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Perspective

Retinoids at the Threshold: Their Biological Significance and Therapeutic Potential

Beverly A. Pawson,*,† Carl W. Ehmann,† Loretta M. Itri,† and Michael I. Sherman‡

Roche Research Laboratories, Hoffmann-La Roche Inc., and Roche Institute of Molecular Biology, Nutley, New Jersey 07110.
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Introduction and Historical Perspectives

From our perspective, the chemistry and biology of retinoids (vitamin A and its derivatives) may be likened to a child coming of age—but only just of age—with the prospects of a future full of new learning and opportunities for a long and useful life. The broad spectrum of activity of retinoids has been foretold in the literature almost from the time of their discovery and synthesis. Despite research efforts in the last decade, in which there has been a renaissance in the chemistry and biology of the retinoids, the precise mechanisms of action of vitamin A and its analogues remain unknown.

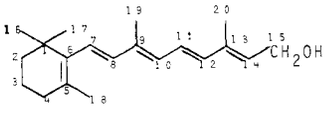
At present, retinoids have found clinical utility in the treatment of severe cystic acne, psoriasis, and other disorders of keratinization. Possible uses of retinoids are being explored in the prophylaxis and treatment of cancer.

Although the knowledge of these compounds has early roots in the Egyptian writings (ca. 1500 B.C.), which described the benefits of liver as a treatment for night blindness,[1] the discovery of the substance vitamin A and the understanding of its biological role and that of its congeners are a result of research efforts within this century. In 1909, a fat-soluble extract from egg yolk was found to be essential for life.[2,3] This substance, initially called fat-soluble A,[4] was also found in animal fats and fish oils and was subsequently named vitamin A.[5] In 1931, Karrer et al.[6,7] isolated and purified vitamin A (retinol) from halibut liver oil and determined its structural formula (Chart I).

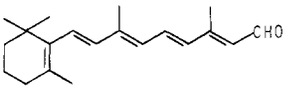
In the same time period (1928–1930), the relationship of vitamin A to the carotenoid pigments, many of which were isolated and described during the 19th century, was discovered.[8-12] The high vitamin A activity of carotene, its metabolism to vitamin A, and its storage in the liver were clarified with the structural assignment by Karrer's group in 1930.[13]

In 1935, Wald demonstrated the importance of another vitamin A derivative, retinene, in the visual cycle.[14,15] Morton's work established the identity of retinene as vitamin A aldehyde (retinal), the product of enzymatic cleavage of β-carotene, through synthesis from retinol.[16,17]

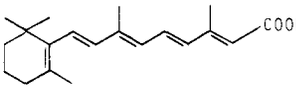
Chart I. Structures of Vitamin A and Related Molecules



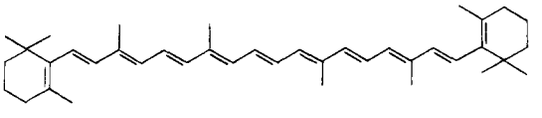
VITAMIN A, RETINOL



VITAMIN A ALDEHYDE, RETINAL

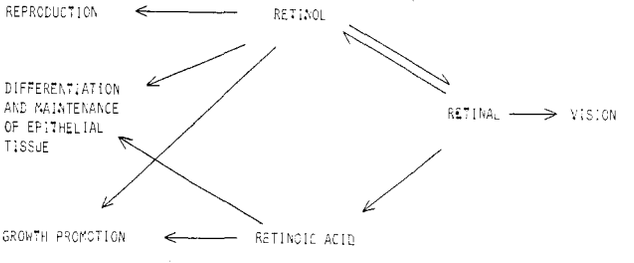


VITAMIN A ACID, RETINOIC ACID



β-CAROTENE

Chart II. Biological Functions of Vitamin A



In 1946, vitamin A acid (retinoic acid) was synthesized[18] and shown to be important for growth. For approximately

† Roche Research Laboratories.
‡ Roche Institute of Molecular Biology.

(1) Mandel, H. G.; Cohn, V. H. In "The Pharmacological Basis of Therapeutics"; Gilman, A. G.; Goodman, L. S.; Gilman, A., Eds.; Macmillan: New York, 1980; pp 1583–1592.

0022-2623/82/1825-1269$01.25/0

the next 20 years, chemical efforts centered on syntheses of the natural retinoids, their double-bond isomers, and derivatives such as esters. From this research, several industrial processes were developed[19] that afforded the capability of world-wide production of large quantities of vitamin A for therapeutic use and as a nutritional supplement.

The biological roles of retinol, retinal, and retinoic acid, as well as their interrelationship, are shown in Chart II. In a reversible process, retinol is oxidized in vivo to the aldehyde retinal, which is important in vision. Retinoic acid is a major oxidative metabolite of retinol; unlike retinol, which is stored in the liver, retinoic acid is not stored and is rapidly excreted. Retinol is necessary for growth, for differentiation and maintenance of epithelial tissue, and for reproduction. Retinoic acid can substitute for retinol in vitamin A deficient animals in growth promotion and in epithelial differentiation and maintenance. However, it cannot substitute completely for retinol in maintaining reproductive function, nor can it replace retinal in the visual cycle.

Evidence for the utility of vitamin A in oncology and dermatology was obtained shortly after the discovery of the vitamin itself. The relationship between low levels of vitamin A and dyskeratotic skin conditions was recognized as early as 1925, when epithelial changes in vitamin A deficient animals were reported.[20,21] Early investigators described the use of vitamin A clinically in the treatment of skin diseases due to abnormal keratinization.[22-24] Systemic treatment with vitamin A acid was also reported to interfere with keratinization in human skin.[24-28]

The connection between vitamin A and cancer was initially reported in 1926, when rats fed a vitamin A deficient diet were found to develop carcinomas of the stomach.[29] The similarity of the squamous metaplastic changes in epithelial tissue due to vitamin A deficiency appeared by light microscopy to be morphologically similar to the changes found in certain precancerous lesions caused by carcinogen administration.[30] Several investigators have confirmed and extended the early observations and suggested the use of vitamin A to prevent or reverse these changes.[31] For example, one such study by Saffioti and collaborators[32] demonstrated the preventive effect of vitamin A palmitate on the formation of tracheo-bronchial squamous metaplasias and tumors, induced by intratracheal instillations of the carcinogen benzo[a]pyrene and iron oxide, in hamsters.

These early studies were done with retinol, or one of the retinyl esters (the acetate and palmitate are the derivatives found in commerce), which is stored in the liver and which is toxic at high doses. The use of retinoic acid, which was also studied clinically in the 1960's,[24-28,33] is limited by the toxicity known as hypervitaminosis A. In humans, these side effects appear as changes in the skin and mucous membranes and in the form of musculoskeletal pains and headaches. Thus, to facilitate the search for new retinoids with greater potency and lower toxicity, begun in 1968, two screening tests were developed in mice. In one test, symptoms of hypervitaminosis A could be produced;[34] in the other, skin papillomas in mice were induced in 3 to 8 months with 7,12-dimethylbenz[a]anthracene as the initiator and croton oil as the promoter. A dose-dependent regression of these tumors was observed with retinoic acid given intraperitoneally over a 14-day period, while control animals showed a progression.[35,36] A therapeutic index could then be obtained as the ratio of the lowest daily intraperitoneal dose causing a defined level of hypervitaminosis A effects in a 14-day experiment and the dose producing a 50% regression of papillomas when administered intraperitoneally once a week for 2 weeks. Thus, the higher the therapeutic index achieved, the greater the potential clinical effectiveness of the retinoid. In studies of structure–activity relationships in these in vivo assays, it was found that a number of structural modifications in the ring, side chain, or polar terminal group were possible, which led to an improved therapeutic index over *all-trans*- and 13-*cis*-retinoic acid, whereas other alterations in the structure lowered or eliminated the therapeutic value. In Table I, the structures and activities in these tests of a selected group of retinoids are shown.

During the decade of the 1970's, many new biological tests—both in vitro and in vivo—were developed that addressed different aspects of the biological functions of retinoids, in particular their ability to affect cellular differentiation and proliferative properties. The results of

(2) Stepp, W. *Biochem. Z.* **1909**, *22*, 452-460.
(3) Stepp, W. *Z. Biol.* **1911**, *57*, 135-171.
(4) McCollum, E. V., Kennedy, C. *Z. Biol.* **1916**, *24*, 491-502.
(5) Drummond, J. C. *Biochem. J.* **1920**, *14*, 660.
(6) Karrer, P.; Mörf, R.; Schöpp, K. *Helv. Chim. Acta* **1931**, *14*, 1036-1040, 1431-1436.
(7) Euler, H. V.; Karrer, P. *Helv. Chim. Acta* **1931**, *14*, 1040-1044.
(8) Isler, O. In "Carotenoids"; Isler, O., Ed.; Birkhäuser Verlag: Basel, 1971; pp 11-27.
(9) Euler, B. V.; Euler, H. V.; Hellström, H. *Biochem. Z.* **1928**, *203*, 307-384.
(10) Euler, B. V.; Euler, H. V.; Karrer, P. *Helv. Chim. Acta* **1929**, *12*, 278-285.
(11) Moore, T. *Biochem. J.* **1930**, *24*, 692-702.
(12) Moore, T. *Lancet* **1929**, *2*, 380-381.
(13) Karrer, P.; Helfenstein, A.; Wehrli, H.; Wettstein, A. *Helv. Chim. Acta* **1930**, *13*, 1084-1099.
(14) Wald, G. *J. Gen. Physiol.* **1935**, *18*, 905-915.
(15) Wald, G. *J. Gen. Physiol.* **1935**, *19*, 351-371.
(16) Morton, R. A. *Nature (London)* **1944**, *153*, 69-71.
(17) Morton, R. A.; Goodwin, T. W. *Nature (London)* **1944**, *153*, 405-406.
(18) Arens, J. F.; van Dorp, D. A. *Recl. Trav. Chim. Pays-Bas* **1948**, *67*, 973-979.
(19) Isler, O. *Pure Appl. Chem.* **1979**, *51*, 447-462.
(20) Wolbach, S. B.; Howe, P. R. *J. Exp. Med.* **1925**, *42*, 753-778.
(21) Mori, S. *J. Am. Med. Assoc.* **1922**, *79*, 197-200.
(22) Peck, S. M.; Chargin, L.; Sobatka, H. *Arch. Dermatol. Syphilol.* **1941**, *43*, 223-229.
(23) Porter, A. D. *Br. J. Dermatol.* **1951**, *63*, 123-127.
(24) Burgoon, C. F., Jr.; Graham, J. H.; Urbach, F.; Musgnug, R. *Arch. Dermatol.* **1963**, *87*, 63-80.
(25) Beer, P. *Dermatologica* **1962**, *124*, 192-195.
(26) Storck, H. *Praxis* **1964**, *53*, 550-560.
(27) Thomson, J.; Milne, J. A. *Br. J. Dermatol.* **1969**, *81*, 452-455.
(28) Stuttgen, G. *Dermatologica* **1962**, *124*, 65-80.
(29) Fujimaki, Y. *J. Cancer Res.* **1926**, *10*, 469-477.
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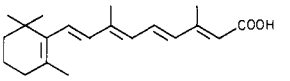
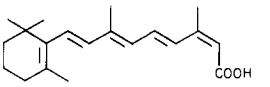
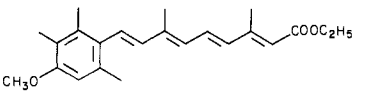
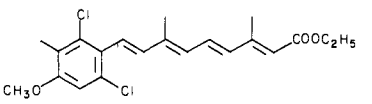
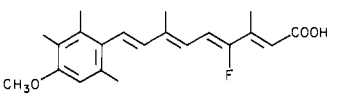
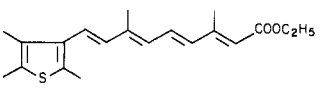
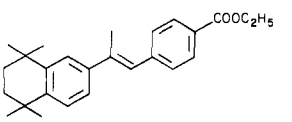
(31) For a review, see Bollag, W. *Cancer Chemother. Pharmacol.* **1979**, *3*, 207-215.
(32) Saffioti, U.; Montesano, R.; Sallakumar, A. R.; Borg, S. A. *Cancer*, **1967**, *20*, 857-864.
(33) Bollag, W. *Int. J. Vitam. Res.* **1970**, *40*, 299-314.
(34) Bollag, W. *Eur. J. Cancer* **1974**, *10*, 731-737.
(35) Bollag, W. *Experientia* **1971**, *27*, 90-92.
(36) Bollag, W. *Schweiz. Med. Wochenschr.* **1971**, *101*, 11-17.

Table I. Effect of Retinoids with Differing Chemical Structures on Hypervitaminosis A and Antipapilloma Activities

name	structure	hypervitaminosis A dose,[a] mg/kg	papilloma act. ED_{50},[b] mg/kg	therapeutic index[c]
all-trans-retinoic acid (tretinoin)		80	400	$\frac{80}{400} = 0.2$
13-*cis*-retinoic acid (isotretinoin)		400	800	$\frac{400}{800} = 0.5$
trimethylmethoxyphenyl analogue of ethyl retinoate (etretinate)		50	25	$\frac{50}{25} = 2$
dichloromethylmethoxyphenyl analogue of ethyl retinoate		12	6	$\frac{12}{6} = 2$
4-fluoro-9-trimethylmethoxyphenyl analogue of retinoic acid		25	11.5	$\frac{25}{11.5} = \sim 2$
trimethyl-3-thienyl analogue of ethyl retinoate		200	75	$\frac{200}{75} = \sim 3$
arotinoid		0.1	0.05	$\frac{0.1}{0.05} = 2$

[a] Lowest daily intraperitoneal dose causing hypervitaminosis A symptoms in a 2-week period. [b] Dose given intraperitoneally once a week for 2 weeks, which causes a 50% regression. [c] Ratio of hypervitaminosis A dose to the ED_{50} dose.

these studies have not yet revealed the exact mechanisms by which retinoids modulate these processes; however, they have demonstrated that retinoids have profound, complex, often confusing, and sometimes apparently inconsistent effects upon many different types of cells.

Biological Effects of Retinoids

Before different aspects of the biological functions of retinoids are addressed, it is worthwhile to consider briefly how they are transported in biological systems and how they are metabolized. Chart III, summarized from the review by Lotan,[37] outlines the circuitous route by which retinol reaches target cells. As shown in this chart, retinol normally circulates as a complex with a unique serum-binding protein (sRBP) and prealbumin.[38] It has been claimed that cells of the retinal pigment epithelium,[39-41] the intestinal mucosa,[42] and the testis[43] possess a receptor for the retinol–sRBP complex and that such cells are unable to accumulate free retinol.[40,41] It is not clear whether other cell types possess receptors for the retinol–sRBP complex. Chen and Heller[41] failed to find evidence for the presence of such molecules on the surface of leukocytes, erythrocytes, and retinal photoreceptor cells. Despite contentions by several investigators that free retinoids would be unable to penetrate cells (e.g., ref 38 and 41), evidence exists from in vitro studies that retinoids not complexed with sRBP can penetrate cells passively and relatively rapidly (e.g., ref 44–46). Of course, if circulating retinol is present only as a complex with its binding protein, little or none might be available in vivo to cells lacking the appropriate receptor.

Although retinoic acids can bind to the sRBP in vitro, when administered to animals they appear to circulate as a complex with albumin.[47,48] Such compounds might, therefore, be able to enter cells in vivo by a mechanism not involving sRBP. However, cells appear to take up retinoic acid much less effectively than free retinol,[46] and the physiological concentration of retinoic acid in serum is normally very low or undetectable (see ref 49). Taken together, these studies suggest that accessibility and uptake of retinoids in vivo might be of critical importance in the potential responsiveness of cells to these compounds. Much remains to be learned about this topic.

As mentioned above, retinol can be oxidized by several tissues to retinal and retinoic acid.[50] However, many other

(37) Lotan, R. *Biochim. Biphys. Acta* **1980**, *605*, 33–91.
(38) Ganguly, J.; Rao, M. R. S.; Murthy, S. K.; Sarada, K. *Vitam. Horm. (N.Y.)* **1980**, *38*, 1–54.
(39) Heller, J. *J. Biol. Chem.* **1975**, *250*, 3613–3619.
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(41) Chen, C. C.; Heller, J. *J. Biol. Chem.* **1977**, *252*, 5216–5221.
(42) Rask, L.; Peterson, P. A. *J. Biol. Chem.* **1976**, *251*, 6360–6366.
(43) Bhat, M. K.; Cama, H. R. *Biochim. Biophys. Acta* **1979**, *587*, 273–281.
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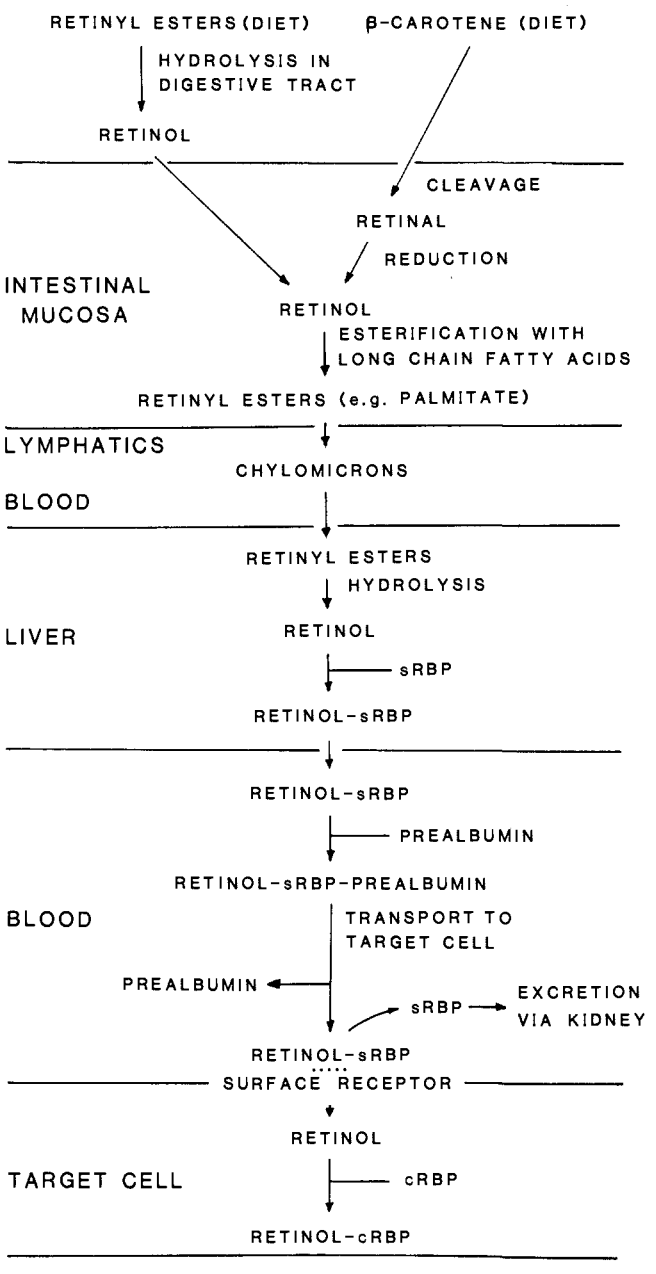
(49) Shidoji, Y.; Hosoya, N. *Anal. Biochem.* **1980**, *104*, 457–463.

Chart III. Delivery of Retinol to Target Cells



metabolites have been observed and several remain to be identified. Some metabolites, such as those with shortened side chains, the glucuronide of retinoic acid, and the 4-hydroxy or 4-keto derivatives of retinoic acid, appear to be produced for purposes of excretion.[50,51] The reason for the formation of other derivatives with reduced biological activity, e.g., the 5,6-epoxide of retinoic acid,[50,51] is not yet clear. Other metabolites, such as 13-*cis*-retinoic acid, retain biological activity in at least some systems.[52] Obviously, it is this latter group of retinoids that is of greatest interest; it is as yet unknown whether they or the parental compounds are normally responsible for the various biological effects of retinoids.

One pathway of retinoid metabolism might be of special functional significance. De Luca, Wolf, and their colleagues have discovered that both retinol and a hydroxylated derivative of retinoic acid can be phosphorylated and subsequently glycosylated, primarily with mannose.[53,54] As will be discussed below, these derivatives appear to serve as intermediates in glycosylation reactions.

In addition to sRBP, which is involved in retinoid transport, several intracellular proteins have been described that bind various retinoids specifically and with relatively high affinity. This subject has been reviewed in detail by Chytil and Ong.[55,56] Briefly, most (though not all) normal cell types appear to possess a 14 600-dalton protein (cRBP) that binds retinol but not retinal or retinoic acid. There also exists a second binding protein (cRABP) that interacts specifically with retinoic acids but not with retinol or retinal. This protein is immunologically different from cRBP, although it has a similar molecular weight and a related amino acid sequence.[57] Although cRABP is detectable in many tissues late in embryogenesis, its distribution in normal adult tissues is much more limited.[58,59] cRBP and cRABP each appear to have a single specific retinoid binding site.[56] Futterman et al.[60] have described a specific retinal binding protein that is restricted to cells of the retina and appears to be structurally unrelated to the other retinoid binding proteins. Further binding proteins have been described[55,61] but are as yet poorly characterized.

cRBP and cRABP are typically detected in high-speed supernates of cell extracts. It has been claimed that cRABP is associated with the plasma membrane,[62] but others have not found this to be the case.[63] Sani[64] reported the presence of free or ligand-exchangeable cRABP in nuclei from chick embryo skin cells. Studies with retinoblastoma[44] and embryonal carcinoma[52] cells suggest that cRABP translocates to the nucleus after it is charged with ligand. The intracellular fate of cRBP is not completely clear: Takase et al.[65] have observed that the purified retinol–cRBP complex has specific binding sites on or in isolated rat liver nuclei, and Shinde et al.[66] reported finding cRBP-like activity in nucleosol and chromatin fractions of rat testis cells. On the other hand, efforts to demonstrate translocation of the retinol–cRBP complex from the cytoplasm to the nucleus by cultured retinoblastoma cells were not successful.[44]

Retinoids have been reported to modulate the growth and progression of tumors and premalignant lesions, to affect the immune system, to play a role in inflammatory processes, to regulate differentiation of tissues (especially

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(51) Frolik, C. A. *Ann. N.Y. Acad. Sci.* **1981**, *359*, 37–44.
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(53) De Luca, L. M. *Vitam. Horm. (N.Y.)* **1977**, *35*, 1–57.
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(61) Muto, Y.; Omori, M. *Ann. N.Y. Acad. Sci.* **1981**, *359*, 91–103.
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(65) Takase, S.; Ong, D. E.; Chytil, F. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2204–2208.
(66) Shinde, R.; Das, R. C.; Sarada, K.; Ganguly, J. *Ind. J. Biochem. Biophys.* **1980**, *17*, 135–138.

epithelial) and organs, and to influence cellular adhesiveness and cellular interactions. From a cell-biological point of view, these effects can all be explained by retinoid action at three interrelated levels: proliferative capacity, differentiation state, and cell surface composition. Not surprisingly, different investigators have championed one or another of these levels of action as *the* primary means by which retinoids act. However, based upon available evidence, it is probably safe to state that no single function of retinoids can explain their widespread effects and that some observed retinoid-induced alterations in cellular behavior involve changes at more than one level.

Attempts to clarify the underlying mechanisms of retinoid action have been frustrated in no small part by inconsistencies of effect. For example, Lotan[37] has summarized data that illustrate that retinoids slow the growth of many cell types, particularly malignant ones, but increase the proliferative capacity of some others. Paradoxically, retinoids can potentiate the stimulatory effect of growth factors on many different cell types even though they might inhibit proliferation of those same cells in the absence of the growth factors.[67] Anchorage-independent growth, an indication of cell transformation in response to various agents, can be influenced in some instances by retinoids; once again, however, retinoids can increase the incidence of transformation of some cells but inhibit the process in others.[67]

In several systems, retinoids function to convert cells from a relatively unspecialized phenotype to an overtly differentiated state. An excellent example of this effect is the irreversible conversion by many retinoids of teratocarcinoma stem cells (i.e., embryonal carcinoma cells) to differentiated progeny.[52,68,69] Retinoids can also influence the direction of differentiation: in skin, for example, vitamin A deficiency leads to the keratinization of normally mucus-secreting epithelia, whereas retinoid excess has the converse effect.[70] This relatively reliable "transdifferentiation" effect has led to important applications for retinoids in dermatological disorders.

The effects of retinoids upon differentiation are not without confusing aspects. Recent reports suggest that retinoids can have a *negative* effect upon differentiation of mesodermal elements, e.g., in chondrogenesis and osteogenesis[71-73] and in the conversion of fibroblasts to adipocytes.[74] There have also been contradictory reports within similar systems. For example, retinoid treatment of a human melanoma line shifted the cells toward a more differentiated phenotype (increased production of melanin, increased tyrosinase activity),[75] whereas the opposite effect was observed with another human line[76] and with a hamster melanoma line.[77] Similarly, retinoids have been found to promote differentiation of human promyelocytic leukemia cells[78,79] but appear to suppress differentiation in a murine line.[80] As with any complex system, these discrepancies could be due to subtle differences in experimental conditions, types and dosages of retinoids used, and the criteria for assessing differentiation.

Cell-surface alterations are commonly observed when cells are exposed to retinoids. These changes are often reflected by increased adhesiveness of the treated cells.[81-83] Direct biochemical analyses have revealed alterations in surface protein profiles,[77,83,84] increased protein glycosylation,[53,85,86] modulation of glycosaminoglycan synthesis,[81,87] and quantitative changes in surface protein receptors.[67]

With the exception of the role of retinal in vision, the mechanisms by which retinoids elicit their effects are not clearly understood. The following results implicate cRABP in the in vitro promotion of differentiation of at least some cell types by retinoids: (a) retinoic acid is generally more effective than retinol in promoting differentiation;[37] (b) a correlation exists between the ability of various retinoid metabolites or synthetic analogues to promote differentiation and to compete for sites on the cRABP;[37,52,88] (c) human promyelocytic leukemia cells, which differentiate in response to retinoic acid, possess cRABP activity, whereas unresponsive mouse myelocytic leukemia cells do not;[89] and (d) embryonal carcinoma cell mutants lacking cRABP activity fail to differentiate in response either to retinoids or to other promoters of differentiation.[63,90] The reported translocation of the cRABP holoprotein from the cytoplasm to the nucleus raises the possibility that differentiation is initiated directly by interaction of the complex with chromatin. The observation that retinoic acid is a more potent teratogen than retinol (see below) might imply that cRABP is normally involved in differentiation of embryonic cell types. It is not clear what role, if any, cRBP plays in the promotion of cellular differentiation.

Since differentiated cells tend to proliferate less rapidly than their undifferentiated progenitors, retinoids can influence growth rates by promoting differentiation. Consistent with this view, retinoic acid tends to be a more potent inhibitor of cell proliferation than retinol.[91,92]

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However, it has now been demonstrated that retinoids can reduce proliferation rates in some instances without inducing differentiation or interacting with cRABP. Convincing evidence that this is so derives from demonstrations that (a) the correlation between retinoid affinity for cRABP and suppression of growth of tumors or cells in culture is qualitative at best, clearly not quantitative;[84,88] (b) the proliferation rates of several cell types lacking cRABP, cRBP, or both are, nevertheless, reduced by retinoids;[37,93] and (c) growth properties in mutant embryonal carcinoma cells lacking the ability to differentiate in response to retinoids are affected by retinoic acid.[63] Proliferation rates of different cell types might be altered by one or more of the pleiotropic effects of retinoids.

Just as the promotion of differentiation by retinoids can influence growth rates, so too can it affect cell-surface properties.[69] Once again, however, it is apparent that other actions of retinoids can provoke cell-surface changes. De Luca and his colleagues have proposed that promotion of glycosylation by glycosyl retinyl phosphates can affect the properties of cell-surface glycoproteins.[53,67] The observation that retinoids can alter membrane microviscosity[94] has led Jetten to propose that retinoids might act directly by intercalating into the cell membrane.[67]

Although endogenous retinoids are essential for normal reproduction and embryonic development, these same compounds are highly teratogenic in excessive amounts. Almost all embryonic tissues are susceptible to teratogenic insult by retinoids (see, e.g., ref 95 and 96). Retinoids at high concentrations are cytotoxic, presumably because they disrupt membranes via a detergent-like action.[37] However, their teratogenic effects do not seem to reflect a nonspecific killing of cells. Though many teratogenic agents elicit damage by mutagenesis, retinoids appear to have little, if any, mutagenic activity.[97] The following generalities of retinoid teratogenicity emerge from a summary of teratological studies by Geelen[95] and particularly from a comprehensive analysis of golden hamster embryos by Shenefelt:[96] (a) dramatic embryonic abnormalities can be detected at retinoid concentrations that are toxic neither to the embryo as a whole nor to the mother; (b) tissues are differentially damaged with regard to severity and even qualitatively, depending upon the time of the insult; (c) the most drastic effect on a tissue is often seen when the administration of the retinoid takes place prior to, or at the onset of, the appearance of the anlage for that particular tissue; (d) the types of abnormalities seen commonly reflect the failure of tissues to reach their normal size and shape; and (e) retinoic acid is a more potent teratogen than retinol.

A consideration of these observations leads to the impression that retinoids cause embryonic abnormalities by the same actions attributed to them in other studies: alterations of growth rate, state of differentiation, and cell-surface structure. It is reasonable to expect that disruption of these properties would affect a spectrum of different tissues at different times, particularly during the anlagen stages when precise proliferative and differentiative activities are essential. Retinoic acid might be a particularly potent teratogen because of its strong ability to promote differentiation. Premature differentiation of cells with a concomitant diminution of proliferative capacity could explain the reduced size of affected tissues. Finally, differential accessibility to, and handling of, retinoids by different parts of the embryo might also be important in the patterns of teratogenicity.

Malignant cells share with early embryonic cells a marked capacity for rapid proliferation. Numerous studies have indicated that cell-surface properties are altered during cell transformation. Furthermore, malignant cells, like early embryonic cells, are often thought of as being relatively undifferentiated; embryonal carcinoma cells provide a valuable model system in which it can be demonstrated that induction of differentiation leads to a conversion from a malignant to a benign phenotype.[63,98,99] It is therefore understandable that agents which suppress growth and induce differentiation of embryonic cells should have analogous effects upon malignant cells.

Research on the antitumor effects of retinoids has mushroomed in recent years. Although it is difficult to summarize such a large number of studies and dangerous to draw generalizations, particularly with pleiotropic agents such as retinoids, some reproducible observations have been made, and these have been reviewed recently by Sporn and Newton[100] and by Lotan.[37] First, retinoids seem most potent as preventive agents: they are effective in combating preneoplastic lesions, they antagonize chemical and photoinduced tumor promotion in vivo, and they can suppress the induction of cellular transformation in vitro due to carcinogens, radiation, viruses, or growth factors. It has been reported that retinoids can prevent conversion of some procarcinogens to carcinogens;[101,102] Dickens and Soroff proposed that retinoids might not suppress tumor promotion per se but rather interfere with the metabolism of agents that require such action in order to become effective promoters.[102] It is inconsistent with this view, however, that 7,12-dimethylbenz[*a*]anthracene, a procarcinogen antagonized by retinoids in the murine mammary gland system,[102] retains its ability in the presence of retinoids to induce tumors in mouse epidermis.[103]

Although retinoids can interfere with proliferation of several malignant cell lines in vitro,[91] with few exceptions[88,98,99] they show limited success in reversing the growth and metastasis of established tumors in vivo.[104] To some degree, the problem might be one of numbers. Lotan et al.[105] have observed that the effectiveness of retinoic acid in suppressing growth of subcutaneously injected murine melanoma cells is inversely related to the size of the cell inoculum. In other words, the tumors might contain, or come to acquire in response to treatment, subpopulations of cells that differ in their susceptibility to retinoid action; if the number of tumor cells is sufficiently high at the time of retinoid administration, then a refractory subpopulation will expand and effects on tumor growth will be minimal. Even if the idea of genetically resistant subpopulations

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proves to be incorrect, should the highest permissible dose of retinoid fail to kill 100% of tumor cells, progressively higher inocula will increase the probability that a minimum essential proliferative mass of the cells will persist.

The role of retinoid binding proteins in neoplasia and its suppression are far from clear. Surveys indicate that cRBP and cRABP levels in tumors can resemble those of adjacent normal tissue or they can be very different; the tendency appears to be toward increased levels of cRABP and decreased levels of cRBP in tumor cells.[37,56,106] Although it is now fashionable to compare binding protein levels in tumors vs. adjacent tissues, relationships between the presence or absence of these proteins and response of the malignant cells to retinoids have not been clearly drawn in most instances.

One enzyme that has figured prominently in studies on antitumor effects of retinoids is ornithine decarboxylase (ODC). The increase in activity of this enzyme, which is the first in the pathway of polyamine biosynthesis, is one of the earliest detectable responses to tumor promoters. Retinoids suppress the rise in ODC activity by mouse epidermal tissue in instances wherein tumor promoter effects are counteracted; however, when retinoid treatment is ineffective in preventing tumor promotion, the rise in ODC activity is not blocked.[103] Russell and Haddox[107] have reviewed widespread evidence that a rise in ODC activity during the G_1 phase of the cell cycle is related to stimulation of growth. From their studies on Chinese hamster ovary cells, they have concluded that retinol prevents the rise of ODC activity during G_1 and thereby blocks progression through the cell cycle; that the block occurs by direct or indirect action of the retinoid at the level of transcription of the ODC gene; and that overall RNA synthesis is also suppressed.[107] Retinoids suppress the rise in ODC activity as they block proliferation of other cell types as well.[72,108,109]

It must be noted that several reports have now accumulated that indicate that retinoids are either ineffective in controlling the proliferation of certain types of malignant cells or that they actually stimulate growth.[37,103] This is not unexpected in view of the aforementioned inconsistencies of retinoid effects on proliferation and differentiation. Perhaps the approach of pretesting retinoids on biopsy material in vitro[110,111] will help to minimize the possibility of negative therapeutic effects.

There is much to be said in favor of studying the antitumor effects of retinoids on cells or tissues in culture. Results can be obtained relatively quickly and inexpensively, many different cell types can be studied, and in vitro systems are often more straightforward than in vivo models. On the other hand, in vitro studies cannot take into account accessibility and delivery of retinoids to tumor cells, metabolism of the retinoids by other tissues, and immune effects. Many studies have now been carried out on the consequences of retinoid treatment on the different components of the immune system, and it is apparent that immune cells are also targets of vitamin A effects. It is likely that retinoids modulate behavior of these cells in the same way as they do many others, i.e., by influencing growth rates, differentiation state, and/or cell surface properties.[112,113] In this case, however, the results can be profound at an organismal level, since the immune response can be modified. In general, retinoids stimulate immune responses, both humoral and cell mediated: Lotan[37] has reviewed data suggesting that retinoids can act as adjuvants in antibody production and can also enhance allogeneic graft rejection. Evidence is accumulating that in some instances the antitumor activity of retinoids includes immunological responses at either or both of these levels.[114-116] Details of the mechanisms by which retinoids influence immune responsiveness have yet to be agreed upon. Several retinoids promote immune effects, although retinol and retinyl palmitate appear to be the most potent.[37]

Among the biochemical manifestations of retinoids, alterations in the levels of two protease activities have been repeatedly documented. Plasminogen activators are a group of proteases that convert the serum zymogen plasminogen to plasmin. Plasmin, in turn, can catalyze fibrinolysis. It appears as though stimulation of differentiation of embryonal carcinoma cells by retinoids leads invariably to increased secretion of plasminogen activator.[63,68,69,98,117,118] Plasminogen activator production is promoted in response to retinoids in other instances in which differentiation does not appear to be involved.[119-123] The significance of this increased enzyme activity is not yet clear, although it has been proposed that the enzyme might be involved directly or indirectly in tissue remodeling.[119,120]

The second protease, collagenase, often shows an opposite response to retinoids. In synovial fibroblasts and skin, retinoids suppress collagenase levels.[124-127] Suppression of collagenase activity might play a role in countering inflammation and in wound healing. An effect of retinoids has been demonstrated in both instances.[128,129] However, Coffey et al.[128] failed to find evidence that sup-

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pression of collagenolytic activity was involved in their antiinflammatory properties. It has been suggested that retinoids might be of therapeutic value in the treatment of rheumatoid arthritis,[124,125] although, as Hamilton[123] has pointed out, concomitant elevation of plasminogen activator levels might be detrimental.

Gerber and Erdman[130,131] have reported that rats on a retinol-supplemented diet possess elevated levels of triglycerides in both the very low density lipoprotein fraction and the high density lipoprotein fraction of serum. Some tissues were found to possess decreased levels of lipoprotein lipase activity,[130,131] raising the possibility that retinoids induce hyperlipidemia in part by modulating lipid catabolism.

Clinical Application of Retinoids

As noted above, the profound effects of vitamin A on keratinization of epithelial cells led to its early therapeutic use in dermatological disorders of keratinization. In 1941, Peck et al.[22] reported its usefulness in Darier's disease. Subsequently, vitamin A was tested in cases of ichthyosis,[132] pityriasis rubra pilaris,[133] acne,[134] psoriasis,[135] mycosis fungoides,[136] and other major dermatological conditions. However, the therapeutic doses required (300 000 to 1 000 000 IU/day) for an extended period of time limited its clinical usefulness.

Stuttgen[28] reported that oral and topical *all-trans*-retinoic acid was useful in patients with pityriasis rubra pilaris, and Beer[25] reported its efficacy in psoriasis and acne. Topically this retinoid has shown efficacy for ichthyosis, psoriasis,[137] acne,[138] actinic keratosis,[139] and acne vulgaris.[140] When *all-trans*-retinoic acid is administered orally, its profile of toxicity and therapeutic ratio are similar to that of oral vitamin A, and thus it was not clinically acceptable for treating chronic cutaneous diseases. Another topical retinoid, motretinide (Ro 11-1430), has shown efficacy in European reports in the treatment of acne vulgaris[141] and selected disorders of keratinization.

In 1971, Bollag[142] first reported evidence on the therapeutic efficacy of 13-*cis*-retinoic acid in acne vulgaris. Since then several hundred severe cystic acne patients have been treated with the drug under several clinical protocols. These studies have demonstrated remarkable clearing of the previously treatment-resistant disease after one or two 15- to 20-week courses of 13-*cis*-retinoic acid administration. Prolonged remissions (often for more than 3 years) were seen after that relatively brief treatment.[143]

In May 1982, 13-*cis*-retinoic acid became the first oral retinoid in the U.S., indicated for the treatment of severe cystic acne in patients unresponsive to previously available forms of therapy. In a review by Windhorst and Nigra,[144] of more than 500 patients treated for cystic acne and other dermatological conditions with 13-*cis*-retinoic acid, all patients experienced one or more of the following adverse reactions: mucocutaneous dryness in the form of cheilitis, cutaneous desquamation, epistaxis, hair thinning, and/or conjunctivitis. Musculoskeletal symptomatology was noted in 15% of patients, lethargy in 10%, headache in 10%, and mild gastrointestinal symptomatology in 20% of patients. Laboratory examinations showed elevated sedimentation rates in 50% of patients and elevated triglycerides in 25%. While side effects (including these and others) were common, relatively few patients required discontinuation of therapy because of them.

In 1973, Runne[145] and Koch and Schettler[146] demonstrated the efficacy of 13-*cis*-retinoic acid in psoriasis and leukoplakia, respectively. Since that time, efficacy for this drug in several other dermatological conditions has been shown clinically. Etretinate, an aromatic retinoid (Table I), was the third oral drug in this series to be studied for dermatological therapy. In 1975, Ott and Bollag[147] first reported a good response of psoriatic patients to treatment with etretinate. In a subsequent review[148] of data from 12 published studies on more than 600 patients, etretinate produced significant clearing in most patients with psoriasis. The combination of etretinate with various other standard forms of therapy (ultraviolet light, topical steroids, tars, etc.) demonstrated the superiority of combination therapy to either therapy used alone. Etretinate has recently been approved for clinical use for the treatment of psoriasis in some European countries. The incidence and types of side effects are similar to those seen with 13-*cis*-retinoic acid; however, the former retinoid appears to cause a somewhat greater incidence of hair thinning, palmoplantar desquamation, paronychia, and musculoskeletal complaints.[149] A major difference between the two retinoids is the prolonged elimination half-life seen with etretinate.[150]

While indications for both etretinate and isotretinoin are presently limited, the wide variety of cutaneous disorders that have responded to one or both of these retinoids indicates that the future potential use of these drugs in treating dermatological diseases is great. Some dermatological diseases have demonstrated unique specificity in efficacy to one retinoid compared to another. Peck reviewed some of the efficacy differences between the two oral retinoids seen in various cutaneous disorders.[149] While many of these disease indications need additional study, there does appear to be some targeting of efficacy with the various retinoids.

The development of oral retinoids represents the most significant advance in dermatological therapy since the introduction of corticosteroids. Perhaps the development

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of new retinoids should be aimed at a clinical activity profile so as to target the efficacy of a retinoid to a specific cutaneous disorder with a hope of decreasing the incidence and spectrum of toxicity seen. The hope would thus be to develop a retinoid more selective to either sebaceous glands, keratinocyte proliferation, or cutaneous inflammation.

While the usefulness of retinoids in dermatologic disorders has been verified, the validity of this class of compounds in cancer treatment and prevention remains to be established. Sporadic reports of clinical efficacy in a variety of premalignant and malignant disorders have recently caused renewed interest in vitamin A and its analogues for these indications.

As mentioned previously, the probable relationship between vitamin A and cancer had been noted as early as 1925 when Wolbach and Howe[20] observed that the metaplastic epithelial lesions characteristic of vitamin A deficiency had features in common with those of neoplastic lesions. Since that time, extensive in vitro and in vivo results with human, as with animal, cells have suggested that vitamin A and its natural and synthetic derivatives can modulate differentiation and proliferation of both normal and abnormal cells. While the exact mechanism of action remains elusive, the recent finding that retinoids are capable of inducing terminal differentiation in a variety of animal and human malignant cell lines is of special significance. Since some malignant disorders have been viewed as diseases involving a block in normal cellular maturation, the ability of the retinoids to encourage terminal differentiation may provide an alternative therapeutic approach to the conventional method of drug-induced cytotoxicity used in the management of cancer patients. Although there have been only limited clinical trials conducted to date, the therapeutic activity of certain retinoid derivatives has been reported in several proliferative disorders, including the preleukemic syndromes,[151] squamous carcinoma of the head and neck, carcinoma of the lung, malignant melanoma, kerato-acanthoma,[152] and oral leukoplakia.[153] Studies are in progress to define more precisely the activity of the retinoids in these and other malignant and premalignant disorders.

Since this class of compounds may exert its antitumor effect via other mechanisms than conventional cytotoxic chemotherapy, clinical studies should be designed that exploit these differences. From the available preclinical and clinical data, it appears unlikely that the retinoids tested to date will be useful as single-agent therapy in the treatment of advanced cancer, with few exceptions.

Perhaps the most exciting therapeutic potential of vitamin A and its derivatives is in the area of cancer chemoprevention. As mentioned in previous sections, extensive investigations have demonstrated that retinoids can block the promotional step of carcinogenesis whether the process is initiated by chemical or physical agents. A recent publication[154] has indicated the ability of the aromatic retinoid etretinate to reverse metaplastic lesions in the lungs of heavy smokers. Evidence has accumulated that suggests that persons with low serum retinol levels are at a higher risk for the development of cancer. Kark et al.[155] studied 3012 individuals in Evans County, GA, for periods ranging from 12 to 14 years. He reported that the 129 persons who eventually developed cancer had significantly lower mean serum retinol levels at least 12 months before the cancer diagnosis. In another study, Shekelle et al.[156] noted that the intake of dietary provitamin A (carotene) was inversely related to the 19-year incidence of lung cancer in a prospective epidemiologic study of 1954 middle-aged men. Hirayama conducted a prospective epidemiologic study of prostate cancer in Japan. This 10-year study of 122 261 men aged 40 years and above revealed a significantly lower age-standardized death rate for prostate cancer in men who ate green and yellow vegetables daily.[157] Several other large epidemiologic studies have reported similar results, and the National Cancer Institute has recently announced plans to fund large-scale clinical studies of the relationship between retinoids and cancer prophylaxis.

It is clear that the derivatives chosen for use in chemoprevention must have minimal toxicity or be effective at low and nontoxic dose levels. Since it appears likely that treatment would be of long duration, careful consideration must be given to the possible long-term effects on lipid metabolism and reproductive function. Despite these limitations, however, the retinoids remain the prototype agents presently available for evaluation in the prevention of cancer. Analysis of structure–function relationships may reveal directions for the synthesis of newer retinoid derivatives with a therapeutic–toxicity profile suitable for widespread use by a high-risk but otherwise well patient population.

The future of vitamin A and its derivatives in cancer therapy and prophylaxis is still largely undetermined, but the available evidence would suggest that cautious optimism is warranted.

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