I. Introduction

Vitamin A (retinol) and its naturally occurring and synthetic derivatives, collectively referred to as retinoids, exert a wide variety of profound effects in embryogenesis, reproduction, vision, and regulation of inflammation, growth, and differentiation of normal and neoplastic cells in vertebrates (Sporn et al., 1994; Blomhoff, 1994; Becherel et al., 1994).

Vitamin A was first reported to be an essential nutrient (“fat soluble A”) in the beginning of this century (Drummond, 1920). The importance of retinoids in dermatology dates back to Wolbach and Howe in 1925, who identified epidermal changes as abnormal keratinization (“fat soluble A”) in the beginning of this century (Drummond, 1920). The importance of retinoids in dermatology dates back to Wolbach and Howe in 1925, who identified epidermal changes as abnormal keratinization (Sporn et al., 1994; Blomhoff, 1994; Becherel et al., 1994).

Vitamin A was first reported to be an essential nutrient (“fat soluble A”) in the beginning of this century (Drummond, 1920). The importance of retinoids in dermatology dates back to Wolbach and Howe in 1925, who identified epidermal changes as abnormal keratinization in vitamin A-deficient animals (Sporn et al., 1994; Blomhoff, 1994; Becherel et al., 1994).

These observations were followed by numerous studies focused on the metabolism and pharmacological action of retinoids in the skin leading to the establishment of retinoic acid treatment for various skin diseases (Stuettgen, 1962; Baer, 1962; Frost and Weinstein, 1969; Fredriksson, 1971; Schumacher and Stüttgen, 1971; Günther, 1973; Runne et al., 1973). Up to now, far more than 5000 retinoic acid analogs have been synthesized, out of which the next three generations have been established for systemic and topical treatment of various skin disorders: first, the nonaromatic retinoids β-carotene (provitamin A), all-trans-retinoic acid (RA) (tretinoin), and 13-cis-RA (isoretinoin); second, the monoaromatic retinoid derivatives trimethyl-methoxyphenyl analog of RA (etretinate) and 9-(4-methoxy-2,3,6-trim-
ethylenyl)-3-2,4,6,8-nonatetraenoic acid (acitretin); and third, the polyaromatic retinoid derivatives tazarotenic acid and 6-[3-(1-adamantyl)-4-methoxy-phenyl]-2-naphtholic acid (adapalene) (see fig. 1) (Orfanos et al., 1987, 1997; Shalita et al., 1996).

Retinoids mediate their biological effects through binding to nuclear receptors known as RA receptors (RARs) and retinoid X receptors (RXRs), which belong to the superfamilies of ligand-inducible transcriptional regulators that include steroid hormone receptors, thyroid hormone receptors, and vitamin D₃ receptors (reviewed in: Giguere, 1994; Mangelsdorf et al., 1995; Gronemeyer, 1994). RARs and RXRs act via polymorphic responsive elements, the RA responsive elements (RAREs), and retinoid X responsive elements (RXREs), present in the promoters of retinoid-responsive genes (Giguere, 1994; Mangelsdorf et al., 1995; Gronemeyer and Laudet, 1996). The functional interactions of retinoid receptors in the skin were reviewed by Fisher and Voorhees (1996) and Chambron (1996).

Although all-trans- and 9-cis-RA are only minor metabolites of retin (ROL) and β-carotene, they display 100- to 1000-fold higher biological activity (Breitman et al., 1980; Strickland and Mahdavi, 1978). Whereas all-trans-RA binds only to RARs, 9-cis-RA binds both RARs and RXRs. The stereoisomer of all-trans-RA, 13-cis-RA, exhibits a much lower affinity for RARs and RXRs and exerts its molecular effects mostly through its isomerization into all-trans-RA (Allenby et al., 1993).

Retinoids display key regulatory functions in epidermal growth and differentiation but the cellular, immunologic, and biochemical alterations associated with them are not understood completely (Fisher et al., 1991; Fisher and Voorhees, 1996). Furthermore, the metabolic pathways of retinoids operative in skin physiology and pharmacotherapy remain to be defined.

In this review, the metabolic pathways of retinoids in skin are reviewed focusing on the following subjects:

1. The enzymes and binding proteins that mainly are involved in the activation, modulation, and cleavage of retinoids in human skin. The involvement of these enzymes/binding proteins in the pathogenesis of skin disorders, especially malignancies and disorders of keratinization, will be emphasized.
2. The xenobiotics that are capable of modulating the steady-state of tissue retinoid concentrations, and their impact on the enzyme systems that regulate the metabolic pathways of retinoids. Here, the “check points” in the metabolic pathway of retinoids whereby xenobiotics can influence these agents are of major interest with regard to clinical retinoid therapy.

II. Absorption, Transport, and Storage

Major sources of natural retinoids are animal fats, fish liver oil (retinylesters), and yellow and green vegetables (carotenoids) (fig. 2). Ingested retinylesters (RE) are hydrolyzed to ROL by enteral hydrolases in the intestine. ROL and carotenoids are absorbed by intestinal mucosa cells. Of the carotenoids, β-carotene is the most potent ROL precursor, yet it is six-fold less effective than preformed ROL, which results from incomplete resorption and conversion (One ROL equivalent is equal to 1 μg of ROL, 6 μg of β-carotene, or 12 μg of mixed carotenoids) (Blomhoff et al., 1971).

After intestinal absorption, retinoid production from carotenoids can occur by two pathways: First, retinal (RAL) can be synthesized by oxidative cleavage of the central double bond followed by reduction to ROL by a microsomal retinal reductase (Kakkad and Ong, 1988). Here, the cellular retinol binding protein-II (CRBP-II) protects RAL from oxidation into RA. Second, apo-carotenoids are formed through eccentric cleavage followed by transformation of the apo-carotenoids into RAs (Wang et al., 1991).

In the intestinal cell, ROL also forms complexes with CRBP-II. This ROL-CRBP-II complex serves as a substrate for the esterification of ROL to RE by a lecithin: retinol acyltransferase (LRAT) (MacDonald and Ong, 1988) with long-chain fatty acids, which are incorporated into chylomicrons (Blomhoff et al., 1990). The fatty acids reach the general circulation where they undergo several biochemical changes via the lymph RE-chylomicron complexes. This leads to the formation of several chylomicron remnants, which in turn are cleared primarily by the liver, although extrahepatic chylomicron uptake has been shown also in bone marrow and spleen, and to a lesser degree in testes, lungs, kidneys, fat, and skeletal muscle (Blomhoff, 1994; Blomhoff et al., 1991).

In the parenchymal hepatocytes, chylomicron-RE complexes are hydrolyzed and free ROL binds to retinol binding protein (RBP), its serum transport protein. Excess ROL undergoes a paracrinic transfer from the hepatocytes to the perisinusoidal stellate cells, called vitamin A storage or Ito cells, for storage (Hirosawa and Yamada, 1973). Approximately 50 to 80% of the total body vitamin A in humans is stored in the stellate cells in the liver in the form of REs. Depending on their lipophilic character, exogenous and endogenous RA derivatives accumulate in the human body with highly variable elimination half-lives. This has to be considered especially for the use of synthetic RA derivatives in clinical therapy (Chien et al., 1992). To maintain constant physiological ROL concentrations in the plasma of approximately 2 μmol/L, ROL can be released from the stellate cells. The RA concentration in the plasma and other body fluids is approximately 100-fold lower (7 to 14 nmol/L) (Napoli et al., 1985; De Leenheer et al., 1982; Tand and Russel, 1990; Eckhoff and Nau, 1990).

ROL-RBP complexes released from the liver bind to transthyretin, a serum protein named for its ability to bind and transport simultaneously but independently
<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Chemical Structure</th>
<th>Mode of Administration</th>
<th>Principal Indication</th>
<th>Other Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (Vitamin A)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>Oral</td>
<td>Vitamin Supplement</td>
<td></td>
</tr>
<tr>
<td>Retinyl Palmitate</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>Topical 0.5-5% Emulsions</td>
<td>Cosmetic Agents</td>
<td></td>
</tr>
<tr>
<td>β-Carotene (Provitamin A)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>Topical</td>
<td>Hypopigmentation, Hyperpigmentation, Radical Protection</td>
<td>Nutrient Color</td>
</tr>
<tr>
<td>Tretinoin</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>Topical 0.025-0.1% Gels or Creams</td>
<td>Acne vulgaris, Parakeratosis, Hyperkeratosis</td>
<td>Photoaging, Actinic Keratosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Chemical Structure</th>
<th>Mode of Administration</th>
<th>Principal Indication</th>
<th>Other Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotretinoin</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>Topical 0.05% cream</td>
<td>Cystic Acne, Recalcitrant Nodular Acne</td>
<td>Rosacea, Gram-negative Folliculitis, Pyoderma faciale, Hidradenitis suppurativa, Cancer Prevention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral 0.25-1.0 mg/kg/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etretinate</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>Oral 0.25-1.0 mg/kg/d</td>
<td>Generalized pustular Psoriasis, Exfoliative Psoriasis, Plaque Psoriasis</td>
<td>Palmoplantar keratoderma, Pustulosis palmoplantaris, Ichthyosis, Darier’s Disease, Pityriasis rubra pilaris, Lichen ruber planus</td>
</tr>
<tr>
<td>Acitretine</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>Oral 0.25-1.0 mg/kg/d</td>
<td>Psoriasis (erythrodemic, pustular, and severe recalcitrant)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Indications and mode of administration of commercially available retinoids in dermatological therapy.
from both the thyroid hormone and the ROL-RBP complex (Blomhoff et al., 1991).

The plasma carrier of ROL, RBP, as well as the plasma carrier of RA, albumin, are present in the intercellular spaces of the epidermis (Vahlquist et al., 1997; Rabilloud et al., 1988). In human skin, besides ROL, β-carotene, RE, 3,4-didehydro-retinoids, RAL, all-trans-RA, and some of their metabolites have been identified in vitro and in vivo (Vahlquist, 1982; Vahlquist et al., 1982). Cultured epidermal keratinocytes maintained in medium containing a physiological concentration of ROL exhibit a retinoid composition that is similar to intact epidermis (Randolph and Simon, 1993).

As of today, the mechanisms of ROL uptake by target cells are not understood completely. Several possibilities have been proposed: RBP receptor-mediated uptake, nonspecific spontaneous transfer of ROL and RA, and fluid phase endocytosis (Heller, 1975; Rask and Peterson, 1976; Bavic et al., 1991; Dew and Ong, 1995). Orally administered all-trans-RA, 13-cis-RA, and etretinate undergo first-pass absorption directly into the portal blood and circulate in the plasma, mainly bound to albumin. The uptake of these retinoids by target cells is regulated by unknown factors. Similarly, neither the mechanism of transcutaneous absorption of topical retinoids nor their transfer into target cells is well understood.

Topically applied all-trans-RA is isomerized partially to 9-cis-RA, 13-cis-RA, and other metabolites within the epidermis (Lehmann and Malany, 1989). Approximately 80% of the all-trans-RA applied remains on the skin surface, whereas its penetration through the stratum corneum and the hair follicle is vehicle-dependent (Lehmann et al., 1988). After the initial diffusion into the stratum corneum that occurs within a few minutes, further diffusion into epidermis and dermis proceeds more slowly (Schaefer, 1993; Tavakkol et al., 1994). Our findings have shown that topically applied all-trans-RA and 13-cis-RA poorly penetrates into or through the skin: gel-based formulations tend to trap the drug and the RA remains on the surface, whereas cream formulations enhance penetration to a small extent (less than 5% of the applied amount within 30 min). On the other hand, 13-cis-RA penetrates rapidly into normal human keratinocytes in vitro (unpublished data).
The storage of ROL in human skin occurs through esterification of ROL into RE (Kang et al., 1995). Skin cells contain transferases, LRAT and acyl CoA:acyltransferase (ARAT). These two enzymes catalyze RE synthesis (Torma and Vahlquist, 1987; Kurlandsky et al., 1996) (table 1). The hydrolysis of RE to ROL is regulated by a specific RE hydrolase. In cultured keratinocytes, LRAT activity is inducible by retinoids after 12 h incubation (Kurlandsky et al., 1996). Simultaneously, biosynthesis of all-trans-RA is reduced, whereas inhibition of LRAT by phenylmethylsulfonyl fluoride restores all-trans-RA synthesis. The regulation of LRAT activity provides a mechanism of autoregulation of RA synthesis through feedback regulation of substrate availability. The esterifying activity in human skin in vivo is four-fold greater, on a per cell basis, in keratinocytes in the basal layer of the epidermis than in keratinocytes in the upper layers (Kurlandsky et al., 1996), suggesting that retinoid levels are higher in the lower epidermis, which is closer to the perfusate from capillaries in the dermis. Furthermore, this implies that more REs are stored in the lower than in the upper epidermal keratinocyte layers. During migration from the lower to the upper cell layers, these stored REs may provide keratinocytes with a source of RA and thus, a source of RA, which maturing keratinocytes are able to synthesize from ROL (Siegenthaler et al., 1990a). Human keratinocytes incubated with all-trans-RA exhibit time- and concentration-dependent increases in RE mass, increases in the rate of RE synthesis, and decreases in RE utilization (Randolph and Simon, 1996). This clearly demonstrates that keratinocytes respond to exogenous RA by initiating feedback inhibition of endogenous production of active retinoids, sequestering extracellular substrate ROL as RE, and decreasing RE utilization. How these reactions are mediated, and to what extent nuclear retinoid receptors are involved, is not understood completely, but it is obvious that the steady-state system of intracellular retinoids is regulated by a complex feedback control system involving retinoids, several enzymes, and retinoid binding proteins.

III. Retinoid Biosynthesis

Whereas extensive and elegant work has been performed on the family of retinoid receptors, a relatively large gap exists in the knowledge of how ROL is metabolized to form active ligands.

Because ROL produces changes in skin, in vivo, similar to those produced by RA but without measurable levels of RA or irritation, ROL generally is considered a prohormone of RA, implying that ROL-induced responses in human keratinocytes are mediated by its tightly regulated conversion to RA (Kang et al., 1995). These responses include increased epidermal thickening because of increased keratinocyte proliferation, expansion of intercellular spaces, compaction of the epidermal barrier, and induction of CRBP, CRABP-II, and RA 4-hydroxylase activity. From these findings it is possible that ROL may be a more efficient and natural way to deliver RA to the correct subcellular location within skin cells than direct treatment with RA (Fisher et al., 1991). In support of this, it has been shown that ROL and also REs must be converted to RA to exhibit biological activity in human keratinocytes, in vitro (Kurlandsky et al., 1994; Chen et al., 1995a). Because of a tight enzymatic regulation of the conversion of ROL and RAL to RA, all-trans-RA is minimally detectable in untreated and ROL-treated human skin (Kang et al., 1995). Very low levels of RA apparently are required to function as ligands to bind and activate nuclear RARs and RXRs (for review see Giguere, 1994), and the RA that is formed from ROL is hydroxylated rapidly by RA 4-hydroxylase to the metabolites 4-OH-RA and 4-oxo-RA, which exhibit a much lower retinoid receptor binding affinity (see Section V.A.).

Numerous enzymes involved in retinoid metabolism have been identified. These enzymes are members of four distinct families: Alcohol/ROL dehydrogenase (ADH/RolDH), short-chain dehydrogenase/reductase (SDR), aldehyde/RAL dehydrogenase (ALDH/RalDH), and several cytochrome P450-isoenzymes.

A. Alcohol / Retinol Dehydrogenases and Short-Chain Dehydrogenases / Reductases

The conversion of ROL to RA consists of a two-step process: First, members of the alcohol dehydrogenase (ADH I, II, and IV) (Boleda et al., 1993; Yang et al., 1994; Kedishvili et al., 1995) and short-chain dehydrogenase/ reductase enzyme families (SDR) catalyze the reversible interconversion of ROL and RAL, the rate-limiting step (Kim et al., 1992; Blaner and Olson, 1994; Chen et al., 1995c) (fig. 3, table 1). These ADH-isoforms metabolize all-trans-, 9-cis-, and 13-cis-retinoid isomers with reduced nicotinamide adenine dinucleotide (NAD) as co-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Locus</th>
<th>Reaction</th>
<th>Binding protein/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinyl ester hydrolase (REH)</td>
<td>Microsomes</td>
<td>Retinyl esters → retinol</td>
<td>Apo-CRBP/activator</td>
</tr>
<tr>
<td>Alcohol dehydrogenase classes I, II, IV, and VII (RolDH)</td>
<td>Cytosol</td>
<td>Retinol → retinal</td>
<td>Holo-CRBP-retinol/substrate, Apo-CRBP/inhibitor</td>
</tr>
<tr>
<td>Short-Chain dehydrogenase/reductase, microsomal retinol dehydrogenase types I, II, and III</td>
<td>Microsomes</td>
<td>Retinol → retinal</td>
<td>Holo-CRBP-retinol/substrate</td>
</tr>
</tbody>
</table>
enzyme, whereas the SDRs use all-trans-ROL and all-trans-RAL, either free or bound to CRBP-I with reduced NAD phosphate as phosphorylated coenzyme, but are unable to oxidize 9-cis-ROL or 13-cis-ROL (Boerman and Napoli, 1995). Similar ADH enzymes metabolizing ROL into RAL were identified in differentiating keratinocytes (Siegenthaler et al., 1990a) and other cell types (Posch et al., 1992; Tsujita et al., 1994; Chen et al., 1994) in vitro, as well as in human psoriatic epidermis and at very low levels in normal human skin (Siegenthaler et al., 1990b).

Recent studies have revealed that mammalian ADH is part of a complex enzyme family composed of seven evolutionarily conserved classes, each with unique properties and sites of gene expression (Jörnvall et al., 1995; Duester et al., 1995). In mouse skin, the enzyme catalyzing ROL oxidation has been identified as an isoenzyme of ADH class IV (Connor et al., 1987; Zgombic-Knight et al., 1995). To what extent the other ADH classes are active in retinoid metabolism in murine and human skin is unknown.

Three forms of rat liver microsomal ROL dehydrogenases (ROLDH, types I, II, and III) revealed sequence homology with members of the SDR family (fig. 3, table 2). The ADH and SDR enzyme families are related evolutionarily, sharing similar coenzyme binding domains, but differ in that ADH has a greater subunit molecular weight and is zinc-dependent, whereas SDR has a shorter subunit and no metal requirement (Persson et al., 1995). Whether these SDRs are involved in retinoid metabolism in human skin has not yet been determined.

### TABLE 2

<table>
<thead>
<tr>
<th>Enzyme Locus</th>
<th>Reaction</th>
<th>Binding protein/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde dehydrogenases class I (RalDH) Cytochrome P450-isoenzymes</td>
<td>Retinal → retinoic acid</td>
<td>Holo-CRBP-retinal/substrate</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Cytosol</td>
<td>Retinal → retinoic acid</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Microsomes</td>
<td>Retinal → 4-OH-retinal</td>
</tr>
<tr>
<td>CYP2A6</td>
<td></td>
<td>9-cis-Retinal → 4-OH-9-cis-retinal</td>
</tr>
<tr>
<td>CYP1A2</td>
<td></td>
<td>4-oxo-9-cis-retinal</td>
</tr>
<tr>
<td>CYP1A2</td>
<td></td>
<td>9-cis-Retinal → 9-cis-retinoic acid</td>
</tr>
<tr>
<td>CYP2B4, CYP2C3</td>
<td></td>
<td>9-cis-RAL → 4-OH-retinal</td>
</tr>
</tbody>
</table>

B. Aldehyde/Retinal Dehydrogenases and Cytochrome P450

In the second step, members of the aldehyde/RAL dehydrogenases (ALDH/RalDH) and cytochrome P450-isoenzyme families (CYP) catalyze the irreversible oxidation of RAL into RA (Duester, 1996) (fig. 3, table 2). This explains why the administration of RA to vitamin A-deficient animals results in no increase in ROL and RAL production needed for retinoid storage nor does it induce the production of the visual pigment 11-cis-retinal (Dowling and Wald, 1960, 1982). In mouse epidermis topical RAL is transformed into all-trans-RA and exerts biological activity in vivo as measured by messenger ribonucleic acid (mRNA) levels of filaggrin and loricrin (Didierjean et al., 1996). Out of three characterized classes, class I ALDH showed the highest activity for the oxidation of all-trans-RAL and 9-cis-RAL to the corresponding RA isomers (Lee et al., 1991; Roberts et al., 1992; Labrecque et al., 1995). Whether this is also true for human skin is unknown.

Whereas some members of the CYP superfamily are involved in RA synthesis, they seem to be much more important for the catabolism of active retinoid ligands, as discussed below (fig. 3, table 2). Several CYP-isoenzymes derived from rabbit liver catalyze the oxidation of RAL to RA (Roberts et al., 1993; Tomita et al., 1993; Raner et al., 1995). Here, the most important CYP-isoenzymes in human skin apparently are CYP1A1 and CYP1A2, which are both able to oxidize all-trans- and 9-cis-RAL into the corresponding RA isomers (Roberts et al., 1992; Raner et al., 1995). Furthermore, the basal expression of CYP1A2 and 1A1 can be inhibited by RA in human epidermis (Li et al., 1995), which suggests feedback inhibition by one of the products of these enzymes.
The synthesis of RA does not require ROL as a substrate (Napoli and Race, 1988). Because the cleavage of \( \beta \)-carotene involves RAL as an intermediate product, it can function as an alternative precursor for RA in epidermal cells and several other tissues (fig. 4) (Vahlquist, 1982; Vahlquist et al., 1982; Lakshman et al., 1989; Krinsky et al., 1993).

Although 9-cis-RA is known as a ligand for RXRs and RARs, the pathway of its synthesis has not been determined completely. Evidence exists that it arises from nonenzymatic isomerization of all-trans-RA (El Akawi and Napoli, 1994). Other naturally occurring 9-cis-retinoid derivatives such as 9-cis-ROL or 9-cis-\( \beta \)-carotene, which have been identified in several tissues, also could function as precursors of 9-cis-RA (fig. 5) (Stahl et al., 1993). In rat liver, high 9-cis-ROL dehydrogenase activity has been observed (Napoli, 1996), and the major RAL dehydrogenase equally efficiently converts all-trans-RAL and 9-cis-RAL into their respective acids, whereas it discriminates against 13-cis-RAL (Giguère, 1994). This 9-cis-RAL dehydrogenase could convert 9-cis-RAL, which is produced from dietary 9-cis-ROL or from 9-cis-\( \beta \)-carotene, into 9-cis-RA. Because the mechanism of all-trans- to 11-cis-isomerization is comparable with all-trans- to 9-cis-isomerization, 9-cis-RA also could originate from the generation of 9-cis-ROL from RE, as observed with 11-cis-ROL (Cañada et al., 1990). In human plasma, 9-cis-RA is converted rapidly but reversibly into 9,13-cis-RA (Horst et al., 1995). Recently, we showed that this RA derivative is present in human epidermal keratinocytes and fibroblasts, in vitro (unpublished data). However, the function of 9,13-cis-RA has yet to be determined. This interconversion may represent a mechanism for 9-cis-RA clearance, or similarly to the 13-cis-/all-trans-RA interconversion, for a circulating less toxic derivative of 9-cis-RA as a depot for later use (Napoli, 1996).

Little is known about the isomerization of retinoids, although this reaction apparently is of great importance for the maintenance of appropriate intracellular levels of active RA. The cis-isomers of all-trans-ROL, all-trans-RAL, and all-trans-RA may be produced by nonenzymatic isomerizations (El Akawi and Napoli, 1994; Kojima et al., 1994; Urbach and Rando, 1994), by cytochrome P450-isoenzymes modifying the \( \beta \)-ionone ring, or by other yet unknown enzymatic conversions. The all-trans- to 9-cis-isomerization, generating the major ligand for the RXRs, occurs not only among RA isomers but also from all-trans-RAL to 9-cis-RAL, driven by a specific ADH (Labrecque et al., 1995). Remarkably, 9-cis-RA levels in human skin are much lower than all-trans-RA, and 9-cis-RA applied topically to human skin is isomerized rapidly to all-trans-RA (Duell et al., 1996a), suggesting the existence of an isomerase that preferentially produces all-trans-RA. Alternatively, 9-cis-RA is formed from 9-cis-\( \beta \)-carotene (Nagao and Olson, 1994; Wang et al., 1994). The physiological significance of this reaction is unknown.

We observed that 13-cis-RA spontaneously isomerizes to all-trans-RA very rapidly in cell-free medium (an equal ratio was reached in less than 24 h), and a ratio of 1:2.1 after 54 h was measured. In human keratinocytes in vitro this isomerization is slowed (the equal ratio was reached within 36 h), and the ratio after 54 h is still less than 1:1.7. Using all-trans-RA as the substrate for these isomerization studies, only small amounts of 13-cis-RA are converted from all-trans-RA, indicating that all-trans-RA is the most stable isomer. 9-cis-RA is converted rapidly into 13-cis- and all-trans-RA in human keratinocytes in vitro. These observations suggest the existence of an enzyme regulating the interconversion of these three isoforms of RA in human keratinocytes (Jugert et al., unpublished results). Investigation is underway to test this hypothesis.

In addition to several RA derivatives, human epidermis also produces 14-OH-4,14-retro-ROL (Duell et al.,...
1996b), which first was shown to be biologically active in B lymphocytes, where it substitutes for ROL in maintaining cell growth in culture (Buck et al., 1991). The role of this retinoid in human skin is not clear. Similarly, the functional significance of other vitamin A derivatives, 3,4-didehydro-ROL (vitamin A₄, Vahlquist, 1980), 9,13-cis-RA (Horst et al., 1995) and 3,4-didehydro-RA, which are synthesized in human keratinocytes, is unknown (Randolph and Simon, 1993). The finding that topical treatment with RA decreases the concentration of 3,4-didehydro-retinoids in skin suggests that these metabolites may function as a retinoid storage reservoir (Randolph, 1996). Thaller and Eichele (1990) speculated that 3,4-didehydro-RA may function as an endogenous morphogen, which is as important as all-trans-RA (Horst, 1980), 9,13-cis-RA (Vahlquist et al., 1995) and 3,4-didehydro-RA, which are synthesized in human keratinocytes, is unknown (Randolph and Simon, 1993). The finding that topical treatment with RA decreases the concentration of 3,4-didehydro-retinoids in skin suggests that these metabolites may function as a retinoid storage form (Randolph, 1996). Thaller and Eichele (1990) speculated that 3,4-didehydro-RA may function as an endogenous morphogen, which is as important as all-trans-RA.

Besides this complex and not yet completely characterized enzyme system, several retinoid binding proteins interacting with both the substrates and the enzymes are also very important regulators of intracellular retinoid metabolism.

IV. Retinoid Binding Proteins

Within the cytoplasm, ROL and RA are bound to specific cellular binding proteins, CRBP-I and -II and CRABP-I and -II, respectively. These proteins are involved in the regulation of the intracellular concentration of ROL, RAL, and RA by acting as both storage or shuttle proteins in retinoid metabolism, and maintain constant cell-specific levels of free ROL and RA (fig. 3, tables 1–3). The concentrations of both CRBP-I/II and CRABP-I/II exceed those of their ligands (Harrison et al., 1987; Donovan et al., 1995) and exhibit affinities for their ligands which are much higher than many enzymes for their substrates (Li et al., 1991; Norris et al., 1994).

A. Cellular Retinoid Binding Proteins-I and -II

CRBPs facilitate the uptake of ROL and present it to LRAT for storage as REs (Ong, 1994) (table 1). Further, they prevent ROL from spontaneous nonenzymatic isomerization and oxidation, which occur rapidly in the absence of CRBP (Napoli et al., 1995). ROL bound to CRBP-I (holo-CRBP) is a substrate for conversion to RA (Posch et al., 1992; Ottonello et al., 1993; Boerman et al., 1995), and unbound CRBP (apo-CRBP) inhibits LRAT (Ong, 1994) (table 1). Thus, the ratio of apo- to holo-CRBP participates in regulation of the balance between oxidation and esterification of ROL (Napoli, 1993). The activity of microsomal ROL dehydrogenase (SDRs) with all-trans-ROL (as ROL dehydrogenase) or with all-trans-RAL (as RAL reductase) is stimulated in both cases by CRBP-I, facilitating the conversion of ROL to RE as shown in human liver (Yost et al., 1988).

The expression of CRBP is up-regulated by both RA and ROL in several tissues, including human skin (Es-kild et al., 1988; Rush et al., 1991; Ong et al., 1994), and it has been suggested that this is caused by the conversion of ROL to all-trans-RA (Kurlandsky et al., 1994), indicating that this induction of CRBP gene transcription by all-trans-RA is a negative feedback regulatory mechanism of RA synthesis (Smith et al., 1991; Mangelsdorf et al., 1991; Wang et al., 1993; Ong et al., 1994), which decreases the levels of free ROL, and thus inhibits the conversion of ROL to RA.

B. Cellular Retinoic Acid Binding Proteins-I and -II

The intracellular levels of CRABP-I protein are similar in dermis and epidermis, whereas CRABP-II levels are much higher in the epidermis (Siegenthaler et al., 1984; 1992b). Furthermore, CRABP-II is up-regulated by treatment with ROL (Kang et al., 1995), all-trans-RA and its analogs (Astrom et al., 1991; Elder et al., 1992), especially in differentiating keratinocytes. These findings have led to the use of the CRABP-II response to retinoid administration in fibroblasts in vitro as a reproducible measure of retinoid bioactivity that may predict human skin responses (Elder et al., 1996). The sources of CRABP-II in human skin are keratinocytes and fibroblasts, whereas the source of CRABP-I in human skin is primarily melanocytes (Sanquer and Gilchrest, 1994). Basal CRABP-I expression is much lower than that of CRABP-II.

CRABP-I and -II display variable RA binding affinities, regulated by a RA-responsive element in their promoters (Darmon and Blumenberg, 1993). By this mechanism, CRABP-I has been implicated in enhancing the metabolism of RA to inactive metabolites (Fiorella and Napoli, 1991) through a transfer of RA from CRABP-I to CYP-Isoenzymes via a bimolecular complex with juxta-posed ligand portals (Thompson et al., 1995), whereas CRABP-II may facilitate transport of RA to the nucleus.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Locus</th>
<th>Reaction</th>
<th>Binding protein/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450-isoenzymes</td>
<td>Microsomal</td>
<td>Retinoic acid → 4-OH-retinoic acid</td>
<td>CRABP-retinoic acid/substrate</td>
</tr>
<tr>
<td>P450RA1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CYP1A1</td>
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<tr>
<td>CYP1A2</td>
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<td>CYP2B4</td>
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<td>CYP2C3</td>
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<tr>
<td>CYP2E1</td>
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<tr>
<td>CYP2G2</td>
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<tr>
<td>CYP3A</td>
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</table>

TABLE 3

The catabolism of retinoic acid to polar metabolites
(Donovan et al., 1995), suggesting that CRABP-II is involved in the regulation of nuclear receptors by RA. F9-cell mutants that overexpress CRABP-I show a much faster RA metabolic activity than wild-type F9 cells, and much higher RA concentrations are required to induce differentiation (Boylan and Gudas, 1991).

Here it should be mentioned that 13-cis-RA is not bound by CRABP-I/II or other binding proteins in the cytosol. Thus it penetrates the cell nucleus after topical or systemic administration more rapidly than all-trans-RA or 9-cis-RA, as demonstrated in a mouse embryo model system, whereas the access of all-trans-RA to the nucleus seems to be limited by its binding to CRABP-I/II (Nau and Elmazar, 1997). These findings may explain the profound teratogenic effects caused by 13-cis-RA after topical or systemic treatment (Orf anos et al., 1997). The kinetics of the nuclear penetration of RA derivatives in human skin cells is currently under study in our laboratory.

A significant decrease of CRABP-I mRNA expression and an increased CRABP-II mRNA expression have been reported in psoriatic skin (Siegenthaler et al., 1990a, 1992a; Elder et al., 1992; Torma et al., 1994). Moreover, the expression of CRBP-I mRNA was also increased (Busch et al., 1992). Whether this altered expression of CRBP-I and CRABPs is an inherent characteristic of psoriasis or simply reflects the fact that psoriatic epidermis contains a higher proportion of undifferentiated keratinocytes is unknown.

CRABP-I expression was down-regulated in basal and squamous cell carcinomas, whereas CRBP-I was expressed (Busch et al., 1992). Whether these findings are relevant to the development of these skin tumors is not known.

CRABP-I/II double knockout mice had no apparent phenotype (Lampron et al., 1995), suggesting that these binding proteins may not be essential for normal retinoid metabolism or signaling. Instead, it is possible that these proteins sequester RA during vitamin A deficiency to support the maintenance of retinoid signaling. However, retinoid binding proteins (CRBPs and CRABPs) are involved in the regulation of intracellular retinoid concentrations (tables 1–3) and display atypical patterns in psoriasis and other hyperproliferative skin diseases. To what extent the phenotype of these skin disorders is caused by an inappropriate metabolism of retinoids, and whether the atypical patterns of retinoid binding proteins found are primary or secondary, remains to be elucidated. Further investigation is underway to characterize the function of CRBPs and CRABPs in healthy, psoriatic, and neoplastic skin.

V. Retinoid Catabolism

A. Retinoic Acid Metabolites

The cleavage of active retinoid ligands to inactive metabolites is of great importance for the regulation of nuclear retinoid receptors (fig. 5). Recently, the growth inhibitory effects of RA have been shown to correlate with the activity of RA metabolism (Takatsuka et al., 1996). The enzyme mainly responsible for this reaction is the CYP-dependent 4-hydroxylase that converts the β-ionone ring of RA to 4-hydroxy-RA metabolites (4-OH-RA) which are excreted much faster from the cells than RA (Roberts et al., 1979; Williams and Napoli, 1985; Westin et al., 1993). The CYP dependence of this reaction has been demonstrated in microsomes of rat skin (Van den Bossche et al., 1988), rabbit (Roberts et al., 1992) and human liver (Leo et al., 1989), human keratinocytes (Roos et al., 1996), and human skin (Duell et al., 1994).

Interestingly, all-trans-, 9-cis-, and 13-cis-RA induce a 4-hydroxylase, which seems to metabolize only all-trans-RA (Duell et al., 1994, 1996) in human skin. Conversely, 9-cis- and 13-cis-RA inhibited the 4-hydroxylation of all-trans-RA in human liver (Nadin and Murray, 1996). However, topical application of pharmacological doses of all-trans-RA to human skin induces a 4.5-fold increase in its metabolism to 4-OH-RA and other polar metabolites (Duell et al., 1992). Our findings showing that both 9-cis- and 13-cis-RA are isomerized to all-trans-RA in human keratinocytes, in vitro, may explain why topical application of these substances to human skin induces all-trans-RA 4-hydroxylase, leading to an increase in 4-OH-metabolites in epidermis regardless of the isomer applied (Jugert et al., in press). In contrast, human fibroblasts show no significant RA isomerization activity regardless of the isomer (13-cis), 9-cis, and all-trans-RA, respectively) applied. The major catalytic metabolite identified in fibroblasts is 4-oxo-13-cis-RA, no matter which RA isomer is added to the medium (unpublished results). Other studies have shown that micromosal preparations from mouse liver but not mouse skin can 4-hydroxylate 13-cis-RA in an in vitro assay system (Oldfield, 1990). In contrast, in cooperation with R. Wyss (Roche Laboratories, Basel, Switzerland), we found that RA 4-hydroxylase in liver and skin of National Marine Research Institute mice is cytochrome CYP 2E1 (CYP2E1) dependent. After induction of the CYP2e1-specific para-nitrophenol hydroxylase by the addition of ethanol to drinking water or the application of ethanol to mouse skin, we observed a parallel increase in p-nitrophenol hydroxylase activity and the amount of 4-hydroxylated RA metabolites, indicating that CYP2e1 is a major RA 4-hydroxylating enzyme in murine liver and skin (Jugert et al., 1995, 1996a). An alternative pathway of 13-cis-RA inactivation after topical application has been proposed through absorption into the bloodstream and transport to the liver for conversion to 4-OH-13-cis-RA by hepatic CYP2c8 (Leo et al., 1989).

4-oxo-all-trans-RA is the best characterized RA metabolite. It is approximately half as active as all-trans-RA in promoting cell differentiation in F9 embryonal carcinoma cell lines (Williams et al., 1992) and in producing dysmorphic effects in rat embryonal tis-
Our experiments have shown that the isomers 13-cis-RA and all-trans-RA are predominantly inside the keratinocytes, whereas their 4-OH- and 4-oxo-metabolites are excreted rapidly from the cells, indicating that these metabolites do not bind appreciably to receptors (unpublished observations).

The formation of 4-oxo-RA from 4-OH-RA was shown to require only NAD, whereas reduced NAD phosphate was ineffective, which is inconsistent with the involvement of CYP-isoenzymes (Allenby 1993). Wyss (personal communication) observed that there are no differences in the oxidizing activity of 4-OH-RA in cultured human keratinocytes and fibroblasts compared with the tissue culture medium, which suggests that 4-OH-RA is oxidized spontaneously to 4-oxo-RA depending on the concentration of all-trans-RA added to the culture medium and the induction/inhibition of the RA 4-hydroxylase. Alternatively, the formation of 4-oxo-RA can result from oxidation at the 2 or 3 position of the \( \beta \)-ionone-ring of 4-OH-RAL, catalyzed by a CYP1A2-mediated 4-oxidation of all-trans-RAL and 9-cis-RAL, and not from RA, as is assumed generally (Van Wauwe et al., 1994; Raner et al., 1996). This could be an alternative pathway for generation of 4-oxo-RA but would not completely explain how RA is cleaved.

Another group of RA-metabolites, the retinoyl-\( \beta \)-glucuronides (all-trans-RAG, 13-cis-RAG, and 9-cis-RAG), also may have retinoid receptor binding activity as demonstrated in vivo and in vitro (Mehta et al., 1991; Olson et al., 1992; Sass et al., 1994). Only traces of RA-glucuronides are found in human tissues compared with rodent (Sass et al., 1994). This species difference in glucuronidation could explain the high clearance of 13-cis-RA in rodents compared with humans (Nau et al., 1989; Nau, 1990).

The natural metabolite of RA, 5,6-epoxy-RA, is found in intestinal mucosa, liver, and kidney of rat (Napoli and McCormick, 1981). Although this metabolite inhibits the promotion of skin tumors equipotent with RA (Verma et al., 1980), it is converted to polar metabolites more rapidly than RA (Napoli et al., 1982). The specific enzyme generating 5,6-epoxy-RA is unknown.

Using a Cat-reporter assay (Astrom et al., 1990) and \( ED_{50} \) values for RXR\( \alpha \) and RAR\( \gamma \), a potency grading of retinoid binding activity and receptor inducibility has been evaluated with all-trans-RA > \( dd \)-RA > 4-oxo-RA > 4-OH-RA > 5,6-epoxy-RA (Duell et al., 1992). These metabolites produce epidermal thickening in hairless mouse skin in a rank order similar to that achieved with the Cat assay (Reynolds et al., 1993).

B. Cytochrome P450-Isoenzymes

Several isoenzymes of the cytochrome CYP superfamily are involved specifically in the catabolism of retinoids in rodent liver (Frolik et al., 1979; Roberts et al., 1979; Leo et al., 1984; Martini and Murray, 1993; Raner et al., 1996), trachea (Frolik et al., 1979), intestine (Roberts et al., 1991), and skin (Leo et al., 1984; Van den Bossche et al., 1988) (table 3).

White et al. (1996) reported the identification of RA-inducible all-trans-RA 4-hydroxylase (CYPRAI), encoding a new member of the cytochrome P450 supergene-family in zebrafish. They found that this gene was related closely to a human complementary deoxyribonucleic acid (cDNA) isolated from a human fetal brain library, suggesting that this novel CYP subfamily is highly conserved in fish and humans, and identified the cDNA representing this RA-inducible enzyme in various human tissues as a novel family of the CYP superfamily, named CYP26 (White et al., 1997). To what extent this CYPRAI is involved in the regulation of retinoid signaling in human skin cells is unknown.

Various cytochrome CYP-isoenzymes are involved in the catabolism of RA in rodent and human tissues (table 3): In rabbit liver microsomes, cytochrome CYP-1a2 (CYP1A2) and 2B4 were most effective in metabolizing RA to 4-OH-RA, whereas CYP2e3, 2E1, 2G2, and 2E2 were less effective, and CYP1A1 and 3A6 were ineffective (Roberts et al., 1991). In further studies, Roberts et al. (1992) reported that the CYP1a2 shows high activities in both the 4-hydroxylation of RA and the oxidation of RAL to RA, whereas CYP3A6 catalyzes only the latter, and the CYP2B4 catalyzes only the 4-hydroxylation of RA.

Also in rabbit liver microsomes, Raner et al. (1995) demonstrated that CYP1A1 and CYP1A2, to a lesser extent, are the most active enzymes in the conversion of all-trans-RA, 9-cis-RA, and 13-cis-RA to their corresponding RA isomers. This indicates that CYP1A1 and CYP1A2, to a lesser extent, are more involved in activation than in catalytic oxidation of retinoids. The \( k_{cat}/K_{m} \) value for 4-hydroxylation of all-trans- and 13-cis-RA by CYP1A1 is identical with that for all-trans-RA and 13-cis-RA formation, suggesting a dual role for this cytochrome in the oxidation of all-trans-RA and all-trans-RA.

In human liver, a member of the CYP2C family was reported to catalyze the 4-hydroxylation of RA (Leo et al., 1989; Duell et al., 1992), whereas studies with CYP-inhibiting immunoglobulin G antibodies in rat liver revealed that the CYP3A subfamily is involved in this process but can not be considered the “principal RA 4-hydroxylase” (Martini and Murray, 1993).

Isoenzymes of CYP families 2, 3 (Van Pelt et al., 1990), and 4 (Uchida et al., 1997) were shown to be present in human epidermal foreskin keratinocytes. The role of
these enzyme families especially with regard to retinoid metabolism is unknown.

Cultured human keratinocytes have been shown to contain CYP1A1 (Berghard et al., 1990) and the 4-hydroxylation of RA increases because of induction of CYP1A1 by 3-methylcholantrene (Van den Bossche and Willemsens, 1991; Edes et al., 1991), whereas RA inhibited the catalytic activity of CYP1A1 in human skin (Li et al., 1995), indicating the involvement of CYP1A1 in 4-hydroxylation of RA in this tissue.

The principal RA 4-hydroxylase for the catabolism of RA in human skin is still unknown. Whether it is the 4-hydroxylase described by White et al. (1996, 1997) remains to be proved. Further, it would be interesting to determine whether the basal and inducible activity of this enzyme is altered in skin diseases where RA treatment is effective (e.g., psoriasis, squamous cell carcinoma, Darier’s disease, acne, solar keratosis). In the skin of patients suffering from such diseases, it also would be very interesting to evaluate which xenobiotics induce/inhibit the activity of this CYP-isoenzyme and how this affects the clinical appearance of retinoid-sensitive skin diseases.

Because a range of CYP-isoenzymes seem to possess some capacity to 4-hydroxylate retinoids, attempts to isolate the principal RA 4-hydroxylase on the basis of CYP-isoenzyme activity should be conducted carefully.

The extent to which RARs and RXRs are involved in the regulation of retinoid-metabolizing enzyme activity in human skin is unknown and currently is being studied using dominant negative mutants of several RARs and RXRs.

VI. Modulation of Retinoid Metabolism: Pharmacological Interactions

A. Retinoids and Skin Malignancies

Actinic keratoses were the first skin lesions to be treated topically with all-trans-RA (Stüttgen, 1962). In various clinical trials, retinoids have been shown to be active in chemoprevention and treatment or prevention of skin malignancies (Verma, 1987; Hong et al., 1990; Crowe et al., 1991; Hu et al., 1991; Houle et al., 1991; Jones et al., 1992; Moon and Mehta, 1990; Bertram, 1993; Reynolds et al., 1993; De Luca et al., 1993; Lotan et al., 1995; Craven and Griffiths, 1996; Agarwal et al., 1996). These effects are assumed to relate to RAR-mediated antipromoting (Hill and Grubbs, 1992) and anti-initiating effects. The latter seems to be influenced by interference of several xenobiotics with different steps of the metabolism of retinoids in liver and skin microsomes (Verma, 1992; De Luca et al., 1994).

Some well known skin procarcinogens, such as 3-methylcholanthrene (Kinosita and Gelboin, 1972; Van den Bossche and Willemsens, 1988, 1991) and the polycyclic aromatic hydrocarbon benzo[a]pyrene (Falk et al., 1964; Van den Bossche and Willemsens, 1991; Davies, 1967; Bickers and Kappas, 1978; Edes et al., 1991), can increase RA catabolism in human skin and induce local tissue depletion of retinoids, respectively (Edes et al., 1991). This can be antagonized by high dietary intake of β-carotene (Edes et al., 1991) or RA (Li et al., 1995). This acceleration of retinoid cleavage primarily is caused by the xenobiotic-mediated induction of CYP1A1, which also is involved in the inactivation of RA to 4-OH-RA (Van den Bossche and Willemsens, 1991; Edes et al., 1991; Kizaki et al., 1996). Accordingly, retinoid-induced inhibition of basal as well as coal tar- and glucocorticoid-induced CYP1A1 expression in human skin, as reported by Li et al. (1995), seems to reflect a competitive feedback-inhibition of CYP1A1 activity by RA.

CYP1A1 is one major enzyme that converts the procarcinogens mentioned above into active carcinogenic metabolites in skin (Bickers and Kappas, 1978). The induction of this enzyme, leading to an acceleration of the turnover of RA to inactive metabolites and a local RA deficiency, might explain further the profound effect of these carcinogenic CYP1A1-inducers on cell proliferation and tumor formation. In support of this notion, 7,8-benzoflavone, an inhibitor of CYP1A1 activity, increases local vitamin A concentrations and reduces tumor formation in mouse skin (Gelboin et al., 1970). To what extent the procarcinogenic effects of these substances are caused by their induction of CYP-mediated depletion of retinoid levels in the skin, and which CYP-isoenzyme besides CYP1A1, especially the CYPRAI (White et al., 1996, 1997), are involved is unknown. However, the capacity of RA to down-regulate basal as well as inducible CYP1A1 expression indicates that retinoids also have, besides their well known antipromoting potential, an anti-initiating potential by suppressing CYP1A-dependent procarcinogen activation and subsequent tumor formation in target tissues. This down-regulation may be mediated through a retinoid-responsive element in the promoter region of the human CYP1A1 gene (Vecchini et al., 1994).

RA suppresses the expression of the aryl hydrocarbon receptor (AhR) in high calcium transformed cells (Wanner et al., 1996). To what extent this RA-mediated receptor modulation is of importance for the differentiation of epidermal keratinocytes is not known but suggests that RA is able to influence AhR, the main regulatory element of the procarcinogen-activating enzyme CYP1A1.

The imidazole antimycotics, ketoconazole, clotrimazole, and miconazole are all well known inhibitors of various cytochrome P450-isoenzymes, affecting also the metabolism of retinoids. They first were shown to inhibit the metabolism of RA in F9 embryonal carcinoma cells (Williams and Napoli, 1987). When tested in vitro, liarazole, a potent CYP inhibitor (Van Wauwe et al., 1993), suppressed neoplastic transformation and up-regulated gap junctional communication in murine and human fibroblasts (Acevedo and Bertram, 1995), which appar-
ently was caused by the presence of retinoids in the serum component of the cell culture medium (Rogers et al., 1990; Zhang et al., 1992). Furthermore, liarozole magnified the cancer chemopreventive activity of RA and \( \beta \)-carotene in these experiments by inhibiting RA catabolism as demonstrated by absence of a decrease in RA levels in the culture medium in the presence of liarozole during 48 hours, whereas without liarozole 99% of RA was catabolized. In vivo treatment with liarozole and ketoconazole reduced the accelerated catabolism of retinoids and increased the mean plasma \( \text{all-trans-RA} \) concentration in patients with acute promyelocytic leukemia and other cancers (Rigas et al., 1993).

The use of low-dose \( \text{all-trans-RA} \) in tandem with liarozole may enhance therapeutic retinoid levels in target tissues by inhibiting RA catabolism. Retinoid catabolism is induced after long-term \( \text{all-trans-RA} \) treatment (Lefebvre et al., 1991; Muindi et al., 1992) leading to RA-resistant disease (Warrell et al., 1993).

B. Retinoid Resistance

Two possible explanations for accelerated clearance of retinoids in patients during long-term treatment with retinoids have been suggested (Kizaki et al., 1996).

First, RA-mediated induction of CRABP expression, which lowers the plasma and intracellular levels of active RA by binding RA (see Section IV.B.), and second, the RA-mediated induction and/or constitutive overexpression of \( \Pi \)-glycoprotein, which is encoded by the multidrug resistance gene (MDR1), leading to decreased intracellular levels of RA by enhancing active transport of intracellular retinoids out of the target cells (Hamana and Tsuruo, 1986; Chen et al., 1986; Delva et al., 1993).

The \( \text{RAR} \beta_2 \) seems to be of great importance for the retinoid-mediated regulation of epithelial cell growth and differentiation, tumor formation, and the aging process (Houle et al., 1991, 1993; Gebert et al., 1991; Chen et al., 1995b; Lee et al., 1995; Lotan et al., 1995; Si et al., 1996; Bartsch et al., 1992, 1996). Because it is the most tightly RA-regulated retinoid receptor (Kato et al., 1992), \( \text{RAR} \beta_2 \) appears to be essential for pathological tissue alterations in vitamin A deficiency. Whereas vitamin A deficiency causes significant changes in the expression levels of \( \text{RAR} \alpha \) and \( \text{RAR} \gamma \) mRNAs, the level of \( \text{RAR} \beta \) transcripts is decreased greatly in various tissues of vitamin A-deficient rats and is rapidly inducible by administration of RA (Kato et al., 1992). These findings may indicate that a xenobiotic-driven depletion of retinoids favors the formation of dysplastic tissue formation or even malignant cell growth through the depletion of the \( \text{RAR} \beta_2 \) activity. Because the regulation of the \( \text{RAR} \beta_2 \) in epithelial tissue depends primarily on the expression of other retinoid receptors, especially \( \text{RAR} \alpha \) (Schon and Rheinwald, 1996; Geisen et al., 1997), the possible effects of \( \text{RAR} \beta_2 \) depletion on cell growth and differentiation is difficult to analyze separately from the context of expression patterns of other retinoid receptors or other transcription factors (e.g., AP1).

In addition, vitamin \( \text{D}_3 \) and retinoids can inhibit synergistically the growth and progression of squamous cell carcinomas and actinic keratoses in chronically sun-exposed skin (Majewski et al., 1997). One reason for this synergism may be the direct influence of vitamin \( \text{D}_3 \) on the isomerization and the metabolism of RA, which we observed in human keratinocytes (Jugert et al., 1997). Here, vitamin \( \text{D}_3 \) inhibits the isomerization of \( 13\text{-cis-RA} \) to the more receptor active \( \text{all-trans} \) and \( 9\text{-cis-isomers} \). Moreover, we found that the vitamin \( \text{D}_3 \) derivative secocholestra-trien-1,3,24-triol (tacalcitol), used for the treatment of severe keratinizing disorders, significantly inhibits 4-hydroxylation of \( \text{all-trans-RA} \) (Jugert et al., 1998).

Further investigations are underway to elucidate these mechanisms in the control of retinoid levels in retinoid-responsive malignant skin diseases.

C. Retinoids and Disorders of Keratinization

The use of topical and oral retinoids for the treatment of disorders of keratinization, such as psoriasis and Darier’s disease, has been established (Orfanos et al., 1972, 1973, 1987; Runne et al., 1973; Peck et al., 1978; Happle et al., 1987; Blanchet-Bardon et al., 1991). Systemic retinoid therapy often is combined with topical drugs such as corticosteroids, dithranol, tar, and also ultraviolet A/ultraviolet B phototherapies, in which synergistic effects have been reported (Orfanos et al., 1997).

Ethanol treatment of rats results in enhanced microsomal catabolism of \( \text{all-trans-RA} \) to 4-OH-RA and 4-oxo-RA (50%, \( P < 0.01 \)) accompanied by increased microsomal CYP concentrations (34%, \( P < 0.005 \)) (Sato and Lieber, 1982). This induction in turn significantly decreased the storage of ROL in the liver in baboons and rats (Sato and Lieber, 1981). One potential target of ethanol action may be CYP2E1, which oxidizes ethanol (Ohnishi and Lieber, 1977) and 4-hydroxylates retinoids (Roberts et al., 1991).

Ethanol also inhibits ADH-catalyzed ROL oxidation in vitro (Julia et al., 1986), and ethanol treatment of mouse embryos has been demonstrated to reduce endogenous RA levels (Deltour et al., 1996). The inhibition of cytosolic RoIDH activity and stimulation of microsomal RoIDH activity could explain ethanol-mediated vitamin A depletion, apart from ADH-isoenzymes (Napoli, 1996). Although the exact mechanism of inhibition of retinoid metabolism by ethanol is unclear, these observations are consistent with the finding that patients with alcoholic liver disease have depleted hepatic vitamin A reserves (Leo and Lieber, 1982).

In addition to its influence on psoriasis by inhibition of RA synthesis, ethanol may exert its effects in fetal alcohol syndrome by the same mechanism, because the class IV ADH was found to play a crucial role leading to reduced RA levels after ethanol treatment in cultured mouse embryos (Deltour et al., 1996). Ethanol occasion-
ally provokes acute exacerbations of psoriasis (Poikolainen et al., 1990; Frank and Lentner, 1996). Because retinoids have been very beneficial in the treatment of psoriasis, an ethanol-induced decrease of intracellular ROL and RA could be one explanation for this acute worsening of psoriasis.

Liarazole has been demonstrated to be an active antipsoriatic drug (Dockx et al., 1995; De Doncker et al., 1991). By suppressing the CYP-mediated 4-hydroxylation of RA to 4-OH-RA, liarazole increases serum levels of RA from nearly undetectable levels to 2.9 ± 1 ng/ml serum, which enhances the action of RA in cellular differentiation (Van Wauwe et al., 1994). Because liarazole is 2 to 15 times more potent than clotrimazole, miconazole, and metyrapone in inhibiting RA metabolism, it has been used successfully for the treatment of psoriasis (Dockx et al., 1995).

To what extent imbalances in retinoid metabolism are responsible for the pathogenesis of psoriasis and other keratinizing disorders, and which steps of this metabolic pathway are affected, is unknown. The mechanisms of the effect of retinoid therapy in other keratinizing disorders (e.g., ichthyosis, Darier's disease, palmoplantar keratoderma, and pityriasis rubra pilaris) are unknown. Also, it is possible that the effectiveness of systemic and topical retinoids in acne could be influenced by the concomitant administration of liarazole.

D. Other Modulators of Retinoid Metabolism

The corticosteroid dexamethasone, the macrolide antibiotic triacetyloleandomycin, and phenobarbital are all well established inducers of the CYP3A subfamily (Waxman et al., 1985; Wrighton et al., 1985; Hostetler et al., 1987; Jugert et al., 1994) and can increase microsomal 4-hydroxylation of RA in rat liver (Martini et al., 1993). Whether the CYP3A subfamily and its modulation by xenobiotics is important for retinoid metabolism in human skin remains to be clarified. However, CYP3A mRNA is strongly inducible in human hepatocytes with retinoid treatment in vitro (Jurima-Romet et al., 1997).

Glucocorticoids (clobetasol) also induce the expression of CYP1A1 in human skin (Li et al., 1995). This is mediated through glucocorticoid receptor responsive elements that have been identified in the first intron of the rat and human CYP1A1 genes (Hines et al., 1988). These findings suggest the possibility that skin changes caused by long-term treatment with topical or systemic glucocorticoids could be mediated by a steroid-induced depletion of active retinoids. Therefore, we hypothesize that tandem treatment of patients with both glucocorticoids and low-dose RA may prevent some steroid side effects. This idea already has been confirmed in a mouse model (Schwarz et al., 1994). Retinoids may have a steroid-sparing effect (Orfanos et al., 1997). Investigation is underway to test whether this is related to corticoste-

VII. Conclusions and Perspectives

A. Retinoid-Drug Combinations in Dermatologic Therapy

A broad spectrum of drugs is used in combination with retinoids for the treatment of dermatological disorders to enhance the efficacy of either agent. Especially in the treatment of psoriasis, several strategies have been developed whereby retinoids are combined with other agents such as selective ultraviolet irradiation, ultraviolet A irradiation with concomitant psoralen treatment, cyclosporin, vitamin D$_3$-derivatives, azole derivatives, urea, tar, salicylic acid, and dithranol (Gollnick, 1996; Orfanos et al., 1997).

These regimens additively or synergistically may modulate the disease process and also provide opportunities to alter the regimens, especially during long-term treatment to decrease drug toxicities or to enhance efficacy. At least three types of combination strategies for retinoid-drug combinations in dermatological therapy can be described.

First, combinations of drugs displaying distinct effects on cell proliferation/differentiation and immunomodulation (e.g., retinoids and chemotherapy in advanced cutaneous T-cell lymphoma (Gollnick et al., 1981; Thesstrup-Petersen et al., 1988)).

Second, a combination of retinoids with ultraviolet A or B radiation (and other drugs). For example, ultraviolet A irradiation with concomitant retinoid and psoralen treatment therapy (and psoralen and ultraviolet A combination) is currently one of the most effective regimens for recalcitrant severe psoriasis (Saurat et al., 1988; Tanew et al., 1991).
Third, drugs with metabolic interactions that can enhance the half-life of active compounds. An example of this regimen is the interaction between azole (Kato et al., 1992; Van Wauwe et al., 1993; Dockx et al., 1995; Majewski et al., 1997) and vitamin D derivatives (Gollnick, 1996; Jugert et al., 1997) that inhibit the metabolism of retinoids in skin cells leading to increased intracellular amounts of active RA isomers. Further study and the identification of novel interactions of this type of drug interaction is of great clinical interest because they may decrease the dose of retinoids required for efficacy, thereby also reducing the risk of side effects of the retinoids.

The complexity of the metabolic pathways for retinoids and the likelihood that these are altered by diseases affecting the skin suggest that such novel strategies will be forthcoming. Multiple studies are underway to define the steps of retinoid metabolism where the use of modulating drugs might influence the results of dermatological therapy thereby leading to the most profound effects with regard to the clinical outcome.

B. Retinoid Receptor Agonists/Antagonists

Synthetic retinoid receptor-selective agonists/antagonists offer another new approach. This concept of drug development is based on the findings that retinoid receptors (RARs and RXRs) can target different genes depending on the activated retinoid receptor complexes in human skin (Fisher and Voorhees, 1996, 1996). The multiplicity of these retinoid signaling pathways affords potential for therapeutic opportunity as well as undesirable side effects associated with retinoid therapy. It is possible that the indiscriminate activation of all pathways by nonspecific retinoid ligands could lead to unacceptable side effects so that any enhanced efficacy would be obtained at the cost of enhanced toxicity. The development of ligands selective for individual receptor subtypes relevant to a targeted disease could decrease these toxic effects and thereby improve the therapeutic index. Two new arotinoids are now available for topical use in skin diseases. These are tazarotenic acid (tazarotene) and 6-[3-(1-adamantyl)]-4-methoxyphenyl-2-naphtoic acid (adapalene) (fig. 2); other synthetic retinoid derivatives are being developed (Klein et al., 1996; Majewski et al., 1997).

The first of these synthetic receptor-selective ligands available for topical treatment of psoriasis is tazarotene (fig. 2), an acetylenic third-generation retinoid derivative (Ésgleyes-Ribot et al., 1994). It is a poorly absorbed, nonisomerizable arotinoid, which is metabolized rapidly to its free carboxylic acid, tazarotenic acid, binding with high affinity to RARs, with the rank order of affinity being RARβ > RARγ > RARα (Nagpal et al., 1995). It does not bind to any of the RXRs. This retinoid derivative is said to have lower cytotoxic effects than other retinoids, but it achieves sustained therapeutic efficacy in the treatment of plaque-type psoriasis (Chandraratna, 1996; Weinstein, 1996).

The second synthetic receptor-selective retinoid ligand is adapalene (fig. 2), a newly stable naphthoic acid arotinoid with lipophilic properties. It does not bind to CRABP, although it enhances its synthesis, and its rank order of retinoid receptor affinity appears to be RARβ > RARγ > RARα (Bernard, 1993; Griffith et al., 1993; Shalita et al., 1996).

Future generations of such receptor subtype-selective retinoids may provide clinicians with more specific and less toxic drugs for dermatological therapy. These arotinoids, which first were introduced for the treatment of skin diseases, also may have potential as anticancer drugs (Tsambaos and Orfanos, 1982, 1983; Dreno, 1993; Orfanos et al., 1997; Duvic et al., 1997).

C. Future Directions

The steadily increasing knowledge concerning ligand-receptor interaction and the metabolism and molecular actions of retinoids portends new approaches for managing dermatological diseases through pharmacological modulation of the retinoid metabolic pathway. The future of retinoid therapy of these disorders seems to be moving in two directions.

First, the development of drugs that modulate retinoid metabolism by interacting with retinoid-metabolizing enzymes and/or binding proteins, and second, more retinoid receptor subtype-specific synthetic retinoid derivatives.

Drugs from the first category may permit reduction of the amount of the agent administered, thereby increasing therapeutic benefit and reducing the toxic side effects of treatment. The efficacy of the azole derivative liarazole as an inhibitor of RA 4-hydroxylase for the treatment of psoriasis in combination with RA demonstrates the usefulness of this approach. Investigation is underway to evaluate the clinical significance of our in vitro findings of increased intracellular RA levels after treatment with vitamin D₃ or its synthetic derivative tacalcitol (Jugert et al., 1997).

It is important to emphasize that retinoids are also very effective drugs for preventing or treating cancer (Lippman et al., 1997), especially skin malignancies, which present the most frequent type of human cancer (Khuri et al., 1997). New retinoid regimens could lead to innovative therapy options in cutaneous cancers.

Drugs from the second category are selective retinoid receptor agonists/antagonists. As discussed above, they could offer more specific approaches by targeting specific retinoid receptors uniquely relevant for the treatment of specific skin disorders. Furthermore, this approach could reduce the occurrence of retinoid side effects.

The experimental systems used for study are crucial for defining retinoid action in the skin. For example, retinoids were reported to display effects in cultured keratinocytes that were opposite to those in vivo (for
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The knowledge concerning the molecular action of retinoids in the skin has increased dramatically, but the majority of steps of retinoid metabolism especially retinoid inactivation still are not fully understood. The interaction of retinoids as the central agent with other drugs represents a new dimension of dermatological therapy providing us with more specific and less toxic therapy approaches to influence cell proliferation and differentiation. Perhaps in no other area of pharmacology is the concept of using drug-drug interactions as a rationale for therapy more advanced than with retinoids in dermatology. It is likely that this strategy will prove useful in other areas as well.

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