Modulation of constitutive cytochrome P-450 expression *in vivo* and *in vitro* in murine keratinocytes as a function of differentiation and extracellular Ca^{2+} concentration

(xenobiotic metabolism/monooxygenases)

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ABSTRACT A procedure was developed for the per cell estimation of cytochrome P-450-dependent monooxygenase activities in cultures and whole cell suspensions of murine epidermal keratinocytes (MEKs). Murine keratinocytes cultured in medium containing ≤ 0.04 mM Ca^{2+} can be induced to differentiate by raising medium Ca^{2+} concentrations to 1.2 mM. The per cell activities of the monooxygenases 7-ethoxyresorufin *O*-deethylase (7-ER) and 7-ethoxycoumarin *O*-deethylase (7-EC) were elevated $\geq 2090\%$ and $\approx 460\%$, respectively, within 13–24 hr of Ca^{2+} shift. These increases could be completely suppressed by supplementation of culture medium with actinomycin D or cycloheximide immediately prior to Ca^{2+} shift. After prolonged culture in low Ca^{2+} medium, some MEKs detached from the monolayer. These detached cells had the characteristics of differentiating MEKs but did not have elevated 7-EC or 7-ER activities. Percoll gradient centrifugation of freshly isolated dorsal skin MEKs was used to prepare four subpopulations that differed in their stages of terminal differentiation. 7-EC and 7-ER activities varied among these subpopulations and correlated with the degree of MEK differentiation. Specifically, the lowest and highest per cell activities (>7 -fold difference) were in the basal and most differentiated spinous cell populations, respectively. Collectively, the current studies demonstrate that *in vivo* P-450 activities are markedly different in proliferating and differentiating MEKs and suggest that constitutive P-450 expression may be modulated as a function of changes in Ca^{2+} concentration that occur during keratinocyte terminal differentiation.

The epidermis is not an impenetrable barrier. Many xenobiotics can be absorbed by the skin. In many cases the biological consequences of absorption are mediated by metabolites of the parent chemical. Numerous studies demonstrate that murine epidermis has the capacity to metabolize a variety of xenobiotics (1–4) and implicate the cytochrome P-450 system as the source of metabolism (4–8). However, biochemical characterization and quantitation of the epidermal P-450 system are rudimentary at best due to the extremely low levels of activity in the skin.

Although the epidermis is principally composed of keratinocytes (9), there is considerable heterogeneity in the keratinocyte population due to the dynamic nature of skin differentiation. The specific activities of several epidermal enzymes vary with the stage of differentiation (10, 11). The current study was designed to investigate whether cytochrome P-450 expression and content also vary as a function of the stage of keratinocyte differentiation. This study was stimulated by our recent demonstration of enhanced polycyclic aromatic hydrocarbon metabolism in cultures of differentiating mouse epidermal keratinocytes (MEKs) rela-

tive to proliferating MEKs (12). Kinetic analyses of hydrocarbon-dependent mutagenesis in the cultures led us to hypothesize that the differences in metabolism reflected the induction of enzymes capable of hydrocarbon metabolism. However, we were unable to quantitate differences in P-450-dependent monooxygenase activities due to lack of appropriate assay procedures for use in cultured MEKs.

In the current report, we describe a procedure for estimation of the per cell activities of 7-ethoxycoumarin *O*-deethylase (7-EC) and 7-ethoxyresorufin *O*-deethylase (7-ER) in MEKs. Two systems were employed to assess whether the constitutive activities of these P-450-dependent monooxygenases varied with the stage of MEK differentiation *in vivo* and in culture. First, we took advantage of the findings that keratinocyte buoyant density decreases during terminal differentiation (13–16) and that subpopulations of MEKs differing in their stages of differentiation can be prepared by Percoll gradient centrifugation (13–16). Second, we used a culturing procedure in which the proliferation-differentiation potential of cultured MEKs can be modulated as a function of extracellular Ca^{2+} concentration (17). Results obtained in these two systems demonstrate that constitutive P-450 activities in MEKs vary with the stage of differentiation.

MATERIALS AND METHODS

Chemicals. D-Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, NADP⁺, 7-ethoxycoumarin, 7-hydroxycoumarin, and Percoll were from Sigma. 7-Ethoxyresorufin and 7-hydroxyresorufin were obtained from Pierce and Aldrich, respectively. Eagle's minimum essential medium (MEM) containing no Ca^{2+} was purchased from Whittaker M.A. Bioproducts. Fetal bovine serum was obtained from GIBCO.

Preparation of Keratinocytes. Epidermal cell suspensions were prepared from the dorsal skins of adult female SEN-CAR mice (National Cancer Institute, Frederick, MD) as described (11). Cell suspensions were filtered through nylon mesh to remove large squamous sheets and then centrifuged to pellet the cells. The cell pellet was resuspended in low Ca^{2+} MEM containing chelexed 8% fetal bovine serum, overlaid on 15 ml of 30% Percoll [diluted in phosphate-buffered saline (PBS)] and centrifuged for 15 min at $600 \times g$. This step removes debris, small squamous clumps, and granular cells. The resulting pellet was washed once with PBS, counted, and subsequently used for P-450 assays, culture, or further subfractionation. For P-450 analyses the MEKs were repelleted and then resuspended in P-450 buffer (1.6 mg of bovine serum albumin per ml/5 mM MgSO_4 /100 mM Hepes, pH 7.8).

Multiple 0.75-ml samples were transferred to 35-mm tissue culture plates and stored at -70°C . For cell culturing, the MEKs were suspended in low Ca^{2+} MEM supplemented with growth factors and 8% chelexed fetal bovine serum (18). Cells were plated in 60-mm collagen-coated dishes (18) and grown in a humidified 5% CO_2 in air chamber at 37°C . Medium was changed ≈ 48 hr after plating. Nonattached and loosely attached cells were removed 72 hr after plating by washing with PBS. After the addition of fresh medium, MEK terminal differentiation was induced in some cultures by raising medium Ca^{2+} concentration to 1.2 mM by the addition of CaCl_2 . Cultures were pulled at various times relative to medium change. Nonattached and loosely attached MEKs were removed by two PBS washings and pooled with the spent medium. After counting, the detached MEKs were either processed for differentiation assays or washed and suspended in P-450 buffer prior to being stored at -70°C . MEKs remaining attached to the culture dishes were either processed for P-450 analyses or treated with 0.01% trypsin at 37°C and counted.

Percoll Gradient Separation of Keratinocytes. MEKs recovered after the 30% Percoll step were resuspended in 50% Percoll (diluted in PBS) and separated into subpopulations by centrifugation as described (11). Gradient fractions were pooled, diluted with PBS, and centrifuged to pellet the MEKs. The pellets were resuspended in P-450 buffer, counted, and transferred (3 to 8×10^6 cells) to 35-mm tissue culture dishes and stored at -70°C until the time of P-450 analysis.

Keratinocyte Differentiation Assays. MEK terminal differentiation was monitored by determining the percentage of keratinocytes that had formed disulfide cross-linked keratin filaments and cornified envelopes. The former are detected by their insolubility in 1% sodium dodecyl sulfate (SDS) and the latter are detected by their insolubility in 1% SDS and 1 mM dithiothreitol. The procedure we employed has been described in detail (13). For analyses of MEKs attached to culture dishes, the detergent solutions were added directly to washed monolayers.

P-450 Monooxygenase Assays. 7-EC and 7-ER activities were measured by modifications of published protocols (19, 20). Monooxygenase assays were performed in 60- or 35-mm culture dishes containing either cultured MEKs or suspensions of MEKs that had been subjected to a cycle of freeze-thawing. Per cell activities in freeze-thawed cells were at least 20% higher than those measured in nonfrozen MEKs. Final reaction volume was 1.25 ml and contained glucose 6-phosphate (5 mM), NADP^+ (1 mM), glucose-6-phosphate dehydrogenase (2 units), 0.1 mM 7-ethoxycoumarin or 1.5 μM 7-ethoxyphenoxazone (ethoxyresorufin), 1.6 mg of bovine serum albumin per ml, 5 mM MgSO_4 , and 100 mM Hepes (pH 7.8). A cocktail containing all components except substrate was preincubated with the keratinocytes in the culture dishes at 37°C in a humidified chamber for 10–15 min. Reactions were initiated by the addition of 0.125 ml of substrate. 7-EC and 7-ER assays were terminated at various times by the addition of 1.0-ml reaction mixture to 0.4 ml of 5% trichloroacetic acid or 2.0 ml of methanol, respectively. Precipitated protein was removed by centrifugation and 7-hydroxyresorufin was measured in the supernatant fluid by fluorescent detection (550-nm excitation, 585-nm emission) on an SLM Aminco SPF-500C spectrofluorometer. 7-Hydroxycoumarin was measured by fluorescent detection (390-nm excitation, 440-nm emission) after mixing 900 μl of supernatant fluid with 1.8 ml of 1.6 M glycine (pH 10.3). Quantitation was by comparison with standard curves constructed of authentic product. As a control, complete reaction cocktails containing no keratinocytes were processed in parallel with the experimental samples. The fluorescence of

these reactions did not vary with time and were subtracted from the experimental values.

7-EC and 7-ER specific activities were calculated from measured rates of product formation. Rates of product formation were calculated by linear regression analyses of plots of product versus reaction time. These rates were then divided by cell number to yield a specific activity that is expressed as pmol of product per hr per 10^6 MEKs.

RESULTS

Measurement of 7-EC and 7-ER Activities. Measurement of monooxygenase activities in MEKs did not require prior mechanical disruption of the cells. Cultured MEKs and whole cell suspensions of MEKs became permeable to trypan blue after being subjected to a cycle of freeze-thawing. 7-EC and 7-ER activities could be quantitated in these cells (Fig. 1 *a* and *b*, respectively). Product production was linear with time for at least 60 min except in the case of 7-ER activity in MEKs cultured in low Ca^{2+} -containing medium. We were unable to detect any activity in these cells. For those cell systems in which we were able to detect monooxygenase activities, product production was proportional to the number of MEKs used in the assay, and activities were dependent upon the inclusion of a NADPH-generating system and were inhibited by metyraprone and α -naphthoflavone in manners that correlated with the specificities of the inhibitors (J.J.R., unpublished data).

Monooxygenase Activities in Cultured Keratinocytes. The data in Fig. 1 are for cultured MEKs that were Ca^{2+} shifted 16–18 hr prior to harvesting and suggest that 7-EC and 7-ER activities in differentiating MEKs (high Ca^{2+} medium) are greater than the activities found in proliferating MEKs (low Ca^{2+} medium). Detailed analyses of 7-EC and 7-ER activities at various times after Ca^{2+} shift are presented in Fig. 2 *a* and *b*, respectively. Increases in 7-EC specific activities were first noted 10 hr after Ca^{2+} shift and reached a maximum 4–14 hr later. Thereafter, 7-EC specific activities declined. 7-EC activities in MEKs cultured in low Ca^{2+} -containing medium initially increased with time but never approached the activities measured in Ca^{2+} -shifted cultures. The kinetics of

FIG. 1. 7-EC- and 7-ER-dependent product formation as a function of incubation time. 7-Hydroxycoumarin (*a*) and 7-hydroxyresorufin (*b*) formation were measured in suspensions of murine dorsal skin keratinocytes (■) or keratinocytes cultured in low (○) or high (●) Ca^{2+} -containing medium for 14–16 hr. Cell numbers used in the studies were as follows: 7-EC and 7-ER low and high Ca^{2+} cultures, 2.24×10^6 ; 7-EC MEK suspensions, 5.94×10^6 ; 7-ER MEK suspensions, 3.25×10^6 .

FIG. 2. Kinetics of increases in 7-EC (a) and 7-ER (b) specific activities in cultured keratinocytes following Ca^{2+} shift. Adult MEKs were cultured for 3 days in low Ca^{2+} -containing medium prior to medium change and Ca^{2+} shift (indicated by arrow). $\circ$, Low Ca^{2+} attached MEKs; $\triangle$, low Ca^{2+} detached MEKs; $\bullet$, high Ca^{2+} attached MEKs.

increase and decline of 7-ER specific activities in Ca^{2+} -shifted cultures paralleled 7-EC activity. However, 7-ER activity was detected sooner (by 6 hr). In general, maximum 7-EC and 7-ER specific activities were measured 12–24 hr after Ca^{2+} shift, and 7-EC activities were increased 5-fold (Table 1). Because of our inability to measure 7-ER activity in MEKs cultured in low Ca^{2+} , it is difficult to assign a fold increase for 7-ER (Fig. 2b, Table 1). However, based upon the limits of product detection and the numbers of cells we employed, we should have been able to detect 0.2 pmol/hr per 10^6 cells. If this value is used as an upper limit of 7-ER activity for MEKs grown in low Ca^{2+} medium, 7-ER specific activities were increased at least 21-fold following Ca^{2+} shift (Table 1).

Very few MEKs detached from the culture dishes following Ca^{2+} shift (Fig. 3a). In the attached population of Ca^{2+} -shifted cultures the increases in 7-EC and 7-ER activities precede the formation of either disulfide cross-linked keratin filaments or cornified envelopes, two markers of late-stage MEK differentiation (compare Fig. 2 with Fig. 3b and c). Indeed, 7-EC and 7-ER activities had appreciably declined by the time 50% of the MEKs had formed cornified envelopes. These results suggest that the elevations and declines in monooxygenase activities detected in Ca^{2+} -shifted MEK cultures occur during the early and late stages of differentiation, respectively.

In our culturing protocol, MEKs rapidly proliferate at 37°C in low Ca^{2+} medium during the first 3 days of culturing.

Table 1. 7-EC and 7-ER specific activities in cultured MEKs following Ca^{2+} shift

P-450	Time of maximum,* hr	Specific activity, pmol/hr per 10^6 cells		Fold increase
		Low Ca^{2+}	High Ca^{2+}	
7-EC	14–22	52.5 ± 13.5	281.8 ± 72.9	5.6 ± 0.7
7-ER	13–24	ND [†]	4.4 ± 1.1	$\geq 21.9 \pm 5.8^\ddagger$

Adult MEKs were cultured for 3 days in low Ca^{2+} -containing medium prior to medium change and Ca^{2+} shift. Data are mean $\pm$ SEM of five to nine experiments.

*Time after Ca^{2+} shift.

[†]Activity not detectable with $\leq 4 \times 10^6$ cells.

[‡]Calculated using an empirically derived detection limit for 7-hydroxyresorufin of 0.2 pmol/hr per 10^6 cells for 4×10^6 cells.

FIG. 3. Kinetics of differentiation of murine keratinocytes cultured in low and high Ca^{2+} -containing medium. Adult MEKs were cultured for 3 days in low Ca^{2+} -containing medium prior to medium change and Ca^{2+} shift. Cells insoluble in SDS and SDS plus dithiothreitol (DTT) represent MEKs having formed disulfide cross-linked keratins and cornified envelopes, respectively. Ca^{2+} was added at the time of medium change. Values are the mean $\pm$ SEM of a minimum of three experiments. $\triangle$, Low Ca^{2+} attached MEKs; $\circ$, low Ca^{2+} detached MEKs; $\bullet$, high Ca^{2+} attached MEKs; $\square$, high Ca^{2+} detached MEKs. All values were calculated as a percentage of the total number of cells per plate (attached plus detached).

Thereafter, proliferation markedly decreases and the cells detach from the monolayer (Fig. 3a). These detached cells were spherical and had buoyant densities characteristic of suprabasal cells (J.J.R., unpublished data). Some of these cells expressed a basic 67-kDa cytokeratin (21) and with time became insoluble in 1% SDS solutions (Fig. 2b). These characteristics suggest that they have undergone a form of terminal differentiation (13, 21–23). The failure of this MEK population to cornify (Fig. 3c) is possibly due to the Ca^{2+} requirement of epidermal transglutaminase, an enzyme involved in the cornification process (24). 7-EC and 7-ER specific activities in this detached low Ca^{2+} MEK population were equivalent to, or less than, the values measured in the attached MEKs cultured in low Ca^{2+} -containing medium (Fig. 2).

Inhibition of 7-ER and 7-EC Induction. Supplementation of culture medium with actinomycin D or cycloheximide at the time of Ca^{2+} shift prevented the increases in MEK 7-EC and 7-ER specific activities that normally occurred following Ca^{2+} shift (Table 2). Activities were suppressed to levels either equivalent to or less than the values measured in cultures maintained in low Ca^{2+} medium. Consequently, *de novo* RNA synthesis and protein synthesis are necessary for the increases in monooxygenase activities that occur following Ca^{2+} shift.

In Vivo Monooxygenase Activities. Keratinocyte buoyant density decreases as MEKs terminally differentiate (13–16). Four subpopulations of MEKs differing in their stages of differentiation were prepared by Percoll gradient centrifuga-

Table 2. Inhibition of Ca^{2+} -dependent elevation of 7-EC and 7-ER activities in cultured keratinocytes by actinomycin D and cycloheximide

Ca^{2+} in medium	Treatment	Dose, μM	% of Ca^{2+} -induced activity	
			7-EC	7-ER
—	—	—	16.0	ND
+	—	—	100.0	100.0
+	Act-D	0.01	39.7	43.8
+	Act-D	0.05	12.3	—
+	Act-D	0.1	11.5	ND
+	Act-D	1.0	8.2	—
—	—	—	16.0	ND
+	—	—	100.0	100.0
+	Chx	0.35	1.9	67.2
+	Chx	3.50	ND	ND

Adult MEKs were cultured in low Ca^{2+} -containing medium for 3 days prior to medium change and Ca^{2+} shift. Actinomycin D (Act-D) and cycloheximide (Chx) were added to the medium 10–15 min prior to the time of Ca^{2+} shift. Analyses are of cultures pulled 16 hr after medium change or Ca^{2+} shift. ND, not detected.

tion (Table 3). Based upon a variety of criteria, our P_4 fraction consisted of basal cells (13–16). The P_3 fraction contained basal and spinous cells, whereas the P_2 and P_1 fractions contained spinous cells. The P_1 fraction also contained nucleated squames and granular cells (<25% of total cell count). Gradients of 7-EC and 7-ER specific activities were measured in these four populations (Table 3). The lowest specific activities for either monooxygenase were measured in the P_4 fraction, and the highest specific activities were measured in the P_1 fraction, which contained the most differentiated spinous MEKs. Mathematical reconstitution of the four gradient fractions yielded composite specific activities that were very similar to unfractionated MEK activities (Table 3). These studies demonstrate that 7-EC and 7-ER activities in unseparated MEKs reflect the additive activities of the various MEK subpopulations and that constitutive P-450 expression varies with the stage of MEK differentiation.

DISCUSSION

In 1983 Coomes *et al.* (25) suggested that there were subpopulations of MEKs that differed in their P-450 activities. These investigators used a Percoll step gradient to prepare a differentiated MEK subpopulation and an enriched basal cell population ($\approx 30\%$ basal cells). Per cell 7-EC activity in the enriched basal cell population was $\approx 36\%$ of the activity measured in the differentiated cell fraction. In a later study,

Table 3. Variation in keratinocyte 7-EC and 7-ER activities as a function of the stage of terminal differentiation

Percoll fraction	Density, g/ml	Specific activity, pmol/hr per 10^6 cells			
		7-EC	7-EC	7-ER	7-ER
Unfractionated	—	27.56	15.47	0.39	1.03
P_1	≤ 1.055	64.35	24.52	1.75	1.66
P_2	1.055–1.062	20.50	17.57	0.63	0.50
P_3	1.062–1.100	18.40	6.98	0.17	0.13
P_4	≥ 1.100	5.48	3.3	ND*	ND*
Reconstituted†	—	36.63	15.34	0.52	0.82

Murine keratinocytes isolated from the dorsal skins of SENCAR mice were separated on 50% Percoll gradients into four fractions that differed in their buoyant densities. Analyses were performed on $3.2\text{--}9 \times 10^6$ cells.

*Not detectable with 3.6×10^6 MEKs per dish.

†Calculated as the sum of the products of specific activities times the percentage of the gradient population for the four subpopulations.

Pohl *et al.* (6) used metrizamide gradients and elutriation to separate dorsal MEKs into populations differing in size, and hence the stage of differentiation. A 2- to 4-fold gradient of 7-EC specific activities (calculated as pmol/min per mg of DNA) was measured in cell sonicates. The lowest and highest specific activities were measured in the smallest (least differentiated) and largest cells, respectively. Unfortunately, interpretation of the data was complicated by contamination of the larger cell populations with clumps of smaller cells and varying DNA recoveries in the subpopulations. The current study used a procedure that gives highly purified preparations of basal cells and demonstrates a continuous gradient of per cell 7-EC and 7-ER activities in four subpopulations of dorsal MEKs that differed in their stages of differentiation. Per cell activities in a basal cell fraction were minimally 7-fold less than the activities measured in the most differentiated preparation of spinous MEKs. Similar results were obtained in a culturing model in which MEK differentiation was modulated as a function of extracellular Ca^{2+} concentration.

Ion capture cytochemical analyses of fixed sections of skin suggest that there is a Ca^{2+} gradient *in vivo* in the epidermis, with little or no Ca^{2+} in the cytoplasm of basal cells (26). Given the *in vivo* gradient of P-450 activities in the epidermis and the modulation of P-450 expression in culture as a function of Ca^{2+} concentration, it is conceivable that constitutive P-450 expression *in vivo* in the epidermis may be modulated as a function of changes in Ca^{2+} concentration that occur during keratinocyte differentiation. On the other hand, the correlation between Ca^{2+} and P-450 gradients may be coincidental and constitutive P-450 expression in the epidermis may be a characteristic of the differentiating phenotype, and independent of Ca^{2+} . We have several findings that are relevant to these two possibilities. First, some of the detached MEKs that accumulated with time in low Ca^{2+} -maintained cultures had characteristics of differentiating MEKs. 7-EC and 7-ER activities in these cells were comparable to the more basal-like cells that remained attached to the culture dishes (Fig. 2). *A priori*, this suggests that maximal constitutive P-450 expression in culture may be dependent upon intracellular Ca^{2+} concentration. Second, MEKs cultured at 32°C can be induced to differentiate by Ca^{2+} shift in a manner analogous (but slower) to that occurring at 37°C (J.J.R., unpublished data). We have been unable to measure 7-EC activity in MEKs grown at 32°C in low Ca^{2+} -containing medium and measured only marginal activity in Ca^{2+} -shifted cultures. This would suggest that changes in Ca^{2+} concentration and the induction of differentiation, in some circumstances, are insufficient for constitutive P-450 expression in MEKs. These seemingly incongruous findings can be rationalized on the basis of the proliferative status of the cultures and the timing of the Ca^{2+} shift in our studies. MEKs plated in our low Ca^{2+} medium at 37°C proliferate for only 3 days. Thereafter, the cells round-up, detach, and acquire some features that are characteristic of differentiating MEKs. In the current study cultures were Ca^{2+} shifted at a time when a majority of the cells had just ceased dividing. In contrast, a majority of the MEKs cultured at 32°C were actively proliferating at the time of Ca^{2+} shift. We recently determined that shifting cultured MEKs from 32°C to 37°C terminates cell proliferation and results in a time-dependent responsiveness to Ca^{2+} and expression of 7-EC activity that are comparable to MEKs cultured at 37°C from the onset (J.J.R., unpublished data). Collectively, these studies suggest that the Ca^{2+} -dependent modulation of P-450 expression in cultured MEKs is dependent upon the occurrence of an event at the time of, or shortly after, the cessation of proliferation. Once this event occurs, Ca^{2+} shift can facilitate maximal constitutive P-450 expression. Presumably there is marginal P-450 expression in MEKs cultured at 32°C following Ca^{2+} shift because this early event is bypassed and does

not occur. Relevant to this hypothesis is the recent report of Yuspa *et al.* (27), who reported that the induction of MEK differentiation by adjustment of medium Ca^{2+} concentration to 1.2 mM bypasses many of the differentiation-related events that occur during normal spinous cell maturation and the basal to spinous cell transition. Based upon our Percoll studies, we predict that constitutive P-450 expression would occur specifically during these times. Collectively, our studies suggest that constitutive P-450 expression in MEKs is a component of the differentiation program that can be influenced by Ca^{2+} .

The Percoll-derived subpopulation of MEKs having the lowest per cell P-450 activities was highly enriched for cells having a basal cell phenotype and the capacity for proliferation *in vitro* (13–16). The correlation we observed between P-450 activities and the proliferation/differentiation status of MEKs is not unique to the epidermis. Hepatic P-450 levels and activities vary markedly with the tissues' rate of proliferation. P-450s are virtually absent in the rapidly proliferating fetal liver up until just before birth (28, 29) and are dramatically decreased on a per mg of protein basis following partial hepatectomy (30, 31). P-450 species and activities are also dramatically reduced in rapidly growing spontaneous and chemically induced hepatic nodules and hepatomas (32, 33). Collectively these studies suggest that constitutive P-450 expression in epithelial cells may be regulated as a function of the tissues proliferation/differentiation status.

The demonstration that the monooxygenase activities of MEKs vary with their stage of differentiation *in vivo*, and with medium Ca^{2+} concentration *in vitro*, has several implications. (i) The current study provides an enzymatic basis for the observed differences in the metabolic activation of chemicals by MEKs cultured in low and high Ca^{2+} -containing medium (12). These findings are relevant to investigators using cultured MEKs for the bioassaying of chemical genotoxicity in MEK mutagenesis and transformation assays. (ii) The 67-kDa cytochrome K1 is considered to be the earliest marker of MEK differentiation (22, 23). K1 is found in the spinous layer and in some basal cells that have committed to differentiation but have not yet migrated into the suprabasal layer (22, 23, 34). Our Percoll studies (Table 3) suggest a comparable distribution of 7-EC and 7-ER activities. Maximum accumulation of K1 mRNA and protein in cultured MEKs occurs 12 hr and 24 hr, respectively, after Ca^{2+} shift (27). We have detected increases in 7-ER activity within 6 hr of Ca^{2+} shift, with a maximum being reached as early as 13 hr after induction of differentiation. Consequently, 7-EC expression and 7-ER expression in MEKs are very early markers for keratinocyte commitment to differentiation. (iii) Our studies suggest that modulation of epidermal differentiation *in vivo* may markedly alter epidermal xenobiotic metabolism. Indeed, treatment of murine epidermis with retinoic acid prior to treatment with polycyclic aromatic hydrocarbons increased the numbers of papillomas that developed in experimental initiation-promotion and complete carcinogenesis protocols (35, 36). The basis for this potentiation of papilloma development is unknown. However, retinoic acid is a potent modulator of epithelial differentiation (37, 38). This third point may have human health significance since a variety of agents capable of modulating epidermal differentiation are used topically in clinical and cosmetic applications.

Note Added in Proof. Since the submission of this manuscript, a report investigating aryl hydrocarbon hydroxylase activity in rat epidermal cells has been published (39) which has findings and conclusions similar to our own.

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