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Retinoid Metabolism in the Skin

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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 in vitamin A-deficient animals (Wolbach and Howe, 1925). 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)[b] (tretinoin), and 13-*cis*-RA (isotretinoin); second, the monoaromatic retinoid derivatives trimethyl-methoxyphenyl analog of RA (etretinate) and 9-(4-methoxy-2,3,6-trim-

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[b] Abbreviations: ADH, alcohol dehydrogenase; AhR, arylhydrocarbon receptor; ALDH, aldehyde dehydrogenase (RalDH); AP1, activator protein 1; cDNA, complementary deoxyribonucleic acid; CRBP, cellular retinol binding protein (apo- and holo); CRABP, cellular retinoic acid binding protein (apo- and holo); CYP, cytochrome P450-isoenzymes; LRAT, lectin:retinol acyltransferase; mRNA, messenger ribonucleic acid; NAD, nicotinamide adenine dinucleotide; RA, retinoic acid; RAL, retinal; RalDH, retinal dehydrogenase; RAR, retinoic acid receptor; RBP, retinol binding protein; RE, retinylesters; REH, retinylester hydrolase; ROL, retinol; RolDH, retinol dehydrogenase; RXR, retinoid X receptor; RXRE, retinoid X responsive element; SDR, short-chain dehydrogenase/reductase.

ethylphenyl)-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-naphthoic 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 superfamily of ligand-inducible transcriptional regulators that include steroid hormone receptors, thyroid hormone receptors, and vitamin D_3 receptors (reviewed in: Giguere, 1994; Mangelsdorf *et al.*, 1994; Chambon, 1996). RARs and RXRs act via polymorphic *cis*-acting 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 Chambon (1996).

Although *all-trans-* and *9-cis*-RA are only minor metabolites of retinol (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

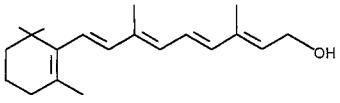
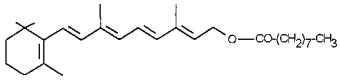
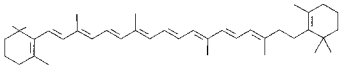
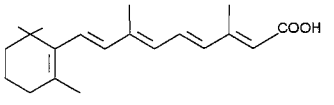
Major sources of natural retinoids are animal fats, fish liver oil (retinylesters), and yellow and green vegetables (carotenoids) (fig. 2). Ingested retinylesters (RE) are hydrolyzed to ROL by enteral hydrolases in the intestine. ROL and carotenoids are absorbed by intestinal mucosa cells. Of the carotenoids, 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 excentric cleavage followed by transformation of the apo-carotenoid acids into RAs (Wang *et al.*, 1991).

In the intestinal cell, ROL also forms complexes with CRBP-II. This ROL-CRBP-II complex serves as 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 by chylomicrons (Bloomhoff *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; Tang 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

Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Retinol (Vitamin A)		Oral	Vitamin Supplement	
Retinyl Palmitate		Topical 0.5-5% Emulsions	Cosmetic Agents	
β-Carotene (Provitamin A)		Topical	Hypopigmentations, Hyperpigmentations, Radical Protection	Nutritient Color
Tretinoin		Topical 0.025-0.1% Gels or Creams	Acne vulgaris, Parakeratosis, Hyperkeratosis	Photoaging, Actinic Keratosis

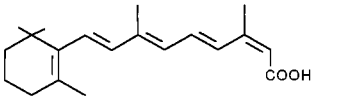
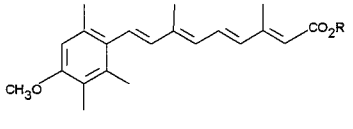
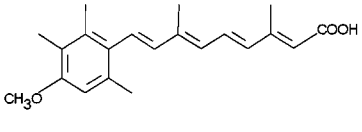
Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Isotretinoin		Topical 0.05% cream Oral 0.25-1.0 mg/kg/d	Cystic Acne, Recalcitrant Nodular Acne	Rosacea Gram-negative Folliculitis Pyoderma faciale Hidradenitis suppurativa Cancer Prevention
Etretinate		Oral 0.25-1.0 mg/kg/d	Generalized pustular Psoriasis, Exfoliative Psoriasis, Plaque Psoriasis	
Acitretine		Oral 0.25-1.0 mg/kg/d	Psoriasis (erythrodermic, pustular, and severe recalcitrant)	Palmoplantar keratoderma Pustulosis palmoplantaris, Icthyosis, Darier's Disease, Pityriasis rubra pilaris, Lichen ruber planus

FIG. 1. Indications and mode of administration of commercially available retinoids in dermatological therapy.

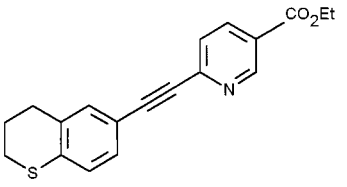
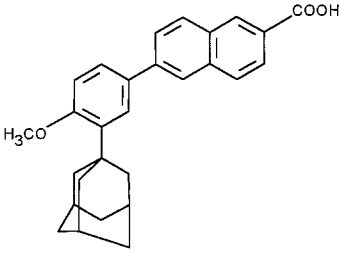
Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Tazarotene		Topical 0.05-0.1% gels	Psoriasis vulgaris	
Adapalene		Topical 0.1% gel	Acne vulgaris	

FIG. 1. Continued.

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).

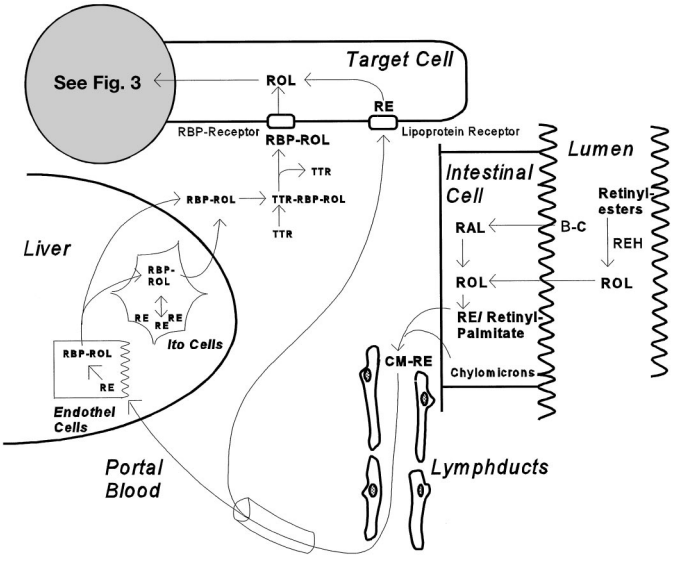


FIG. 2. Retinoid absorption, transport, and storage. B-C, β-carotenoids, CM-RE, chylomicron-RE complexes; RBP-ROL, RPB-ROL complexes; TTR, transthyretine.

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 ROL 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 1
The reversible retinol/retinal interconversion

Enzyme	Locus	Reaction	Binding protein/function
Lecitin:retinol-acyltransferase (LRAT)	Microsomes	Retinol → retinyl esters	Holo-CRBP-retinol/substrate, Apo-CRBP/inhibitor
Retinyl ester hydrolase (REH)	Microsomes	Retinyl esters → retinol	Apo-CRBP/activator
Alcohol dehydrogenase classes I, II, IV, and VII (RolDH)	Microsomes Cytosol	Retinol → retinal	Holo-CRBP-retinol/substrate, Apo-CRBP/inhibitor
Short-Chain dehydrogenase/reductase, microsomal retinol dehydrogenase types I, II, and III	Microsomes	Retinol → retinal	Holo-CRBP-retinal/substrate

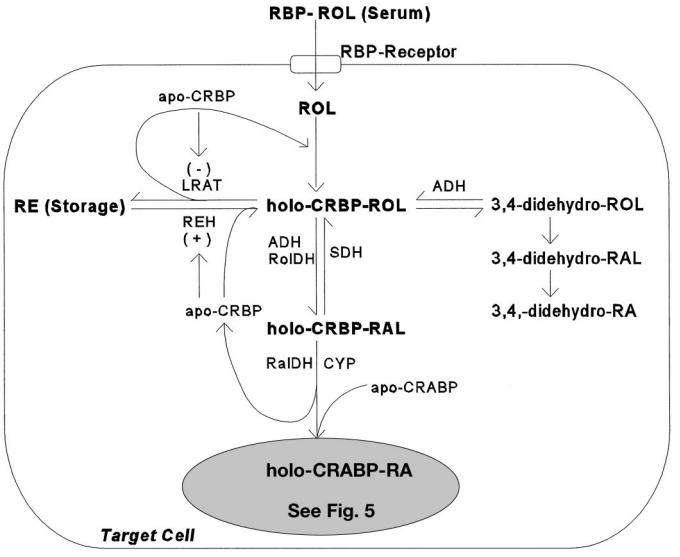


FIG. 3. Storage and metabolism of retinol in the target cell. SDH, short-chain dehydrogenase.

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 1). 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.

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.

TABLE 2
The irreversible oxidation of retinal to retinoic acid

Enzyme	Locus	Reaction	Binding protein/function
Aldehyde dehydrogenases class I (RalDH)	Cytosol	Retinal → retinoic acid	Holo-CRBP-retinal/substrate
Cytochrome P450-isoenzymes	Microsomes		
CYP1A1		Retinal → retinoic acid	Holo-CRBP-retinal/substrate
CYP1A1		Retinal → 4-OH-retinal	
CYP3A6			
CYP1A2		9-*cis*-Retinal → 4-OH-9-*cis*-retinal → 4-oxo-9-*cis*-retinal	
CYP1A2		9-*cis*-Retinal → 9-*cis*-retinoic acid	
CYP2B4, CYP2C3		9-*cis*-RAL → 4-OH-retinal	

The synthesis of RA does not require ROL as a substrate (Napoli and Race, 1988). Because the cleavage of β-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*-β-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*-β-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 maintainance 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 β-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*-β-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.*,

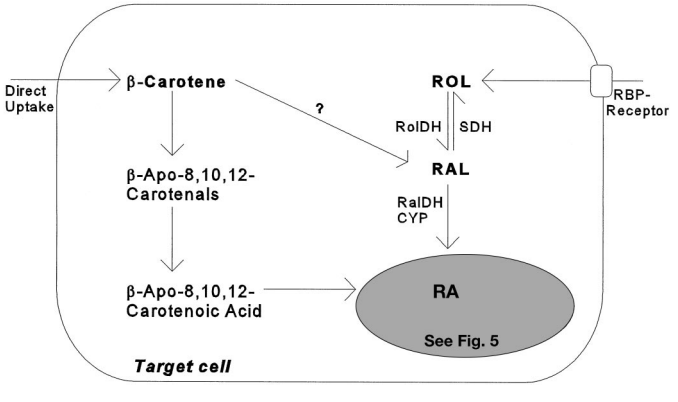


FIG. 4. Metabolism of β-carotene in the target cell.

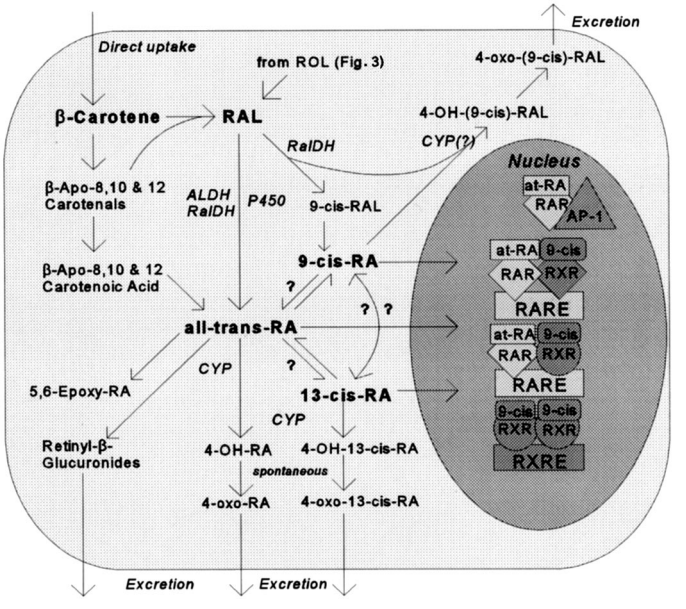


FIG. 5. Intracellular pathways of retinoid metabolism. at-RA, *all-trans*-RA.

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 A_2) (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 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 (Harrisson *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 (Eskild *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 juxtaposed ligand portals (Thompson *et al.*, 1995), whereas CRABP-II may facilitate transport of RA to the nucleus

TABLE 3
The catabolism of retinoic acid to polar metabolites

Enzyme	Locus	Reaction	Binding protein/function
Cytochrome P450-isoenzymes	Microsomal		
P450RAI		Retinoic acid → 4-OH-retinoic acid	CRABP-retinoic acid/substrate
CYP1A1		Retinoic acid → 4-OH-retinoic acid	CRABP-retinoic acid/substrate
CYP1A2			
CYP2B4			
CYP2C3			
CYP2E1, 2			
CYP2G2			
CYP3A			

(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 (Orfanos *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 also was 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 maintainance 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 microsomal 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 dysmorphogenic effects in rat embryonal tis-

sue (Kraft *et al.*, 1992). Likewise, 4-oxo-RA binds RARβ with an affinity similar to that of *all-trans*-RA (Pijnappel *et al.*, 1993), but binds poorly to RARγ (Reddy *et al.*, 1992). 4-oxo-RA also binds to CRABP with an affinity slightly lower than that of *all-trans*-RA in vitro (Fiorella *et al.*, 1993)

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 β-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-β-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 rodents (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 RXRα and RARγ, 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 CYP2c3, 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*-RAL, 9-*cis*-RAL, and 13-*cis*-RAL 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*-RAL 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*-RAL 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-methylcholantrene (Kinoshita 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, liarazole magnified the cancer chemopreventive activity of RA and β-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 liarazole during 48 hours, whereas without liarazole 99% of RA was catabolized. In vivo treatment with liarazole and ketoconazole reduced the accelerated catabolism of retinoids and increased the mean plasma *all-trans*-RA concentration in patients with acute promyelocytic leukemia and other cancers (Rigas *et al.*, 1993).

The use of low-dose *all-trans*-RA in tandem with liarazole may enhance therapeutic retinoid levels in target tissues by inhibiting RA catabolism. Retinoid catabolism is induced after long-term *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 P-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 RARβ$_2$ 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), RARβ$_2$ appears to be essential for pathological tissue alterations in vitamin A deficiency. Whereas vitamin A deficiency causes no significant changes in the expression levels of RARα and RARγ mRNAs, the level of RARβ 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 RARβ$_2$ activity. Because the regulation of the RARβ$_2$ in epithelial tissue depends primarily on the expression of other retinoid receptors, especially RARα (Schon and Rheinwald, 1996; Geisen *et al.*, 1997), the possible effects of RARβ$_2$ 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 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 D$_3$ on the isomerization and the metabolism of RA, which we observed in human keratinocytes (Jugert *et al.*, 1997). Here, vitamin D$_3$ inhibits the isomerization of 13-*cis*-RA to the more receptor active *all-trans* and 9-*cis*-isomers. Moreover, we found that the vitamin D$_3$ derivative seco-cholestra-trien-1,3,24-triol (tacalcitol), used for the treatment of severe keratinizing disorders, significantly inhibits 4-hydroxylation of *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 *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 (Julià *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 RolDH activity and stimulation of microsomal RolDH 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., icthyosis, Darier's disease, palmoplantar keratodermas, and pityriasis rubra pilaris (Borok and Lowe, 1990; Peck and Yoder, 1976; Happle *et al.*, 1987] 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 corticosteroid-induced inhibition of CRABP-II expression (Piletta *et al.*, 1994).

Studies on ADH inhibitors have revealed further evidence that this enzyme functions in ROL oxidation for RA synthesis. The ADH inhibitor 4-methylpyrazole can inhibit the conversion of ROL to RA in mouse embryos in vivo (Collins *et al.*, 1992), whereas microsomal ROL dehydrogenases (SDHs) are not inhibited by 4-methylpyrazole (Chai *et al.*, 1995).

Exogenous fatty acids may be another remarkable way to alter the metabolism of active retinoids in cultured human epidermal keratinocytes. Randolph and Simon (1995) demonstrated that unsaturated 16- and 18-carbon fatty acids exert the following effects on intracellular retinoid metabolism: The total cell retinoid mass increases up to 50% because of RE accumulation corresponding to the added fatty acid, whereas the utilization of endogenous RE decreased up to 80%. Furthermore, the steady-state cellular concentrations of ROL, 3,4-didehydro-ROL, and their respective carboxylic acids decreased up to 80%, whereas the RA metabolism was not altered.

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; Thestrup-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; Dockxs *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 undesired 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; Duvic *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 (Esgleyes-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 $\gg$ 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 new highly stable naphtoic 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 $\gg$ 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 D_3 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

review see Fisher and Voorhees, 1996). Monolayer in vitro systems exhibit responses to retinoid treatment that differ from the in vivo situation. Using keratinocytes grown on a dermal substrate without direct contact with culture medium has helped to solve this dilemma (Asselineau, 1989).

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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REFERENCES

Acevedo P and Bertram JS (1995) Liarazole potentiates the cancer chemopreventive activity of and the up-regulation of gap junctional communication and connexin 43 expression by retinoic acid and beta-carotene in 10T1/2 cells. *Carcinogenesis* **16:**2215–2222.

Agarwal R, Mohan RR, Ahmad N and Mukhtar H (1996) Protection against malignant conversion in SENCAR mouse skin by all *trans* retinoic acid: Inhibition of the ras p21-processing enzyme farnesyltransferase and Ha-ras p21 membrane localization. *Mol Carcinog* **17:**13–22.

Allenby G, Bocquel MT, Saunders M, Kazmer S, Speck J, Rosenberger M, Lovey A, Kastner P, Grippo JF, Chambon P and Levin AA (1993) Retinoic acid receptors and retinoid x receptors: Interactions with endogenous retinoic acids. *Proc Natl Acad Sci USA* **90:**30–34.

Asselineau D, Bernard BA, Bailly C and Darmon M (1989) Retinoic acid improves epidermal morphogenesis. *Dev Biol* **133:**322–335.

Astrom A, Pettersson U, Krust A, Chambon P and Voorhees JJ (1990) Retinoic acid and synthetic analogs differentially activate retinoic acid receptor dependent transcription. *Biochem Biophys Res Commun* **173:**339–345.

Astrom A, Tavakkol A, Petersson U, Cromie M, Elder JT and Voorhees JJ (1991) Molecular cloning of two human cellular retinoic acid binding proteins (CRABP): Retinoic acid induced expression of CRABP-II but not CRABP-I in adult human skin in vivo and skin fibroblasts in vitro. *J Biol Chem* **266:**17662–17666.

Baer R (1962) Untersuchungen über die wirkung von vitamin-A-Säure. *Dermatologica* **124:**192–195.

Bavic CO, Eriksson U, Allen RA and Peterson PA (1991) Identification and partial characterization of a retinal pigment epithelial membrane receptor for plasma retinol binding protein. *J Biol Chem* **266:**14978–14985.

Becherel PA, Mossalayi MD, Le Goff L, Frances C, Chosidow O, Debre P and Arock M (1994) Mechanism of antiinflammatory action of retinoids on keratinocytes NO-synthase activation. *Lancet* **344:**1570–1571.

Berghard A, Gradin K and Toftgard R (1990) Serum and extracellular calcium modulate induction of cytochrome P4501A1 in human keratinocytes. *J Biol Chem* **265:**21086–21090.

Bernard BA (1993) Adapalene, a new chemical entity with retinoid activity. *Skin Pharmacol* **6**(Suppl)**:**61S–69S.

Bertram JS (1993) Cancer prevention by carotenoids: Mechanistic studies in cultured cells. *Ann NY Acad Sci* **691:**177–191.

Bickers DR and Kappas A (1978) Human skin aryl hydrocarbon hydroxylase induction by coal tar. *J Clin Invest* **31:**147–151.

Blanchet-Bardon C, Nazzaro V, Rognin C, Geiger JM and Puissant A (1991) Acitretin in the treatment of severe disorders of keratinization: Results of an open study. *J Am Acad Dermatol* **24:**982–986.

Blaner WS and Olson JA (1994) Retinol and retinoic acid metabolism, in *The Retinoids: Biology, Chemistry, and Medicine* (Sporn MB, Roberts AB and Goodman DS eds) ed 2, pp 229–255, Raven Press, New York.

Blomhoff R (1994) *Vitamin A in Health and Disease,* Marcel Dekker, New York.

Blomhoff R, Green MH, Berg T and Norum KR (1990) Transport and storage of vitamin A. *Science (Wash. DC)* **250:**399–404.

Blomhoff R, Green MH, Green JB, Berg T and Norum KR (1991) Vitamin A metabolism: New perspectives on absorption, transport, and storage. *Physiol Rev* **71:** 951–990.

Boerman MHEM and Napoli JL (1995) Characterization of a microsomal retinol dehydrogenase: A short-chain alcohol dehydrogenase with integral and peripheral membrane forms that interacts with holo-CRBP (type I). *Biochemistry* **34:**7027–7037.

Boleda MD, Saubi N, Farres J and Pares X (1993) Physiological substrates for rat alcohol dehydrogenase classes: Aldehydes of lipid peroxidation, omega-hydroxy fatty acids, and retinoids. *Arch Biochem Biophys* **307:**85–90.

Borok M and Lowe NJ (1990) Pityriasis rubra pilaris: Further observations of systemic retinoid therapy. *J Am Acad Dermatol* **22:**792–795.

Boylan JF and Gudas LJ (1991) Overexpression of the cellular retinoic acid binding protein-I (CRABP-I) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells. *J Cell Biol* **112:**965–969.

Breitman TR, Selonick SE and Collins SJ (1980) Induction of differentiation of human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* **77**:2936–2940.

Buck J, Derguini F, Levi E, Nakanishi K and Hammerling U (1991) Intracellular signaling by 14-hydroxy-4,14-retro-retinol. *Science (Wash. DC)* **254:**1654–1656.

Busch C, Siegenthaler G, Vahlquist A, Nordlinger H, Sundelin J, Saksena P and Eriksson U (1992) Expression of cellular retinoid-binding proteins during normal and abnormal epidermal differentiation. *J Invest Dermatol* **99:**795–802.

Cañada FJ, Law WC, Rando RR, Yamamoto T, Derguini F and Nakanishi K (1990) Substrate specificities and mechanism in the enzymatic processing of vitamin A into 11-cis-retinol. *Biochemistry* **29:**9690–9697.

Chai X, Boerman MHEM, Zhai Y and Napoli JL (1995) Cloning of a cDNA for liver microsomal retinol dehydrogenase: A tissue-specific, short chain alcohol dehydrogenase. *J Biol Chem* **270:**3900–3904.

Chambon P (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J* **10:**940–954.

Chambon P (1995) The molecular and genetic dissection of the retinoid signaling pathway. *Recent Prog Horm Res* **50:**317–332.

Chandraratna RAS (1996) Tazarotene: First of a new generation of receptor selective retinoids. *Br J Dermatol* **135:**18–25.

Chen C, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM and Roninson IB (1986) Internal duplication and homology with bacterial transport proteins in the mdr-1 (P-Glycoprotein) gene from multidrug resistant human cells. *Cell* **47:**381–389.

Chen H, Namkung MJ and Juchau MR (1995a) Biotransformation of all-trans retinol and all-trans retinal to all-trans retinoic acid in rat conceptual homogenates. *Biochem Pharmacol* **50:**1257–1264.

Chen JY, Penco S, Ostrowsky J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P and Gronemeyer H (1995b) RAR-specific agonists/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO J* **14:**1187–1197.

Chen M, Achkar C and Gudas LJ (1994) Enzymatic conversion of retinaldehyde to retinoic acid by cloned murine cytosolic and mitochondrial aldehyde dehydrogenase. *Mol Pharmacol* **46:**88–96.

Chen S, Darling IM, Yu KL, Starrett JE Jr, Mansuri MM, Whiting G and Tramposch KM (1995c) In vivo activity of retinoid esters in skin is related to in vitro hydrolysis rate. *J Pharm Pharmacol* **47:**626–631.

Chien DS, Sandri RB and Tang-Liu DS (1992) Systemic pharmacokinetics of Acitretin, etretinate, isotretinoin, and acetylenic retinoids in guinea pigs and obese rats. *Drug Metab Dispos Biol Fate Chem* **20:**211–217.

Collins MD, Eckhoff C, Chahoud I, Bochert G and Nau H (1992) 4-Methylpyrazole potentially ameliorated the teratogenicity of retinol and reduced the metabolic formation of all-trans retinoic acid in the mouse. *Arch Toxicol* **66:**652–659.

Connor MJ and Smith MH (1987) Terminal group-oxidation of retinol by mouse epidermis: Inhibition in vitro and in vivo. *Biochem J* **244:**489–492.

Craven NM and Griffiths CE (1996) Topical retinoids and cutaneous biology. *Clin Exp Dermatol* **21:**1–10.

Crowe DL, Gudas LJ and Rheinwald JG (1991) Variable expression of retinoic acid receptor (RARbeta) mRNA in human oral and epidermal keratinocytes: Relation to keratin 19 expression and keratinization potential. *Differentiation* **48:**199–208.

Darmon M and Blumenberg M (1993) Retinoic acid in epithelial and epidermal differentiation, in *Molecular Biology of the Skin: The Keratinocyte* (Darmon M ed) pp 181–206, Academic Press, San Diego.

Davies RE (1967) Effect of vitamin A on 7, 12-dimethylbenz(a)anthracene-induced papillomas in rhino mouse skin. *Cancer Res* **27:**237–241.

De Doncker P, Stoppie P and Van Wauwe JP (1991) Inhibitors of the metabolism of retinoic acid in the skin: A novel approach in the treatment of acne and psoriasis. *Proc Natl Acad Dermatol Dallas* **3:**7–12.

De Leenheer AP, Lambert WE and Claeys I (1982) All-trans retinoic acid: Measurement of reference values in human serum by high performance liquid chromatography. *J Lipid Res* **23:**1362–1367.

De Luca LM, Darwiche N, Celli G, Kosa K, Jones C, Ross S and Chen LC (1994) Vitamin A in epithelial differentiation and skin carcinogenesis. *Nutr Rev* **52:**45–52.

De Luca LM, Sly L, Jones CS and Chen LC (1993) Effects of dietary retinoic acid on skin papilloma and carcinoma formation in female SENCAR mice. *Carcinogenesis* **14:**539–542.

Deltour L, Ang HL and Duester G (1996) Ethanol inhibition of retinoic acid synthesis as a potential mechanism for fetal alcohol syndrome. *FASEB J* **10:**1050–1057.

Delva L, Cornic M, Balitrand N, Guidez F, Miclea JM, Delmer A, Teillet F, Fenaux P, Castaigne S, Degos L and Chomienne C (1993) Resistance to all-trans retinoic acid (ATRA) therapy in relapsing promyelocytic leukemia: Study of in vitro ATRA sensitivity and cellular RA binding protein levels in leukemic cells. *Blood* **82:**2175–2181.

Dew SE and Ong DE (1995) Specificity of the retinol transporter of the rat small intestine brush border. *Biochemistry* **321:**434–441.

Didierjean L, Carraux P, Grand D, Sass JO, Nau H and Saurat JH (1996) Topical retinaldehyde increases skin content of retinoic acid and exerts biological activity in mouse skin. *J Invest Dermatol* **107:**714–719.

Dockx P, Decree J and Degreef H (1995) Inhibition of the metabolism of endogenous

retinoic acid as treatment for severe psoriasis: An open study with oral liarozole. *Br J Dermatol* **133:**426–432.
Donovan M, Olofsson B, Gustafson AL, Dencker L and Eriksson U (1995) The cellular retinoic acid binding proteins. *J Steroid Biochem Mol Biol* **53:**459–465.
Dowling JE and Wald G (1960) The role of vitamin A acid. *Vitam Horm* **18:**514–541.
Dreno B (1993) Roferon-A (interferon alpha 2a) combined with Tegison (etretinate) for treatment of cutaneous T cell lymphomas. *Stem Cells* **11:**269–275.
Drummond JC (1920) Nomenclature of the so-called accessory food factors (vitamins). *Biochem J* **14:**660–661.
Duell EA, Astrom A, Griffith CEM, Chambon P and Voorhees J (1992) Human skin levels of retinoic acid and cytochrome P-450-derived 4-hydroxyretinoic acid after topical application of retinoic acid in vivo compared to concentrations required to stimulate retinoic acid receptor-mediated transcription in vitro. *J Clin Invest* **90:**1269–1272.
Duell EA, Astrom A, Kang S, Griffith CEM and Voorhees J (1994) All-trans, 9-cis and 13-cis retinoic acid each induce a cytochrome P450 4-hydroxylase which causes all-trans but not 9-cis retinoic acid to self metabolize [abstract]. *J Invest Dermatol* **102:**641A.
Duell EA, Derguini F, Kang S, Elder JT and Voorhees JJ (1996a) Extraction of human epidermis treated with retinol yields retro retinoids in addition to free retinol and retinyl esters. *J Invest Dermatol* **107:**178–182.
Duell EA, Kang S and Voorhees JJ (1996b) Retinoic acid isomers applied to human skin in vivo each induce a 4-hydroxylase that inactivates only all-trans retinoic acid. *J Invest Dermatol* **106:**316–320.
Duester G (1996) Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis. *Biochemistry* **35:**12221–12227.
Duester G, Ang HL, Deltour L, Foglio MH, Hayamizu TF and Zgombic-Knight M (1995) Class I and class IV alcohol dehydrogenase (retinol dehydrogenase) gene expression in mouse embryos. *Adv Exp Med Biol* **372:**301–313.
Duvic M, Breneman D, Kuzel T, Strausd, Stevens V and Locum R (1996) LGD1069, a novel RXR-selective retinoid analogue, in the treatment of cutaneous T-cell lymphoma [abstract]. *International Society for Cutaneous Lymphomas Conference*, Sydney, Australia.
Eckhoff C and Nau H (1990) Identification and quantitation of all-trans- and 13-cis-retinoic acid and 13-cis-4-oxo-retinoic acid in human plasma. *J Lipid Res* **31:**1145–1154.
Edes TE, Gysberg DG, Buckley CS and Thornton WH (1991) Exposure to the carcinogen benzpyrene depletes tissue vitamin A: Beta-carotene prevents depletion. *Nutr Cancer* **15:**159–166.
El Akawi Z and Napoli JL (1994) Rat liver cytosolic dehydrogenase: Comparison of 13-cis-, 9-cis- and all-trans-retinal as substrates and effects of cellular retinoid-binding proteins and retinoic acid on activity. *Biochemistry* **33:**1938–1943.
Elder JT, Astrom A, Petersson U, Tavakkol A, Griffith CE, Krust A, Kastner P, Chambon P and Voorhees JJ (1992) Differential regulation of retinoic acid receptors and binding proteins in human skin. *J Invest Dermatol* **100:**356–359.
Elder JT, Kaplan A, Cromie MA, Kang S and Voorhees JJ (1996) Retinoid induction of CRABP-II mRNA in dermal fibroblasts: Use as a retinoid bioassay. *J Invest Dermatol* **106:**517–521.
Esgleyes-Ribot T, Chandraratna RA, low-key DA, Sefton J and Duvich M (1994) Response of psoriasis to a new topical retinoid, AGN 190168. *J Am Acad Dermatol* **30**:581–590.
Eskild W, Oyen O, Beebe S, Jahnsen T and Hansson V (1988) Regulation of mRNA levels for cellular retinoid binding protein in rat Sertoli cells by cyclic AMP and retinol. *Biochem Biophys Res Commun* **152:**1504–1510.
Falk HL, Kotin P and Mehler A (1964) Polycyclic hydrocarbons as carcinogens for man. *Arch Environ Health* **8:**721–731.
Fiorella PD, Giguère V and Napoli JL (1993) Expression of retinoic acid-binding protein (type II) in *Escherichia coli*: Characterization and comparison to cellular retinoic acid-binding protein (type I). *J Biol Chem* **268:**21545–21552.
Fiorella PD and Napoli JL (1991) Expression of cellular retinoic acid binding protein (CRABP) in *Escherichia coli*. *J Biol Chem* **266:**16572–16579.
Fisher GJ and Voorhees JJ (1996) Molecular mechanisms of retinoid actions in skin. *FASEB J* **10:**1002–1013.
Fisher GJ, Esmann J, Griffith CEM, Talwar HS, Duell EA, Hammerberg C, Elder JT, Karabin GD, Nickoloff BJ, Cooper KD and Voorhees JJ (1991) Cellular, immunologic, and biochemical characterization of topical retinoic acid-treated human skin. *J Invest Dermatol* **96:**699–707.
Frank J and Lentner A (1996) Psoriasis und alkoholkonsum. *Acta Derm* **22:**320–322.
Fredriksson T (1971) Antipsoriatic activity of retinoic acid (vitamin A acid). *Dermatologica* **142:**133–136.
Frolik CA, Roberts AB, Tavela TE, Roller PP, Newton DL and Sporn MB (1979) Isolation and identification of 4-hydroxy- and 4-oxo-retinoic acid: In vitro metabolism of all trans retinoic acid in hamster trachea and liver. *Biochemistry* **18**:2092–2097.
Frost P and Weinstein GD (1969) Topical administration of vitamin A acid for ichthyosiform dermatoses and psoriasis. *J Am Med Assoc* **207:**1863–1868.
Gebert JF, Moghal N, Frangioni JV, Sugarbaker DJ and Neel BG (1991) High frequency of retinoic acid receptor beta abnormalities in human lung cancer. *Oncogene* **6:**1859–1868.
Geisen C, Denk C, Gremm B, Baust C, Karger A, Bollag W and Schwarz E (1997) High-level expression of the retinoic acid receptor β gene in normal cells of the uterine cervix is regulated by the retinoic acid receptor alpha and is abnormally down-regulated in cervical carcinoma cells. *Cancer Res* **57:**1460–1467.
Gelboin HV, Wiebel F and Diamond L (1970) Dimethylbenzanthracene tumorigenesis and aryl hydrocarbon hydroxylase in mouse skin: Inhibition by 7,8-benzoflavone. *Science (Wash. DC)* **170:**169–171.
Giguere V (1994) Retinoic acid receptors and cellular retinoid binding proteins: Complex interplay in retinoid signaling. *Endocr Rev* **52:**32–44.
Gollnick HPM (1996) Oral retinoids-efficacy and toxicity in psoriasis. *Br J Dermatol* **135:**6–17.
Gollnick H, Tsambaos D and Orfanos CE (1981) Risk factors promote elevations of serum lipids in acne patients under oral 13-cis-retinoic acid (isotretinoin). *Arch Dermatol Res* **26:**960–967.
Griffith CEM, Elder JT and Bernard BA (1993) Comparison of CD 271 (Adapalene) and all-trans retinoic acid in human skin: Dissociation of epidermal effects and CRABP-II mRNA expression. *J Invest Dermatol* **101:**325–328.
Gronemeyer H and Laudet V (1996) Transcription factors 3: Nuclear receptors, in *Protein Profile*, vol. 2, Academic Press, New York.
Günther S (1973) The therapeutic value of retinoic acid in chronic, acute, guttate, and erythrodermic psoriasis: Clinical observations on twenty-five patients. *Br J Dermatol* **89:**515–517.
Hamada H and Tsuruo T (1986) Functional role for the 170- to 180-kDa glycoprotein specific to drug resistant tumor cells as revealed by monoclonal antibodies. *Proc Natl Acad Sci USA* **83:**7785–7789.
Happle R, van de Kerkhof PCM and Traupe H (1987) Retinoids in disorders of keratinization: Their use in adults. *Dermatologica* **175**(Suppl)**:**107S–124S.
Harrisson EH, Blaner WS, Goodman DS and Ross AC (1987) Subcellular localization of retinoids, retinoid binding proteins, and acyl-CoA retinol acyltransferase in rat liver. *J Lipid Res* **28:**973–981.
Heller J (1975) Interactions of plasma retinol-binding protein with its receptor: Specific binding of bovine and human retinol-binding protein to pigment epithelium cells from bovine eyes. *J Biol Chem* **250:**3613–3619.
Hill DL and Grubbs CJ (1992) Retinoids and cancer prevention. *Annu Rev Nutr* **12:**161–181.
Hines RN, Mathis JM and Jacob CS (1988) Identification of multiple regulatory elements on the human cytochrome P4501A1 gene. *Carcinogenesis* **9:**1599–1605.
Hirosawa K and Yamada K (1973) The localization of the vitamin A in the mouse liver as revealed by electron microscope radioautography. *J Electron Microsc (Tokyo)* **22:**337–346.
Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R, Peters LJ, Dimery IW, Brown BW and Goepert H (1990) Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. *N Engl J Med* **323:**795–801.
Horst RL, Reinhardt TA, Goff JP, Nonnecke BJ, Gambhir VK, Fiorella PD and Napoli JL (1995) Identification of 9,13-cis-retinoic acid as a major circulating retinoid in human plasma. *Biochemistry* **34:**1203–1209.
Hostetler KA, Wrighton SA, Kremers P and Guzelian PS (1987) Immunochemical evidence for multiple steroid-inducible hepatic cytochrome P450 in the rat. *Biochem J* **245:**27–33.
Houle B, Leduc F and Bradley WEC (1991) Implications of RAR beta in epidermoid (squamous) lung cancer. *Genes Chromosom Cancer* **3:**358–366.
Houle B, Rochette-Egly C and Bradley WEC (1993) Tumor suppressive effect of the retinoic acid receptor in human epidermoid lung cancer cells. *Proc Natl Acad Sci USA* **90:**985–989.
Hu L, Crowe DL, Rheinwald JG, Chambon P and Gudas LJ (1991) Abnormal expression of retinoic acid receptors and keratin 19 by human oral end epidermal squamous cell carcinoma cell lines. *Cancer Res* **51:**3972–3981.
Jones G, McLean J, Rosenthal D, Roberts J and Saunder DN (1992) Combined treatment with oral etretinate and electron beam therapy in patients with cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). *J Am Acad Dermatol* **26:**960–967.
Jörnvall H, Danielsson O, Hjelmquist L, Persson B and Shafquat J (1995) The alcohol dehydrogenase system. *Adv Exp Med Biol* **372:**281–294.
Jugert FK, Agarwal R, Kuhn A, Bickers DR, Merk HF and Mukhtar H (1994) Multiple cytochrome P450 isoenzymes in murine skin: Induction of P450 IA, 2β, 2E and 3A by dexamethasone. *J Invest Dermatol* **102:**970–975.
Jugert FK, Notzon I and Merk HF (1996a) Cytochrome P450 dependent metabolism of retinoic acid in cultured human keratinocytes [abstract]. *J Invest Dermatol* **107:**453A.
Jugert FK, Notzon I and Merk HF (1996b) Cytochrome P450 inactivates 13-cis- and all-trans-retinoic acid in human keratinocytes [abstract]. *Skin Pharmacol* **9:**154A.
Jugert FK, Roos TC, Notzon I and Merk HF (1998) Vitamin D3 and its synthetic analogue secocholestra-trien-1,3,24-triol influence the metabolism and the isomerization of retinoic acid in human keratinocytes. *Skin Pharmacol*, in press.
Jugert FK, Wyss R, Bucheli F and Merk HF (1995) Alcohol induces the 4-hydroxylation of isotretinoin [abstract]. *Skin Pharmacol* **8:**277A.
Julià P, Farrés J and Parés X (1986) Ocular alcohol dehydrogenase in the rat: Regional distribution and kinetics of the ADH-1 isoenzyme with retinol and retinal. *Exp Eye Res* **42:**305–314.
Jurima-Romet M, Neigh S and Casley WL (1997) Induction of cytochrome P450 3A by retinoids in rat hepatocyte culture. *Hum Exp Toxicol* **16:**198–203.
Kakkad B and Ong DE (1988) Reduction of retinaldehyde bound to cellular retinol-binding protein (type II) by microsomes from rat small intestine. *J Biol Chem* **263:**12916–12919.
Kang S, Duell EA, Fisher GJ, Datta SC, Wang ZQ, Reddy AP, Tavakkol A, Yi JY, Griffith CEM, Elder JT and Voorhees JJ (1995) Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding protein characteristic of retinoic acid but without measurable retinoic acid levels or irritation. *J Invest Dermatol* **105:**549–556.
Kato S, Mano H, Kumazawa T, Yoshizawa Y, Kojima R and Masushige S (1992) Effect of retinoid status on, and retinoic acid receptor mRNA levels in various rat tissues. *Biochem J* **286:**755–760.
Kedishvili NY, Bosron WF, Stone CL, Hurley TD, Peggs CF, Thomasson HR, Popov KM, Carr LG, Edenberg HJ and Li T-K (1995) Expression and kinetic characterization of recombinant human stomach alcohol dehydrogenase. Active-site amino acid sequence explains substrate specificity compared to liver isoenzymes. *J Biol Chem* **270:**3625–3630.
Khuri FR, Lippman SM, Spitz MR, Lotan R and Hong WK (1997) Molecular epidemiology and retinoid chemoprevention of head and neck cancer (1964) *J Natl Cancer Inst* **89:** 199–211.

Kim CI, Leo MA and Lieber CS (1992) Retinol forms retinoic acid via retinal. *Arch Biochem Biophys* **294:**388–393.
Kinoshita N and Gelboin HV (1972) The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz[a]-anthracene skin tumorigenesis: On the mechanism of 7,8-benzaflavone inhibition in tumorigenesis. *Cancer Res* **32:**1329–1339.
Kizaki M, Ueno H, Yamazoe Y, Shimada M, Takayama N, Muto A, Matsushita H, Nakajima H, Morikawa M, Koeffler HP and Ikeda Y (1996) Mechanisms of retinoid resistance in leukemic cells: Possible role of cytochrome P450 and p-glycoprotein. *Blood* **87:**725–733.
Klein ES, Pino ME, Johnson AT, Davies PJ, Nagpal S, Thacher SM, Krasinski G and Chandraratna RA (1996) Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists. *J Biol Chem* **271:**22692–22696.
Kojima R, Fugimori T, Kiyota N, Toriya Y, Fukuda T, Ohashi T, Sato T, Yoshizawa Y, Takeyama K, Mano H, Masushige S and Kato S (1994) In vivo isomerization of retinoic acids, rapid isomer exchange, and gene expression. *J Biol Chem* **269:** 32700–32707.
Kraft JC, Bechter R, Lee QP and Juchau MR (1992) Microinjections of cultured rat conspectuses: Studies with 4-oxo-all-trans-retinoic acid, 4-oxo-13-cis-retinoic acid and all-trans-retinol-glucuronide. *Teratology* **45:**259–270.
Krinsky NI, Wang XD, Tang G and Russel RM (1993) Mechanism of carotenoid cleavage to retinoids. *Ann NY Acad Sci* **691:**167–176.
Kurlandsky SB, Duell EA, Kang S, Voorhees JJ and Fisher GJ (1996) Auto-regulation of retinoic acid biosynthesis through regulation of retinol esterification in human keratinocytes. *J Biol Chem* **271:**15346–15352.
Kurlandsky SB, Xiao JH, Duell EA, Voorhees JJ and Fisher GJ (1994) Biological activity of all-trans retinol requires metabolic conversion to all-trans retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. *J Biol Chem* **269:**32821–32827.
Labrecque J, Dumas F, Lacroix A and Bhat PV (1995) A novel isoenzyme of aldehyde dehydrogenase specifically involved in the biosynthesis of 9-cis and all-trans retinoic acid. *Biochem J* **305:**681–684.
Lakshman MR, Mychkovsky I and Attlesey M (1989) Enzymatic conversion of all-trans beta-carotene to retinal by a cytosolic enzyme from rabbit and rat intestinal mucosa. *Proc Natl Acad Sci USA* **86:**9124–9128.
Lampron C, Rochette-Egly C, Gorry P, Dolle P, Mark M, Lufkin T, Lemeur M and Chambon P (1995) Mice deficient in cellular retinoic acid binding protein II (CRABP-II) or in both CRABP-I and -II are essentially normal. *Development* **121:**539–548.
Lee M-O, Manthey CL and Sladek NE (1991) Identification of mouse liver aldehyde dehydrogenases that catalyze the oxidation of retinaldehyde to retinoic acid. *Biochem Pharmacol* **42:**1279–1285.
Lee X, Si SP, Tsou HC and Peacocke M (1995) Cellular aging and transformation suppression: A role for retinoic acid receptor β2. *Exp Cell Res* **218:**296–304.
Lefebvre P, Thomas G, Gourmel B, Agadir A, Castaigne S, Dreux C, Degos L and Chomienne C (1991) Pharmacokinetics of oral all-trans retinoic acid in patients with acute promyelocytic leukemia. *Leukemia* **5:**1054–1058.
Lehmann PA and Malany AM (1989) Evidence for percutaneous absorption of isotretinoin from the photoisomerization of topical tretinoin. *J Invest Dermatol* **93:**595–599.
Lehmann PA, Slattery JT and Franz TJ (1988) Percutaneous absorption of retinoids: Influence of vehicle, light exposure, and dose. *J Invest Dermatol* **91:**56–61.
Leo MA and Lieber CS (1982) Hepatic vitamin A depletion in alcohol liver injury. *N Engl J Med* **307:**2015–2023.
Leo MA, Iida S and Leiber CS (1984) Retinoic acid metabolism by a system reconstitued with cytochrome P450. *Arch Biochem Biophys* **234:**305–312.
Leo MA, Lasker JM, Raucy JH, Kim CI, Black M and Lieber CS (1989) Metabolism of retinol and retinoic acid in human liver cytochrome P450IIC8. *Arch Biochem Biophys* **269:**305–312.
Li E, Qian S-J, Winter NS, d'Avignon A, Levin MS and Gordon J (1991) Fluorine nuclear magnetic resonance analysis of the ligand binding properties of two homologous rat cellular retinol-binding proteins expressed in *Escherichia coli*. *J Biol Chem* **266:**3622–3629.
Li XY, Astrom A, Duell EA, Quin L, Griffiths CEM and Voorhees JJ (1995) Retinoic acid antagonizes basal as well as coal tar and glucocorticoid-induced cytochrome P4501A1 expression in human skin. *Carcinogenesis* **16:**519–524.
Lippman SM, Lotan R and Schleuniger U (1997) Retinoid-interferon therapy of solid tumors. *Int J Cancer* **70:**481–483.
Lotan R, Xu X-C, Lippman SM, Ro JY, Lee JS, Lee JJ and Hong WK (1995) Suppression of retinoic acid receptor beta in premalignant oral lesions and its upregulation by isotretinoin. *N Engl J Med* **332:**1405–1410.
MacDonald PN and Ong DE (1988) Evidence for a lecithin-retinol acyltransferase activity in the rat small intestine. *J Biol Chem* **263:**12478–12482.
Majewski S, Skopinska M, Marczak M, Novicka D, Szmurlo A, Bollag W and Jablonska S (1997) Synergistic anticancer action of retinoids, vitamin D3, and cytokines (interferons and interleukin-12) as related to antiangiogenic and anti-proliferative effects. *J Invest Dermatol* **108:**571A.
Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schüth G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P and Evans RM (1995) The nuclear receptor superfamily: The second decade. *Cell* **83:**835–839.
Mangelsdorf DJ, Umesono K and Evans RM (1994) The retinoid receptors, in *The Retinoids*: *Biology, Chemistry and Medicine* (Sporn MB, Roberts AB and Goodmann DS eds) 2nd ed, pp 319–350, Raven Press, New York.
Mangelsdorf DJ, Umesono K, Kliewer SA, Borgmeyer U, Ong ES and Evans RM (1991) A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell* **66:**555–561.
Martini R and Murray M (1993) Participation of P450 3A enzymes in rat hepatic microsomal retinoic acid 4-hydroxylation. *Arch Biochem Biophys* **303:**57–66.
Mehta RG, Barua AB, Olson JA and Moon RC (1991) Effects of retinoid glucuronides on mammary gland development in organ culture. *Oncology* **48:**505–509.
Moon RC and Mehta RG (1990) Chemoprevention of mammary cancer by retinoids. *Basic Life Sci* **52:**213–224.
Muindi JF, Frankel SR, Miller WH Jr, Jakubowski A, Scheinberg DA, Young CW, Dimitrowski E and Warrel RP Jr (1992) Continuous treatment with all-trans retinoic acid causes a progressive decrease in plasma concentrations: implications relapse and "resistance" in acute promyelocytic leukemia. *Blood* **79:**299–303.
Nadin L and Murray M (1996) All-trans retinoic acid 4-hydroxylation in human liver microsomes: In vitro modulation by therapeutic retinoids. *Br J Clin Pharmacol* **41:**609–612.
Nagao A and Olson JA (1994) Enzymatic formation of 9-cis, 13-cis and all-trans retinals from isomers of β-carotene. *FASEB J* **8:**968–973.
Nagpal S, Athanikar J and Chandraratna RAS (1995) Separation of transactivation and AP1 antagonism functions of retinoic acid receptorα. *J Biol Chem* **270:**923–927.
Napoli JL (1993) Biosynthesis and metabolism of retinoic acid: Roles of CRBP and CRABP in retinoic acid homeostasis. *J Nutr* **123:**362–366.
Napoli JL (1996) Retinoic acid biosynthesis and metabolism. *FASEB J* **10:**993–1001.
Napoli JL and McCormick AM (1981) Tissue dependence of retinoic acid metabolism in vivo. *Biochim Biophys Acta* **666:**165–175.
Napoli JL and Race KR (1988) Biogenesis of retinoic acid from β-carotene. Differences between the metabolism of β-carotene and retinal. *J Biol Chem* **263:**17372–17377.
Napoli JL, Boerman MHEM, Chai X, Zhai Y and Fiorella PD (1995) Enzymes and binding proteins affecting retinoic acid concentrations. *J Steroid Biochem Mol Biol* **53:**497–502.
Napoli JL, Khalil H and McCormick AM (1982) Metabolism of 5,6-epoxy retinoic acid in vivo: Isolation of a major intestinal metabolite. *Biochemistry* **21:**1942–1949.
Napoli JL, Pramanik BC, Williams JB, Dawson MI and Hobbs PD (1985) Quantification of retinoic acid by gas-liquid chromatography-mass spectrometry: Total versus all-trans-retinoic acid in human plasma. *J Lipid Res* **26:**387–392.
Nau H (1990) Correlation of transplacental and maternal pharmacokinetics of retinoids during organogenesis with teratogenesis, in *Methods in Enzymology* (Packer L ed) vol 190, part B, pp 437–448, Academic Press, San Diego.
Nau H and Elmazar MME (1997) Subcellular localization of 13-cis and all-trans-retinoic acid in mouse embryo after application of equipotent teratogenic doses of either substance. *European Retinoid Research Group Meeting, Nizza,* poster.
Nau H, Kraft J, Eckhoff C and Lofberg B (1989) Interpretation of retinoid teratogenesis by transplacental pharmacokinetics, in *Pharmacology of Retinoids in the Skin* (Reichert U and Shroot B eds) vol 3, pp 165–173, Karger, Basel.
Norris AW, Cheng L, Giguere V, Rosenberger M and Li E (1994) Measurement of subnanomolar retinoic acid binding affinities for cellular retinoic acid binding proteins by fluorometric titration. *Biochim Biophys Acta* **1209:**10–18.
Ohnishi K and Lieber CS (1977) Reconstitution of the microsomal ethanol-oxidizing system: Qualitative and quantitative changes of cytochrome P450 after chronic ethanol consumption. *J Biol Chem* **252:**7124–7131.
Oldfield N (1990) The metabolic fate of 13-cis-retinoic acid in mouse skin microsomal preparations. *Drug Metab Dispos Biol Fate Chem* **18:**1105–1107.
Olson JA, Moon RC, Anders MW, Fenselau C and Shane B (1992) Enhancement of biological activity by conjugate reactions. *J Nutr* **122(suppl 3):**615–624.
Ong DE (1994) Cellular transport and metabolism of vitamin A: Roles of the cellular retinoid-binding proteins. *Nutr Rev* **52:**24–31.
Ong DE, Newcomer ME and Chytil F (1994) Cellular retinoid binding protein, in *The Retinoids: Biology, Chemistry and Medicine* (Sporn MB, Roberts AB and Goodmann DS eds) pp 283–318, Raven Press, New York.
Orfanos CE, Ehlert R and Gollnick H (1987) The Retinoids. A review of their clinical pharmacology and therapeutic use. *Drugs* **34:**459–503.
Orfanos CE, Schmidt HW, Mahrle G, Gartmann H and Lever WF (1973) Retinoic acid in psoriasis: Its value for topical therapy with and without corticosteroids. Clinical histological and electron microscopical studies on forty-four hospitalized patients with extensive psoriasis. *Br J Dermatol* **88:**167–188.
Orfanos CE, Schmidt HW, Mahrle G and Runne U (1972) Die wirksamkeit von vitamin-A-Säure bei psoriasis. Topische kombinationsbehandlung mit corticoiden. Zwei neue VAS-Präparate zur peroralen therapie. *Arch Dermatol Forsch* **244:**424–426.
Orfanos CE, Zouboulis CC, Almond-Roesler B and Geilen CC (1997) Current use and future potential role of retinoids in dermatology. *Drugs* **53:**358–388.
Ottonello S, Scita G, Mantovani G, Cavazzini G and Rossi GL (1993) Retinol bound to cellular retinol-binding protein is a substrate for cytosolic retinoic acid synthesis. *J Biol Chem* **268:**27133–27142.
Peck GL and Yoder FW (1976) Treatment of lamellar ichthyosis and other keratinizing dermatoses with oral synthetic retinoid. *Lancet* **2:**172.
Peck GL, Yoder FW, Olsen TG, Pandya MD and Butkus D (1978) Treatment of Darier's disease, lamellar ichthyosis, pityriasis rubra pilaris, and basal cell carcinoma with oral 13-cis-retinoic acid. *Dermatologica* **157(suppl 1):**11–12.
Persson B, Krook M and Jörnvall H (1995) Short chain dehydrogenases/reductases. *Adv Exp Med Biol* **372:**383–395.
Pijnappel WW, Hendriks HF, Folkers GE, van den Brink CE, Dekker EJ, Edelenbosch C, van der Saag PT and Durston AJ (1993) The retinoid ligand 4-oxo RA is a highly active modulator of positional specification. *Nature* **366:**340–344.
Piletta P, Jaconi S, Siegenthaler G, Didierjean L and Saurat JH (1994) Topical glucocorticosteroid modulate the expression of CRABP-I and -II in human skin differently. *Exp Dermatol* **3:**23–28.
Poikolainen K, Reunala T, Karvonen J, Lanharatna J and Karkkainen P (1990) Alcohol intake: A risk for psoriasis in young and middle aged men? *Br J Med* **300:**780–783.
Posch KC, Burns RD and Napoli JL (1992) Biosynthesis of all-trans-retinoic acid from retinal. Recognition of retinal bound to cellular retinol binding protein (type 1) as substrate by a purified cytosolic dehydrogenase. *J Biol Chem* **67:**19676–19682.
Rabilloud T, Asselineau D, Bailly C, Miquel C, Tarroux P and Darmon M (1988) Study of the human epidermal differentiation by two dimensional electrophoresis, in *Electrophoresis '88* (Schaefer-Nielsen C ed) pp 95–101, Verlag Chemie, Munich.

Randolph RK and Simon M (1993) Characterization of retinol metabolism in cultured human keratinocytes. *J Biol Chem* **268:**9198–9205.
Randolph RK and Simon M (1995) Metabolic regulation of active retinoid concentrations in cultured human epidermal keratinocytes by exogenous fatty acids. *Arch Biochem Biophys* **318:**6–14.
Randolph RK and Simon M (1996) All-trans-retinoic acid regulates retinol and 3,4-didehydroretinol metabolism in cultured human epidermal keratinocytes. *J Invest Dermatol* **106:**168–175.
Raner GM, Vaz and and Coon MJ (1995) Metabolism of all-trans, 9-cis, and 13-cis isomers of retinal by purified isoenzymes of microsomal cytochrome P450 and mechanism-based inhibition of retinoid oxidation by citral. *Mol Pharmacol* **49:** 515–522.
Rask L and Peterson PA (1976) In vitro uptake of vitamin A from retinol-binding protein to mucosal epithelial cells of the monkey's small intestine. *J Biol Chem* **251:**6360–6366.
Reddy AP, Chen J, Zacharewsky T, Gronemeyer H, Voorhees JJ and Fisher GJ (1992) Characterization and purification of human retinoic acid receptor gamma 1 overexpressed in the baculovirus-insect cell system. *Biochem J* **287:**833–840.
Reynolds NJ, Fisher GJ, Griffith CEM, Tavakkol A, Talwar HS, Rowse PE, Hamilton PA and Voorhees JJ (1993) Retinoic acid metabolites exhibit biological activity in human keratinocytes, mouse melanoma cells, and hairless mouse skin in vivo. *J Pharmacol Exp Ther* **226:**1632–1642.
Rigas Jr., Francis PA, Muindi JF, Kris MG, Huselton C, Degrazia F, Orazem JP, Young CW and Warrell RP Jr (1993) Constitutive variability in the pharmacokinetics of the natural retinoid, all-trans retinoic acid, and its modulation by ketoconazole. *J Natl Cancer Inst* **85:**1921–1926.
Roberts AB, Nichols MD, Newton DL and Sporn MB (1979) In vitro metabolism of retinoic acid in hamster intestine and liver. *J Biol Chem* **254:**6296–6302.
Roberts ES, Vaz AD and Coon MJ (1991) Metabolism of retinoids by rabbit P450 cytochromes. *FASEB J* **5:**514A.
Roberts ES, Vaz AD and Coon MJ (1992) Role of isoenzymes of rabbit microsomal cytochrome P450 in the metabolism of retinoic acid, retinol, and retinal. *Mol Pharmacol* **41:**427–433.
Roberts ES, Vaz AD and Coon MJ (1993) Role of isoenzymes of rabbit microsomal retinoic acid 4-hydroxylation. *Arch Biochem Biophys* **303:**57–66.
Rogers M, Berestecky JM, Hossain MZ, Gou HM, Kadle R, Nicholson BJ and Bertram JS (1990) Retinoid-enhanced gap junctional communication is achieved by increased levels of connexin 43 mRNA and protein. *Mol Carcinog* **3:**335–343.
Roos TC, Jugert FK, Notzon I and Merk HF (1996) Cytochrome P450-mediated 4-hydroxy-metabolites of retinoic acid are produced by human keratinocytes. *J Invest Dermatol* **106:**870A.
Runne U, Orfanos CE and Gartmann H (1973) Perorale applikation zweier derivate der vitamin A-Säure zur internen psoriasis-therapie (13-cis-beta-vitamin A-Säure und vitamin A-säure-äthylamid). *Arch Dermatol Res* **247:**171–180.
Rush MG, Ul-Haq R and Chytil F (1991) Opposing effects of retinoic acid and dexamethasone on cellular retinol-binding protein ribonucleic acid levels in the rat. *Endocrinology* **129:**705–709.
Sanquer S and Gilchrest BA (1994) Characterization of human cellular retinoic acid-binding proteins-I and -II: Ligand binding affinities and distribution in skin. *Arch Biochem Biophys* **311:**86–94.
Sass JO, Forster A, Bock KW and Nau H (1994) Glucuronidation and isomerization of all-trans- and 13-cis-retinoic acid by liver microsomes of phenobarbital- or 3-methylcholanthrene-treated rats. *Biochem Pharmacol* **47:**495–492.
Sato M and Lieber CS (1981) Hepatic vitamin A depletion after chronic ethanol consumption in baboons and rats. *J Nutr* **111:**2015–2023.
Sato M and Lieber CS (1982) Increased metabolism of retinoic acid after chronic ethanol consumption in rat liver microsomes. *Arch Biochem Biophys* **213:**557–564.
Saurat JH, Geiger JM and Amblard P (1988) Randomized double blind study comparing Acitretin-PUVA, etretinate-PUVA ,and placebo-PUVA in the treatment of severe psoriasis. *Dermatologica* **177:**218–224.
Schaefer H (1993) Penetration and percutaneous absorption of topical retinoids. *Skin Pharmacol* **6:**17–23.
Schon M and Rheinwald JG (1996) A limited role for retinoic acid and retinoic acid receptors RARα and RARβ in regulating Keratin 19 expression and keratinization in oral and epidermal keratinocytes. *J Invest Dermatol* **107:**428–438.
Schumacher A and Stüttgen G (1971) Vitamin A-Säure bei hyperkeratosen, epithelialen tumoren, und akn:. Orale und lokale anwendung. *Dtsch Med Wochenschr* **96:**1547–1551.
Schwarz E, Mezick JA, Gendimenico GJ and Kligman LH (1994) In vivo prevention of corticosteroid-induced skin atrophy by tretinoin in the hairless mouse is accompanied by modulation of collagen, glycosaminoglycans, and fibronectin. *J Invest Dermatol* **102:**241–246, 1994.
Shalita A, Weiss JS, Chalker DK, Ellis CN, Greenspan A, Katz HI, Kantor I, Millikan LE, Swinehart T, Swinyer L, Whitmore C, Baker M and Czernielewski J (1996) A comparison of the efficacy and safety of Adapalene gel 0.1% and tretinoin gel 0.1% in the treatment of acne vulgaris: A multicenter trial. *J Am Acad Dermatol* **34:**483–485.
Si SP, Lee X, Tsou HC, Buchsbaum R, Tibaduiza E and Peacocke M (1996) RARβ2-mediated growth inhibition in HeLa cells. *Exp Cell Res* **223:**102–111.
Siegenthaler G, Gumowski-Sunec D and Saurat JH (1990) Metabolism of natural retinoids in psoriatic epidermis. *J Invest Dermatol* **95:**47s–48s.
Siegenthaler G, Saurat JH and Ponec M (1990) Retinol and retinal metabolism. *Biochem J* **268:**371–378b.
Siegenthaler G, Saurat JH, Morin C and Hotz R (1984) Cellular retinol- and retinoic acid-binding proteins in the epidermis and dermis of normal human skin. *Br J Dermatol* **111:**647.
Siegenthaler G, Tomatis I, Chatellard-Gruaz D, Jaconi S, Eriksson U and Saurat JH (1992) Expression of CRABP-I and -II in human epidermal cells. *Biochem J* **287:**383–389b.
Siegenthaler G, Tomatis I, Didierjean L, Jaconi S and Saurat JH (1992) Overexpression of cellular retinoic acid binding protein type II (CRABP-II) and down-regulation of CRABP-I in psoriatic skin. *Dermatology* **185:**251–256a.
Smith WC, Nakshatri H, Leroy P, Rees J and Chambon P (1991) A retinoic acid response element is present in the mouse cellular retinol binding protein I (mCRABP-I) promoter. *EMBO J* **10:**2223–2230.
Sporn MB, Rogerts AB and Goodmann DS (1994) *The Retinoids: Biology, Chemistry and Medicine.* 2nd ed, Raven Press, New York.
Stahl W, Sundquist AR, Hanusch M, Schwarz M and Sies H (1993) Separation of β-carotene and lycopene geometrical isomers in biological samples. *Clin Chem* **39:**810–814.
Strickland S and Mahdavi V (1978) The induction of differentiation in teratocarcinoma cells by retinoic acid. *Cell* **15:**393–403.
Stuettgen D (1962) Zur lokalbehandlung von keratosen mit vitamin A-Säure. *Dermatologica* **124:**65–80.
Takatsuka J, Takahashi N and De Luca LM (1996) Retinoic acid metabolism and inhibition of cell proliferation: An unexpected liaison. *Cancer Res* **56:**675–678.
Tanew A, Guggenbichler A and Hoenigsmann H (1991) Photochemotherapy for severe psoriasis without or in combination with Acitretin: A randomized, double-blind comparison study. *J Am Acad Dermatol* **25:**682–684.
Tang GW and Russell RM (1990) 13-cis-retinoic acid is an endogenous compound in human serum. *J Lipid Res* **31:**175–182.
Tavakkol A, Zouboulis CC, Duell EA and Voorhees JJ (1994) A retinoic acid-inducible skin-specific gene (RIS-1/psoriasin): molecular cloning and analysis of gene expression in human skin in vivo and cultured skin cells in vitro. *Mol Biol Rep* **20:**75–83.
Thaller C and Eichele G (1990) Isolation of 3,4-retinoic acid, a novel morphogenic signal in the chick. *Nature* **345:**815–819.
Thestrup-Petersen K, Hammer R and Kaltoft K (1988) Treatment of mycosis fungoides with recombinant interferon-alpha 2a alone and in combination with etretinate. *Br J Dermatol* **118:**811–818.
Thompson Jr., Bratt JM and Banaszak LJ (1995) Crystal structure of cellular retinoic acid binding protein I shows increased access to the binding cavity due to formation of an intermolecular β-sheet. *J Mol Biol* **252:**433–446.
Tomita S, Tsujita M, Matsou Y and Ichikawa Y (1993) Identification of a microsomal retinoic acid synthase as a microsomal cytochrome P450-linked monooxygenase system. *Int J Biochem* **25:**1775–1784.
Torma H and Vahlquist A (1987) Retinol esterification by mouse epidermal microsomes: evidence for acyl-CoA: Retinol acyltransferase activity. *J Invest Dermatol* **88:**398–402.
Torma H, Lonz W, Liu W, Rollmann O and Vahlquist A (1994) Expression of cytosolic retinoid-binding protein genes in human skin biopsies and cultures keratinocytes and fibroblasts. *Br J Dermatol* **131:**243–249.
Tsambaos D and Orfanos CE (1982) Arotinoid: A new potential retinoid. Preliminary results, in *Psoriasis* (Farber A ed) pp 515–520, Grune & Stratton, New York.
Tsambaos D and Orfanos CE (1983) Antipsoriatic activity of a new synthetic retinoid: The arotinoid Ro 13–6298. *Arch Dermatol* **119:**746–751.
Tsujita M, Tomita S, Miura S and Ichikawa Y (1994) Characteristic properties of retinal oxidase (retinoic acid synthase) from rabbit hepatocytes. *Biochim Biophys Acta* **1204:**108–116.
Uchida Y, Oda Y, Sun P, Largman C, Elias PM and Holleran WM (1997) Cytochrome P450 isoenzyme responsible for α-hydroxylation of very long-chain fatty acids in mammalian epidermis. *J Invest Dermatol* **108:**606A.
Urbach BL and Rando RR (1994) Isomerization of all-trans retinoic acid to 9-cis retinoic acid. *Biochemistry* **299:**459–465.
Vahlqist A (1980) The identification of dehydroretinol (vitamin A2) in human skin. *Experientia* **36:**317–318, 1980.
Vahlquist A (1982) Vitamin A in human skin. I. Detection and identification of retinoids in normal epidermis. *J Invest Dermatol* **79:**89–93a.
Vahlquist A, Lee JB, Michaelsson G and Rollmann O (1982) Vitamin A in human skin. II. Concentrations of carotene, retinol, and dehydroretinol in various components of normal skin. *J Invest Dermatol* **79:**94–97b.
Vahlquist A, Torma H, Rollmann O and Berne B (1991) Distribution of natural and synthetic retinoids in the skin, in *Retinoids, New Trends in Research and Therapy* (Saurat JH ed) pp 159–167, Karger, Basel.
Van den Bossche H, Willemsens G and Janssen PA (1988) Cytochrome P450 dependent metabolism of retinoic acid in rat skin microsomes: Inhibition by ketoconazole. *Skin Pharmacol* **1:**176–185.
Van den Bossche HV and Willemsens G (1991) Retinoic acid and cytochrome P450, in *Retinoids: Ten Years On* (Saurat JH ed) Karger, Basel.
Van Pelt FNAM, Olde-Meierink YJM, Blaauboer BJ and Weterings BJ (1990) Immunohistochemical detection of cytochrome P450 isoenzymes in cultured human epidermal cells. *J Histochem Cytochem* **38:**1847–1851.
Van Wauwe J, Van Nyen G, Coene MC, Stoppie P, Cools W, Goossens J, Borghgraef P and Janssen PA (1994) Liarazole fumarate inhibits the metabolism of 4-keto-all-trans-retinoic acid. *Biochem Pharmacol* **47:**737–741.
Van Wauwe JP, Coene N-C, Cools W, Goossens J, Lauwers W, Le Jeune L, van Hove C and van Nyen G (1993) Liarazole, an inhibitor of retinoic acid metabolism, exerts retinoid-mimetic effects in vivo. *J Pharmacol Exp Ther* **261:**773–774.
Vecchini F, Lenoir-Viale MC, Cathelineau C, Magdalou J, Bernard BA and Shroot B (1994) Presence of a retinoid responsive element in the promotor region of the human cytochrome P4501A1 gene. *Biochem Biophys Res Commun* **201:**1205–1212.
Verma AK (1987) Inhibition of both stage I and stage II mouse skin tumor promotion by retinoic acid and the dependence of inhibition of tumor promotion on the duration of retinoic acid treatment. *Cancer Res* **47:**5097–5101.
Verma AK (1992) Ornithine decarboxylase, a possible target for human cancer prevention, in *Cellular and Molecular Targets for Chemoprevention* (Steele VE, Boone C and Kelloff G eds) pp 207–224, CRC Press, Boca Raton.
Verma AK, Slaga TJ, Werz PW, Mueller GC and Boutwell RK (1980) Inhibition of skin tumor promotion by retinoic acid and its metabolite 5,6-epoxy retinoic acid. *Cancer Res* **40:**2367–2371.
Wang XD, Krinsky NI and Russel RM (1993) Retinoic acid regulates retinol metab-

olism via feedback inhibition of retinol oxidation and stimulation of retinol esterification in ferret liver. *J Nutr* **123:**1277–1285.
Wang XD, Krinsky NI, Benotti PN and Russel RM (1994) Biosynthesis of 9-cis-retinoic acid from 9-cis-β-carotene in human intestinal mucosa in vitro. *Arch Biochem Biophys* **313:**150–155.
Wang XD, Tang GW, Fox JX, Krinsky NI and Russel RM (1991) Enzymatic conversion of beta-carotene into beta-apocarotenals and retinoids by human, monkey, ferret, and rat tissues. *Arch Biochem Biophys* **285:**8–16.
Wanner R, Brommer S, Czarnezki BM and Rosenbach T (1996) The differentiation-related upregulation of aryl hydrocarbon receptor transcript levels is suppressed by retinoic acid. *Biochem Biophys Res Commun* **209:**706–711.
Warrell RP Jr, de The H, Wang ZY and Degos L (1993) Acute promyelocytic leukemia. *N Engl J Med* **329:**177–189.
Waxman DJ, Dannan GA and Guengerich FP (1985) Regulation of rat hepatic cytochrome P450: Age dependent expression, hormonal imprinting, and xenobiotic inducibility of sex specific isoenzymes. *Biochemistry* **24:**4409–4417.
Weinstein GD (1996) Safety, efficacy and duration of therapeutic effect of Tazarotene used in the treatment of plaque psoriasis. *Br J Dermatol* **135:**32–36.
Westin S, Mode A, Murray M, Chen R and Gustafsson JA (1993) Growth hormone- and vitamin A-induced P450 2C7 mRNA expression in primary rat hepatocytes. *Mol Pharmacol* **44:**997–1002.
White JA, Beckett-Jones B, Guo J-D, Dilworth FJ, Bonasoro J, Jones G and Petkovich M (1997) cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450 (CYP26). *J Biol Chem* **272:**18538–18541.
White JA, Guo YD, Baetz K, Beckett-Jones B, Bonasoro J, Hsu KE, Dilworth FJ, Jones G and Petkovich M (1996) Identification of the retinoic acid-inducible all-trans-retinoic acid 4-hydroxylase. *J Biol Chem* **271:**29922–29927.
Williams JB and Napoli JL (1985) Metabolism of retinoic acid and retinol during differentiation of F9 embryonal carcinoma cells. *Proc Natl Acad Sci USA* **82:**4658–4662.
Williams JB and Napoli JL (1987) Inhibition of retinoic acid metabolism by imidazole antimycotics in F9 embryonal carcinoma cells. *Biochem Pharmacol* **36:**1386–1388, 1987.
Williams JB, Shields CO, Brettel LM and Napoli JL (1992) Assessment of retinoid-induced differentiation in F9 embryonal carcinoma cells with an enzyme-linked immunoabsorbent assay for laminin: Statistical comparison of dose response curve. *Anal Biochem* **160:**267–274.
Wolbach SB and Howe PR (1925) Tissue changes following deprivation of fat soluble vitamin A. *J Exp Med* **43:**753.
Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Young B and Guzelian PS (1985) Identification of the cytochrome P450 induced macrolide antibiotics in the rat liver as the glucocorticoid responsive cytochrome P450-p. *Biochemistry* **24:**2171–2178.
Yang ZN, Davies GJ, Hurley TD, Stone CL, Li T-K and Bosron WF (1994) Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. *Clin Exp Res* **18:**587–591.
Yost RW, Harrison EH and Ross AC (1988) Esterification by rat liver microsomes of retinol bound to retinoid-binding protein. *J Biol Chem* **263:**18693–18701.
Zgombic-Knight M, Ang HL, Foglio MH and Duester G (1995) Cloning of the mouse class IV alcohol dehydrogenase (retinol dehydrogenase) cDNA and tissue specific expression patterns of the murine ADH gene family. *J Biol Chem* **270:**10868–10877.
Zhang L, Cooney RV and Bertram JS (1992) Carotenoids upregulate connexin 43 gene expression independent of their pro-vitamin A or antioxidant properties. *Cancer Res* **52:**5707–5712