Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 5130–5134, December 1975
Medical Sciences

Increased concentrations of nonesterified arachidonic acid, 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ in epidermis of psoriasis*

(quantitative mass spectrometry/eicosatetraynoic acid/indomethacin)

SVEN HAMMARSTRÖM†, MATS HAMBERG†, BENGT SAMUELSSON†, ELIZABETH A. DUELL‡, MAREK STAWISKI‡, AND JOHN J. VOORHEES‡

† Department of Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden; and ‡ Departments of Dermatology and Biological Chemistry, The University of Michigan Medical School, Ann Arbor, Mich. 48104

Communicated by Sune Bergström, September 26, 1975

ABSTRACT **Lesional epidermis of psoriasis has a probable reduction in the cyclic AMP/cyclic GMP ratio. This altered ratio may in part be responsible for the characteristic glycogen storage, rapid cell proliferation, and reduced differentiation in lesional epidermis. The concentrations of prostaglandins E_2 and $F_{2\alpha}$, free arachidonic acid, and 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid in specimens of uninvolved and involved epidermis of psoriasis were measured with deuterium-labeled carriers and multiple ion analysis. Snap frozen specimens contained: 1.4 ± 0.4 μg/g (wet weight) of arachidonic acid in uninvolved in contrast to 36.3 ± 16.7 μg/g in involved epidermis ($P = 0.015$); <0.05 ± 0.01 μg/g of hydroxyeicosatetraenoic acid in uninvolved in contrast to 4.1 ± 1.9 μg/g in involved epidermis ($P = 0.015$); 23.6 ± 5.0 ng/g of prostaglandin E_2 in uninvolved in contrast to 33.1 ± 5.7 ng/g in involved epidermis ($P < 0.01$); and 21.0 ± 4.4 ng/g of prostaglandin $F_{2\alpha}$ in uninvolved in contrast to 39.0 ± 5.9 ng/g in involved epidermis ($P < 0.01$). The arachidonic acid and hydroxyeicosatetraenoic acid levels in involved epidermis were strongly correlated ($r = 0.97$). The increased levels of arachidonic acid and 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid in involved epidermis may have diagnostic and pathophysiological importance.**

Psoriasis is a genetic skin disease characterized by glycogen accumulation, excessive cell proliferation, and incomplete differentiation in lesional epidermis (1). The cellular levels of cyclic AMP and cyclic GMP are probably decreased and increased, respectively, in involved lesional epidermis in contrast to clinically uninvolved epidermis of psoriasis (2). It has been suggested that the probable imbalance of these two cyclic nucleotides may play a central role in the pathogenesis of the lesion (3).

Previously, reduced cellular levels of cyclic AMP were demonstrated in transformed cells (4, 5). Increased levels of cyclic GMP as well as increased cyclic GMP and decreased cyclic AMP levels have been reported in cell systems stimulated to proliferate (6, 7). In certain transformed cells, prostaglandin E_2 (PGE_2) can elevate the cellular levels of cyclic AMP (8), whereas in bovine venous strips prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) elevated cyclic GMP levels (9). Most tissues, including epidermis (10, 11), can convert arachidonic acid to PGE_2 and $PGF_{2\alpha}$. Other oxygenated metabolites of arachidonic acid, including 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), have more recently been identified in human platelets (12, 13).

Abbreviations: HETE, 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

* This work was presented at the Annual Meeting of the American Society for Clinical Investigation in Atlantic City, N.J., May 4, 1975. An abstract has been published in *Clin. Res.* 23, 386 A (1975).

In the present investigation the levels of free arachidonic acid, PGE_2, $PGF_{2\alpha}$, and HETE have been quantitatively measured in specimens of uninvolved and involved epidermis, using deuterium-labeled carriers and multiple ion analyses.

MATERIALS AND METHODS

[3,3,4,4-$^2H_4$]PGE_2 and [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ were generously provided by Dr. U. Axen, the Upjohn Co., Kalamazoo, Mich.

Quantitative Determination of Arachidonic Acid. [5,6,8,9,11,12,14,15-$^2H_8$]Arachidonic acid and the corresponding tritium-labeled compound were prepared as described (14). A mass spectrum of methyl octadeuteroarachidonate showed ions of high intensity at m/e 326 (M), 270, 255, 226, 213, 210, 184, 168, 154, 139, 125, 110, 97, 82, 71, 57, and 43, whereas the mass spectrum of unlabeled methyl arachidonate had ions of high intensity at m/e 318 (M), 270, 264, 250, 247, 220, 203, 180, 177, 175, 164, 161, 150, 147, 133, 119, 106, 93, 79, 67, 55, and 43. A small amount of octatritioarachidonic acid was added to the octadeutero compound to give a specific activity of 3.65 Ci/mol. Mixtures of unlabeled and tritium,deuterium-labeled arachidonic acid were prepared, converted to methyl esters, and subjected to multiple ion analysis in an LKB 9000 gas chromatograph-mass spectrometer equipped with an accelerating voltage alternator. The ion intensities at m/e 318 and 326 were recorded against time. A standard curve was obtained by plotting the ratio between the maximum ion intensities at m/e 318 and 326 on the y-axis against the ratio between the amounts of added unlabeled and deuterium-labeled arachidonic acid on the x-axis. A linear relationship was obtained ($y = 0.91x + 0.027$; $0 \leq x \leq 0.4$).

Quantitative Determination of PGE_2 and $PGF_{2\alpha}$ (15). [17,18-$^3H_2$]PGE_2 (specific activity: 22.5 Ci/mmol) was prepared as described (16). [17,18-$^3H_2$]$PGF_{2\alpha}$ was prepared from [17,18-$^3H_2$]PGE_2 by $NaBH_4$ reduction (17). Tritium-labeled prostaglandins were added to the corresponding deuterium-labeled compounds to give specific activities of 23.6 Ci/mol for PGE_2 and 18.4 Ci/mol for $PGF_{2\alpha}$. Mixtures of unlabeled and tritium,deuterium-labeled prostaglandins were prepared and converted to methyl ester, *O*-methoxime, acetate (PGE_2), and methyl ester, acetate ($PGF_{2\alpha}$) derivatives, respectively (15). The ion intensities at m/e 419 and 423 (PGE_2) and m/e 314 and m/e 318 ($PGF_{2\alpha}$) were monitored, and standard curves were plotted as described for arachidonic acid. The relationships were $y = 0.95x +$

0.009 ($0 \leq x \leq 0.25$) for PGE_2 and $y = 0.97x + 0.005$ ($0 \leq x \leq 0.2$) for $PGF_{2\alpha}$.

Quantitative Determination of 12*L*-Hydroxy-5,8,10,14-Eicosatetraenoic Acid (HETE). The preparation of [*1*-^{14}C,*5,6,8,9,11,12,14,15*-$^{2}H_8$]HETE (specific activity 0.93 Ci/mol) and the method for quantitative determination of HETE were recently described (12, 13).

Epidermal Biopsies. The epidermal strips were procured as described with a modified motor-driven keratome (1). Both uninvolved epidermis and involved epidermis were removed after each area was anesthetized by the local infiltration of 1% lidocaine without epinephrine. The involved epidermal strip was removed before the uninvolved strip. The average time for the keratome to cut through uninvolved and involved epidermis was 8 and 5 sec, respectively. The strips were placed immediately in liquid nitrogen. A portion of both uninvolved and involved epidermis was processed for light histology. Only specimens containing full-thickness involved or uninvolved epidermis and minimal dermal contamination as described (1) were analyzed.

Analytical Procedure. Between 50 and 150 mg of keratomed epidermis was powdered in a mortar at −78°. The powder was weighed on an analytical balance at −20°, and 10–100 mg was suspended in either 30 ml of chloroform-methanol (2:1, vol/vol) at −20° or in 10 volumes (volume/weight) of 0.1 M K-PO_4 buffer, pH 8.0 at 0°. Powders suspended in K-PO_4 buffer were incubated at 37° for 10 min, after which 30 ml of chloroform-methanol (2:1, vol/vol) was added. The chloroform-methanol solution that was added contained 10–11 μg of ^{3}H,^{2}H-labeled arachidonic acid, 1.7 μg of ^{14}C,^{2}H-labeled HETE, 0.4–0.8 μg of ^{3}H,^{2}H-labeled PGE_2, and 0.8–1.3 μg of ^{3}H,^{2}H-labeled $PGF_{2\alpha}$ as carriers. In some cases, 10 ml of ethanol containing the same amount of HETE carrier was added to incubated samples instead of chloroform-methanol 2:1. The latter mixtures were diluted with water, acidified, extracted with diethyl ether, and subjected to thin-layer chromatography as described (13). Suspensions in chloroform-methanol were stirred for 1 hr at room temperature, filtered, and partitioned with 8 ml of 1% (vol/vol) aqueous formic acid. For quantitative recovery of $PGF_{2\alpha}$, the upper phase was diluted with water and extracted with diethyl ether. The ether extracts and the lower phase were combined, the solvents were evaporated under reduced pressure, and the residue was subjected to silicic acid chromatography. The column was eluted with hexane, 5%, 10%, and 30% (vol/vol) diethyl ether in hexane, ethyl acetate, and methanol. Eluates of 5% and those of 30% diethyl ether in hexane contained arachidonic acid and HETE, respectively, whereas PGE_2 and $PGF_{2\alpha}$ appeared in the combined ethyl acetate and methanol eluates. The solvents were removed under reduced pressure and the compounds esterified by treatment with diazomethane. HETE methyl ester was purified as described (13). Methyl arachidonate was subjected to thin-layer chromatography on plates coated with silver nitrate-silica gel G (1:15, weight/weight) and with diethyl ether-hexane (15:85, vol/vol) as solvent, whereas PGE_2 methyl ester and $PGF_{2\alpha}$ methyl ester were separated by thin-layer chromatography (silica gel G) with the organic phase of ethyl acetate-methanol-water (160:10:100, vol/vol/vol) as solvent. The positions of the labeled compounds were determined with a Berthold Dünnschicht-scanner II. Methyl arachidonate had an R_F value of 0.03, whereas PGE_2 methyl ester and $PGF_{2\alpha}$ methyl ester had R_F values of 0.65 and 0.45, respectively. The zones containing these compounds were scraped off and eluted with diethyl ether (methyl arachidonate) or methanol (PGE_2 and $PGF_{2\alpha}$ methyl esters). The diethyl ether eluates were evaporated to dryness and subjected to multiple ion analysis (m/e 318 and 326) using a GLC column containing 13% (weight/weight) EGSS-X on 100/120 mesh Gas Chrom Q at 220° (Fig. 1). The methanol eluates were diluted with water and extracted with diethyl ether, and the solvents were removed under reduced pressure. PGE_2 methyl ester was converted to the *O*-methoxime, acetate derivative and $PGF_{2\alpha}$ methyl ester to the acetate derivative (15). The derivatives were finally purified by silicic acid chromatography (18) and subjected to multiple ion analysis (m/e 419 and 423, PGE_2; and m/e 314 and 318, $PGF_{2\alpha}$) using a column containing 1% OV-1 on 60/80 mesh Supelcoport® at 240°.

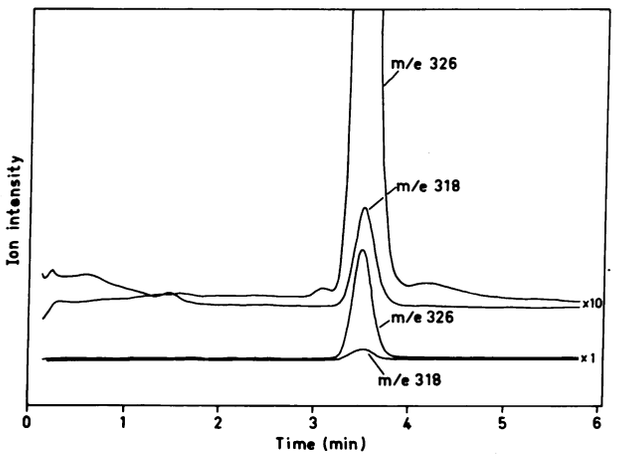

FIG. 1. Multiple-ion analysis recordings from the quantitative determination of free arachidonic acid in a specimen of involved psoriasis epidermis. The ion intensities at m/e 318 and m/e 326 were monitored during gas-liquid chromatography of methyl arachidonate. Column 13% EGSS-X; column temperature, 220°; electron energy, 25 eV; trap current, 120 μA.

The data have been expressed as μg or ng/g wet weight of epidermis. When appropriate, mean values ($\bar{x}$) ± standard error of the mean (SEM) were calculated.

RESULTS

PGE_2 and $PGF_{2\alpha}$ concentrations were measured in eight snap frozen specimens of epidermis (Table 1). The concentration of PGE_2 was 23.6 ± 5.0 ng/g in uninvolved in contrast to 33.1 ± 5.7 ng/g in involved epidermis ($0.001 < P < 0.01$); the concentration of $PGF_{2\alpha}$ was 21.0 ± 4.4 ng in uninvolved in contrast to 39.0 ± 5.9 ng/g in involved epidermis ($0.001 < P < 0.01$). The mean ratios between the levels in involved and uninvolved epidermis were 1.5 ± 0.1 for PGE_2 and 2.3 ± 0.4 for $PGF_{2\alpha}$. Prostaglandin concentrations, and the effects of added arachidonic acid, 5,8,11,14-eicosatetraynoic acid, and indomethacin were determined in incubated samples of epidermis (Table 2). Five specimens incubated at 37° for 10 min in 0.1 M potassium phosphate buffer, pH 8.0 without additions had PGE_2 concentrations of 1.08 ± 0.23 μg/g in uninvolved in contrast to 1.77 ± 0.16 μg/g in involved epidermis ($0.01 < P < 0.025$) and $PGF_{2\alpha}$ concentrations of 0.52 ± 0.09 μg/g in uninvolved and 0.50 ± 0.06 μg/g in involved epidermis ($P < 0.5$). The average ratios of involved to uninvolved epidermis were 1.9 ± 0.4 for PGE_2 and 1.1 ± 0.2 for $PGF_{2\alpha}$. Addition of 2×10^{-5} M arachidonic acid increased the PGE_2 levels 4.5- and 2.5-fold and the $PGF_{2\alpha}$ levels 2.5- and 1.7-fold in an uninvolved and an involved specimen, respectively, whereas 10^{-5} M eicosatetray-

Table 1. Prostaglandin levels in quick-frozen, keratomed samples of uninvolved (U) and involved (I) epidermis from psoriasis patients

Specimen	Prostaglandin E_2 (ng/g wet weight)			Prostaglandin $F_{2\alpha}$ (ng/g wet weight)		
	U	I	I/U	U	I	I/U
502	20	37	1.85	41	62	1.51
503	25	30	1.20	14	62	4.43
495	25	41	1.64	<7	28	>4.00
457 + 497	33	47	1.42	20	46	2.32
449	11	17	1.55	31	36	1.16
500	6	10	1.67	9	18	2.00
515	45	50	1.11	14	22	1.57
498‡	25	§		32	38	1.19
$\bar{x}$ ± SEM	23.6 ± 5.0	33.1 ± 5.7	1.5 ± 0.1	21.0 ± 4.4	39.0 ± 5.9	2.3 ± 0.4
	$(0.001 < P\ 0.01)$*			$(0.001 < P < 0.01)$†		

* Student's *t* test for paired data (6 degrees of freedom) with use of a two-sided hypothesis.
† Student's *t* test for paired data (7 degrees of freedom) with use of a two-sided hypothesis.
‡ Specimen 498 was not included in the statistical analysis for prostaglandin E_2.
§ Not measured.

noic acid inhibited PGE_2 synthesis 28 and 73% and $PGF_{2\alpha}$ synthesis 29 and 26% in a pair of uninvolved and involved specimens, respectively. Indomethacin (10^{-5} M) inhibited PGE_2 and $PGF_{2\alpha}$ synthesis completely.

The metabolic relationships among arachidonic acid, PGE_2, $PGF_{2\alpha}$, and HETE are shown in Fig. 2. Nonesterified arachidonic acid and HETE were measured in snap frozen epidermis specimens from eight psoriasis patients (Fig. 3; specimens 457 and 497 were pooled). The concentration of arachidonic acid was 1.4 ± 0.4 μg/g in uninvolved and 36.3 ± 16.7 μg/g in involved epidermis ($P = 0.015$), and the mean ratio for involved to uninvolved epidermis was 65.8 ± 39.6. Uninvolved epidermis contained <0.05 ± 0.001 μg/g, whereas involved epidermis contained 4.1 ± 1.9 μg/g of HETE ($P = 0.015$; mean ratio of involved to uninvolved 98.5 ± 49.3). Both the arachidonic acid and the HETE levels varied considerably between individual involved specimens (Fig. 3). However, the levels of the two acids were strongly correlated in these specimens ($r = 0.97$). Incubation of epidermis specimens at 37° for 10 min caused the concentrations of arachidonic acid to increase from 3.6 to 8.7 μg/g in a specimen of uninvolved epidermis and from 21.0 to 32.0 μg/g in the corresponding involved specimen (457 + 497). The HETE levels also increased: from <0.04 to 0.33 μg/g in uninvolved and from 3.2 to 3.6 μg/g in involved epidermis. The mean HETE concentration in four incubated specimens was 0.8 ± 0.2 μg/g in uninvolved and 2.3 ± 0.5 μg/g in involved epidermis. Both uninvolved and involved epidermis increased their HETE levels when incubated in the presence of 2×10^{-5} M arachidonic acid; specimen 535 (uninvolved) from 0.73 to 3.66 μg/g and specimen 535 (involved) from 1.72 to 2.60 μg/g. The effects of 10^{-5} M eicosatetraynoic acid and 10^{-5} M indomethacin on arachidonic acid levels and HETE synthesis were determined by incubating the pooled specimens 479 + 520 + 532 in the presence or absence of these inhibitors. Eicosatetraynoic acid markedly in-

Table 2. Prostaglandin levels in incubated samples of uninvolved (U) and involved (I) epidermis from psoriasis patients

Specimen	Additions	Prostaglandin E_2 (ng/g wet weight)			Prostaglandin $F_{2\alpha}$ (ng/g wet weight)		
		U	I	I/U	U	I	I/U
501	None	1780	2100	1.18	690	580	0.84
457 + 497	None	530	1900	3.58	580	390	0.67
521	None	1410	2070	1.47	420	440	1.05
535	None	720	1390	1.93	710	680	0.96
479 + 520 + 532	None	960	1400	1.46	210	390	1.86
$\bar{x}$ ± SEM		1080 ± 229.2	1772 ± 158.1	1.9 ± 0.4	522 ± 93.7	496 ± 57.8	1.1 ± 0.2
		$(0.01 < P < 0.025)$*			$(P > 0.5)$*		
535	2×10^{-5} M 20:4†	3240	3510	1.08	1800	1150	0.64
479 + 520 + 532	10^{-5} M ETA‡	690	380	0.55	150	290	1.93
479 + 520 + 532	10^{-5} M IM§	39	<22	<0.56	<22	<22	—

Powders of epidermis specimens were incubated in 0.1 M $K\text{-}PO_4$ buffer (pH 8.0) at 37° for 10 min with the additions indicated above.
* Student's *t* test for paired data (4 degrees of freedom) with use of a two-sided hypothesis.
† Arachidonic acid.
‡ 5,8,11,14-eicosatetraynoic acid.
§ Indomethacin.

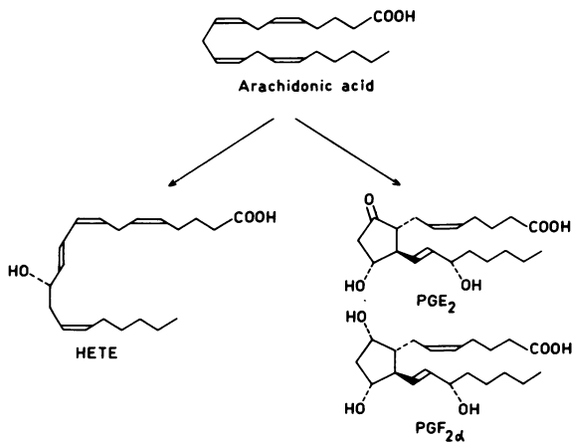

FIG. 2. Metabolic relationships among arachidonic acid, HETE, PGE_2, and $PGF_{2\alpha}$.

hibited HETE synthesis in uninvolved epidermis (0.12 μg/g with in contrast to 1.12 μg/g without the inhibitor). The apparent effect on involved epidermis was less since this tissue contained high concentrations of HETE before incubation (1.00 μg/g with in contrast to 1.92 μg/g without eicosatetraynoic acid). Indomethacin did not inhibit HETE synthesis in either uninvolved or involved epidermis; instead, moderately increased concentrations were observed (uninvolved 1.48 μg/g with and 1.12 μg/g without indomethacin; involved 2.46 μg/g with and 1.92 μg/g without indomethacin). Both eicosatetraynoic acid and indomethacin raised the levels of free arachidonic acid in uninvolved and involved epidermis (uninvolved control: 19.3 μg/g, plus eicosatetraynoic acid: 25.9 μg/g, plus indomethacin: 28.8 μg/g; involved control: 39.4 μg/g, plus eicosatetraynoic acid: 45.4 μg/g, plus indomethacin: 44.7 μg/g).

DISCUSSION

If PGE_2 and $PGF_{2\alpha}$, respectively, are physiological regulators of adenylate and guanylate cyclase in epidermis, increased levels of $PGF_{2\alpha}$ and decreased levels of PGE_2 in involved in contrast to uninvolved epidermis might be expected. The present results indicate that the levels of both PGE_2 and $PGF_{2\alpha}$ were increased in involved in contrast to uninvolved epidermis on a wet weight basis. Virus-transformed cells share certain properties with epidermal cells of psoriasis lesions. Both cell types show increased rates of cell division, decreased differentiation, increased levels of cyclic GMP, and decreased levels of cyclic AMP. It is of interest in this context that polyoma virus-transformed BHK cells had considerably higher concentrations of PGE_2 than ordinary BHK cells (18). One possibility is that the PGE_2 concentrations in involved epidermis and polyoma-transformed BHK cells are increased secondarily to a decreased or lost sensitivity to PGE_2 of adenylate cyclase in these cells. It has been shown that polyoma virus transformation of some cells renders their adenylate cyclase insensitive to E-type prostaglandins (19). Furthermore, it has been claimed that the sensitivity of adenylate cyclase to E-type prostaglandins is reduced in involved compared to uninvolved epidermis (20).

Upon incubation of epidermis specimens at 37° for 10 min, the PGE_2 and $PGF_{2\alpha}$ concentrations increased to about the same extent in uninvolved and involved epidermis. These results disagree with a previous report on decreased conversion of arachidonic acid to PGE_2 and $PGF_{2\alpha}$ by homogenates of involved compared to homogenates of uninvolved epidermis (21). In view of the present results on free arachidonic acid levels in involved and uninvolved epidermis, it seems likely that the discrepancy can be explained by different degrees of dilution of the ^{14}C-labeled arachidonic acid used with endogenous acid. Indomethacin inhibited the synthesis of PGE_2 and $PGF_{2\alpha}$ completely during incubations of epidermis specimens. The lower inhibitory effect of 5,8,11,14-eicosatetraynoic acid may have been due to a requirement of preincubation of this inhibitor with fatty acid cyclo-oxygenase before addition of the substrate (in this case the endogenous arachidonic acid).

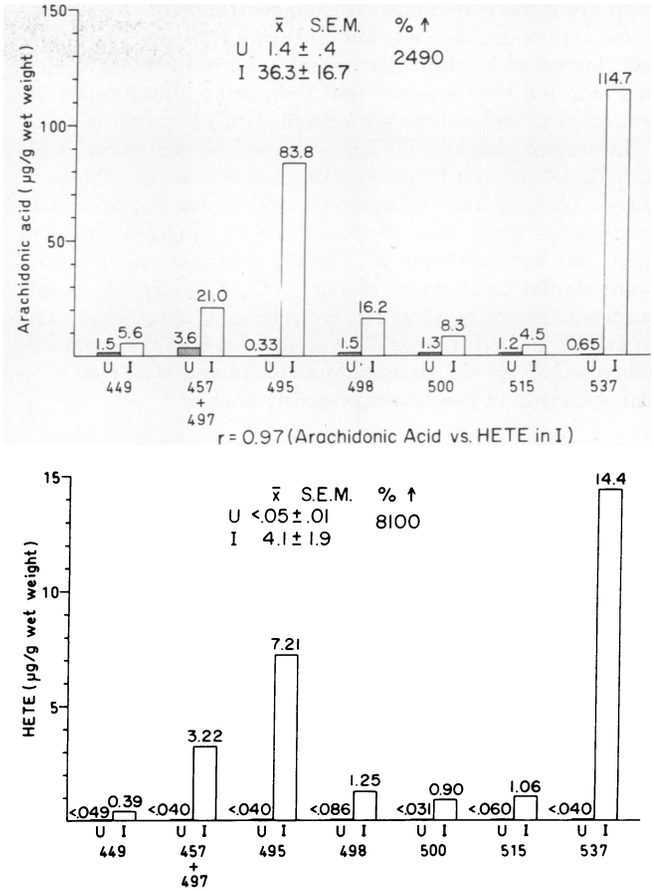

FIG. 3. Endogenous levels of arachidonic acid (*upper panel*) and HETE (*lower panel*) in quick-frozen, keratomed specimens of uninvolved (U, hatched bars) and involved (I, open bars) epidermis from psoriasis patients. "*r*" is the correlation coefficient between the increase in HETE and the increase in arachidonic acid in tissue samples. Both arachidonic acid and HETE levels of all seven specimens were increased in involved in contrast to uninvolved epidermis ($P = 0.015$; sign test with use of a two-sided hypothesis). For the statistical analysis of HETE in uninvolved epidermis, the values used were those at the limits of detection for each individual sample. These values are those shown above the U bars in the lower panel.

The endogenous levels of arachidonic acid and HETE were markedly elevated in involved compared to uninvolved epidermis. On the other hand uninvolved and involved specimens incubated in the presence of added arachidonic acid had similar HETE concentrations, suggesting that the elevated HETE levels in involved epidermis were secondary to the increased arachidonic acid concentrations in the epidermis. There was also a strong correlation between HETE and arachidonic acid levels in involved epidermis,

supporting this contention. The concentration of free arachidonic acid is usually very low in tissues (22), and the markedly increased levels of arachidonic acid in involved epidermis reported here suggests that there is an alteration in the control of these levels in lesional epidermis of psoriasis.

In human platelets, HETE is formed by reduction of 12-*L*-hydroperoxy-5,8,10,14-eicosatetraenoic acid, which in turn is formed from arachidonic acid by the action of a lipoxygenase (12). The effects of 5,8,11,14-eicosatetraynoic acid and indomethacin on HETE synthesis in epidermis were similar to those in platelets (12, 13), suggesting that similar enzymes catalyze the conversion of arachidonic acid to HETE in both tissues. The significance of the markedly increased levels of free arachidonic acid and HETE in lesional epidermis of psoriasis is presently unclear.

We thank Mrs. Saga Elwe, Miss Mona Gyllinger, and Miss Lena Hammar for excellent technical assistance. Noelle M. Acculto, MPH, Department of Dermatology, and M. Anthony Schork, Ph.D., Department of Biostatistics, University of Michigan Medical School, provided statistical analysis of the data. The work was supported by grants from the Swedish Medical Research Council (03X-217) and in part by the National Institute of Arthritis and Metabolic Diseases, research Grant 2P01AM-15740-04.

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