Alterations of prostaglandin E_2-9-ketoreductase activity in proliferating skin[1]

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Abstract The activity of an NADPH-dependent PGE_2-9-ketoreductase has been demonstrated in rat and human skin. This activity is localized in the high speed supernatant fraction, indicating the presence of an active PGE_2-9-ketoreductase associated with the cytoplasmic fraction of the skin. Transformation of PGE_2 into $PGF_{2\alpha}$ is enhanced by skin specimens from psoriatic plaques and EFA-deficient rats, both characterized by excessive cellular proliferation and increased NADPH production. Incubations of the 105,000 *g* supernatant fractions from normal and EFA-deficient rats demonstrated that the activity of the PGE_2-9-ketoreductase was elevated in high speed preparations from EFA-deficient rats. Results from these studies suggest that the increased activity of PGE_2-9-ketoreductase observed in skin from human psoriatic plaques and EFA-deficient rats may be due in part to the increased generation of NADPH by these tissues and in part to alteration of the PGE_2-9-ketoreductase by the excessive proliferation of the tissues.

Supplementary key words Psoriasis · psoriatic plaque · essential fatty acid

The prostaglandins constitute an intriguing family of compounds synthesized by the body from essential fatty acids (1, 2). Recent discoveries and elucidation of their chemistry are leading to exciting developments in pharmacology and clinical medicine. The E and F prostaglandins in many instances do exert diverse and potent effects on many physiological systems (3–5). For example, experiments in vitro with isolated human and guinea pig ileum and colon have demonstrated that the F prostaglandins increase contraction whereas the E prostaglandins inhibit it (6). Recently, it has been reported that vein cGMP levels and the ratio of cGMP to cAMP are increased during venoconstriction elicited by $PGF_{2\alpha}$ whereas cAMP concentrations are increased when vein strips are superfused with PGE_2 (7). These observations underscore the importance of the relative concentrations of these two potent biological substances in tissues. The enzyme systems involved with their interconversion must therefore play a vital role in regulating physiological processes.

The occurrence of PGE_2-9-ketoreductase, the enzyme catalyzing the transformation of PGE_2 into $PGF_{2\alpha}$, has been reported in a variety of animal species and tissues (8–12). The activity of the enzyme is dependent on the availability of reducing equivalents (NADPH) in the tissues. The relative levels of reduced and oxidized pyridine nucleotides in tissues are therefore of vital importance and may regulate the activity of this enzyme and consequently the tissue levels of E and F prostaglandins.

Previous studies in our laboratory have demonstrated increased pentose cycle activity and lipid synthesis in skin of essential fatty acid (EFA)-deficient rats (13). Increased pentose cycle activity has also been reported in plaques obtained from psoriatic patients when compared to normal or uninvolved areas of skin (14, 15). Since the above reports suggested increased production of NADPH via the pentose cycle pathway, it was of interest to determine whether or not the activity of the PGE_2-9-ketoreductase was affected in these tissues. The present studies were therefore undertaken to provide evidence for the occurrence and localization of PGE_2-9-ketoreductase in the skin, and to determine whether or not the activity of the enzyme is altered in skin preparations from EFA-deficient rats and from human psoriatic plaques.

MATERIALS AND METHODS

Materials

[^{3}H]Prostaglandin E_2 (125 μCi/mmole) was purchased from New England Nuclear Corp. (Boston, MA). The radiopurity was ascertained by thin-layer chromatography (TLC) in the solvent system chloroform–methanol–acetic acid–water 90:8.5:1:0.65.

Abbreviations: TLC, thin-layer chromatography; EFA, essential fatty acids. Trivial names are: prostaglandin E_2 (PGE_2), 11,15-dihydroxy-9-oxoprosta-5,13-dienoic acid; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9,11,15-trihydroxy-prosta-5,13-dienoic acid; prostaglandin B_2 (PGB_2), 15-hydroxy-9-oxoprosta-5,8(12)13-dienoic acid.

[1] A preliminary report of this work was presented at the 2nd International Conference on Prostaglandin, Florence, Italy, May 26–30, 1975.

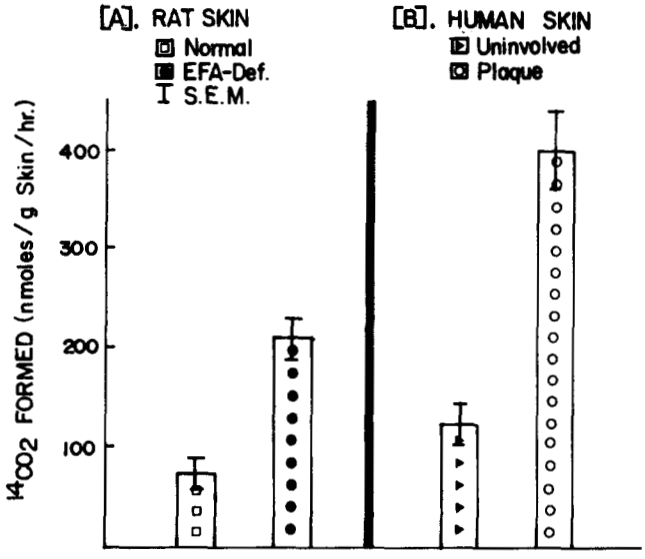

Fig. 1. Oxidation of [1-^{14}C]glucose to $^{14}CO_2$ by human and rat skin. The data are from the means ± SEM of three experiments.

Seventy to eighty percent of the chromatographed ^{3}H was found to have the migration rate of authentic PGE_2. Authentic PGE_2 and $PGF_{2\alpha}$ were supplied by Dr. U. F. Axen of Upjohn Co., Kalamazoo, MI. [1-^{14}C]Glucose (5.6 mCi/mmole) was also obtained from New England Nuclear Corp. and its purity was ascertained by TLC. NADPH was purchased from Sigma Chemical Co., St. Louis, MO. Sephadex (G-25 coarse) was purchased from Pharmacia Fine Chemicals Inc., Piscataway, N. J. Hyamine hydroxide was from Packard Instrument Co., Downers Grove, IL. Reagents were of analytical grade and solvents were distilled before use.

Preparation and incubations of skin specimens with differentially labeled glucose

Paired skin specimens from normal and EFA-deficient rats (25–50 mg) were removed with scissors and the underlying subcutaneous tissue scraped off with a scalpel as reported previously (13). Similarly, skin from uninvolved and psoriatic plaques (20–30 mg) was obtained from patients with signed, informed consent. Our specimens were obtained with the keratome blade set at 0.2 mm; when examined under the microscope, they were found to be mostly epidermis (approximately 80%). It is technically difficult to obtain epidermal specimens for rapid enzymatic work without some contamination by the dermal tissue. However, since previous studies in this laboratory have shown that the dermal tissue contaminant contributes little or nothing to the enzyme activity under study we chose to use the entire specimen. The skin specimens were placed in 2 ml of Krebs-Ringer bicarbonate solution pH 7.4, previously gassed with 95% O_2 and 5% CO_2, in an incubation flask fitted with a rubber cap (serum stopper) and a polyethylene center well attached to the cap. The reaction mixture contained glucose, 10 mM, gentamicin sulfate (200 μg) and [1-^{14}C]glucose (2.5 μCi). The mixture was incubated with shaking at 37°C for 2 hr. At the end of the incubation, 0.3 ml of 1 M hyamine hydroxide was introduced by injection through the rubber cap into the center well, and 0.4 ml of 6N H_2SO_4 was injected into the incubation mixture to stop the reaction and liberate CO_2 as reported previously (16). The vessel was left at 37°C for 4 hr, after which the center well was carefully removed and placed directly into a counting vial for radioassay. In control experiments, the recovery of $^{14}CO_2$ from $NaH^{14}CO_3$ was greater than 90%.

Conversion of PGE_2 into $PGF_{2\alpha}$ by skin specimens

Paired skin specimens from normal and EFA-deficient rats (25–50 mg) and from uninvolved and psoriatic plaques (20–30 mg) were incubated aerobically in 2 ml of Krebs-Ringer phosphate buffer pH 7.4, containing glucose (10 mM), indomethacin (100 μM) to inhibit new biosynthesis of nonradioactive PGE_2, and [^{3}H]PGE_2 (0.5 μCi, 5 pmole), in a Dubnoff shaking incubator at 37°C for 2 hr. In control experiments transformation of [^{3}H]PGE_2 into $PGF_{2\alpha}$ was inhibited with skin previously boiled for 20 min or with buffer containing glucose and indomethacin alone and no tissue. At the end of the incubation, the tissue was removed, rinsed with 0.9% saline and immediately homogenized in 20 ml of a mixture of chloroform–methanol 2:1 (v/v) in a Polytron homogenizer. The incubation mixture was extracted with 20 ml of the same solvent mixture according to Folch et al. (17). In preliminary experiments, after the separation of lipid residues on TLC plates, less than 0.01% of radioactive $PGF_{2\alpha}$ and PGE_2 were associated with the incubated tissues. All the [^{3}H]-$PGF_{2\alpha}$ formed was extracted from the incubation mixture. In subsequent experiments, the incubation mixtures were extracted with chloroform–methanol 2:1 for the [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ estimations.

Thin-layer chromatography and determination of prostaglandins

TLC plates were coated with silica gel G and activated at 110°C for 60 min before use. The labeled lipid extract from the incubation mixture was applied to the activated plate. Small amounts of reference $PGF_{2\alpha}$, PGE_2, and their 15-keto derivatives were also applied to other activated plates. All plates were developed simultaneously in chloroform–methanol–acetic acid–water 90:8.5:1:0.65. Reference $PGF_{2\alpha}$,

PGE_2, 15-keto PGE_2, and 15-keto $PGF_{2\alpha}$ were visualized after the plates were placed in a tank of iodine vapor. The solvent system is effective for separating $PGF_{2\alpha}$ and PGE_2 from their 15-keto derivatives. The silica gel on the plate containing the ^3H-labeled extract corresponding to $PGF_{2\alpha}$ was scraped into a sintered funnel and the ^3H-labeled material was eluted with chloroform–methanol 1:1. An aliquot of the eluate was assayed for radioactivity with a Packard TriCarb model 2002 liquid scintillation counter as reported previously (13).

For further purification, reference $PGF_{2\alpha}$ and PGE_2 and the remaining radioactive material were treated with 3 ml of 0.5 N KOH in 50% aqueous methanol and incubated at room temperature for 30 min. Under these conditions, the E prostaglandins form products containing the $\Delta^{8(12)13}$-9-keto chromophore (18, 19). The samples were diluted with water and then acidified with 6 N HCl to pH 2–3. The acidified samples were extracted with dichloromethane, dried under nitrogen, and chromatographed on thin-layer plates as reported previously (20). This transformation of PGE_2 to PGB_2 and the TLC removed any further PGE_2 contamination of the $PGF_{2\alpha}$ fration.

Conversion of PGE_2 into $PGF_{2\alpha}$ by a 105,000 *g* supernatant fraction from rat skin

In order to localize the activity of the PGE_2-9-ketoreductase, skin specimens were removed from the shaved area of the posterior dorsum from normal and EFA-deficient male Spraque-Dawley rats as reported previously (21). The skin specimens were homogenized with a Polytron homogenizer (PT-20, Kinematica, Lucern, Switzerland), in 5 vol of ice-cold 0.1 M phosphate buffer pH 7.4 containing 4 mM $MgCl_2$ and 0.1 mM dithiothreitol. Preparation of the 105,000 *g* supernatant fraction by differential centrifugation was as described previously (21). In order to remove endogenous pyridine nucleotides and other small molecular weight substances, the 105,000 *g* supernatant fraction was partially purified and concentrated by filtering through Sephadex G-25 (coarse). The protein in the concentrated 105,000 *g* supernatant fraction was determined by the method of Lowry et al. (22), with bovine albumin used as standard.

Enzyme assay

The enzymatic reduction of PGE_2 into $PGF_{2\alpha}$ was measured, unless otherwise stated, in a final volume of 1 ml containing $MgCl_2$ (4 mM), NADPH (2.0 mM), dithiothreitol (0.1 mM), 105,000 *g* supernatant fraction (2–4 mg protein) and [^3H]PGE_2 (0.1 μCi, 1 pmole). The mixture was incubated aerobically with shaking in a Dubnoff incubator at 37°C for various intervals of time. Control experiments contained [^3H]PGE_2 and subcellular fractions previously boiled for 15 min. The incubations were terminated by the addition of 15 ml of chloroform–methanol 2:1 (v/v) to the incubation vial. The mixture was extracted twice with 15 ml of the same solvent mixture. The combined lipid extracts were evaporated to dryness under a stream of N_2 gas. The residue was dissolved in a small volume of chloroform–methanol 1:1 (v/v) and then chromatographed on silica gel G thin-layer plates as described above. The activity of the PGE-9-ketoreductase was calculated from the amount of radioactivity that cochromatographed with authentic $PGF_{2\alpha}$ as a percentage of the total radioactivity recovered.

RESULTS

Formation of CO_2 from [1-^{14}C]glucose by rat and human skin specimens

A previous study in this laboratory demonstrated the formation of CO_2 from glucose carbons by normal rat skin (23). For comparison, these experiments were again carried out with skin from normal and EFA-deficient rats and with uninvolved and plaque areas from human skin. The data in **Fig. 1** show that the formation of CO_2 from glucose was enhanced by skin specimens from EFA-deficient rats and by human psoriatic plaque.[2] These results indicate an increased activity of the pentose cycle pathway, which would generate increased amounts of NADPH. Since NADPH has been reported to be obligatory for the conversion of PGE_2 into $PGF_{2\alpha}$ (9), skin specimens from rat and human were incubated with [^3H]PGE_2 to ascertain whether or not transformations by skin specimens from EFA-deficient rats and human psoriatic plaques were altered.

Conversion of [^3H]PGE_2 into [^3H]$PGF_{2\alpha}$ by rat and human skin

Since the [^3H]PGE_2 used in these experiments contained impurities, as indicated by TLC chromatography under Methods, it was questionable whether

[2] Although some investigators choose DNA content as a basis for comparison between psoriatic and normal specimens, we chose to use the wet weight because of the variability and problems associated with measurements of DNA in small skin specimens. Despite the small contamination of our psoriatic specimens with dermal or parakeratotic scale, the general pattern of results was not altered. If a 20% correction (24) were applied to our results, the results of the psoriatic specimens would be more marked.

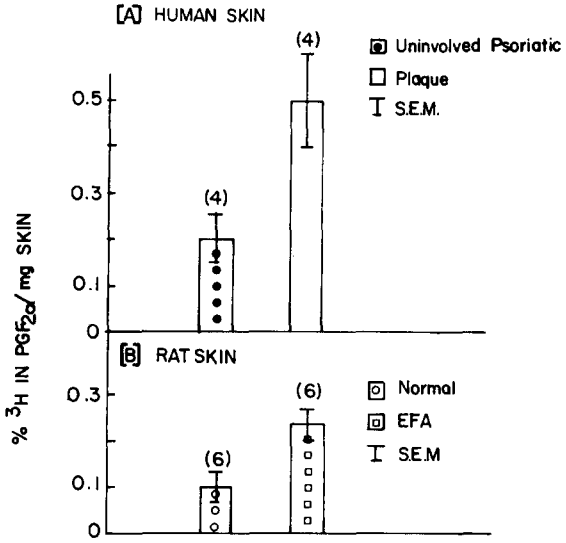

Fig. 2. Conversion of [^{3}H]PGE_2 into $PGF_{2\alpha}$ by human and rat skin specimens. Results are expressed as percentages per mg protein of added substrate after correction for boiled control. The data are from the means±SEM of the number of experiments indicated in parentheses.

or not the radioactive contaminants might also be precursors of $PGF_{2\alpha}$. In preliminary experiments to test these possibilities, the recovered radioactivity from the thin-layer plate other than that in the fraction corresponding to authentic PGE_2 was incubated with rat skin specimens and 105,000 *g* supernatant fractions as described under Methods. The products of incubation were analyzed by TLC, and less than 0.01% of the radioactivity was associated with the $PGF_{2\alpha}$ fraction.

In preliminary experiments, normal rat skin specimens (25–50 mg) were incubated with [^{3}H]PGE_2 as described under Methods for various time intervals in order to determine the maximal time of incubation for the tissues. Our results indicate that the reaction remained linear for 2 hr; consequently all subsequent incubations with skin specimens were carried out for this time period. The data in **Fig. 2** show that, when compared to controls, the formation of $PGF_{2\alpha}$ from PGE_2 was enhanced by skin specimens from psoriatic plaques [A] and from EFA-deficient rats [B]. These results suggest that the increased transformation of PGE_2 into $PGF_{2\alpha}$ by skin specimens from EFA-deficient rat and from psoriatic plaques may be due to increased availability of NADPH generated via increased pentose cycle activity (Fig. 1). To ascertain whether the increased transformation of PGE_2 into $PGF_{2\alpha}$ by skin from EFA-deficient rats was also due to increased activity of the PGE_2-9-ketoreductase, cell-free extracts from rat skin were prepared as described under Methods and the activity of the enzyme determined.

Localization of PGE_2-9-ketoreductase activity in subcellular fractions of rat skin

In order to carry out the exhaustive studies with subcellular fractions, skin from normal rats was homogenized and subjected to differential centrifugation to obtain 105,000 *g* particulate and supernatant fractions. Incubations and assays of radioactive metabolites were carried out as indicated under Methods. The data in **Fig. 3** demonstrate that the activity of the NADPH-dependent PGE_2-9-ketore-

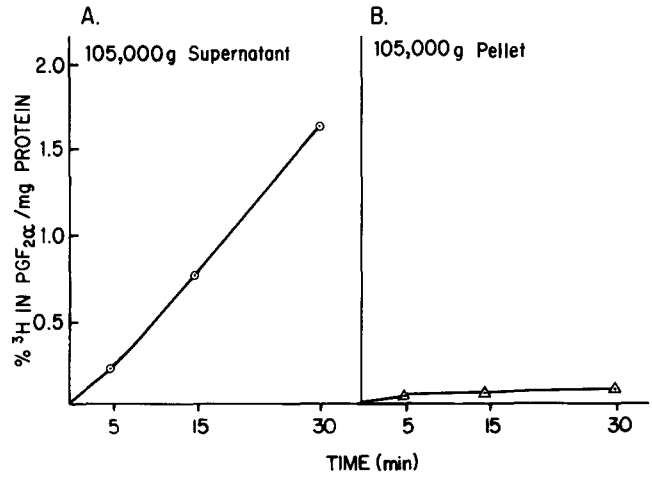

Fig. 3. Intracellular localization of the conversion of [^{3}H]PGE_2 into [^{3}H]$PGF_{2\alpha}$ in rat skin homogenates. Results are expressed as percentages per mg protein of added substrate after correction for boiled enzyme control. The data are averages of duplicate experiments.

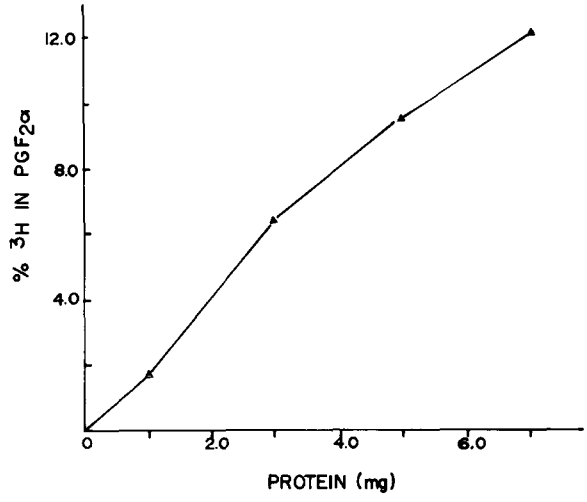

Fig. 4. Effect of increasing 105,000 *g* supernatant protein on the conversion of [^{3}H]PGE_2 into [^{3}H]$PGF_{2\alpha}$. [^{3}H]PGE_2 (0.1 μCi, 1.0 pmole) was incubated for 30 min with increasing amounts of partially purified 105,000 *g* supernatant protein. The results are expressed as percentages of added substrate. The data are averages of duplicate experiments.

ductase in rat skin is localized in the 105,000 *g* supernatant fraction.

Effects of increasing 105,000 *g* supernatant protein on the transformation of [^{3}H]PGE_2 into [^{3}H]$PGF_{2\alpha}$

The relationship between percentage conversion of PGE_2 into $PGF_{2\alpha}$ and the amount of protein from the 105,000 *g* supernatant fraction is shown in **Fig. 4**. The amounts of [^{3}H]$PGF_{2\alpha}$ formed from [^{3}H]PGE_2 increased with increasing amounts of protein from the 105,000 *g* fraction.

Effects of reducing equivalents on the transformation of [^{3}H]PGE_2 into $PGF_{2\alpha}$

Addition of increasing amounts of NADPH to the partially purified 105,000 *g* supernatant fraction resulted in the stimulation of the activity of the PGE_2-9-ketoreductase, whereas NADH produced little effect (**Fig. 5**), even at higher concentrations.

Effects of EFA-deficiency on the activity of skin PGE_2-9-ketoreductase

As reported under Methods, 105,000 *g* supernatant fractions from normally fed and EFA-deficient rats were incubated with [^{3}H]PGE_2. The data in **Fig. 6** reveal consistently higher activity of PGE_2-9-ketoreductase in high speed supernatant fractions from EFA-deficient rats. These results indicate that the increased activity of the PGE_2-9-ketoreductase by the EFA-deficient rats was also due in part to enhanced activity of the enzyme since endogenous NADPH in the 105,000 *g* preparations was removed by chromatography on Sephadex G-25 before incubations.

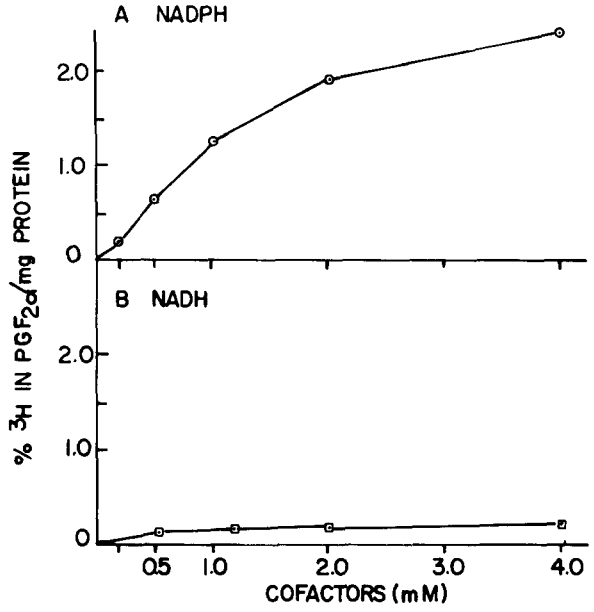

Fig. 5. The effects of reducing equivalents (NADPH and NADH) on the conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$. [^{3}H]PGE_2 (0.1 μCi, 1.0 pmole) was incubated for 30 min with partially purified 105,000 *g* supernatant fraction (3.7 mg protein) and varying amounts of NADPH or NADH. The results are expressed as percentages per mg protein of added substrate.

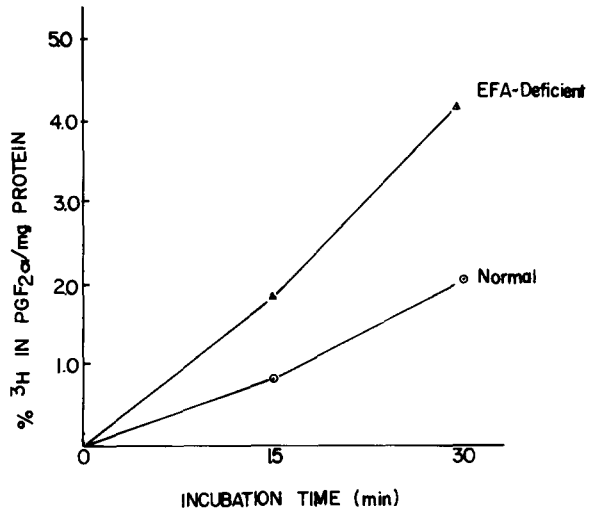

Fig. 6. Effect of essential fatty acid deficiency on the activity of skin PGE_2-9-ketoreductase. [^{3}H]PGE_2 (0.1 μCi, 1.0 pmole) was incubated with partially purified 105,000 *g* supernatant fractions prepared, respectively, from skin of normal and EFA-deficient rats. Details of incubation and identification of radioactive products are described in the text. The results are expressed as percentages per mg protein of added substrate. The data are averages of duplicate experiments.

In order to determine whether or not the increased activity of the PGE_2-9-ketoreductase was associated with the development of EFA deficiency and proliferation of the tissues, 105,000 *g* supernatant fractions were prepared from normal rats and from rats fed the EFA-deficient diet. Specimens were taken at various times during a 12-week period when signs of EFA deficiency were fully visible on the skin. In addition to the incubations of the 105,000 *g* fraction with [^{3}H]PGE_2, portions of the skin specimens were also taken for histologic evaluation to observe the acanthosis of the epidermal layer and for measuring the incorporation of [^{3}H]thymidine in DNA. The data in **Fig. 7** demonstrate consistently increased activity of PGE_2-9-ketoreductase from 4 weeks after feeding the animals a diet deficient in essential fatty acids. These data are consistent with increased incorporation of [^{3}H]thymidine into DNA and hyperproliferation of the skin at this same time.

DISCUSSION

In these studies, we have demonstrated the activity of NADPH-dependent PGE_2-9-ketoreductase in rat

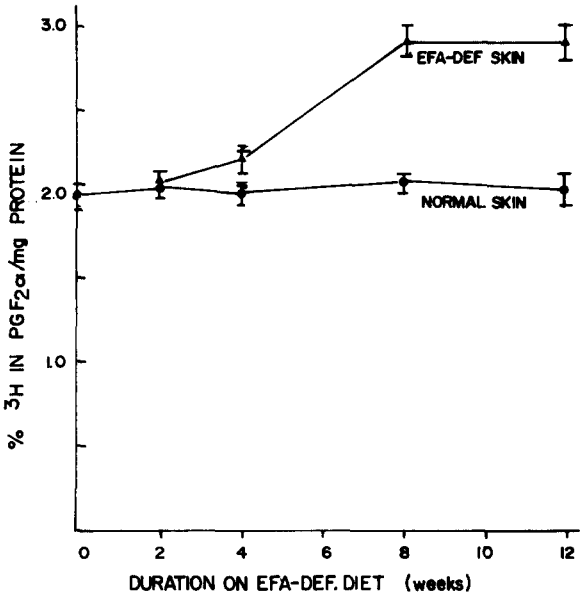

Fig. 7. Alteration of the activity of PGE_2-9-ketoreductase with the development of EFA-deficiency syndrome. [^{3}H]PGE_2 (0.1 μCi, 1.0 pmole) was incubated with partially purified 105,000 g supernatant fractions prepared from skin of rats before and during the feeding of a diet deficient in essential fatty acids. Specimens were taken at intervals over a period of 12 weeks. Details of incubation and identification of radioactive products are described in the text. The results are expressed as percentages per mg protein of added substrate. The data are averages of duplicate experiments.

and human skin. The enzyme's activity is localized in the 105,000 g supernatant fraction. The skin PGE_2-9-ketoreductase is therefore similar to that reported in other tissues (9).

The data in Fig. 2 show that the transformation of PGE_2 into $PGF_{2\alpha}$ is enhanced by skin specimens from psoriatic plaques and from EFA-deficient rats in which the tissues are characterized by excessive cell proliferation. This observation is interesting in view of a recent report by Hammarstrom et al. (25) that indicated increased concentrations of PGE_2 and $PGF_{2\alpha}$ in epidermis of psoriatic plaques. We have also observed elevated levels of PGE_2 and $PGF_{2\alpha}$ in the plaques of a small number of psoriatic patients.[3] Although other factors such as Cu^{2+} (26), dihydrolipoamide (27), and L-epinephrine (28) have been reported to influence the formation of $PGF_{2\alpha}$ by the prostaglandin synthetase system, our results (Figs. 1 and 2) suggest that the endogenous generation of NADPH may at least in part be an important regulator of $PGF_{2\alpha}$ formation in tissues.

Our studies with the high speed supernatant fractions from EFA-deficient rats also demonstrated that the activity of the PGE_2-9-ketoreductase is increased in this syndrome. Incubations of partially purified high speed preparations from normal and EFA-deficient rats in the presence of 2 mM NADPH resulted in increased activity of the PGE_2-9-ketoreductase in the high speed supernatant fraction from EFA-deficient rats (Fig. 6). Another interesting observation in the present studies is the gradual elevation in activity of the PGE_2-9-ketoreductase along with increased proliferation of the tissues (Fig. 7) as the animal developed symptoms of EFA-deficiency. These results suggest that the formation of $PGF_{2\alpha}$ from PGE_2 is associated with the onset of the proliferation of the skin induced by EFA-deficiency. Whether this mechanism of action resulting in the accumulation of $PGF_{2\alpha}$ is fundamental to cellular proliferation is presently unknown. Further studies are necessary to delineate whether the alteration of the tissue levels of E and F prostaglandins by a PGE_2-9-ketoreductase plays a central role in the pathophysiology of proliferating tissues or cells.

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