour after U. V. irradiation.

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