Metabolism of all-*trans*-retinoic acid by cultured human epidermal keratinocytes

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Abstract The uptake and metabolism of exogenous all-*trans*-retinoic acid by human epidermal keratinocytes in submerged culture was examined. Retinoic acid presented to keratinocytes in physiological form bound to albumin was rapidly taken up. Peak cellular concentrations were achieved within 1 h and were 50- to 100-fold higher than that in medium. Retinoic acid metabolism was less rapid but was vigorous, exhibiting a half-life of 6 h. Neither uptake nor metabolism was saturable at medium retinoic acid concentrations up to 1 μM. Eighty-five percent of retinoic acid was metabolized to unidentified polar compounds which were excreted from cells to the medium. The production and clearance of the polar metabolites was inhibited 60% by 10 μM ketoconazole. Fifteen percent of intracellular retinoic acid was converted to 3,4-didehydroretinoic acid, was proportional to cellular retinoic acid concentration, and was not affected by ketoconazole. Cellular retinyl ester content, which is sensitive to exogenous retinoic acid, was correlated with both the concentration and the quantity of retinoic acid in the culture system. These results suggest that the metabolism of retinoic acid in keratinocytes is substrate limited and has potential to limit the availability of exogenous retinoic acid for retinoid signaling.—**Randolph, R. K., and M. Simon.** Metabolism of all-*trans*-retinoic acid by cultured human epidermal keratinocytes. *J. Lipid Res.* 1997. **38:** 1374–1383.

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Retinoic acid (RA) is an active form of vitamin A, retinol (Roh), that is essential for normal epithelial homeostasis (1). This signaling molecule exerts its regulatory actions in target cells by binding to nuclear retinoid receptors, thus influencing the transcription of retinoid-responsive genes (reviewed in refs. 1, 2). Retinoic acid is potentially available to epithelial tissues from two sources. First, RA can be generated in situ after the uptake and local metabolism of plasma Roh that is transported bound to retinol-binding protein (3). In tissues such as bone marrow and adipose tissue, chylomicron retinyl ester also serves as a source of Roh (3). Second, in addition to Roh, RA is also present in the circulation bound to albumin (3) where it is taken up by a variety of cell types (4). Both Roh and RA are present in the plasma under normal physiological circumstances although at different concentrations. Retinol is present in human plasma at 1–2 μM (3, 5) and RA is present at 4–14 nM (6, 7). Interestingly, exogenous RA contributes significantly to tissue RA levels in some tissues (4).

The differentiation program of epidermal keratinocytes is sensitive to exogenous retinoid, including Roh and RA (for reviews see refs. 8–10). Terminal differentiation is alternatively suppressed or augmented by an excess or deficiency, respectively, of these retinoids. For example, Asselineau et al. (11) have demonstrated that keratinocytes maintained on dermal equivalent cultures at the air–liquid interface exhibit a normal pattern of differentiation only when medium contains nanomolar concentrations of RA. Retinoic acid concentrations exceeding this range shifted the pattern of differentiation toward that of hyperproliferation and suppressed differentiation. Lower concentrations of RA led to the formation of an orthokeratotic epithelium. Two conclusions follow these and other similar observations. One, RA is essential for normal keratinocyte differentiation. Two, normal differentiation is observed when extracellular RA concentration is in the low nanomolar range, concentrations either above or below this do not support normal differentiation.

Retinoic acid has indeed been detected in intact epidermis (12–14). Normal epidermal RA concentrations have been reported to be 20 nM or lower (13, 14), similar to that found in the plasma (6, 7). The maintenance of nanomolar concentrations of RA in normal epidermis is thought to be due, at least in part, to a low rate of synthesis from Roh (13). However, uptake and me-

Abbreviations: RA, retinoic acid; 4-oxo-RA, 4-oxo-retinoic acid; Roh, retinol; 3,4-ddRoh, 3,4-didehydroretinol; 3,4-ddRA, 3,4-didehydroretinoic acid.

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tabolism of exogenous RA could also contribute to the maintenance of suitably low concentrations of RA in the epidermis. The present work was undertaken to characterize the uptake and metabolism of RA by epidermal keratinocytes. We have chosen to approach this problem initially by examining RA metabolism in the submerged culture model which exhibits many features of wound healing epithelium (15).

MATERIALS AND METHODS

[11,12-^{3}H(N)]-all-*trans*-retinoic acid, 50 Ci/mmol, was purchased from New England Nuclear. All-*trans*-retinoic acid and fatty acid-free bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Ketoconazole was obtained from Research Diagnostics, Inc. (Flanders, NJ). The authentic retinoid standards, 3,4-didehydro-all-*trans*-retinoic acid (3,4-ddRA) and 4-oxo-all-*trans*-retinoic acid (4-oxo-RA), were the generous gift from Hoffmann-La Roche (Nutley, NJ).

Experimental protocol

The overall experimental protocol for labeling and analyzing keratinocyte-associated retinoids has been described in detail previously (16). Brief descriptions of the individual experimental elements are described below.

Cell culture

Human epidermal keratinocyte strains were obtained from biopsies of three healthy adults. Cells were grown in submerged culture conditions utilizing gamma-irradiated 3T3 feeders (17) in a basal growth medium consisting of a 3:1 (v/v) mixture of Dulbecco's Minimum Essential Medium and Ham's F-12 medium with supplements as described previously (16). Stock cultures of keratinocytes were maintained and passaged for experiments in this basal medium containing 5% fetal bovine serum (FBS; Hyclone).

Keratinocytes for experiments were of passage six or less and were allowed to grow to confluence (7–8 days after plating) with medium changes every other day prior to initiation of experiments.

[^{3}H]retinoid substrates

All retinoids were stored in the dark under an atmosphere of N_2 at −20°C and when out of storage were kept on ice under amber fluorescent lighting (GE F40 Gold). [^{3}H]retinoic acid-containing experimental medium was prepared on the day of experiments. Both unlabeled and radiolabeled RA were purified by normal phase high performance liquid chromatography (HPLC; described below). On the day of experiments freshly purified [^{3}H]RA was dried under a stream of nitrogen and was then dissolved in 40 µl dimethylsulfoxide and added to a sterile 100 mg/ml solution of fatty acid-free bovine serum albumin (BSA). The [^{3}H]RA–albumin preparation was added to basal medium containing 5% FBS such that the final added albumin concentration was 1 mg/ml. Together the added BSA and the native albumin present in 5% FBS yielded a final medium albumin concentration of 45 µM. This albumin concentration compares to an albumin concentration in dermal interstitial fluid of 300 µM (18). The binding and stability of [^{3}H]RA in the BSA preparations and in experimental medium was evaluated two ways. First, [^{3}H]RA-BSA preparations were applied to a Sephadex G-150 column and protein and tritium were monitored in the column eluate. Protein and radioactivity coeluted in the retained volume of the column. When [^{3}H]RA was added to protein-free medium, no [^{3}H]RA was eluted from the column. Second, cell-free incubations of medium containing [^{3}H]RA, BSA (1 mg/ml), and 5% FBS were conducted in parallel with experiments (see control in Fig. 1). In such incubations, approximately 85% of the ^{3}H was recovered as authentic [^{3}H]all-*trans*-RA. The final concentration of radiolabeled RA added to cells was approximately 3.8×10^5 cpm/ml (see below). Except as indicated, experiments were performed with a [^{3}H]RA concentration of 1 nM. In these studies, the albumin (BSA) to RA mole ratio was approximately 45,000, which compares to an albumin to RA mole ratio range of 43,000–150,000 in human plasma, based on a plasma RA concentration range of 4–14 nM (6, 7) and a plasma albumin concentration of 600 µM (18).

Experimental treatments and measurements

After purification, [^{3}H]RA complexed with BSA was added to the basal medium described above for maintenance of keratinocyte cultures. Medium containing [^{3}H]RA was prepared on the day experiments were initiated. Experiments were initiated by the addition of medium containing [^{3}H]RA to cultures. Where indicated, ketoconazole was added to cultures dissolved in dimethylsulfoxide. For this a 10 mM stock solution was added to cultures at a volume:volume ratio of 1000 to yield a final ketoconazole concentration of 10 µM.

Retinoid analysis

After experimental incubations with [^{3}H]RA, keratinocytes and medium were harvested separately; [^{3}H]retinoids were extracted and analyzed by HPLC. To 1-ml aliquots of a sonicated cell suspension or medium were added 50 pmol each of the following internal standards: 4-oxo-RA, 3,4-ddRA, and RA. The latter two retinoids have been previously shown to be present in cultured human epidermal keratinocytes (16). Total

[^{3}H]retinoids were extracted by the method described by Barua (19). The organic solvents for this extraction all contained butylated hydroxytoluene (0.05%). The recovery of ^{3}H and internal standards by this extraction method was consistently greater than 96%. Extracts were dried under N_2 and dissolved in 100 μl of mobile phase, all of which was injected for HPLC analysis.

Two separate HPLC protocols were utilized during these studies. The first used a reverse phase (Waters 3.9 mm × 300 mm C18 NovaPak analytical column) gradient system and was utilized for quantitation of the polar RA metabolites produced by or present in keratinocytes (16, 19). The second HPLC protocol, a normal phase system, was used for the purification of all-*trans*-RA (19). It consisted of a Waters NovaPak 3.9 × 150 mm silica analytical column and an isocratic mobile phase consisting of hexanes, dichloromethane, and glacial acetic acid (170:30:0.1; v:v:v) with a flow rate of 1.5 ml/min.

The identity of all-*trans*-RA and all-*trans*-3,4-ddRA was checked by rechromatographing the methylated derivatives (16) by reverse phase HPLC as described above.

Column effluent was monitored for ^{3}H by scintillation counting of 0.25-min fractions. Fractions for scintillation counting were collected directly into 7-ml scintillation vials, mixed with 3 ml Scintiverse BD (Fisher) and counted in an LKB Rackbeta scintillation counter. The efficiency of counting in this system was 35%.

Individual ^{3}H-labeled retinoids were identified by matching retention times of isotope peaks with those of the added internal standards. The individual [^{3}H]retinoids were quantitated by summing the background-corrected cpm in each peak. In some cases, background-corrected cpm were converted to pmol based on the specific activity of the original substrate[^{3}H]retinoid. Typical background radioactivity ranged between 25 and 40 cpm/fraction. Cell protein was determined by the method described by Markwell et al. (21).

As indicated in figure legends, results from typical separate experiments are presented. As intraexperimental variation was always less than 15%, only mean values are shown.

RESULTS

Identification of retinoic acid metabolites

The first objective of these studies was to identify the metabolites of RA that are produced by cultured keratinocytes. The data in **Fig. 1** depict representative metabolites of RA as analyzed by reverse phase HPLC (left hand panels) and normal phase HPLC (right hand panels) which were observed in all experiments. Note that the different Y-axis ranges between the reverse phase and normal phase chromatograms reflect reproducible differences in recoveries in these two chromatography systems. A small quantity of 13-*cis*-RA was consistently observed to form spontaneously upon addition of freshly purified all-*trans*-RA to the medium (see control medium chromatograms). This is in contrast to the recovery of only all-*trans*-RA when this retinoid was reinjected after normal phase purification (data not shown). Although 13-*cis*-RA accounted for up to 15% of total ^{3}H in control medium, this retinoid was apparently not taken up by cells to any great extent and its concentration did not change appreciably in either medium or cells with additional incubation time.

After the uptake of [^{3}H]all-*trans*-RA, two major peaks of tritium-labeled metabolites were produced by confluent keratinocyte cultures. One, all-*trans*-3,4-ddRA, was present predominantly in cells at levels up to 15% of those observed for RA. In this reverse phase chromatography system, 3,4-ddRA exhibits a retention time approximately one-half minute earlier than 13-*cis*-RA. By normal phase chromatography 3,4-ddRA was clearly resolved from other retinoids. Although not evident in these data, this metabolite was also observed in the medium in some experiments. Two, a significant quantity of unretained radioactivity was observed at the column void volume (reverse phase chromatography, V_O) in extracts of medium. This polar material was also present in cell extracts but in lower quantities than that in medium. The V_O material was completely retained in the normal phase HPLC system.

In addition to matching retention times in reverse and normal phase HPLC systems, the metabolite exhibiting chromatographic behavior similar to 3,4-ddRA was subjected to methylation with diazomethane and was rechromatographed by reverse phase HPLC. Under these conditions, the metabolite in question underwent methylation as a single peak and exhibited a retention time identical to authentic methyl-3,4-didehydroretinoate (data not shown).

The radioactive material eluting from the reverse phase column at the column void was methylated and rechromatographed by reverse phase HPLC. Approximately 80% of the radioactivity was methylated as suggested by its subsequent retention on the reverse phase column. However, the methylated V_O material eluted as a broad smear of radioactivity between 6 and 14 min (data not shown). These metabolites were not further characterized.

It is noteworthy that in no instance were carbon 4 metabolites of RA, such as 4-oxo-RA, observed in cells or medium. This metabolite exhibits a retention time

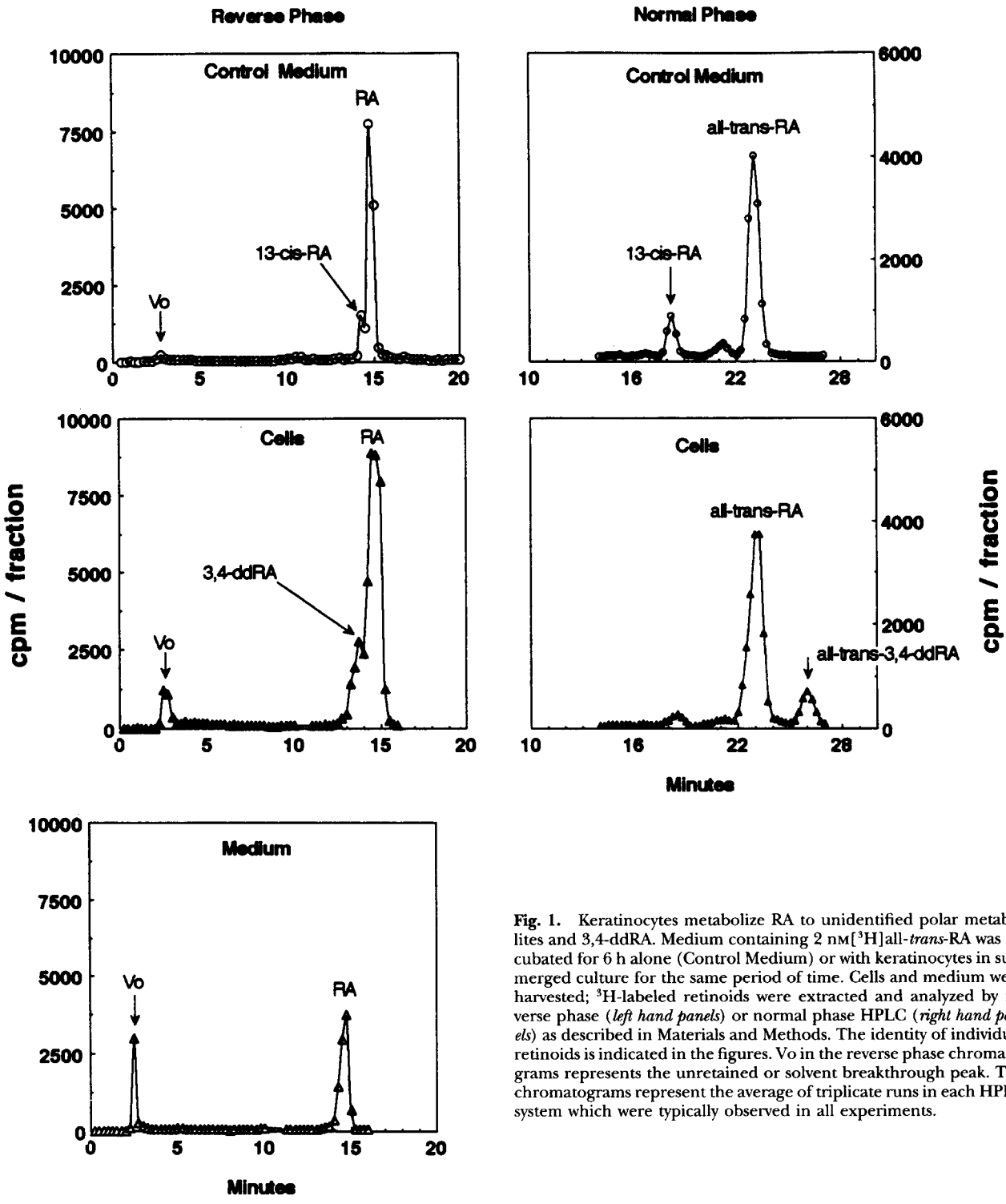

Fig. 1. Keratinocytes metabolize RA to unidentified polar metabolites and 3,4-ddRA. Medium containing 2 nM[^{3}H]all-*trans*-RA was incubated for 6 h alone (Control Medium) or with keratinocytes in submerged culture for the same period of time. Cells and medium were harvested; ^{3}H-labeled retinoids were extracted and analyzed by reverse phase (*left hand panels*) or normal phase HPLC (*right hand panels*) as described in Materials and Methods. The identity of individual retinoids is indicated in the figures. Vo in the reverse phase chromatograms represents the unretained or solvent breakthrough peak. The chromatograms represent the average of triplicate runs in each HPLC system which were typically observed in all experiments.

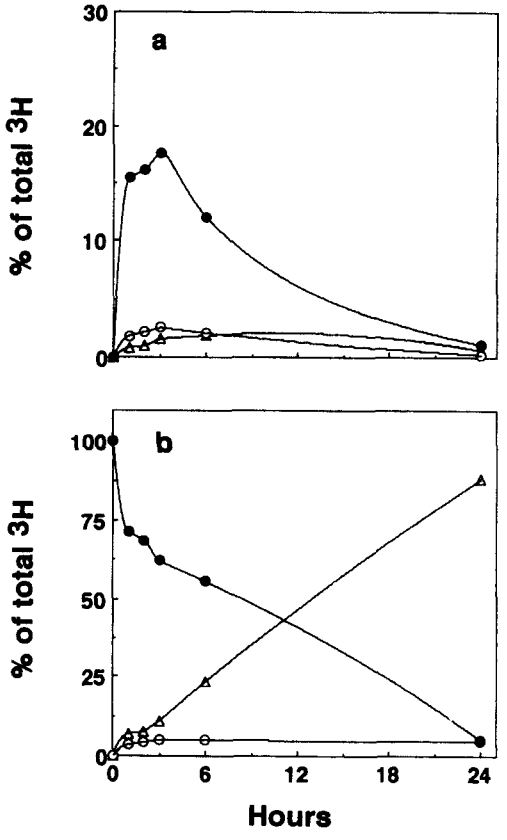

Fig. 2. Cultured keratinocytes rapidly and completely metabolize RA. Keratinocytes were incubated with medium containing 1 nM [^{3}H]RA for the indicated periods of time. Cells (a) and medium (b) were harvested and analyzed by reverse phase HPLC. Filled circles (●) represent RA; open circles (○) 3,4-ddRA; open triangles (△) polar metabolites eluting in the column Vo. Data represent the percentage of total ^{3}H present in the dish during the experiment. This experiment was conducted in 100-mm dishes, each containing 6 mL of medium. Each data point represents the average of triplicate samples from one experiment which is representative of three.

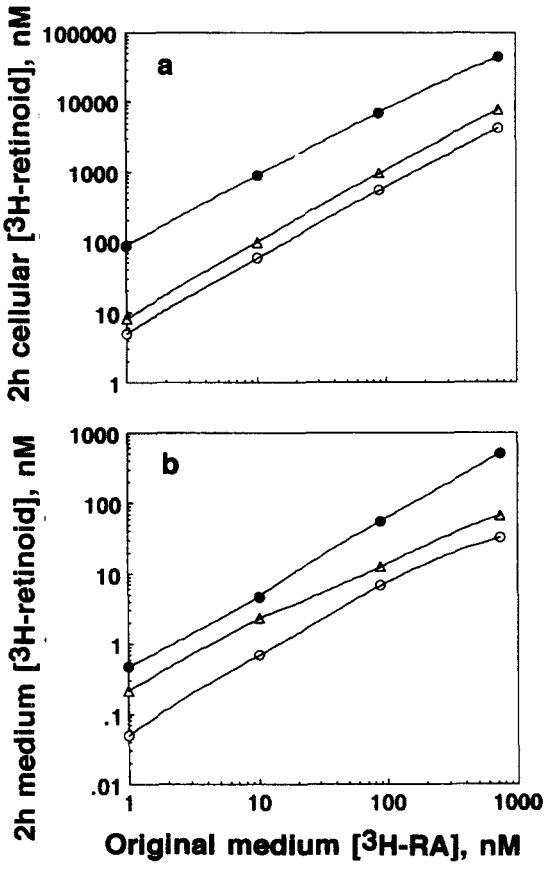

Fig. 3. Very high capacity uptake and metabolism of RA by keratinocytes is not saturated at RA concentrations up to 1 μM. Keratinocytes were incubated with medium containing the indicated concentrations of [^{3}H]RA for 2 h. Cells (a) and medium (b) were harvested and analyzed by reverse phase HPLC. Filled circles (●) RA; open circles (○) 3,4-ddRA; open triangles (△) polar metabolites eluting in the column Vo. Data are plotted as the nM concentrations measured at 2 h in the cells or medium versus the original medium [^{3}H]RA concentration. Each data point represents the average of triplicate samples from one experiment which was repeated with similar results.

of 6.5 min in this reverse phase chromatography system. As indicated in Materials and Methods, authentic 4-oxo-RA was added to cell and medium extracts and was recovered with high efficiency. In addition, a radioactive metabolite exhibiting a retention time similar to retinoyl glucuronide (approximately 10 min) on reverse phase HPLC was not observed.

Kinetics of retinoic acid uptake and metabolism

As shown in **Fig. 2a,** RA was rapidly taken up from the medium. Peak cellular levels were achieved within 1–2 h and decreased thereafter with a half life of 6 h. Cellular RA was rapidly metabolized to very polar metabolites (V_O), almost all of which were excreted from the cells into the medium (Fig. 2b). Concomitant with the increase in medium polar metabolites was the disappearance of medium RA, almost all RA was metabolized by 24 h. Cellular levels of 3,4-ddRA increased gradually to 6 h and decreased thereafter (Fig. 2a).

Retinoic acid metabolism as a function of retinoic acid concentration

The cellular concentrations of RA and its metabolites that were sustained under physiological to pharmacological conditions of exogenous RA availability were also determined. Exogenous RA was strikingly concentrated in keratinocytes 50- to 100-fold relative to the original medium RA concentration (**Fig. 3a**). Moreover, cellular levels of RA were linearly related to original medium RA over a concentration range extending between 1 nM and 1 μM. Retinoic acid uptake was not saturated over this concentration range.

Cellular concentrations of polar metabolites and 3,4-ddRA were 10- to 50-fold lower than those for RA but

were likewise linearly related to cellular and medium RA concentration over the entire concentration range tested.

Medium concentrations of polar metabolites and 3,4-ddRA increased steadily as a function of original medium RA concentration, neither showed signs of saturation (Fig. 3b).

In work published previously (22) the potential for RA induction of metabolism was examined. Under the culture conditions used, the capacity for RA metabolism in keratinocytes was not further induced by exposure to pharmacological doses of RA.

Retinoic acid metabolism is mediated in part by cytochrome P450-linked enzymes

The role of cytochrome P450-linked enzymes in RA metabolism was examined. For this, cells were pulse-labeled with radiolabeled-RA, the radioactive medium was removed, and the isotopically labeled retinoids were analyzed from cells and medium after chase incubations in growth medium alone or growth medium containing 10 μM ketoconazole, an inhibitor of cytochrome P450-linked enzymes (23; **Fig. 4**). Consistent with previous observations (20, 22), and with the data in Fig. 2, the half-life of RA in keratinocytes was 6 h. The metabolism of RA as measured by its disappearance from cells and the appearance of V_O metabolites in medium was inhibited by 60% in incubations containing ketoconazole. Production of 3,4-ddRA from RA was not affected by ketoconazole.

Control of retinyl ester homeostasis by exogenous RA is limited by RA metabolism

Previous work from this laboratory has demonstrated that keratinocyte retinyl ester homeostasis is regulated by exogenous RA (22); both retinyl ester synthesis and hydrolysis appear to be subject to retinoid-mediated control. In this earlier work, 2 days of exposure to medium containing 50 nM RA, with fresh medium changes each day (6 ml per 100 mm dish), was necessary to increase keratinocyte content of retinyl ester by 25%. In light of the current work, the requirement for daily medium RA renewal may have reflected the rapid metabolic depletion of RA from medium. Together these observations suggest that the regulation of keratinocyte retinyl ester homeostasis by exogenous RA may be limited by its own metabolism. To test this possibility, cells in 175 cm^2 flasks were incubated in different volumes of medium containing 1 nM [^{3}H]RA. The quantities of [^{3}H]RA and its metabolites in cells and medium were determined at 3, 12, and 24 h. The results of this type of experiment are shown in **Fig. 5.** Increasing the medium volume from 10 ml to 90 ml resulted in almost a 5-fold increase in peak cellular levels of RA (Fig. 5, top panel). The cellular concentrations of RA present at 3 h in the incubations containing 10 ml, 30 ml, or 90 ml were 96 nM, 240 nM, and 450 nM, respectively. Medium RA was significantly depleted (less than 10% of original RA) in the 10 ml and 30 ml incubations by 3 h. Total quantity of RA in the 90 ml incubation was not depleted to this level until 24 h. Coinciding with the disappearance of RA from the medium and cells with continued incubation was a progressive appearance of polar metabolites in the medium (bottom panel). The quantity of polar metabolites produced varied by 5-fold as a function of a 9-fold difference in medium volume (Fig. 5, bottom panel). The higher rate of metabolism was clearly driven by the higher cellular concentrations of RA that were sustained in incubations with the higher medium volumes.

The effect of differing quantities of RA, as a function of medium volume and concentration on cellular retinyl ester mass, was determined in cells after a 24-h incubation (**Fig. 6**). There was no change in retinyl ester content of cells incubated in medium lacking RA, or in incubations containing 10 ml of either 1 nM RA or 10 nM RA. There was a 20% increase in retinyl ester content in cells incubated with 30 ml 1 nM RA or 10 nM RA. When 90 ml medium was supplied, however, there was a dose-dependent increase in retinyl ester mass for cells incubated with 1 nM RA or 10 nM RA. Increasing the RA concentration further to 100 nM had no additional effect on retinyl ester mass.

DISCUSSION

Epidermal keratinocytes in submerged culture avidly take up exogenous RA and metabolize it via a high capacity pathway. Metabolism of exogenous RA by keratinocytes is so efficient that elevated intracellular RA concentrations can be sustained only by supplying a very large extracellular supply of RA (Fig. 5), conditions that might mimic a situation in vivo where there is exogenous RA available from the plasma bound to albumin. Assuming a plasma albumin concentration of 600 μM, and an RA concentration of 14 nM, at the upper end of the normal range in humans (6, 7), the albumin to RA mole ratio is 43,000. Under culture conditions where the RA is supplied to keratinocytes at a similar albumin to RA mole ratio of 45,000 (45 μM albumin and 1 nM RA), and after equilibrium is reached, the intracellular RA concentration can be sustained at about 20 nM for up to 24 h. This concentration is similar to the low nanomolar RA concentrations reported in vivo in the epidermis (13, 14). Assuming that keratinocytes in vivo exhibit a similar capacity to take up and

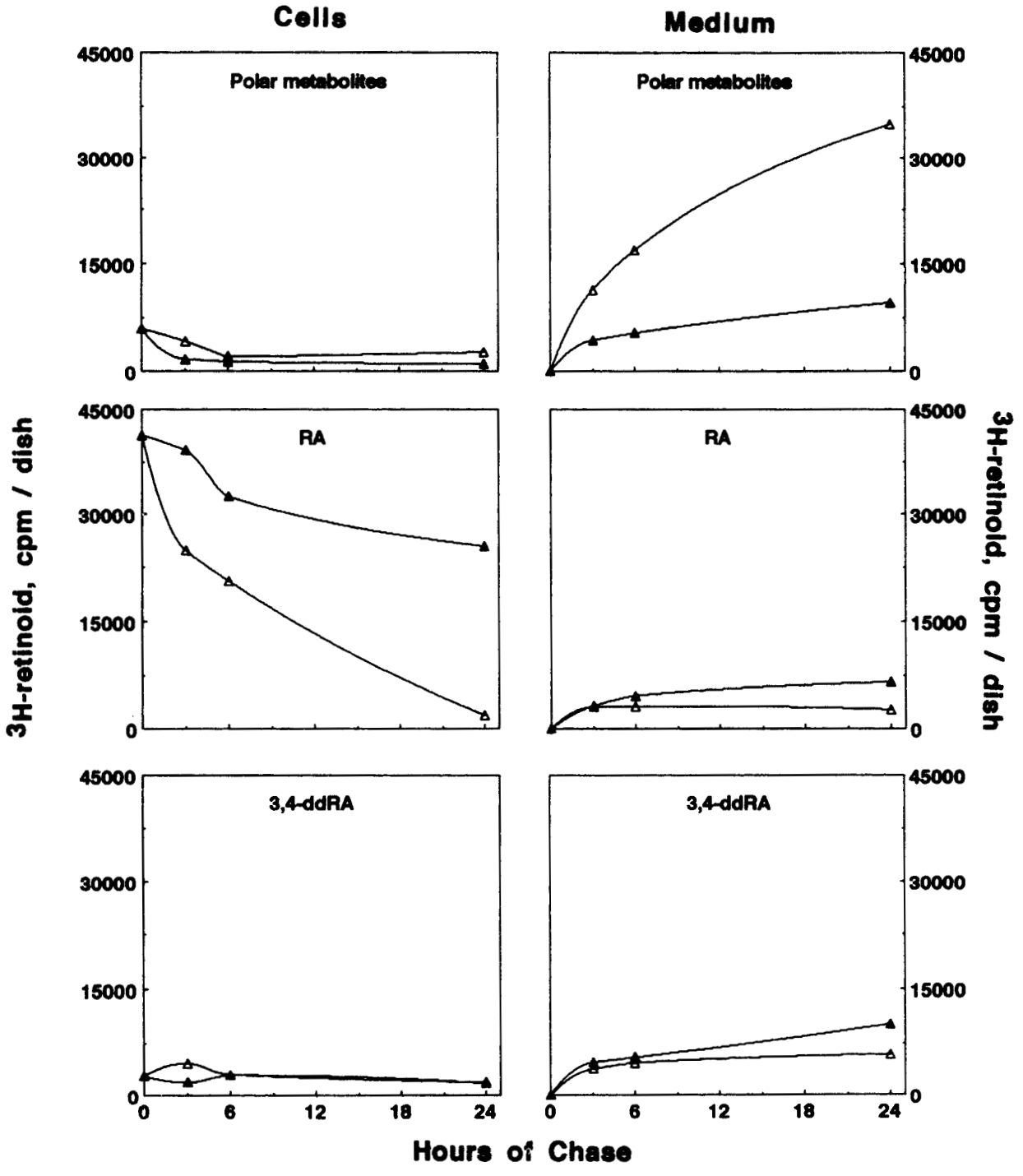

Fig. 4. Ketoconazole inhibits metabolism of RA by keratinocytes. Keratinocytes were pulsed with medium containing 0.5 nM [^{3}H]RA for 1 h. Pulse medium was removed and cells received fresh medium lacking RA alone (open symbols) or containing 10 μM ketoconazole (filled symbols). Chase incubations were continued for the indicated periods of time. Cells and medium were harvested and analyzed for [^{3}H]retinoid by reverse phase HPLC. Each data point represents the average of triplicate samples from one experiment which was repeated with similar results.

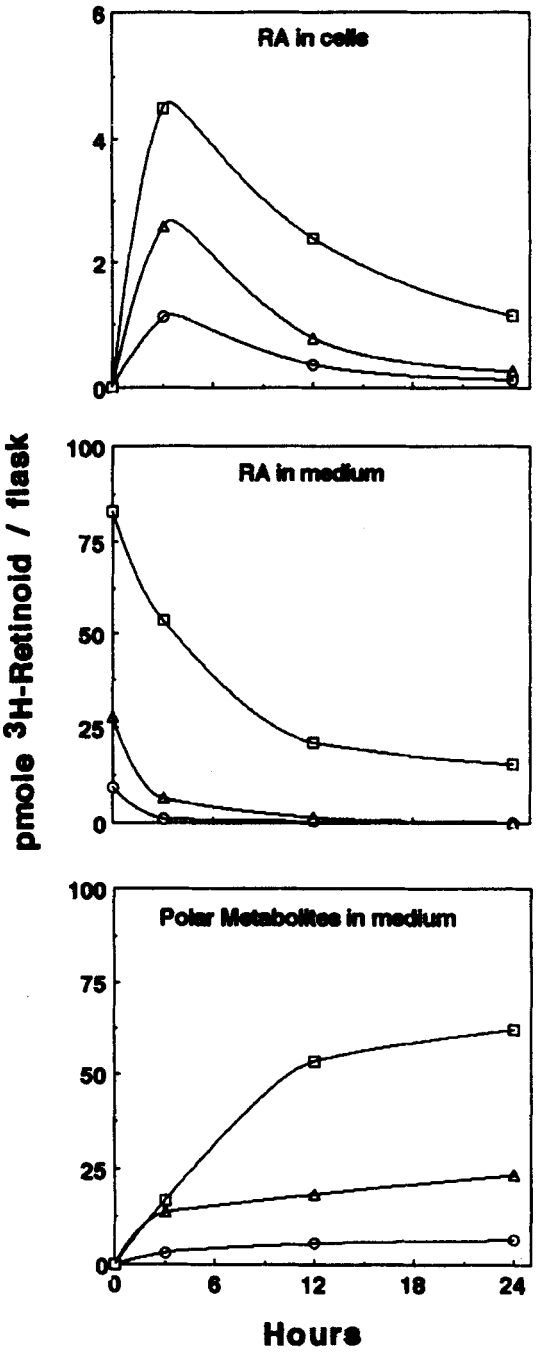

Fig. 5. Cellular content of exogenous RA is limited by RA metabolism. Keratinocytes were grown to confluence in growth medium as indicated in Materials and Methods in 175 cm^2 flasks. The experiment was initiated by incubating cells for the indicated times with 10 ml (○), 30 ml (△), or 90 ml (□) of medium containing 1 nM [^{3}H]RA. At the indicated times, cells and medium were harvested separately and [^{3}H]retinoids were analyzed by reverse phase HPLC. For reference, cellular concentrations of RA (top panel) at 3 h were 96 nM, 240 nM, and 450 nM, respectively. There were no differences in cell protein in flasks with different medium volumes.

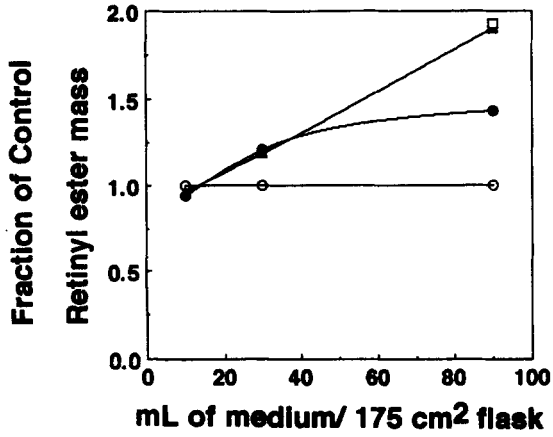

Fig. 6. Stimulation of retinyl ester accumulation in keratinocytes by RA is limited by RA metabolism. Keratinocytes were grown to confluence in growth medium as indicated in Materials and Methods in 175 cm^2 flasks. The experiment was initiated by incubating cells for 24 h with the indicated volumes of medium lacking RA (5% FBS; control; ○) or containing 1 nM RA (●), 10 nM RA (△), or 100 nM RA (□). The experimental condition with 100 nM RA was conducted only with 90 ml medium. Cells were harvested separately and retinyl ester mass was analyzed by reverse phase HPLC. Retinyl ester mass in control cells averaged 10.2 pmol/mg cell protein and did not differ in incubations with different medium volumes. Cell protein per flask was not different among any of the treatment groups.

metabolize RA as they do in vitro, the RA concentration available to the epidermis from plasma must be limited. The availability of plasma RA to the epidermis could be modulated by a variety of physiological parameters including albumin to RA mole ratio (24), local blood flow, metabolic activity, and density of cells in the dermis.

Previous work from this laboratory has shown that cultured keratinocytes maintain steady state intracellular active retinoid (RA and 3,4-ddRA together) concentrations in the 20–50 nM range (16) when grown in medium containing 25 nM Roh (originating from 5% fetal bovine serum) and no detectable RA (less than nM). These active retinoids are synthesized from substrates derived from intracellular ester stores of vitamin A_1 and A_2 (16), the production of which is subject to down-regulation by exposing the cells to exogenous RA (22). Maximal down-regulation was achieved by incubating the cells with 50 nM RA for 3 days. Under these conditions, intracellular concentrations of endogenously produced active retinoids were reduced only 50%; this occurred without any change in the rate of RA metabolism. This suggests that synthesis of active retinoids in keratinocytes proceeds, even in the presence of pharmacological concentrations of exogenous RA. It follows from this that in situ synthesis of RA has potential to contribute to tissue active retinoid in the epidermis regardless of the supply of exogenous RA.

Approximately 60% of intracellular RA is metabo-

lized to polar metabolites by cytochrome P450-linked enzymes. The metabolites produced are not retained by the cells and are released back into the medium. This result repeats previous observations in other laboratories (24, 25). Similarly, carbon 4 metabolites were not detected in vitro in the present studies, in work by Hodam and Creek (24), or in incubations of untreated human epidermal microsomes with RA (25). The carbon 4 metabolites are, however, observed in incubations of RA with microsomes from RA-treated skin (14, 25). This result does not indicate that RA metabolism at carbon 4 does not occur in untreated cells or epidermis. Rather, RA metabolism under these conditions probably involves multiple sequential reactions that modify the retinoid extensively, making it very polar. It is noteworthy that in incubations with ketoconazole, no downstream metabolites of RA were observed to increase. This suggests that the initial reaction in RA metabolism by keratinocytes is cytochrome P450-dependent. The successful methylation of RA polar metabolites suggests that the metabolism of RA by keratinocytes does not involve a decarboxylation reaction.

A surprising result was the finding that 3, 4-ddRA is a metabolite of RA. We have previously provided evidence that 3,4-ddRA is a metabolite of 3,4-didehydroretinol (3,4-ddRoh) metabolism in cultured keratinocytes (16, 22). The mole ratio of RA to 3,4-ddRA in confluent cultures is 2.5 when radiolabeled Roh is the initial substrate. In the present work with radiolabeled RA as the initial substrate, the mole ratio of RA to 3,4-ddRA averaged 10. A comparison of these two ratios suggests that approximately one-fourth of the 3,4-ddRA present in cultured keratinocytes under these conditions is derived from RA. This result is significant in that signaling mediated by both RA and 3,4-ddRA could occur when exogenous RA is added to keratinocyte experimental systems. This consideration is particularly important in work seeking to delineate the function of 3,4-ddRA in keratinocytes.

The metabolism of RA is one of two predominant pathways of retinoid metabolism in cultured keratinocytes. The second of these, Roh esterification, has been described previously by this and other laboratories (13, 16, 20, 22, 26–28). Both of these pathways exhibit very high apparent affinity and capacity for their respective substrates, RA and Roh. Robust esterification of Roh and metabolism of RA are observed at nanomolar substrate concentrations and neither exhibits saturation at substrate concentrations exceeding their respective physiological ranges. Moreover, both pathways are subject to autoregulation by active retinoids. Together these pathways position the proliferating epidermal keratinocyte to efficiently sequester and store or inactivate the two most abundant retinoids present in plasma, Roh and RA, thus achieving very tight control of epidermal active retinoid signaling and thereby the differentiation program.

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