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EFFECTS OF X-IRRADIATION ON THE SKIN

I. Histochemistry of acute changes in the epithelial structures*

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Interest in the biologic effects of irradiation and methods of their detection has become of importance during recent decades because of increasing use of and exposure to radioactive substances. The superficial anatomical position of the skin makes this organ accessible to accidental as well as intentional irradiation from a variety of sources. Furthermore, the skin is readily available for observation and study and thus lends itself well to the evaluation of irradiation effects.

There are many gaps in our knowledge of the mechanisms of tissue response to ionizing radiation. Consequently the study of cellular and metabolic changes may help in the understanding of the biological effects of irradiation. The present study is concerned with the histological and histochemical changes induced in the epithelial structures of the skin following a single dose of X-irradiation.

Materials and Methods

Guinea pigs, about 500 gms each were shaved with electric clippers. Each animal received a single dose of 4000 r (HVL 1.18 mm Al) delivered by a skin contact X-ray unit[1] at 50 KV, 2 ma, 4.2 cm FSD and 1 mm Al filter. Similar non-irradiated guinea pigs served as controls.

Biopsy specimens were removed from irradiated skin sites and from comparable areas of the skin of control animals at 3-hourly intervals for the first 24 hours and then every 24 hours for 3 to 4 weeks. The following procedures were performed on each biopsy specimen:

I. *Paraffin Sections*

a. Hematoxylin and Eosin (H.E.).
b. Toluidine Blue 0.5 M pH 4.0 (T.B.).
c. Periodic Acid Schiff—Alcian Blue with and without pretreatment of the tissue sections with diastase (PAS-AB) (16).
d. Barnett-Seligman's Dihydroxy-Dinaphthyl Disulfide (DDD) method for Sulfhydryl (—SH) and Disulfide (S=S) groups utilizing blockage of $^-$SH groups with iodoacetate and then reduction of S=S to $^-$SH by potassium cyanide (1).
e. Feulgen Reaction for Desoxyribonucleic Acid (DNA) before and after treatment of tissue sections with Desoxyribonuclease (DNase) in veronal buffer pH 7.5 (16).
f. Methyl green—Pyronin Reaction for Ribonucleic Acid (RNA) before and after treatment of tissue sections with Ribonuclease (RNase) (16).
g. Acridine Orange (A.O.) before and after treatment of tissue sections with DNase and RNase (17).

[1] Phillips.

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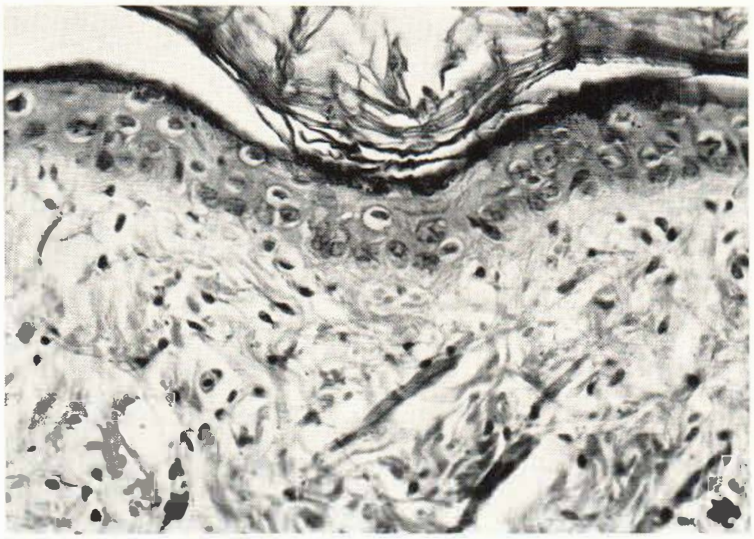


Fig. 1. Guinea pig skin. Epidermal changes 3 days after irradiation. HE ×280.

h. Thioflavin (T.T.) before and after treatment of tissue sections with DNase and RNase (8).

II. *Reactions for Enzymatic Activities*

a. Alkaline phosphatase—using sodium alpha naphthyl phosphate and Fast Blue Salt B at pH 10.5–11.0 (9).
b. Acid phosphatase—using sodium alpha naphthyl phosphate and Fast Blue Salt B at pH 5.0 (16).
c. Beta Glucuronidase—using ferric hydroxyquinoline method (16).
d. Succinic Dehydrogenase using sodium succinate and Nitro BT at pH 6.7 (16).
e. Glucose-6-phosphatase using a substrate of potassium glucose-6-phosphate and lead nitrate at pH 6.7 (16).
f. NADP[2]-specific Glutamate Dehydrogenase (GluDH/NADP) (6).
g. NAD[2]-specific Glutamate Dehydrogenase (GluDH/NAD) (6).
h. Malate Dehydrogenase (MHD) (6).
i. NADP-specific Malate Enzyme (ME) (6).
j. Glycerine-1-phosphate Dehydrogenase (GDH/NAD) (6).
k. Lactate Dehydrogenase (LHD) (6).
l. NADP-specific Isocitrate Dehydrogenase (ICDH/NADP) (6).
m. NAD-specific Isocitrate Dehydrogenase (ICDH/NAD) (6).
n. Glucose-6-phosphate Dehydrogenase (G6PDH) (6).

Appropriate controls were studied in all the above histochemical reactions.

Results

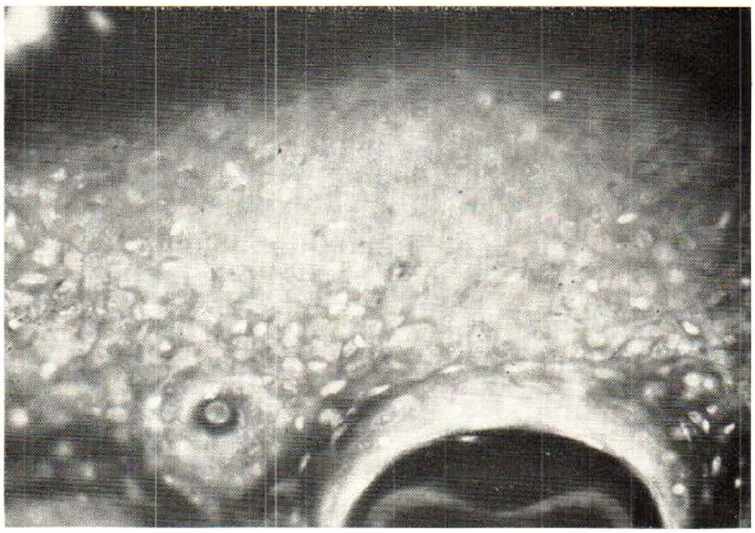
A. *Changes during the first post-irradiation week*

a. Epidermis

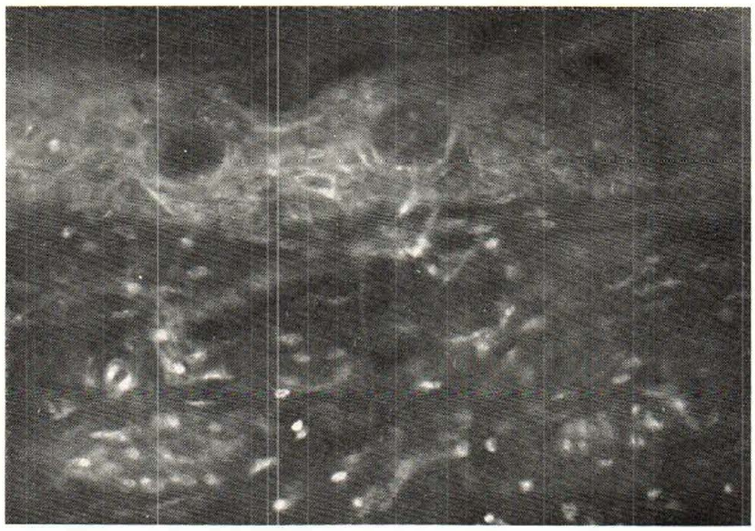
Definite changes in the epidermis had become obvious by the 3rd to 4th post-irradiation day. The basal cell layer showed progressive degeneration and the prickle cell layer displayed abnormal mitoses, pyknotic nuclei, micronuclei, dispersed chromatin granules and swollen cytoplasm. The granular cell layer became more prominent. These abnormalities are readily demonstrable with the H. & E. stain (Fig. 1).

There was a decreased reaction for the sulfhydryl (⁻SH) groups, but no significant change in the disulfide linkages (S=S). The epidermal nuclei showed a decreased fluorescence with Thioflavin T (TT) (fig. 2). With Acridine Orange (AO) the nuclei

[2] NAD and NADP stand for Nicotinomide-Adenine-Dinucleotide and Nicotinomide-Adenine-Dinucleotide Phosphate respectively.



2 a.

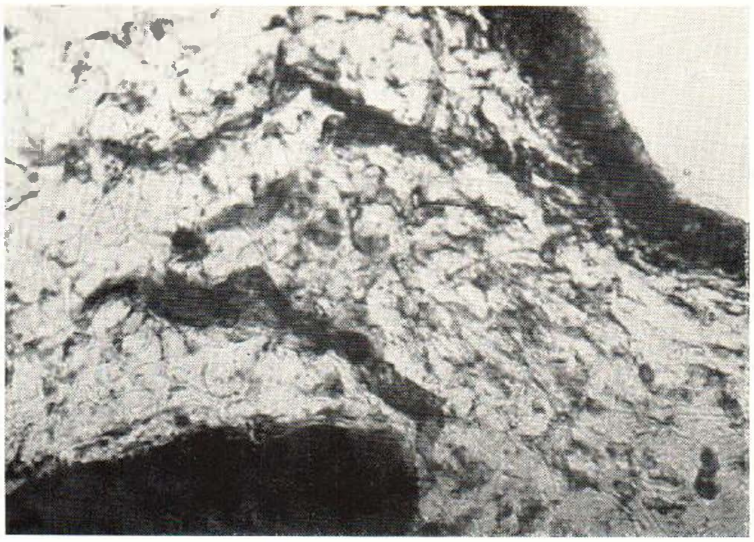


2 b.

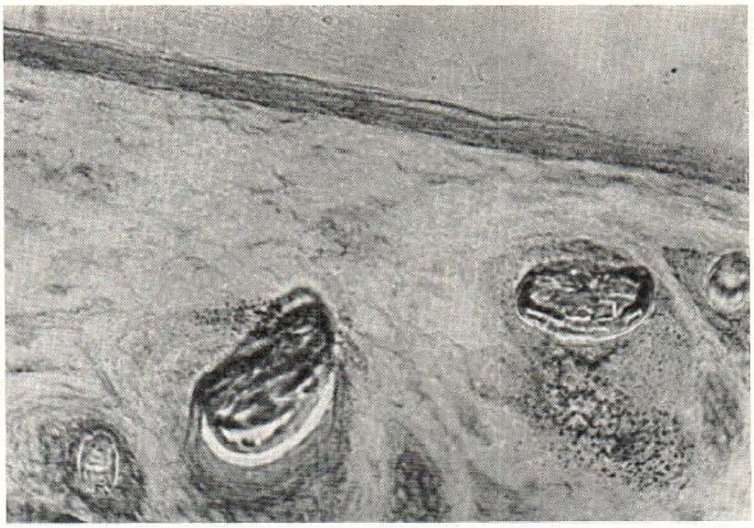
Fig. 2. Guinea pig skin. a) unirradiated, showing bright fluorescence of nuclei in the epidermis, b) decreased fluorescence of epidermal nuclei 6 days after irradiation. T.T. ×450.

appeared as bizarre-shaped empty spaces. The nuclear desoxyribonucleic acid (DNA) gave a decreased reaction with the Feulgen technique. Around the 3rd post-irradiation day desoxyribonuclease (DNase) failed to digest completely the nuclear material. The cytoplasmic ribonucleic acid (RNA) showed a transient decreased reactivity with methyl green-Pyronin stain. Ribonuclease (RNase) however, retained its ability to digest the RNA material.

Forty-eight hours following irradition there was a sharp decrease in the Beta-glucuronidase activity of the epidermal cells. This reactivity, however, slowly returned to the pre-irradiation intensity by the end of the first week. GluDH/NADP, GluDH/NAD, MDH, ME, GDH/NAD, LDH and ICDH/NAD showed a markedly reduced activity in the epithelial structures as of the fourth post-irradiation day (Fig. 6).



3 a.



3 b.

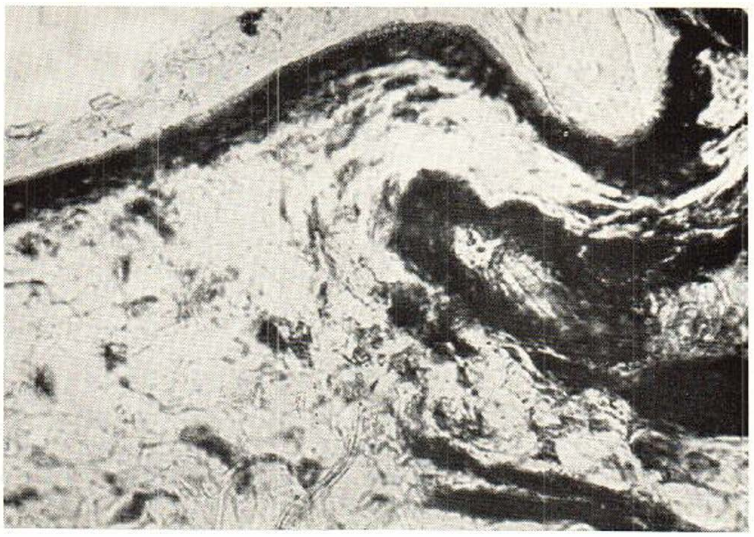
Fig. 3. Guinea pig skin. a) unirradiated, b) 7 days after irradiation. GDH/NAD ×280.

b. Stratum Corneum

The changes in the stratum corneum during the first post-irradiation week were seen to advantage with the AO stain. Normally this layer fluoresces with a bright yellow orange color. By the 2nd day after irradiation a band of bluish fluorescence appeared in the lower part of the stratum corneum. By the 3rd to the 6th day the entire stratum corneum fluoresced with a light blue color.

c. Appendages

The effects of irradiation on the hair follicles were comparable to those described for the epidermis. The cells of the matrix had degenerated and accumulated PAS-positive, diastase-labile material. The bulbs became shrunken and the pigment granules became coarse. The PAS-positive vitreous membrane appeared thickened and irregular. The Feulgen reactivity of the cells of the matrix was decreased and there was a mild diminution in the stainability with Methyl-green-Pyronin. Beta-glucuronidase reaction showed a sharp decrease. The guinea pig sebaceous glands manifested an



4 a.

4 b.

Fig. 4. Guinea pig skin. a) unirradiated, b) 7 days after irradiation. LDH ×280.

initial increase in the activity of alkaline phosphatase followed by a decrease and eventual disappearance as the glands degenerated.

2. *Changes during the sceond post-irradiation week*

There was a gradual progression of the alterations in the epidermis. Marked atrophy and hyperkeratosis were observed. Subepidermal vesicles may form. In the hairs the bulb was reduced to a thin cord of cells and the vitreous membrane was thicker and more coiled. Sebaceous glands were hardly seen.

3. *Changes after the second post-irradiation week*

By the 3rd week, there was superficial ulceration with epidermal hypertrophy at the edges of the ulcers. There was strong PAS reaction at the ulcer base. Repair of the ulceration started from the hyperplastic epidermal borders and was complete by the

3rd to the 4th week. There was no regrowth of hair or of sebaceous glands with the X-ray dosage employed.

There were no detectable changes in the acid phosphatase, succinic dehydrogenase, glucose-6-phosphatase, G6PDH and ICDH/NADP reactions in the guinea pig skin during the first three weeks after irradiation.

Discussion

The results have been presented in relation to defined periods of time following irradiation for the sake of convenience. Undoubtedly no sharp delineation exists, since the effects are smoothly progressive throughout the period of observation.

Several workers have reported on the gross and histologic changes in the skin following irradiation (5, 14, 15, 19, 10, 12). Our findings are in agreement with these workers and confirm the two-phase ulceration in the guinea pig skin described by Mellet *et al.* (12). It is evident that the epithelial structures of the skin are quite susceptible to the effects of ionizing irradiation and early gross changes in the cells become manifest. The more subtle changes, as detected by histochemical means, comprise changes in the DNA-RNA structures in the epithelial cells as well as in the enzymatic processes therein.

The change in the DNA and RNA of the epithelial cells following irradiation is evidenced by the decreased reactivity with the Feulgen and Methyl-green Pyronin stains respectively; as well as the marked change in the fluorescence of the epithelial cells with both T.T. and A.O. The inability of DNase to act on the nuclear DNA following irradiation may signify a major change in the DNA molecular structure.

Even though hyperkeratosis ensues as a result of the irradiation, the keratin formed seems to be different from the normal keratin as evidenced by the altered pattern of fluorescence with A.O. The early marked decrease in sulfhydryl groups (^{-}SH) in the epithelial cells may be due to either a masking effect on these radicals or to an oxidation from ^{-}SH to ^{-}SS bonds. Barron *et al.* (2), studying the *in vitro* effects of X-rays on various enzymes, postulate a possible oxidation of ^{-}SH groups of proteins by products of water irradiation. Daniels and coworkers (4) studying the effects of ultraviolet irradiation on human skin found that degenerated cells show an increased reaction for $S=S$ and ^{-}SH groups.

Few investigators have reported the effects of irradiation on enzyme systems. Barron *et al.* (2) have studied the effects *in vitro*. Mollura and coworkers (13) found no demonstrable change in the activity of alkaline phosphatase in the duodenum, spleen, kidney, liver and mammary glands of the mouse following total body irradiation. Rich *et al.* (18) on the other hand, showed a decrease in the activity of alkaline phosphatase, acid phosphatase and Glucose-6-phosphatase of the rat kidney following abdominal X-irradiation, and Wilkins *et al.* (20) demonstrated decreased alkaline phosphatase activity in bone following irradiation. Woodard and Spiers (21) also showed decreased alkaline phosphatase activity in mouse bone following irradiation and they concluded that the decreased activity was due to a depression in the production of the enzyme. Karcher (7) demonstrated in rabbit skin a decrease in the activity of succinic dehydrogenase, DPN diaphorase and non-specific estarases. Bruni and Mazza (3, 11), on the other hand, showed increased activity of alkaline phosphatase, and alpha-naphthyl and Tween esterases in guinea pig skin following irradiation.

With these varied reports, the interpretation of the changes in the different enzyme systems following irradiation remains obscure. In addition, it is not clear whether the ionizing irradiation affects the enzymes directly or indirectly by inhibition of their formation or by non-specific blockage of the enzyme systems by the damaged cells. One would also keep in mind the possibility of tissue and species differences as far as the effects on any one enzyme. The varied responses in different enzymes may signify derangements in specific metabolic pathways in the irradiated cells.

SUMMARY

The acute changes in the epithelial structures of the guinea pig skin following a single dose of X-rays are demonstrable with routine histological techniques as well as histochemical methods. The epidermal basal cell layer and the matrix of the hair follicles show the earliest degenerative changes. Both DNA and RNA structures in the epithelial cells show disturbances, but the changes are more apparent with DNA. Even though there is an early effect on the quantity of reactive ^{-}SH groups in the epidermis, the ultimate effect on the epidermal function is the formation of keratin that seems to differ chemically from the normal.

Different enzyme systems respond differently to irradiation. In particular, the activities of beta-glucuronidase and most of the dehydrogenases in epithelial cells show an early marked decrease. Alkaline phosphatase activity in the sebaceous gland shows an initial increase and then decrease.

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