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Induced Biosynthesis of Cutaneous Prostaglandins by Ionizing Irradiation[1] (41362)

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Abstract. This study has demonstrated that exposure of white domestic pig skin to a single treatment of X-ray irradiation (300–1000 rad) results in a rapid release of epidermal arachidonic acid followed by its transformation via the cyclooxygenase pathway into prostaglandin E_2 (PGE_2). Beyond 24 hr the epidermal PGE_2 decreased steadily. The time-dependent biosynthesis of epidermal PGE_2 can be blocked by the immediate topical application of 2.5% indomethacin in ethanol to the irradiated skin area. In order to seek an explanation for the progressive decrease of epidermal PGE_2 beyond 24 hr we assayed for the activity of the NADPH-dependent PGE_2-9-ketoreductase (an enzyme which catalyzes the transformation of PGE_2 into $PGF_{2\alpha}$) in the 105,000*g* supernatant fractions prepared from the irradiated and nonirradiated epidermal specimens. Our data revealed that the activity of this interconverting enzyme increased steadily beyond 24 hr. These results indicate that PGE_2 formed as a result of exposure of skin to X rays is metabolized into $PGF_{2\alpha}$. The biological significance of this interconversion and the role of $PGF_{2\alpha}$ in X-ray-induced injury to the skin remains to be determined.

Prostaglandins (PGs)[3] and related metabolites constitute a family of 20-carbon fatty acids whose initial synthesis involves the release of precursor arachidonic acid (AA) from membrane phosphoglycerides by tissue phospholipase A_2 and its subsequent oxygenation either by a cyclooxygenase into potent cyclized endoperoxides (PGG_2 and PGH_2) which can be enzymatically transformed into prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2), prostacyclin (PGI_2), and thromboxane A_2 (TxA_2) or by a series of lipoxygenases into a variety of noncyclized hydroperoxy and hydroxy fatty acids (1).

The biosynthesis of prostaglandins from arachidonic acid by skin preparations is now well documented (2–5). When the skin was exposed to ultraviolet (uv) irradiation, prostaglandin-like compounds were detected in increased concentrations in areas of delayed inflammation (6, 7). Topical application of indomethacin to the skin immediately after exposure to uv irradiation resulted in suppression of erythema (8). These reports suggest at least in part, that a relationship exists between uv irradiation, formation of prostaglandins, and the development of erythema in the skin.

Several deleterious effects of ionizing radiation on skin have been reported (9–11). Of particular interest is the initiation of erythema and ulceration in pig skin at a dose range of 1240 to 500 rad (12). Since prostaglandin E_2 (PGE_2), a metabolite of arachidonic acid is known to induce erythema in man (13), we decided to test the effects of x-ray irradiation on the release of cutaneous arachidonic acid and prostaglandin E_2 formation in the skin of domestic pigs, reportedly with gross and histological resemblance to man.

Materials and Methods. Three 8- to 12-week-old domestic pigs weighing approximately 8–20 kg were used in these studies. [^{3}H]PGE_2 (specific activity, 125Ci/mmole) was purchased from New England Nuclear, Boston, Massachusetts. Radiopurity was ascertained by TLC on silica

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[3] Abbreviations used: PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TLC, thin-layer chromatography; AL, aluminum.

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gel G in solvent system ethyl acetate:acetic acid (99:1, v/v). Approximately 80% of the chromatographed ^3H was found to have similar chromatographic mobility as authentic PGE_2. Nonradioactive PGE_2 and $PGF_{2\alpha}$ were gifts from Dr. U. F. Axen of Upjohn Company, Kalamazoo, Michigan. Reagents were of analytical grade, and solvents were redistilled before using.

X-ray irradiation of pig skin. White domestic pigs varying from 8 to 12 weeks of age and weighing 8 to 20 kg were anesthetized with sodium pentobarbital (25 mg/kg), given intravenously. Epidermis from these white pigs contained no melanin. Posterior flanks were shaved, and an area 5 × 10 cm was marked off on both sides of the pig. The superficial irradiation used had the following characteristics: 100 kV, 20 mA, half-value layer in aluminum (AL) of 1.0 mm, skin target distance of 30 cm, and delivered through a 5-cm cone. The exposure rate was measured with each treatment and was 317 R/min. Quantities of 1000 or 3000 R were given in single treatments of the 5 × 10-cm areas on the posterior flanks of pigs. For evaluation of dose–response studies, quantities of 300, 600, 1000, 1500, and 2000 R of irradiation were given to 5 × 10-cm areas within the 5 × 10-cm sites on both posterior flanks. Lead shielding was used to protect previously exposed areas.

Preparation of tissue specimens and analysis for PGE_2. To test whether the cyclooxygenase pathway is enhanced after exposure of skin to X-ray irradiation, one exposed site was treated with a single topical application of 2.5% indomethacin in ethyl alcohol (vehicle) while the opposite site was treated with the vehicle alone immediately following irradiation.

Skin specimens were removed immediately from pigs with a Castroviejo keratome set at 0.3–0.5 mm. These specimens contained approximately 75% epidermis with 25% dermal contamination. The specimens were immediately placed in vials containing a mixture of chloroform:methanol (2:1) and stored at −70° until processed for prostaglandin E_2. The irradiated pigs were housed in separate cages and treatment sites were examined daily. When needed, the epidermal strips were homogenized in the same solvent mixture with a Polytron homogenizer Model PT 20 from Kinematica, Lucerne, Switzerland. Aliquots were taken for protein assay by the method of Lowry *et al.* (14), with bovine serum used as standard. The extract was evaporated in a rotary evaporator. The residue was redissolved in chloroform:methanol (1:1), applied to silica gel-coated thin-layer plates (TLC), and developed in the solvent system chloroform:methanol:acetic acid:water (90:8:1:0:8). This system separates total lipids into several fractions including phospholipids, prostaglandins (PGs), and neutral lipids (triglycerides and sterol esters).

The fraction which contained the mixture of prostaglandins was scraped and eluted as described previously (15). After drying under N_2 the residue was redissolved in the same solvent mixture and developed twice in the solvent system ethyl acetate:acetic acid (99:1). The portion of the TLC gel which contained PGE_2 was eluted and transformed into PGB_2 with ethanolic NaOH as reported previously (16). PGE_2 in skin specimens was estimated from a standard curve of authentic PGE_2 which had been similarly transformed into PGB_2.

Estimation of arachidonic acid in phospholipids and neutral lipid fractions. The phospholipid and neutral lipid fractions were also scraped and eluted separately from the TLC plates, dried under N_2 and subjected to transesterification by refluxing under N_2 with 30 ml of a 5% solution of HCl in methanol for 60 min as described previously (17). The resulting fatty acid methyl esters were analyzed on an F and M Model 402 gas chromatograph equipped with a flame ionization detector (FID) system as reported previously (17). Identification of arachidonic acid was by comparison with the methyl ester of an authentic arachidonic acid obtained from the Hormel Institute. Quantitation was by triangulation of the chromatographic peak and comparison with known peak of the reference arachidonic acid.

Subcellular preparation of skin specimens and conversion of [^3H]PGE_2 into

[^3H]$PGF_{2\alpha}$. Skin specimens taken at various times after irradiation were homogenized with a Polytron homogenizer in 5 vol of ice-cold 0.1 *M* phosphate buffer, pH 7.4, containing 4 m*M* $MgCl_2$ and 0.1 m*M* dithiothreitol. Preparation of the 105,000*g* supernatant fraction by differential centrifugation was as described previously (18). The high-speed supernatant fraction was partially purified and concentrated by filtering through Sephadex G-25 (coarse) in order to remove endogenous pyridine nucleotides and other small molecular weight substances. The amount of protein in the concentrated supernatant fraction was determined by the method of Lowry *et al.* (14), with bovine albumin used as standard.

Enzymatic conversion of [^3H]PGE_2 *into* [^3H]$PGF_{2\alpha}$. The enzymatic reduction of [^{3}H]PGE_2 into [^{3}H]$PGF_{2\alpha}$ was determined in a final volume of 1 ml of 0.1 *M* phosphate buffer, pH 7.4, containing $MgCl_2$ (4 m*M*), NADPH (2.0 m*M*), dithiothreitol (0.1 m*M*), partially purified 105,000*g* supernatant fractions (2–4 mg protein) from irradiated skin areas and [^{3}H]PGE_2 (0.1 μCi, 1 pmole). The mixture was incubated aerobically with shaking in a Dubnoff incubator at 37° for 30 min. Control experiments contained [^{3}H]PGE_2 and 105,000*g* supernatant fractions from skin removed from the nonirradiated side. Fractionation and assay of [^{3}H]$PGE_{2\alpha}$ were as reported previously (18). The activity of the PGE-9-ketoreductase was calculated as percentage of radioactivity from incubated [^{3}H]PGE_2 that cochromatographed with authentic $PGF_{2\alpha}$.

Results. In our preliminary experiments, we tested whether or not the exposure of the domestic pig skin to X-ray irradiation would result in the release of epidermal arachidonic acid (a substrate for PG biosynthesis). Figure 1 shows a time course of the release of this substrate from the right and left dorsal areas of the pig skin after exposure to 1000 rad of X irradiation. The slight decrease of the endogenous fatty acid during the first hour after irradiation is unclear. Nonetheless, after one hour, the release of free arachidonic acid from both dorsal skin areas increased and remained elevated for 24 hr. These results stimulated us to determine whether or not the release of arachidonic acid can be correlated to the formation of prostaglandins, particularly the E type (PGE_2).

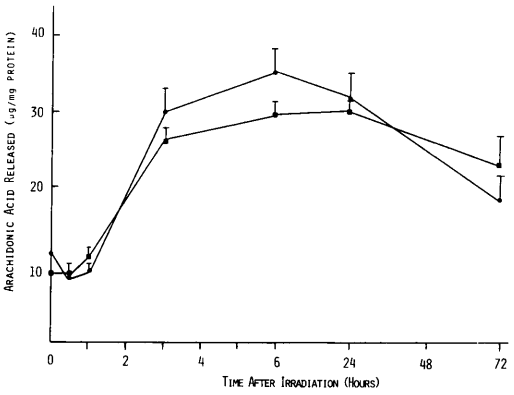

FIG. 1. Time course of X-ray-induced release of arachidonic acid by pig epidermis. Right and left dorsal areas of pig skin (5 × 10 cm) were exposed to a single treatment of X-ray irradiation (1000 rad). Removal of epidermal strips and identification of arachidonic acid by gas–liquid chromatography are as described under Methods. Each value point represents the mean ± SEM of duplicate determinations from three experiments. ●, Pig right dorsal side; ■, pig left dorsal side.

In one series of experiments, we tested whether or not the release of endogenous arachidonic acid correlated with an increase in endogenous epidermal prostaglandins. In another, we tested the effect of topical indomethacin (2.5% in vehicle) applied immediately after irradiation on the release of the endogenous epidermal PGE_2. Figure 2 (A and B) shows a time course of the formation of epidermal PGE_2 after exposure of skin to 1000 rad of X-ray irradiation. Endogenous PGE_2 corresponded with the release of arachidonic acid as shown in Fig. 1. Topical application of indomethacin to the skin immediately after exposure to X-ray irradiation resulted in a decrease of the epidermal PGE_2. These results suggest that the transformation of released arachidonic acid into PGE_2 via the cyclooxygenase pathway is inhibited by the applied indomethacin (a cyclooxygenase inhibitor).

The effects of increasing doses of X-ray irradiation and the formation of epidermal PGE_2 after 24-hr were also examined. Results are shown in Fig. 3. These data

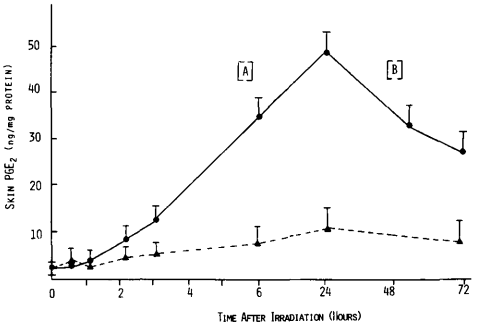

FIG. 2. Time course of X-ray-induced formation of PGE_2 and its inhibition by indomethacin in pig epidermis. Posterior areas (5 × 10 cm) of the pig skin were exposed to one single treatment with X-ray irradiation (1000 rad). Selected exposed areas were treated topically with 2.5% indomethacin in ethanol. Removal of epidermal strips after various time intervals and identification of PGE_2 are as described under Methods. [A] portion of the curve represents increased formation of PGE_2 during the first 24 hr, while [B] represents decrease of the PGE_2 beyond 24 hr. Each value point represents the mean ± SEM of duplicate determinations from three experiments. ●, X-ray irradiation + vehicle; ▲, X-ray irradiation + vehicle + indomethacin.

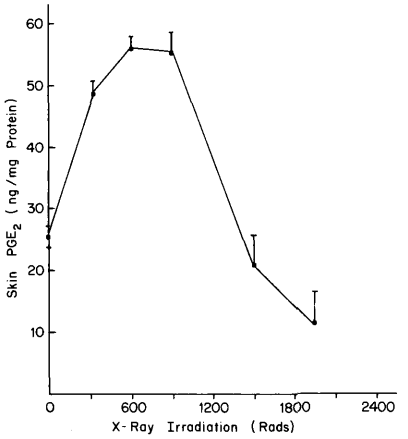

FIG. 3. Effect of exposure of pig skin to increasing dose of X-ray irradiation. Selected dorsal areas of pig skin (5 × 10 cm) were exposed respectively to varying doses of a single treatment with X-ray irradiation. Epidermal strips were removed after 24 hr from irradiated areas and subjected immediately to extraction, chromatography, and estimation of PGE_2 as described under Methods. The data are averages of duplicate experiments. The bars represent ranges.

showed that the formation of PGE_2 was induced when skin is exposed to a single dose of 300 to 1000 rad of X-ray irradiation. Above the 1000-rad single dose the formation of PGE_2 was decreased.

Conversion of [^3H]PGE_2 into [^3H]$PGE_{2\alpha}$ by 105,000g supernatant fractions from skin exposed to X-ray irradiation. The decrease of epidermal PGE_2 with time after X-ray irradiation as shown in Fig. 2 was puzzling, but it did stimulate us to ascertain whether or not PGE_2 was being metabolized, particularly into $PGF_{2\alpha}$ by the NADPH-dependent PGE_2-9-ketoreductase (an enzyme known to catalyze the transformation of PGE_2 in $PGF_{2\alpha}$). The activity of this enzyme has been reported to increase after exposure of skin to ultraviolet irradiation (19). Incubations of high-speed supernatant preparations from non- and irradiated skin with [^{3}H]PGE_2 and NADPH resulted in the formation of [^{3}H]$PGF_{2\alpha}$. The data in Fig. 4 show that the activity of the PGE_2-9-ketoreductase in skin specimens removed after various time intervals after one dose of X-ray irradiation increased moderately the first 6 hr, then continues steadily up to 48 hr. This elevation in activity correlates with the diminished level of endogenous PGE_2 after 24 hr (Fig. 2).

When the PGE_2 formed and the activity of the PGE_2-9-ketoreductase in the pig epidermis after irradiation are compared (Fig. 5), a relationship between the two biochemical events seems to emerge. A rapid synthesis of PGE_2 was evident within the first hour, continued up to 24 hr, and then decreased. On the other hand, activity of the PGE_2-9-ketoreductase was evident after one hour, and increased activity continued even at 48 hr when PGE_2 was decreasing.

Discussion. This study has demonstrated that exposure of domestic pig skin to X-ray irradiation (300–1000 rad) results in a rapid release of epidermal arachidonic acid. Although the mechanism of this release cannot be delineated from the present studies, it is nonetheless likely that the ionizing radiation of X rays may have initiated the hydrolysis of arachidonic acid from epi-

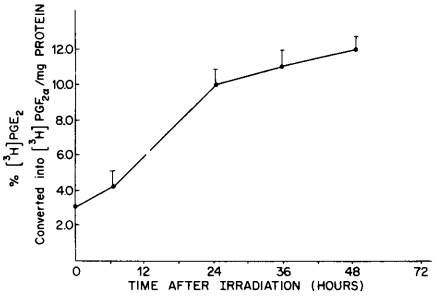

FIG. 4. Time course of the conversion of [^{3}H]PGE_2 into [^{3}H]$PGF_{2\alpha}$ by X-ray irradiated pig skin. Partially purified high-speed 105,000*g* supernatant fractions were prepared from nonirradiated and irradiated skin (100 rad) taken after various hours. One milliliter of each high-speed preparation (4 mg protein) in 0.1 *M* phosphate buffer (pH 7.4) and containing $MgCl_2$ (4 m*M*), dithiothreitol (0.1 m*M*), and NADPH (2 mM) was incubated with [^{3}H]PGE_2 (0.1 μCi, 1 pmole) at 37° for 30 min. Identification of radioactive $PGF_{2\alpha}$ was by TLC in silica gel coated plates described under Methods. Each value represents the mean ± SEM of triplicate determinations from two experiments.

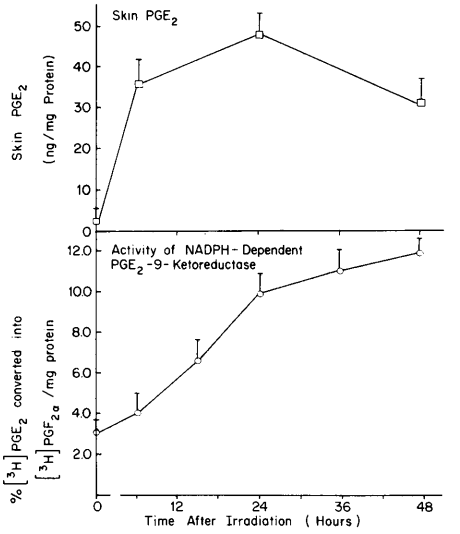

FIG. 5. Relationship of X-ray-induced skin PGE_2 formation and PGE-9-ketoreductase activity. Experimental procedures for respective determinations are as described in legends for Figs. 2 and 3.

dermal membrane phospholipids via the activation of the skin phospholipase A. Activation of the activity of this enzyme by ultraviolet irradiation has previously been reported (19). Furthermore, ionizing radiations such as that generated by X rays are known to elaborate free radicals in tissues (20), and it is likely that they do contribute to the early initiation step of a free radical fatty acid formation from arachidonic acid released in the epidermis which then serves as a reactive intermediate for the attachment of O_2. This free radical–oxygen complex is then transformed enzymatically by the cyclooxygenase into the cyclic endoperoxide and then ultimately converted into the stable prostaglandins products. The formation of the E and F prostaglandins (Figs. 2 and 4) after X-ray irradiation is consistent with the above suggestion of an X-ray-induced arachidonic acid release in the skin, and its eventual transformation into prostaglandins.

Of particular interest in this study is the increased activity of the NADPH-dependent PGE_2-9-ketoreductase with increasing time after X-ray irradiation while the endogenous level of PGE_2 which had increased in the first 24 hr decreased with elapsed time. It is, therefore, likely that the increased activity of PGE_2-9-ketoreductase may explain at least in part the decrease in endogenous PGE_2 as shown in Fig. 2. One other pathway of PGE_2 inactivation in the epidermis is via its metabolism by the 15-hydroxy-PGE_2-dehydrogenase (4). The activity of this enzyme was not determined in these studies. On the other hand, homogenates from spleen of mice which have previously been exposed to X rays were reported to inhibit the activity of the 15-hydroxy-prostaglandin E_1-dehydrogenase (21).

The mechanism of epidermal PGE_2-9-ketoreductase activation by X rays is presently unclear. The biological significance of the increased transformation of the proinflammatory PGE_2 into $PGF_{2\alpha}$ several hours after irradiation can only at this time be speculative, but it is not unreasonable to suggest that the increased formation of $PGF_{2\alpha}$ may, at least in part, be a response of the irradiated skin to inhibit the proinflammatory effects of PGE_2 which has been generated by the X-ray irradiation. This view is consistent with reports by Crunkhorn *et al.* (22), who demonstrated that the

inflammatory response to PGE_2 in guinea pig skin could be suppressed by injecting $PGF_{2\alpha}$ before the onset of the PGE_2. Furthermore, Willoughby (23) reported that $PGF_{2\alpha}$ suppressed the inflammatory reaction induced by tissue injury by a variety of permeability factors, including histamine and serotonin. Velo *et al.* (24), in a time–sequence study of the distribution of PGE_2 and $PGF_{2\alpha}$ in the inflammatory exudates of carrageenan peritonitis, observed that as the exudate is developing (6 hr) the ratio of PGE_2:$PGF_{2\alpha}$ shifts to 1:2. However at 24 hr when the exudate process waned and looked more as that seen in the resting peritoneal cells, the ratio of PGE_2:$PGF_{2\alpha}$ shifts to 1:6. Thus, the use of the domestic pig and its exposure to ionizing radiation could provide a useful model for further explorations into the mechanisms of action of various sources of ionizing radiation on the skin.

1. Samuelsson B, Granstrom E, Green K, Hamberg M, Hammarstrom S, Malmsten C, Goldyne E. Annu Rev Biochem **47**:997–1029, 1978.
2. Ziboh VA, Hsia SL. Prostaglandin E_2: Biosynthesis and effects on glucose and lipid metabolism in rat skin. Arch Biochem Biophys **146**:100–109, 1971.
3. Van Dorp D. Recent developments in the biosynthesis and the analysis of prostaglandins. Ann NY Acad Sci **180**:181–199, 1971.
4. Jonsson DC, Angaard E. Biosynthesis and metabolism of prostaglandin E_2 in human skin. Scand J Clin Lab Invest **29**:289–296, 1972.
5. Greaves MW, McDonald M. Inhibition of prostaglandin biosynthesis by corticosteroids. Brit Med J **2**:83–84, 1972.
6. MacArthur GP, Gandhi VM. Prostaglandin in human and albino rat skin. Invest Dermatol **58**:291–295, 1972.
7. Greaves MW, Sondergaard J. Pharmacologic agents released in ultraviolet inflammation studied by continuous skin perfusion. Invest Dermatol **54**:365–367, 1970.
8. Snyder DS, Eaglstein W. Intradermal antiprostaglandin agents and sunburn. Invest Dermatol **62**:47–50, 1974.
9. Archambeau JO, Fairchild RG, Commerford SL. Response of the skin of swine to increasing exposures of 250-KVP X-ray. In: Bustad LK, McClellan RO, eds. Swine in Biomedical Research. Pacific Northwest Laboratory, Richland, Wash, p 463, 1966.
10. Archambeau JO, Gerber A, Ayout R. Brenneis HJ. Epidermal cell population changes produced by exposure to 2,300 R. Radiology **103**:191–196, 1972.
11. Berry RJ, Wiernik G, Petterson RJS, Hopewell JW. Excess late subcutaneous fibrosis after irradiation of pig skin, consequent upon the application of NSD formula. Brit J Radiol **47**:277–281, 1974.
12. Lippincott SW, Wilson JD, Montour JL. Radiation effects on pig skin. Arch Pathol **99**:105–110, 1975.
13. Sondergaard J, Pekka H, Jorgensen H. Human cutaneous inflammation induced by prostaglandin E_1. J Pathol **190**:239–243, 1973.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagent. J Biol Chem **193**:265–275, 1951.
15. Ziboh VA, Miller AM, Yunis AA, Jimenez JJ, Kursunoglu I. Arachidonic acid metabolism by rat chloroleukemia cells in culture. Cancer Res **41**:12–17, 1981.
16. Ziboh VA. Biosynthesis of prostaglandin E_2 in human skin: Subcellular localization and inhibition by unsaturated fatty acids and antiinflammatory drugs. J Lipid Res **14**:377–384, 1973.
17. Ziboh VA, Vanderhoek JY, Land WEM. Inhibition of sheep vesicular gland oxygenase by unsaturated fatty acids from skin of essential fatty acid deficient rats. Prostaglandins **4**:233–239, 1974.
18. Ziboh VA, Lord JT, Penneys NS. Alterations of prostaglandin E_2-9-ketoreductase activity in proliferating skin. J Lipid Res **18**:37–43, 1977.
19. Lord JT, Ziboh VA, Poitier J, Legget G, Penneys NS. The effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins. Brit J Dermatol **95**:397–406, 1976.
20. Little JB. Cellular effects of ionizing radiation. N Eng J Med **278**:308–315, 1968.
21. Eisen V, Walker DI. Effect of ionizing radiation on prostaglandin-like activity in tissues. Brit J Pharmacol **57**:527–532, 1976.
22. Crunkhorn P, Willis AL. Interaction between prostaglandins E and F given intradermally in the rat. Brit J Pharmacol **41**:507–512, 1971.
23. Willoughby DA. Effects of Prostaglandins $PGF_{2\alpha}$ and $PGE_{1\alpha}$ on vascular permeability. J Pathol Bacteriol **96**:381–387, 1968.
24. Velo GP, Dunn CJ, Giroud JP, Timsit J, Willoughby DA. Distribution of prostaglandins in inflammatory exudate. J Pathol **111**:149–158, 1973.

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