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Final Report on the Safety Assessment of Retinyl Palmitate and Retinol

Retinol is the naturally occurring form of vitamin A; Retinyl Palmitate is the ester of Retinol and Palmitic Acid. In acute oral studies, Retinol was slightly toxic to mice, and Retinyl Palmitate was practically nontoxic in mice and rats. Large single doses can be lethal. It is recognized that Retinol is essential for reproduction; however, high intake of Retinol has produced adverse effects on several reproductive functions. Vitamin A was nonmutagenic in several in vitro tests. There is no evidence that vitamin A is carcinogenic. However, the vitamin has both enhanced and inhibited responses to viral or chemical carcinogens. Cosmetic products containing 0.1–1% Retinyl Palmitate were, at most, slightly irritating and nonsensitizing when tested on a total of 607 subjects. Results of cumulative irritation tests of two products containing 0.1% Retinyl Palmitate indicated that the products were nonirritating and nonsensitizing. On the basis of the available animal and clinical data presented in this report, it is concluded that Retinyl Palmitate and Retinol are safe as cosmetic ingredients in the present practices of use and concentration.

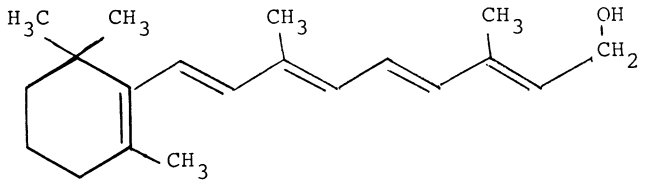
INTRODUCTION

The literature on Retinol and Retinyl Palmitate is voluminous. That literature dating from 1920 to 1980 has been previously reviewed in a series of GRAS (generally recognized as safe) reports and evaluations and is only briefly summarized here.[1–3] More recently, Sporn et al.[4] have reviewed the chemistry and biology of the retinoids. Unpublished cosmetic industry data have also been included.

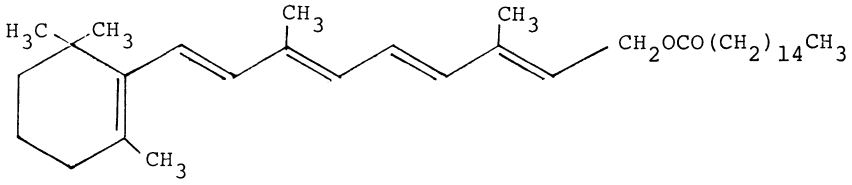
CHEMICAL AND PHYSICAL PROPERTIES

Retinol, chemically known as 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol, is the primary naturally occurring form of vitamin A.[5] Retinyl Palmitate is the ester of Retinol and palmitic acid, also

known as vitamin A palmitate (when used as a nutrient or drug).[6] These two compounds conform to the following structures:



Retinol[6]



Retinyl Palmitate[7]

Retinol occurs as a pale yellow, crystalline compound or viscous liquid, and Retinyl Palmitate is a yellow to yellow-red solid or oily substance. Both compounds have a faint characteristic odor and are soluble in most organic solvents while insoluble in water.[5,7,8–10] Retinol and Retinyl Palmitate have ultraviolet absorption maxima in the range of 324 to 328 nm.[5,9,10] The physicochemical properties of Retinol and Retinyl Palmitate are presented in Table 1.

A large number of processes have been developed for the synthesis of Retinol and its esters. The most important commercial methods today are based on the work of Isler et al.[11] at Hoffmann-La Roche and of Pommer et al.[12–14] at the BASF laboratories in Ludwigshafen, Germany. Retinyl acetate is the end product of both these processes (see Frickel[15] for more details). Retinol can be esterified by various methods, usually under very mild conditions. The imadazolide method is useful because of its nonacidic reaction conditions.[15] Retinol can also be obtained by concentration from animal fats and fish liver oil. This is accomplished by molecular distillation, saponfication, and crystallization of the distillate and conversion to the desired ester.[10]

Retinol oxidizes readily although it is quite stable in oil solutions. Ultraviolet (UV) light inactivates the vitamin and its solutions, which give off a characteristic green fluorescence. Retinol is relatively heat stable and is more stable in alkaline than acid solution. The acetic and palmitic acid esters of Retinol are commercially important due to their considerably greater degree of stability when compared to the alcohol.[5,10,15,18,19]

TABLE 1. Physiochemical Properties.

Property	*Retinol*	*Retinyl Palmitate*	*Reference*
Physical appearance	Yellow viscous liquid/yellow crystals; odorless or with a faint characteristic odor	Light yellow to yellow-red solid or oily substance with faint characteristic odor	5, 7, 8, 10, 16
Molecular formula	$C_{20}H_{30}O$	$C_{36}H_{60}O_2$	5, 9
Molecular weight	286.44	524.87	5
Melting point	62–64°C	27–20°C (all-trans form)	5, 9, 17
Boiling point	137–138°C		8, 16
Distillation point at 0.005 mm Hg	120–125°C		5, 9
Absorption max (UV)	324–325 nm (ethanol) ($E^{1\%}_{1cm}$ = 1835); 328 m	325–328 nm (ethanol) ($E^{1\%}_{1cm}$ = 940–975)	1, 5, 9, 10
Solubility[a]			
Water	I	I; S	5, 8, 9, 17, 18
Glycerol	I	I	5, 18
Alcohol	S	S	5, 8, 18
Methanol	S		5, 8, 18
Ethanol	S		16
Acetone	S		16
Benzene	S		16
Chloroform	S	S	5, 8, 18
Ether	S	S	5, 8, 18
Most organic acids, fats, and oils	S	S (most vegetable oils)	5, 8, 9, 18
Light ptroleum	S		8
Refractive index (22°C)	N_D-1.6410 (calculated from refraction indices of 20–70% solutions in mineral oil)		1
Optical rotation	±0°		1

[a]S, soluble; I, insoluble.

The analysis of these retinoids* depends on certain inherent chemical properties: their intense absorption in the near UV region, their ability to fluoresce, their light sensitivity, and their ready conversion to charged, intensely colored complexes in the presence of certain acids. High-pressure liquid chromatography (HPLC) has become the preferred method in retinoid analysis because of the stability of retinoids on most HPLC columns, its high resolution and sensitivity, and the rapidity of most analyses. HPLC also has the ability to separate a wide range of retinoids that may differ only in their isomeric configuration. Other analytical methods used include conventional column, thin-layer, and gas–liquid chromatographies, fluorescence and the UV-VIS (visible light) absorption of the retinoids themselves or of their colored products. Nuclear magnetic resonance (NMR) spectroscopy is used routinely to establish the chemical structure, whereas mass spectroscopy is useful for determining the molecular weight and structural information.[15,10,20]

*Retinoids, designated term for the natural forms of vitamin A and their synthetic derivatives.

The reader is referred to Frolik and Olson[20] for a detailed presentation of the analytical methods used to identify Retinol and Retinyl Palmitate.

USE

Cosmetic Use

Retinol and Retinyl Palmitate are used primarily in hair, facial makeup, and skin care preparations. They are generally used at concentrations $\leq 1\%$.[21] Table 2 presents the FDA product formulation data for Retinol and Retinyl Palmitate.[21] These computerized data, made available by the FDA, are compiled through voluntary filing in accordance with Title 21 part 270.4 (d)(1) of the Code of Federal Regulations.[22] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration

TABLE 2. Product Formulation Data.[21]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*			
			Unreported concentration	*> 1–5*	*> 0.1–1*	*≤ 0.1*
Retinol						
Baby lotions, oils, powders, and creams	56	1	—	—	1	—
Eye makeup remover	81	1	—	—	—	1
Hair conditioners	478	2	2	—	—	—
Hair sprays (aerosol fixatives)	265	1	—	—	—	1
Hair rinses (noncoloring)	158	1	1	—	—	—
Hair shampoos (noncoloring)	909	4	3	—	—	1
Tonics, dressings, and other hair grooming aids	290	2	—	—	1	1
Wave sets	180	4	—	—	—	4
Other hair preparations (noncoloring)	177	1	—	—	1	—
Blushers (all types)	819	1	1	—	—	—
Face powders	555	1	1	—	—	—
Makeup foundations	740	4	—	—	—	4
Lipstick	3319	5	5	—	—	—
Makeup bases	831	5	2	—	—	3
Nail creams and lotions	25	1	1	—	—	—
Other manicuring preparations	50	1	—	—	—	1
Personal cleanliness products	227	1	1	—	—	—

TABLE 2.

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*			
			Unreported concentration	*> 1–5*	*> 0.1–1*	*≤ 0.1*
Aftershave lotions	282	1	1	—	—	—
Preshave lotions (all types)	29	1	1	—	—	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	7	2	—	2	3
Face, body, and hand skin care preparations (excluding shaving preparations)	832	20	10	1	2	7
Hormone skin care preparations	10	3	—	—	3	—
Moisturizing skin care preparations	747	39	13	—	6	20
Night skin care preparations	219	11	4	—	2	5
Paste masks (mud packs)	171	4	2	—	—	2
Skin fresheners	260	4	—	—	—	4
Wrinkle smoothers (removers)	38	1	—	—	—	1
Other skin care preparations	349	8	—	—	1	7
Suntan gels, creams, and liquids	164	3	—	—	1	2
1981 TOTALS		138	50	1	20	67
Retinyl Palmitate						
Bubble baths	475	1	—	—	—	1
Other bath preparations	132	1	—	—	—	1
Eye makeup preparations	230	4	1	—	1	2
Hair conditioners	478	2	1	—	—	1
Tonics, dressings, and other hair grooming aids	290	2	—	—	2	—
Blushers (all types)	819	2	—	—	1	1
Face powders	555	1	—	—	—	1
Makeup foundations	740	7	—	—	—	7
Lipstick	3319	14	—	—	3	11
Makeup bases	831	1	—	—	—	1
Rouges	211	2	—	—	1	1
Other makeup preparations (not eye)	530	1	—	—	—	1
Nail creams and lotions	25	1	—	—	—	1
Nail polish and enamel	767	1	—	—	—	1
Face, body, and hand skin care preparations (excluding shaving preparations)	832	12	1	—	5	6
Moisturizing skin care preparations	747	28	5	—	11	12
Night skin care preparations	219	9	2	1	2	4
Paste masks (mud packs)	171	4	1	—	1	2
Wrinkle smoothers (removers)	38	1	—	—	1	—
Other skin care preparations	349	7	2	—	4	1
Suntan preparations	28	1	—	—	1	—
1981 TOTALS		102	13	1	33	55

range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

In 1981, Retinol reportedly was used in a total of 138 formulations, most of which were hair (noncoloring), makeup (not eye), and skin care preparations. Of these 138, 49% incorporated Retinol at concentrations $\leq 0.1\%$; 14% at concentrations of $> 0.1–1\%$; less than 1% at concentrations of $> 1–5\%$; and 36% at unreported concentrations.[21]

Retinyl Palmitate reportedly was used in a total of 102 formulations in 1981, most of which were makeup (not eye) and skin care preparations. Of these 102, 54% incorporated Retinyl Palmitate at concentrations $\leq 0.1\%$; 32% at concentrations of $> 0.1–1\%$; 1% at concentrations of $> 5–10\%$; and 13% at unreported concentrations.[21]

The formulation data presented in Table 2 indicate that cosmetic products containing Retinol and Retinyl Palmitate may contact all external body surfaces and hair, as well as oral, ocular, and vaginal mucosae. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application could result in a continuous exposure.

Retinol and Retinyl Palmitate are both approved for use in cosmetics in Japan.[23]

Noncosmetic Use

Retinol and Retinyl Palmitate are both affirmed as GRAS (generally recognized as safe) food ingredients when used in accordance with good manufacturing practices.[24,25] Their functional uses in foods are as nutrients and dietary supplements.[18] Retinol is also used as a nutrient and dietary supplement in the veterinary field.[5,15,26]

Vitamin A is used in pharmaceutical products for the treatment of diaper rash and hemorrhoids (internal and external creams), in corn and callus removers and vaginal creams, and as dietary supplements for the prevention of vitamin A deficiency. Retinyl Palmitate is also used in products for the treatment of diaper rash.[27,28]

Retinol administered orally has been used as therapy for a variety of dermatoses for the past 40 years. However, since the hypervitaminosis A syndrome (see section Clinical Assessment of Safety, Hypervitaminosis A) has interferred with chronic therapeutic use of vitamin A, current research is searching for synthetic derivatives that are less toxic and yet as efficacious as vitamin A. Two synthetic retinoids, isotretinoin and etretinate, have been introduced into the clinical practice of dermatology.[29] Some clinicians still advocate the use of oral Retinol in the treatment of severe acne.[30]

BIOLOGY

Retinol is essential for the growth, health, and life of higher animals. It is required for vision, reproduction, and the maintenance of differentiated epithelia and of mucous secretion. Although its role in the visual process is

well understood, little is known about the molecular mechanisms of these other biological effects.[31]

A synopsis of the biology of Retinol and Retinyl Palmitate is presented in the following sections.

Biosynthesis and Absorption

Natural sources of Retinol in the diet include certain plant carotenoid pigments, particularly β-carotene, and the long-chain retinyl esters found in animal tissues. β-Carotene is converted to Retinol primarily in the intestinal mucosa, although conversion also occurs in the liver and other tissues.[31,32] The overall biological efficiency of conversion of β-carotene to Retinol is about 50% at the maximum; this level of efficiency occurs at lower concentrations of carotene intake and declines as intakes rise.[33]

Dietary retinyl esters, Retinol, and provitamin A carotenoids are dispersed and emulsified in the stomach during the gastric phase of lipid digestion. Extensive hydrolysis of the retinyl esters is carried out in the intestinal lumen by an enzyme derived from the pancreas, commonly called the "pancreatic nonspecific lipase or cholesterol esterase." Repeated hydrolysis and reesterification of retinyl ester occur during its digestion and assimilation.[32]

Retinol obtained from the diet and that resulting from the hydrolysis of retinyl esters is solubilized in mixed micelles, transported across the aqueous diffusion barrier (the unstirred water layer), and absorbed by the mucosal cells. Here, it is reesterified with long-chain mainly saturated, fatty acids (predominantly palmitic acid).[32] An acyl-Coenzyme A retinol acyltranferase is believed to be responsible for the intestinal esterification of Retinol.[34] The retinyl esters are then incorporated along with other lipids and apolipoproteins into chylomicrons and transported via the lymph into the general circulation. The chylomicrons are metabolized in extrahepatic tissues by the lipolytic removal of most of the triglyceride, leaving a smaller, cholesterol-rich particle that contains essentially all of the chylomicron retinyl esters. These chylomicron remnants are removed from the circulation almost entirely by the liver.[32]

The reader is referred to Hollander et al. for an in-depth review of the intestinal absorption of Retinol and β-carotene.[35–39]

Lee[40] has studied the disposition of topically applied vitamin A in the cornea, conjunctiva, iris-ciliary body, and aqueous humor of healthy male albino rabbits using radiotracer techniques. Single doses (25 μl volume) of a 0.1% solution of vitamin A (all trans[^{3}H] and nonradioactive) in arachis oil were instilled directly into the cornea of the test rabbits. Both eyes of the test animals were used, but the dosing time was staggered so that the eyes could be evaluated at different time points. Rabbits were sacrificed at various times by intravenous (IV) injection of 30% sodium phenobarbitol solution into a marginal ear vein and the ocular tissues were obtained. Concentrations of vitamin A were studied for up to 120 min after instillation. The highest concentration of vitamin A was found in the cornea and conjunctiva; these tissues had peak times early, suggesting a rapid uptake of vitamin A from the

tear pool. A sustained concentration of vitamin A was evident in all ocular tissues studied beginning at 30 min postinstillation. Lee found that vitamin A was available to the conjunctiva and the cornea from topical dosing, since these two tissues are known to be affected by vitamin A deficiency and dry-eye states.

Hepatic Metabolism and Storage

The uptake of chylomicron remnants by the liver appears to occur primarily by receptor-mediated endocytosis, followed by lysosomal degradation. The retinyl esters are again hydrolyzed, reesterified, and stored in both the parenchymal liver cells and in the nonparenchymal fat-storing cells. The enzyme catalyzing the esterification of Retinol in the liver is found in the microsomal fraction and appears to qualify as a fatty acyl-Co A: retinol acyltransferase.[32,41-43]

The retinyl esters may be stored in several forms, as lipid droplets or granules or as a high molecular weight lipid-protein aggregate in the cytosol of the liver cell. Futterman and Andrews[44] studied the composition of retinyl ester isolated from the livers of a number of vertebrate species (calf, sheep, rabbit, rat, human, frog, trout, and cat). With the exception of the cat, livers of all the species contained substantial amounts of Retinol, of which 95% was present as long-chain retinyl esters. Retinyl Palmitate was the predominant ester in all species, comprising 66% of the retinyl esters in the human. Retinyl stearate and oleate were the next most common esters. These hepatic stores normally represent over 90% of the total body reserves of vitamin A.[32,42]

The hepatic parenchymal cell is the major cell type responsible for the uptake and initial metabolism of newly absorbed Retinol. Recent evidence suggests that Retinol is then transferred from parenchymal cells to nonparenchymal fat-storing cells for storage (as retinyl ester in lipid droplets).[45-47] The hepatic parenchymal cells also plays an essential role in the mobilization of Retinol in that it synthesizes and secretes the specific plasma transport protein retinol-binding protein (RBP).[31,32,42]

The mammalian liver is capable of storing varying quantities of dietary vitamin A. However, on an individual basis, plasma vitamin A concentrations remain remarkably constant over a wide range of dietary intakes and liver stores.[31] The hepatic fat-storing cells appear to act as a reservoir for excessive vitamin A intake and are capable of storing Retinol up to a point, beyond which hypervitaminosis occurs.[48]

McKenna and Bieri[49] have used the total parenteral nutrition (TPN) rat and its sham-operated control as a model to compare the storage and fate of vitamin A (and E) when administered intravenously or orally. Plasma concentrations of Retinol were the same for the TPN rats (infused through the jugular vein) as for the controls (orally administered). However, hepatic storage was much higher in the infused rats. This could be due to the different physical state in which the vitamin was delivered to the liver: in chylomicrons when given orally and in micellar form when given IV.

Mobilization, Transport, and Delivery of Retinol

From the hepatic stores of retinyl esters, vitamin A is mobilized and transported in plasma to peripheral target tissues as Retinol bound to retinol-binding protein (RBP). In humans, RBP is a single polypeptide chain with a molecular weight of approximately 21,000 and a single binding site for one molecule of Retinol. In plasma, RBP normally circulates complexed with Retinol.[31,50]

RBP also interacts with another protein, plasma transthyretin (TTR) (more commonly known as plasma prealbumin) and usually circulates as a 1:1 molar RBP–TTR complex. The formation of this RBP–TTR complex reduces the glomerular filtration and renal catabolism of RBP.[31,50] The reader is referred to Goodman[50] and Rask et al.[51] for an extensive review of the chemistry and biology of RBP.

The mobilization and delivery of Retinol are highly regulated and are particularly controlled by the processes that regulate the hepatic rates of RBP synthesis and secretion (one of which is the nutritional Retinol status). There is also evidence that the delivery of Retinol to peripheral target tissues involves specific cell-surface receptors that recognize RBP.[32,50]

A specific intracellular binding protein for Retinol has been identified and designated as cellular retinol-binding protein (CRBP). CRBP is a single polypeptide chain with a molecular weight of approximately 14,600 and differs from plasma RBP in a number of ways. CRBP may (1) play a direct role in the biological expression of vitamin A activity, (2) facilitate the specific interaction of Retinol with binding sites for Retinol in the cell nucleus, or (3) serve as an intracellular transport protein, transporting Retinol from one locus to another between metabolic events in the liver.[32,52] Information available on CRBP has been summarized in several reviews.[53–56]

Oxidative Metabolism

Retinoic acid is a natural physiological metabolite of Retinol, although it is not known if the acid is at the same time an obligatory metabolite. Most structural studies on retinoid metabolism have been conducted with retinoic acid; therefore, since it is probable that at least some Retinol is metabolized through retinoic acid, many of the metabolites identified for retinoic acid would also be metabolites of Retinol.[41]

The overall metabolism of Retinol has been examined in a number of studies, with emphasis placed on the urinary and fecal or biliary excretion of radioactive metabolites.[41] The results of administration to rats of either [^{14}C] Retinol or [^{3}H] retinyl acetate indicated that 4–15% of the dose was excreted in the urine during the first 24–48 h. The amount of dose excreted in the urine depended on the position of the radioactive atom in the starting retinoid. These data suggest that decarboxylation of retinoic acid, a metabolite of Retinol, is occurring. Some of the urinary Retinol metabolites have been partially characterized by Wolf et al.,[57] although none has been ascribed a chemical structure. These metabolites are mainly water soluble and contain

no detectable free Retinol or retinyl esters. Clark et al.[58] have reported that a protein-bound form of Retinol, or of a metabolite of Retinol that is different from retinoic acid, was excreted in the urine of rats administered a large (23.1 mg) dose of Retinol. A similar substance has been detected in human urine.

The biliary and fecal metabolites of Retinol have not been extensively characterized. With the exception of Retinol, retinoic acid, and their conjugates, the structures of these compounds have not been clarified. The amount of the administered dose recovered varies depending on the position of the radioactivity, the mode of administration, and the quantity administered. Several investigators have reported that 18–25% of a 10–26 μg dose of Retinol can be recovered in the bile of rats 24 h after administration. A small portion of the metabolites has been identified as free or conjugated retinoic acid, whereas almost no free Retinol has been detected. An O-ether derivative of Retinol, retinyl β-glucosiduronate, has been identified in the bile of rats given a 3 mg dose of Retinol. The fecal metabolites are believed to originate from the bile. Recovery values range from 0.3% of a 10 μg dose in 24 h to 26% of a 2 μg dose in 5 days. An initial delay in the fecal excretion of metabolites after injection of Retinol has been noted.[41]

Interactions

Micronutrients

Retinol has long been known to interact with other micronutrients, including vitamin E, ascorbic acid (vitamin C), iron, and zinc. Vitamin E is generally believed to have a nonspecific antioxidant role. Studies have confirmed that the tocopherols (with vitamin E activity) provide protection from oxidation to Retinol. This results in increased hepatic content of Retinol.[33]

The synthesis of ascorbic acid in the liver of rats was impaired during both a deficiency and an excess of Retinol. Feeding ascorbic acid to the Retinol-deficient rats prevented the decrease in hepatic ascorbic acid. However, this had no effect on the rats with an excess of Retinol. Retinol may influence the hepatic synthesis of ascorbic acid, whereas the latter acts as an antioxidant for excess hepatic Retinol.[33]

Iron acts as a prooxidant and may facilitate oxidative destruction of vitamin A-active compounds in the intestine. Other studies in rats have demonstrated that the intestinal absorption of iron is not altered during vitamin A deficiency and that the vitamin appears to facilitate the mobilization of stored iron and its incorporation into erythrocytes.[33]

Zinc is essential for many enzyme systems in the body, some of which are directly or indirectly critical to the metabolism of Retinol. Liver alcohol dehydrogenase catalyzes the reactions for several primary alcohols, including Retinol, and a deficiency in zinc reduces the activity of this enzyme. Some zinc-dependent enzymes are involved in protein synthesis and metabolism and may include the synthesis of RBP and CRBP. In several studies, zinc deficiency has ben accompanied by decreased plasma concentrations of Retinol. The hepatic mobilization of Retinol may be impaired by zinc deficiency.[33] Solomons and Russell[59] have reviewed the impact on human nutrition of vitamin A and zinc interactions.

Hormones—Gonadal Steroid and Adrenocortical

Estradiol exerts a stimulatory effect on the hepatic synthesis of RBP and subsequently increases the mobilization of Retinol as the Retinol–RBP complex. This is supported by results of studies with synthetic estrogen-containing anovulatory hormones: plasma concentrations of Retinol and RBP increased in both animals and humans due not to a variation in dietary vitamin A but to increased mobilization of hepatic reserves.[33]

Adrenocortical hormones have also increased the hepatic mobilization of Retinol in animals, thus increasing plasma Retinol levels.[33]

Drugs, Xenobiotics, and Alcohol

Results of animal studies have indicated that DDT and other chemicals are capable of reducing hepatic stores of Retinol.[33] A decrease in the hepatic content of vitamin A has also been noted after the administration of certain xenobiotics, including polychlorinated biphenyls (PCBs),[60,61] polybrominated biphenyls (PBBs),[62] and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[63] Cullum and Zile[64] studied the effects of a single oral dose of 3,3′,4,4′,5,5′-hexabromobiphenyl (HBB), the most potent of the PBB congeners, on the steady-state metabolism of vitamin A in rats. HBB produced an abnormal twofold increase in the metabolic output of degraded vitamin A in the urine and feces for at least 8 days. The investigators suggested that these effects most likely reflect a stimulation of renal vitamin A metabolism and a deregulation of vitamin A metabolism in the liver, possibly leading to an increased dietary vitamin A requirement.

Lower concentrations of vitamin A have been found in the livers of human drug addicts than in the livers of those dying from multiple injuries and asphyxiation.[33]

The effects of acute and chronic ingestion of alcohol on the metabolism of Retinol are complex and not totally defined. Under conditions of acute hepatic toxicity (due to acute ingestion of alcohol), it was reported that plasma levels of Retinol, RBP, and TTR were all reduced, although hepatic stores may not have been affected. Chronic alcohol ingestion associated with all stages of alcoholic liver disease results in lowered hepatic storage and lowered plasma levels of Retinol. Studies conducted with rats have also suggested that ethanol potentiates the hepatotoxicity due to vitamin A supplementation.[33,65,66]

Cellular and Biochemical Effects

The effects of retinoids on cell differentiation and proliferation have been studied extensively in the past 10 years. Much of this study has been conducted in search of therapeutic roles for retinoids in the treatment of cancer. These studies show that the cellular effects of retinoids in the induction and control of various biochemical processes are extremely variable. High and low concentration of retinoids have produced opposite effects even within the same experimental system. Furthermore, results obtained a short time after the administration of the retinoid may be entirely opposite those obtained at a later time.[67]

Retinoids have been shown to modify the differentiation of nonneoplastic cells in culture, both epithelial cells (keratinocytes) and mesenchymal cells. Similar effects have been observed both in vivo and in organ culture in vitro. Only growth is affected in certain other cells.[67]

Retinoids have been shown to have marked effects on the growth and differentiation (in culture) of many neoplastic cell types, including epithelial and mesenchymal cells as well as cells derived from neural and primitive ectoderm. Retinoids have inhibited growth in monolayer and anchorage-independent growth in semisolid medium. They have promoted the terminal differentiation of certain fully neoplastic embryonal carcinoma and promyelocytic leukemia cells to nonneoplastic differentiated cell types. Although many of the effects of retinoids on the growth of neoplastic cells are reversible, the transformation of phenotype from neoplastic to nonneoplastic is fairly stable. These effects on differentiation may be able to induce remission of disease, whereas the inhibitory effects of retinoids on the growth of neoplastic cells could be valuable when used in conjunction with other therapeutic treatments. Retinoic acid and its analogs, in most cases, were considerably more active than Retinol, retinyl esters, or retinaldehyde in modulating the behavior of cultured cells.[67]

The effects of retinoids on the activity and synthesis of cellular enzymes and effectors have also been studied. Various cell responses were seen with respect to orinthine decarboxylase, transglutaminase, cyclic AMP, cyclic AMP-dependent protein kinases, plasminogen activator, collagenase, and prostaglandins. Due to the variety of responses, these particular enzymes and effectors are not believed to play a major role in the mechanism of action of the retinoids.[67]

Retinoids profoundly influence the biosynthesis of all types of glycoconjugates, including glycoproteins, glycolipids, and proteoglycans. Evidence has been provided for a scheme involving direct participation of retinoid phosphate derivatives in glycosyl transfer reactions. Others have suggested that retinoids might affect the biosynthesis of the lipid glycosyl carrier dolichol or the activity of specific glycosyltransferases.[67]

There exist two dominant theories of retinoid mechanism: the proposed cofactor role of retinoids in glycosyl transfer and the proposed steroid model for retinoid control of gene expression. In the first theory, retinoids are believed to participate directly in the transfer of mannose to glycoconjugates, which control cell growth, differentiation, and transformation. However, no evidence has demonstrated conclusively a function for retinoids separate from that of dolichol. The second hypothesis suggests the retinoids act in a way similar to the accepted model for steroid hormone action. Thus, retinoids would bind to a specific intracellular receptor (CRBP for Retinol and CRABP for retinoic acid), be translocated to the nucleus, bind to chromatin, and lead to altered genomic expression. Data exist to support this hypothesis and also suggest that there are differences in the modes of action of Retinol and retinoic acid.[67,31]

For a complete review of the cellular biology and biochemistry of the retinoids, the reader is referred to Roberts and Sporn,[67] Sporn and Roberts,[68] Lotan,[69] Schroder et al.,[70] Wolf,[71,72] and Zile and Cullum.[73]

Physiological Effects

Morley et al.,[74] using both in vivo and in vitro techniques, studied the effects of the administration of pharmacological doses of vitamin A (as Retinyl Palmitate) on multiple parameters of thyroid function. Three groups of 8 rats each were administered vitamin A orally for 2 weeks at doses of 30 mg (100,000 IU) three times weekly, 45 mg (150,000 IU) three times weekly, or 45 mg (150,000 IU) five times weekly. Vitamin A decreased total T_4 (thyroxine) and T_3 (triiodothyronine) levels. The serum-free T_3 and T_4 levels as measured by dialysis were normal in these vitamin A-treated rats. Treatment with vitamin A in vitro (10^{-4}–$10^{-6}M$) resulted in a marked increase in the percentage dialyzable T_3 and T_4. Total T_4 levels were still decreased in thyroidectomized rats (administered 15 mg vitamin A daily for 7 days) maintained on T_4, suggesting that vitamin A produced its effects by increasing peripheral clearance of T_4. Vitamin A did not alter basal thyroid-stimulating hormone (TSH) release or its release in response to thyroid-releasing hormone, indicative of a normal hypothalamic–pituitary–thyroid axis in vitamin A-treated (45 mg three times weekly for 2 weeks) rats. Decreased Na-K-ATPase activity in the livers from vitamin A-treated (15 mg daily for 7 days) rats and decreased growth hormone (GH) response to T_3 in GH_3 pituitary cultures indicated that tissue responsiveness to thyroid hormones may be decreased by vitamin A. Vitamin A significantly decreased thyroid weight and increased ^{125}I thyroidal uptake in vivo. In vitro, vitamin A (10^{-4}–$10^{-6}M$) enhanced T_4 to T_3 conversion in hepatic homogenates. The investigators stated that the results of this study, as well as previous studies, suggest that there is an inverse relationship between plasma thyroxine and vitamin A levels. A different relationship may exist in humans, where the major binding protein is thyroxine-binding globulin compared to rat thyroid hormone-binding prealbumin (TBPA).

Chertow et al.[75] evaluated the effects of Retinyl Palmitate on IV glucose disposition and insulin secretion in man. Intravenous glucose tolerance tests (IVGTT) with 26 g glucose were performed on 10 healthy subjects before and after two intramuscular injections of Retinyl Palmitate (25,000 IU) 18 h apart. Glucose disposition was impaired after treatment with Retinyl Palmitate in 9 of the 10 subjects. In vitro, 10^{-4}–$10^{-6}M$ Retinyl Palmitate did not affect the binding or displacement of insulin ^{125}I from IM-9 cultured human lymphocytes. Other studies have also indicated that Retinyl Palmitate decreases glucose tolerance.[76,77] The effect of vitamin A appears to be mediated through stimulation of adrenal cortisol secretion.[78–82] However, in this study Retinyl Palmitate did not affect serum cortisols. Another proposal is that Retinyl Palmitate has a direct effect on hepatic glucose metabolism, therefore limiting hepatic glucose uptake and the disposition of an IV glucose load.[77,79,80,83]

Ultraviolet Light Effects on Cutaneous Retinol

Barne et al.[84] investigated the effect of UV irradiation on the concentration of the cutaneous retinoids Retinol and 3-dehydroretinol in rabbit skin in vivo and human skin in vitro. The irraditation source consisted of four Philips

SP500 W water-cooled high-pressure mercury lamps with water-cooled filters. Irradiations were performed by applying the light aperture (2.2 cm in diameter) directly to the skin for periods varying between 30 sec and 15 min depending on the wavelength and irradiation dose studied. The ears of the rabbits were irradiated with either 313, 334, 365, or 405 nm at a dose of 3 J/cm^2 (five rabbits), 334 nm at a single dose of 0.5, 1.0, or 3.0 J/cm^2 or 6 repeated doses of 0.5 J/cm^2 (4 rabbits), or 334 nm at a single dose of 3 J/cm^2, with skin samples removed at 1, 24, 72, and 168 h. Fresh human skin was irradiated in vitro with 334 nm at a dose of 3 or 10 J/cm^2, and frozen skin (on dry ice) was irradiated with 313, 334, 365, or 405 nm at a dose of 3 J/cm^2. The irradiated skin was cut out and separated into epidermis and dermis using 1% acetic acid at 4°C for rabbit skin and heat treatment for human skin. Analysis was performed by HPLC.

Dose-dependent reductions of Retinol where noted in the epidermis and dermis of humans and rabbits, with the maximal effect obtained at 334 nm (a wavelength that approximates the absorption maximum of Retinol in organic solutions). Results of pilot studies indicated that irradiation of frozen human skin at 334 nm reduced the concentration of epidermal Retinol to 88, 74, and 51% of the control value at 1, 5, and 10 J/cm^2, respectively. A similar reduction was noted in irradiated fresh skin, suggesting independence of cellular metabolism. 3-Dehydroretinol was not significantly reduced. The photodecomposition of Retinol in human skin was most extensive in the epidermis and progressively less so in the dermis, presumably reflecting the extent to which 334 nm radiation penetrates the skin. Significant reductions in the concentration of Retinol were noted down to the middermis of irradiated human skin, indicating that the effect of 334 nm extends to a depth of about 1 mm. In rabbit ear skin, similar reductions of Retinol were observed in the epidermis and dermis. The investigators suggested that the thin and nonpigmented rabbit epidermis may allow more radiation to penetrate into the underlying dermis.[84] These investigators had previously found that the concentration of epidermal Retinol was significantly reduced in humans treated for uremic pruritis with a series of 12 UV irradiations (UVA and UVB) over a period of 2–3 months.[85] The nutritional and biological significance of these findings has not been established. Berne et al.[84] are interested in the possibility that radiation-induced depletion of cutaneous vitamin A and formation of photosensitizing retinoid intermediates may promote UV carcinogenesis.

ANIMAL TOXICOLOGY

Single high doses and multiple low doses of Retinol are toxic to laboratory animals. Single large doses can be lethal, whereas chronic intoxication has adverse effects on many tissue and organ systems.[86,87] Characteristic effects of chronic hypervitaminosis A in animals include weight loss, erythema, hair loss, internal hemorrhage, and fractures. Many of these effects are reversible upon cessation of administration.[88]

Theory of Retinol Toxicity

Excess Retinol both in vivo and in vitro results in increased lability of biological membranes. This is attributed to its surface-active membranolytic properties. Excess Retinol increases the synthesis and release of lysosomal enzymes, which play a major role in the effects of vitamin A on cartilage and limb–bone rudiments. Retinol bound to RBP as the Retinol–RBP complex does not appear to exert membranolytic effects. Therefore, the mode of transport of Retinol is believed to be the critical factor in the development of the symptoms of hypervitaminosis A.[50]

This theory has been supported by studies in rats and humans. Rats given excessive doses of vitamin A had large increases in serum vitamin A, due to higher circulating concentrations of retinyl esters and decreased concentrations of serum RBP. Practically all of the serum vitamin A and retinyl esters were found in association with serum lipoproteins, thus suggesting that they play an important role in the transport of excess vitamin A. Similarly, 3 patients with chronic hypervitaminosis A had increased plasma concentrations of total vitamin A, particularly of retinyl esters; plasma RBP levels were normal. These data suggest that vitamin A toxicity occurs only when, due to excessive intake of vitamin A, Retinol begins to circulate in the plasma in a form other than bound to RBP.[50]

Acute Toxicity

Oral and Subcutaneous

The reactions of mice and rats to single large cutaneous or oral doses of Retinol have been described by Nieman and Obbink.[86] Death is preceded by convulsions and paralysis, and those animals that survived had signs of malaise, decreased motor activity, stupor, muscular weakness, and occasionally changes in the pelt. The animals that survived recovered with no apparent residual toxic effects.

Retinol had an oral LD_{50} of 2570 mg (8.6 million IU) per kg body weight in mice.[89] Retinyl Palmitate had oral LD_{50}s of 6060 mg (11 million IU) per kg in mice and 7910 mg (14.4 million IU) per kg in rats.[89]

Intraperitoneal

Intraperitoneal (IP) injection of 200–500 mg (667,000–1.6 million IU*) Retinol per kg body weight to 20 adult Sprague-Dawley rats produced 90% mortality in 3 days. Similarly, IP injection of 425 mg (1.4 million IU) Retinol per kg body weight to 11 weanling rats produced 64% mortality. Survival time was inversely related to plasma Retinol and retinyl ester concentrations. However,

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*Vitamin A or Retinol values are commonly expressed in international units (IU) or both as IU and as retinol equivalents (RE).[33]

1 IU = 0.3 μg preformed retinol.

1 RE = 1 μg retinol or 6 μg β-carotene.

no signs of toxicity were observed over a period of 2 months in 7 adult rats injected IP with 370 mg (673,000 IU) Retinyl Palmitate per kg body weight.[3]

Retinol had an IP LD_{50} of 1510 mg (5 million IU) per kg in mice.[89]

Dermal Irritation

Two lots of a moisturizer containing 0.1% Retinyl Palmitate were evaluated for dermal irritation in albino rabbits. A 0.5 g sample of each lot was applied to one side of the shaved back of each of 3 rabbits. A control lot was applied to the other side of the back. Applications were repeated daily for 4 days. Irritation indices for the experimental and control lots were 3.5 and 3.4, respectively, in one group, and 3.3 and 3.1, respectively, in the other group. These differences were considered slight and insignificant, although it was noted that the first experimental lot was slightly more irritating in 2 of the 3 treated rabbits.[90]

A body lotion containing 0.1% Retinyl Palmitate was evaluated for dermal irritation in 3 albino rabbits. A 0.5 ml sample of the body lotion was applied daily for 4 days to the shaved back of each rabbit. A well-defined erythema and edema developed within 48 h and persisted throughout the 7-day study, resulting in subsequent dehydration and desquamation. The body lotion had an irritation index of 3.1 (max = 8.0).[91]

Ocular Irritation

Two cosmetic products, a moisturizer and a body lotion, each containing 0.1% Retinyl Palmitate, were evaluated for ocular irritation in two groups of 6 albino rabbits (12 total). A 0.1 ml sample of each product was instilled into 1 eye of each rabbit. Slight conjunctival redness was noted in all treated eyes at 1 h; those eyes treated with the moisturizer cleared within 24–48 h, whereas those treated with the body lotion cleared within 24 h. Corneal and iridial membranes were unaffected.[90,91]

Short-Term and Subchronic Toxicity

General

The subchronic toxicity of Retinol and vitamin A preparations as reported in the literature up to the early 1950s has been extensively reveiwed by Nieman and Obbink.[86] The rat was the primary test animal, although a variety of animal species were used. Retinol was administered in doses ranging from 3000 to 180,000 RE (10,000–600,000 IU)/day (approximately 2–120 times the recommended dietary allowance) for periods of time from a few days up to several weeks. Hypervitaminosis A was produced by doses as low as 3000 RE (10,000 IU)/day. The time to first appearance of symptoms depended on the route of administration, the species and age of the animal, duration of treatment, the symptom in question, and the dose.[88]

Hypervitaminosis A is characterized by the following,[86] as summarized by Kamm et al.[88].

1. General signs of toxicity: Anorexia, weight loss, emaciation, anemia, cachexia, and death. Spontaneous fractures (particularly in young rats) that

heal and do not recur after treatment is stopped. Extensive subcutaneous and/or intramuscular hemorrhages and sometimes inflammation of nasal passages, gut, and conjunctivae.[86]

2. Dermal effects: Hair loss, localized hyperemia and/or erythema and thickened epithelium. Topical administration of Retinol is irritating and may produce peeling skin, reduced keratinization, and hyperplasia of the dermal papillae and blood vessels.[86]

3. Internal organ effects: Fatty change of the liver, fatty changes in the spleen, heart, and kidney, hemosiderosis of the spleen, and glomerulonephritis and necrotizing nephrosis. Testicular hypertrophy has been noted in adult rats and degenerative testicular changes in weanling rats after prolonged treatment.[86] In addition, degenerative myocardial fibers were noted in rats treated with 3000 or 6000 RE (10,000 or 20,000 IU)/kg/day for 3 months; these were associated with EKG changes.[88]

4. Hematological effects: Hypochromic anemia with hyperplastic bone marrow, decreased hemoglobin concentration, and transient increases in total circulating lipid and serum cholesterol.

5. Skeletal system effects: Bone fractures due to either increased activity of osteoclasts or decreased activity of osteoblasts while osteoclastic activity remains unchanged. Longitudinal bone growth exceeds circumferential bone growth, resulting in a thin, fragile cortex that fractures easily. Young rats are more susceptible than adults. These fractures were observed in mice and rats after 1 week of daily dosing with 10,000 times the amount of Retinol required to sustain growth and in rats after 1 week of daily dosing with 8500–13,600 RE (28,300–45,300 IU)/day (as retinyl acetate). This bone toxicity has also been demonstrated in dogs, cats, calves, and hogs.[88]

6. Other bone effects: Reduced formation of dentine, atrophy of lingual odontoblasts, and degeneration of pulp and odontoblasts accompanied by amorphous calcification of dentine.[88]

The more current literature on subchronic toxicity has been summarized by FASEB.[3] The lowest reported adverse effect level in experimental animals appears to be in the range of 25,000 to 60,000 IU Retinol per kg per day for periods of 3–5 weeks. All observed signs of hypervitaminosis A toxicity were similar to those recorded. Of note was a study in which high doses (500,000 IU/kg/day for 21 days) of Retinol administered as a water-dispersible commercial preparation were exceedingly more toxic to rats than equal doses of Retinol administered as natural esters.[92] It is now recognized that water-miscible vitamin A is more readily absorbed than oil-soluble vitamin A and therefore capable of eliciting toxic effects at lower doses.[3]

Dermal — Specific Studies

Bern et al.[93] studied the influence of Retinol on the epidermis of rats. They divided 61 male Long-Evans rats into 5 groups: (1) 13 untreated rats, (2) 11 rats receiving topical applications of 0.3 ml sesame oil, (3) 17 rats receiving topical applications of Retinol (1000 IU) in 0.3 ml sesame oil, (4) 8 rats receiving subcutaneous injections of 0.3 ml sesame oil, and (5) 12 rats receiving subcutaneous injections of Retinol (1000 IU) in 0.3 ml sesame oil.

Each of the 5 groups was additionally divided into 4 approximately equal sized groups receiving treatment for periods of 10, 20, 30, and 60 days. A 1-inch square felt pad was applied to the shaved dorsal area of each rat (including controls) and taped in place. Topical applications were injected into the pad, and subcutaneous injections were accomplished with a 2-inch 20-gauge hypodermic needle inserted into the skin outside of the pad and extending to a point underneath it. At the end of the treatment periods, the rats were killed, and the skin of the test area was removed and examined. Epidermal thickness also was measured. Topical applications of Retinol resulted in acanthotic responses after all 4 treatment periods. The thickness of the epidermis (including the stratum granulosum) was approximately twice the normal value after 10 and 20 days of treatment. Topical treatment for 30 days with sesame oil alone produced a mild acanthotic response; this response was noticably stronger after 60 days. The investigators, therefore, attributed part of the increased response to Retinol in sesame oil at 30 and 60 days to be a nonspecific response to the vehicle. The acanthotic responses produced by the topical applications of Retinol were maintained even after 60 days, with no signs of adaptation of the epidermis to Retinol. The subcutaneous administration of Retinol in sesame oil produced no significant reactions in the overlying epidermis. Large deposits of the oil solutions were evident in the subcutaneous tissues of these rats. Results of cytochemical tests performed on the skin sections from untreated and topically treated rats indicated no significant differences among these groups. In cytologic observations and electron microscope studies, the tonofibril system appeared unchanged, although a gradual decrease in the number of intercellular bridges to desmosomes was noted.

Bern et al.[93] also studied the effect of vitamin A on the epidermis of the nipple region of male guinea pigs. Twenty guinea pigs were divided into 5 treatment groups: (1) 2 untreated, (2) 3 treated with 0.2 ml sesame oil, (3) 4 treated with estradiol, (4) 5 treated with vitamin A (1500 IU Retinol or retinyl acetate) in 0.2 ml sesame oil, and (5) 6 treated with both estradiol and vitamin A (1500 IU). A bandage was applied to each nipple and taped in place. The test solutions were injected into the bandage once daily for 10 days. The animals were then killed, and the skin of the nipple was examined. Vitamin A induced acanthosis in the nipple epidermis but did not interfere with its response to estrogen.

Sabella et al.[94] conducted a study similar to that of Bern et al.[93] A total of 55 female rats of the Long-Evans S-1 strain were divided into 5 treatment groups: (1) 11 untreated rats, (2) 11 treated with sesame oil, (3) 11 treated with estradiol in sesame oil, (4) 13 treated with Retinol (5000 IU/ml) in sesame oil, and (5) 9 treated with both estradiol and Retinol in sesame oil. All of the rats had been ovariectomized to eliminate any possible effect of cyclic estrogen production. A 1-inch square felt pad was taped onto the shaved back of each rat. Twice daily for 10 days, 0.37 ml of each oil solution was injected into the felt pads. The total daily dose of vitamin A to each of the rats in groups 4 and 5 was 3700 IU. The rats were killed after 10 days, and the treated skin areas were examined. Both the epidermis and the stratum granulosum alone in the Retinol and Retinol–estradiol treated rats were significantly thicker (approxi-

mately twice as thick) than those of rats in the other groups. This was attributed to an increased number of cell layers and an apparent increase in cell size. The investigators also suggested that an increased rate of keratin formulation or an increased rate of keratohyalin formation may have accounted for the increase in extent of the stratum granulosum. These effects were entirely local in nature, and estradiol did not affect the epidermal response to Retinol.

Rodahl[87] applied 1 drop of saponified bear liver oil (approximately 20,000 IU vitamin A) to the shaved back of each of 5 adult mice daily for 14 days. Marked alopecia was observed on and around the test area (approximately 1 cm in diameter) at the end of the treatment period. The mice were observed for an additional 4 weeks. All of the mice had the typical signs and lesions of hypervitaminosis A 2 weeks after the termination of treatment: exophthalmus, alopecia, soreness and swelling of the palpebrae, loss of hair around the eyes, soreness around the mouth and nose, and clinical signs of fractures of the hind legs. One mouse died at about 3 weeks. One month after the termination of treatment, these symptoms were still marked. The mice were then killed and examined. One mouse had an abscess. Similar abscesses were seen in 3/5 mice. At the postmortem examination, lesions were considered as "similar findings in the organs as described for hypervitaminotic rats" (hyperemia, red blood cells in the space of Bowman's capsule, deposits of sudanophil droplets in the liver, and increased deposits of sudanophil droplets in the adrenal cortex). Since these 5 mice had been kept in the same cage during the study, the investigators believed that the mice may have licked the oil off each other, and therefore they repeated the study with 5 mice individually caged. All procedures were the same except that unsaponified bear liver oil was used. These mice had the same signs and histological changes as the first group. At 18 days, 4/5 had died (1 of peritonitis, others unspecified). It was concluded that topical application of bear liver oil produced lesions of hypervitaminosis A in mice, even when no oil was ingested.

Weslaw et al.[95] have reported that the lower limit of toxicity of vitamin A in the rat is 16,000 IU when rubbed daily into the skin of the back, though admitting that losses occur naturally in these circumstances.

Escarras and Paillas[96] observed signs of local hypervitaminosis A in the guinea pig after applying 2 drops of an oily 3.5% solution of vitamin A every other day to the surface of skin wounds. The wounds treated in this manner healed much more quickly than untreated wounds, while showing extensive conjunctival hyperplasia and vascular hyperplasia with a very high degree of capillary and neovessel proliferation.

A body lotion containing 0.1% Retinyl Palmitate was evaluated for dermal toxicity in New Zealand albino rabbits. The 2 test groups, experimental and control, consisted of 5 males and 5 females each. All of the animals were shaved twice weekly. The body lotion was applied daily for 90 days at a dose of 6 mg/cm^2 to the flank skin of each test rabbit. The lotion was applied to over 10% of each animal's total body surface, and the dose was calculated to be three times the anticipated human use of 2 mg/cm^2. No systemic toxicity was observed in any of the parameters studied: individual weight gain, group mean food consumption data, hematology values, clinical chemistry, uri-

nalyses, organ weight/body weight ratios, and microscopic evaluation. One test rabbit and 2 controls died during the study due to causes not considered treatment-related. All of the treated animals developed slight to moderate erythema and edema during the first week of the study. These symptoms persisted throughout the study and were accompanied by slight to moderate desquamation in all animals. These dermal lesions were characterized histologically by mild dermatitis.[97]

Chronic Toxicity

The relatively few reported long-term toxicity studies with retinoids have been conducted by the laboratories of Hoffmann-La Roche and are unpublished except in the form of brief descriptive reports.[88] Randall[98] has described one study on Retinyl Palmitate.

Retinyl Palmitate was administered orally to dogs and rats for 10 months. The high dose of Retinyl Palmitate in the dogs was selected on the basis that it was tolerated by humans and was approximately 250 times higher than the human recommended allowance for Retinyl Palmitate (approximately 100 IU or 0.06 mg/kg/day Retinyl Palmitate). Groups of 3 dogs were given capsules orally containing 0.6, 2.8, or 13.8 mg/kg/day Retinyl Palmitate 5 days a week (doses of ~ 1000 to 25,000 IU kg/day Retinyl Palmitate). Groups of 10 rats were orally intubated with 5.5, 13.8, or 27.5 mg/kg/day Retinyl Palmitate 5 days a week (doses of 10,000 to 50,000 IU/kg/day). Six dogs and 30 male rats were maintained as controls. The animals were observed for general behavior and clinical signs of toxicity; body weights were recorded weekly. Hematological values were measured after 8, 18, 28, and 38 weeks in the dogs and 18 and 35 weeks in the rats. Necropsy was performed only on the rats that died or were killed for humane reasons. No adverse effects were observed in either the dogs or rats; body growth and hematological values were within normal limits in both species.[88,98]

Teratogenicity and Reproductive Effects

Effects on Fertility and Reproduction

Retinol is essential for reproduction. Even so, there are reports suggesting that high intake of Retinol produces adverse effects on several reproductive functions. Decreased sperm motility and decreased sperm survival were noted in male rabbits receiving 60,000–90,000 IU/kg vitamin A intramuscularly.[3,88] Testicular changes were also noted in rats, although these were reversible upon cessation of treatment.[88] High but nontoxic oral doses of Retinyl Palmitate (5000 IU) administered 3 times per week for 9 months to female rats resulted in an inhibition of cyclic ovulatory activity.[3,88]

Teratogenicity

Retinol administered in high oral doses was first demonstrated to be teratogenic in rats in 1953. Retinol produced more than 70 types of malformations in rats, mice, hamsters, guinea pigs, rabbits, dogs, pigs, and monkeys. The type and incidence of malformations depended on the dose (some 100 times the daily requirement) and stage of pregnancy and, to a lesser extent, on

species and strain.[3] Abnormalities of the face, ears, eyes, and nervous system were most commonly observed. Teratological effects have been produced in rat fetuses at doses of Retinol that do not cause overt toxicity in the dam.[88]

FASEB[3] has summarized the available information (as of 1980) on the teratogenicity of vitamin A. Although it has been reported that the stage of pregnancy when the vitamin is administered critically affects the appearance of teratogenicity, there are relatively few studies in which moderate maternal doses of vitamin A have been administered during the most susceptible periods. These indicate that the no-effect level in mice could be as low as 2500 IU vitamin A/kg body weight, 75,000 IU/kg in hamsters, and 135,000 IU/kg in rats. Thus, sensitivity varies with species.

More recently, Kamm[89] briefly described unpublished studies (conducted at Hoffman-La Roche, Inc.) on the teratogenicity of Retinyl Palmitate. Retinyl Palmitate was administered orally to rats on gestational days 6 through 15 at doses of 0, 10, 30, and 90 mg/kg/day (doses of ~ 18,180 to 163,640 IU/kg/day). Mice were similarly administered doses of 0, 5, 15, and 50 mg/kg/day (doses of ~ 9090–90,900 IU/kg/day). Rabbits received oral administrations of 0, 0.5, 2, and 5 mg/kg/day Retinyl Palmitate (doses of ~ 900–9090 IU/kg/day) on gestational days 6 through 18. The highest no-effect levels were 30, 15, and 2 mg/kg/day Retinyl Palmitate in the rat, mouse, and rabbit, respectively. (This corresponds to doses of ~ 54,550, 27,270, and 3,640 IU/kg/day, respectively.) Lower doses, although not teratogenic, were sometimes fetotoxic.

Hayes et al.[99] have studied the teratogenic effects of Retinyl Palmitate in Fischer 344 rats. Groups of 39 or 43 rats were administered 3.2, 32, and 128 mg/kg/day Retinyl Palmitate (in corn oil) by gavage on days 6 through 15 of gestation. (This corresponds to doses of 5820, 58,180, and 232,730 IU/kg/day, respectively.) A control group of 44 rats received an equal volume of corn oil. The high-dose level was maternally toxic, as evidenced by decreased body weight gains and reduced food and water consumption. This dose level was also embryolethal and teratogenic. The incidence of fetal resorptions was significantly increased compared to controls, and 52/60 surviving fetuses had one or more major malformations. Eighty percent of these fetuses (100% of the litters) had malformations of the craniofacial area. The lower doses tested were neither embryolethal or teratogenic, although the middle dose (32 mg/kg/day) produced slight maternal toxicity.

Willhite and Balogh-Nair[100] studied the teratogenicity of all-trans-Retinol and all-trans-retinylidene methyl nitrone (RMN) in Swiss-Webster mice. The retinoids were dissolved in acetone and solubilized in polyoxyethylenesorbitan monolaurate (final acetone concentration of 5%). Single oral doses of 75 mg/kg of Retinol or RMN were administered to groups of approximately 6–10 pregnant mice by gavage on either day 7, 8, 9, 10, or 11 of gestation. The doses were administered at a rate of 1.0 ml/100 g body weight. Similar groups of mice were given oral doses of the vehicle as controls. All of the mice were killed (in excess CO_2) on day 18 of pregnancy, and the fetuses were removed and examined. Maternal weight changes were calculated, and the number of resorptions were counted. All results were analyzed statistically. No signs of retinoid intoxication were noted in any of the dams. Maternal weight gains and average litter fetal body weights of both treated and control groups were

comparable. Retinol significantly increased the number of affected litters (litter with one or more malformed fetuses) on days 7, 8, 9, and 11 of gestation. The total percentage and mean litter frequency of malformed fetuses were greater after treatment with Retinol than with RMN. Treatment on day 8 produced the highest incidence of embryonic death. Both Retinol and RMN induced malformations of the palate, head, eyes, ears, jaw, and ribs. Retinol treatment on day 9 produced malformations of the tail and head. Retinol treatment on day 10 did not significantly increase the number of affected litters. Retinol treatment on day 11 produced forelimb reduction deformities, polydactyly, and oligodactyly. The mean numbers of bipartite/asymmetrical or unossified/reduced sternabrae were normal except after Retinol treatment on day 9, where the numbers of unossified sternabrae were significantly increased compared to controls. No significant differences were seen in the numbers of ossification centers in metacarpals and metatarsals or in the numbers of cervical and sacrocaudal vertebrae. The investigators suggested that the similar teratogenic activity of these two retinoids may be related to their in vivo biotransformation to all-trans-retinoic acid and its subsequent interaction with embryonic cellular retinoic acid-binding protein (CRABP).

Vitamin A has been used as the positive control in two studies on the teratogenicity of *p*-aminophenol and oxidative hair dyes, respectively. In the first study, vitamin A suspended in rape oil was administered orally at a dose of 15 mg/kg/day to 23 pregnant Sprague-Dawley albino rats on days 6–15 of gestation. The rats were killed and examined at day 19. Vitamin A produced a marked teratogenic effect. The majority of the malformed fetuses had an exencephaly.[101] In the second study, vitamin A was administered in a single oral dose of 100,000 IU/rat on day 9 of gestation. The rats were killed and examined on day 20. Vitamin A produced a significant increase in the number of abnormal fetuses. Frequency of anomalies ranged from 28 to 95%, with major anomalies including hydrocephaly, exencephaly, prognathia macroglossia, open eye, microphthalmia, cleft palate, hydronephrosis, and agenesis of skull bones.[102]

Ismadi and Olson[103] and Donoghue et al.[104] have studied the fetal–maternal transport of Retinol in rats and sheep, respectively. They found that the fetal–maternal transfer of vitamin A was appreciable and characterized the interaction as a dynamic steady-state relationship. Donoghue et al.[104] also sugested that the carrier system of Retinol in the blood may not be the same in the fetal lamb as in the adults in that fetal plasma Retinol was complexed with RBP and another protein other than prealbumin (TTR).

The reader is referred to Gellen[105] and FASEB[3] for more in-depth reviews of the teratogenic effects of hypervitaminosis A and possible mechanisms of action.

Effects on Perinatal and Postnatal Development

Minor brain defects, growth disturbances, and behavioral abnormalities have been observed in postnatal life after in utero exposure to Retinol even when no growth defects were noted at birth.[88]

Joshi et al.[106] studied the effects of postnatal oral administration of 1000 IU vitamin A (in groundnut oil) on the 4th, 6th, 8th, and 10th days of age on

brain maturation in rat pups. Administration of vitamin A reduced brain weight, free cholesterol, phosphatidal ethanolamine, and the synthesis of myelin sulfatides from $Na_2^{35}SO_4$.

Appreciable amounts of vitamin A are transferred to suckling offspring through the maternal milk. This has been observed in rats, cows, and monkeys. The maternal hepatic reserves of vitamin A are usually the major contributor to the milk supply. Evidence has been obtained that Retinol is transferred from the blood to the milk in preference to retinyl esters. Most of the Retinol is then reesterified in the mammary gland and occurs as retinyl esters in the milk. Concentrations of vitamin A are substantially higher in colostrum and early milk than in mature milk in all species studied.[107]

MUTAGENICITY AND ANTIMUTAGENIC EFFECTS

Vitamin A (as retinaldehyde) was evaluated for mutagenicity in the Ames test using *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. Evaluation was made both with and without metabolic activation and at a maximum vitamin A concentration of 2.0 mg/plate. Vitamin A was nonmutagenic in all strains.[89]

Retinol, at concentrations up to 16 μg/ml, did not increase the frequency of sister chromatid exchanges (SCE) or cell cycle delay in Chinese hamster V79 cells either with or without the metabolic activation of S-9 mix. However, Retinol did inhibit SCE frequencies and cell cycle delay in V79 cells induced by the indirect mutagens cyclophosphamide and aflatoxin B_1. The inhibition was dose and time dependent. Retinol may have no direct effect on the genetic materials but instead may inhibit the metabolic activation of an indirect mutagen or carcinogen.[108]

Retinyl Palmitate has produced strong inhibition of the mutagenic effects of 3-methylcholanthrene and benzo[a]pyrene in human epithelial-like cells. In the same cell line, Retinyl Palmitate also reduced 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene binding to DNA.[109]

Retinol has inhibited the mutagenicity induced by aflatoxin B_1 (0–16 μg Retinol/plate),[110] *ortho*-aminoazotoluene (0–150 μg Retinol/plate),[111] and 2-fluorenamine (0–100 μg Retinol/plate)[112] in the Ames *Salmonella*/mammalian microsome test. Retinyl Palmitate also had an inhibitory effect on the mutagenicity of *ortho*-aminoazotoluene, although its inhibition was approximately 50% that of Retinol.[111] However, in a further study using the Ames test, small amounts of Retinol (2–20 μg/plate) increased the mutagenicity of 2-aminofluorene and 2-acetylaminofluorene, although at higher doses (50–150 μg/plate) the mutagenicity of 2-acetyl-aminofluorene remained unchanged while that of 2-aminofluorene decreased gradually.[113]

CARCINOGENICITY

No long-term carcinogenicity studies in laboratory animals are available on Retinol and Retinyl Palmitate. In the opinion of the Select Committee of GRAS Substances, there is no evidence that vitamin A is carcinogenic.[3]

INHIBITION AND ENHANCEMENT OF CHEMICALLY INDUCED CARCINOGENESIS AND PHOTOCARCINOGENESIS

Vitamin A both enhanced and inhibited responses to viral or chemical carcinogens. Much controversy was engendered by studies in the late 1960s showing that 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced carcinogenesis in the hamster cheek pouch was enhanced by application of a 10% Retinyl Palmitate lotion before, together with, or following application of DMBA.[88] The data actually indicated little effect; the incidence and numbers of carcinomas were the same for the Retinyl Palmitate and non-Retinyl Palmitate treated groups, whereas the only indication of potentiation of DMBA-induced carcinogenesis was the slightly larger tumor size in Retinyl Palmitate-treated hamsters. This potentiation has been attributed to a toxic, irritating effect of Retinyl Palmitate as demonstrated by marked histological changes in the cheek pouches of hamsters repeatedly administered 10% Retinyl Palmitate alone. Subsequent studies have confirmed the toxic effect of large doses of Retinyl Palmitate on the hamster cheek pouch epithelium and strongly suggest that nontoxic topical application of Retinyl Palmitate after the appearance of the first neoplasm (DMBA-induced) results in the regression of these tumors.[114]

Results of many studied have indicated that the retinoids can suppress the process of carcionogenesis in laboratory animals in vivo and the development of malignant phenotypes in vitro. Recently, it has been reported that retinoids have surpressed proliferation and led to terminal differentiation of certain fully neoplastic cells, resulting in a more benign, nonneoplastic phenotype. Most of these studies have been conducted in search of therapeutic roles for retinoids in the treatment of cancer[68] (see also section "Cellular and Biochemical Effects").

The reader is referred to Moon and Itri,[114] Roberts and Sporn,[67] Hill and Grubbs[115] for in-depth reviews of the chemopreventative and anticarcinogenic effects of the retinoids.

Retinol (23 mg/kg/day, or 76,670 IU/kg/day) also was reported to have had no effect on the incidence of squamous cell carcinoma in hairless mice exposed to UV light generated by a solar simulator.[88]

IMMUNOLOGICAL EFFECTS

Retinoids may inhibit or stimulate the immune system. The inhibitory or stimulatory effects of the retinoids on various immune responses are reflected in the histological changes in lymphoid organs. High doses of retinoids may effectively inhibit both humoral (antibody-mediated) and cell-mediated immunity, whereas subtoxic doses have stimulated them. Timing, dose, and mode of administration play a major role in determining the effects of the retinoids in the immune system.[116]

Retinol and Retinyl Palmitate stimulated the humoral immune response to soluble or particulate antigens in experimental animals.[116] Cell-mediated immunity studies have shown that Retinyl Palmitate (optimal dose of 150 μg)

stimulated the delayed-type hypersensitivity reaction (DTH) in mice when injected together with the sensitizing antigen (sheep erythrocytes).[117] Skin grafts from male mice transplanted onto syngeneic females were rejected significantly more quickly when Retinyl Palmitate was administered to the transplanted mice. Moderate subtoxic doses of retinoids have significantly increased thymus weight, stimulated thymic involution, and increased the cellularity of lymph nodes in mice.[116]

No clear hypothesis of how retinoids may regulate the immune system has been formulated. However, retinoids are believed to act in the induction phase of immunity. Retinoids stimulate the induction of T-killer activity in vivo and in vitro to both allogeneic and syngeneic (tumor) cells.[116] Walsh et al.[118] studied the influence of Retinol on human gingival cultures and suggested that low doses of Retinol (5 μg/ml) may alter immune reactions within epithelia via stimulation of both keratinocytes and Langerhans cells. The results of recent studies using tumor models have increasingly indicated that the anticarcinogenic effects of the retinoids are due to immunostimulation.[116,119]

The reader is referred to Dennert[116] for an in-depth review of the immunological effects of the retinoids.

CLINICAL ASSESSMENT OF SAFETY

Recommended Dietary Allowance and Daily Intake

The recommended daily dietary allowance (RDA) of vitamin A varies between scientific groups. In 1974, the National Research Council[120] recommended RDAs of 5000 IU for male and 4000 IU for female adults, 1400–2000 IU for infants, 2000–3300 IU for children up to age 11, 5000 IU for pregnant women, and 6000 IU for lactating women. The NRC reaffirmed these values in 1980.[121] On the other hand, the Food and Agriculture/World Health Organization[122] has recommended lower daily intakes for all groups: 750 μg Retinol (2500 IU) for adults, 300 μg Retinol (1000 IU) for infants, 250–400 μg Retinol (833–1333 IU) for children up to 10, 575–725 μg Retinol (1917–2417 IU) for children 10–15 years of age, 750 μg Retinol (2500 IU) for pregnant women, and 1200 μg Retinol (4000 IU) for lactating women.

The requirement for vitamin A appears to be proportional to body weight. This proportion declines rapidly following birth and increases only slightly during the adolescent growth phase. This is reflected in the RDA on a per kg body weight basis: 100, 85, 65, and 50 μg Retinol/kg at birth, 1, 3, and 5 months of age, respectively, declining to 12 μg Retinol/kg for adults.[122] Due to the wide variability of individual responses to vitamin A and reports from postmortem studies showing a substantial number of people with reduced hepatic reserves (even in developed Western countries), the NRC recommended intake is set considerably above the requirement for normal physiological vitamin A functions.[10,33] The reader is referred to Rodriguez and Irwin[123] for an in-depth review of the literature on human vitamin A requirements, including those for infants and children.

Surveys taken from 1971 to 1974 of representative samples of the United States population (all incomes and both sexes) indicated that the daily intake of vitamin A from all food sources was 4774 IU for ages 1–74 and 4500 IU for ages 20–24 years. Approximately 47% of the latter group was subsequently determined to be consuming less than 3500 IU daily.[3]

Hypervitaminosis A

The International Vitamin A Consultative Group has summarized the literature on hypervitaminosis A dating from 1850 to 1979.[124] During this period, 579 cases were described in 195 separate reports. Great differences were observed in the daily doses given and in the frequency and duration of administration associated with the onset of toxicity, indicative of the wide variability of individual tolerance to Retinol.

Acute Toxicity

Acute hypervitaminosis A frequently occurred in Eskimos and Arctic travelers who ingested polar bear or seal liver because of their high content of Retinol (13,000–18,000 IU/g).[3,88] However, acute hypervitaminosis A is now rarely seen in adults, although it is still fairly common in infants due to accidental ingestion or to unintentional overdosing by parents.[88] Acute toxicity is frequently reported when single doses of 100,000 IU vitamin A or more are given to infants or 300,000 IU or more to young children. Multiple doses of 200,000 IU orally or 100,000 IU intramuscularly on sequential days also may produce acute toxicity.[124] Symptoms usually occur within a few days after consumption and are of a transient nature, causing no permanent adverse effects.[88,124] The most prominent effects are on the central nervous system (increased cerebrospinal fluid pressure, as indicated by pseudotumor cerebri and hyperostosis in children and occipital headaches in adults), followed by gastrointestinal tract effects (anorexia, vomiting, and nausea). Other clinical symptoms have included scaling of skin, dry mucous membranes, cheilitis, hair loss, fever, fatigue, somnolence, vertigo, edema, tenderness of long bones, hepetomegaly, and splenomegaly.[124,125] Laboratory abnormalities reported include elevated plasma concentrations of Retinol and calcium and activities of alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT).[125]

Chronic Toxicity

Chronic hypervitaminosis A generally occurs when high doses, usually greater than 25,000 IU daily (~ 400 IU/kg in adults), have been administered over prolonged periods for the treatment of acne or other dermatological conditions by uninformed parents desirous of healthy children or by health food faddists.[33] The majority of documented cases of chronic hypervitaminosis A have been in children.[88]

The most prominent signs and symptoms of chronic toxicity are cutaneous, including skin scaling, erythema, pruritus, disturbed hair growth, dry mucous membranes, cheilitis, and gingivitis. Headache, nausea, anorexia, vomiting, and bone and joint pain are also consistently noted.[124,125] Other

clinical symptoms have included papillary edema, diplopia, optic atrophy, and blindness in children over long-term administration, edema, fatigue, hemorrhage, hypomenorrhea, psychiatric symptoms, hepatic dysfunction associated with hepatosplenomegaly, ascites, and hypoplastic anemia with leukopenia.[88,125,126] Laboratory abnormalities reported include radiologically detected bone changes in children, increased plasma levels of Retinol (also an elevated ratio of retinyl ester to alcohol form), calcium, alkaline phosphatase, SGOT, and lipids, increased cerebrospinal fluid pressure, and disturbed blood clotting.[33,88,125] Most of these symptoms generally disappear when the administration of vitamin A is discontinued. However, growth retardation caused by premature epiphyseal closure has been reported in children.[88,126]

According to FASEB,[3] the least adverse effect intake in humans appears to be from 700 to 3000 IU Retinol/kg/day for several months, with most estimates skewed toward 3000 IU. Dose comparison is complicated; water-miscible vitamin A, being more readily absorbed, elicits toxic symptoms at much lower doses than oil-soluble vitamin A, and it is not always clear which form of the vitamin was administered. However, daily intakes of 700–3000 IU/kg would probably be attained only through supplementation, as mean daily vitamin A intakes from the usual dietary sources are of the order of 80 IU/kg for adults and 300 IU/kg for infants. The Committee on Drugs and the Committee on Nutrition of the American Academy of Pediatrics have indicated that daily doses of 25,000 IU (400 IU/kg in an adult) or more of vitamin A pose a risk when administered over extended periods of time and should not be used except in cases of severe vitamin A deficiency.

There is some concern in developed countries that the prevalence of chronic hypervitaminosis A may increase in the future. Vitamin A is available without prescription in dosage units of 25,000 to 50,000 IU and is also used to fortify several common foods (such as margarine and milk) in the United States. Therefore, young children who are commonly given concentrated vitamin supplements and who are consuming fortified foods as well may have reserves approaching the toxicity concentration. Furthermore, new possible chemopreventive and therapeutic roles of the retinoids may lead to increased consumption of megadoses.[33,127]

Dermal Irritation and Sensitization

A moisturizer containing 1% Retinyl Palmitate was evaluated for irritation and sensitization using a modified Draize (RIPT). A 0.3 ml sample of the moisturizer was applied to the upper arm of each of 100 subjects using an occlusive patch. Patches remained in place for 24 h and were scored (max = 4) upon removal and 24 h later. Patches were applied on alternate days to the same site for a total of 10 applications. After a 2–3 week rest period, similar 24-h challenge patches were applied to the same as well as untreated sites. Reactions were graded at 24 and 48 h. Ninety-nine subjects had no signs of irritation throughout the study. One subject had well-defined erythema (score, 2 +) after the eighth application and after the ninth application on a different site. The tenth application patch was not applied to this subject, although he

did receive both challenge patches. No reactions were observed at challenge. The investigators concluded that the moisturizer was "most likely" not a primary skin irritant nor a fatiguing agent and that it may possibly be a sensitizer agent.[128] Results of dermal irritation and sensitization tests are summarized in Table 3.

A body lotion containing 0.1% Retinyl Palmitate was evaluated for irritation and sensitization using a Shelanski/Jordan RIPT. Occlusive patches were applied for 24 h to the upper back of each of 210 subjects. Patches were applied on alternate days for a total of 10 applications. Sites were examined upon patch removal (max = 4). After a 10–14 day rest period, a 48-h challenge patch was applied, and the reactions were graded upon removal. The subjects received an additional 48-h challenge patch 7–10 days later. Sites were graded at 48 and 72 h. All but 2 of the subjects had completely negative scores throughout the study. One subject had erythema and papules (score, 2) after induction patches 9 and 10; this was considered to be irritation due to occlusive patching, since the subject had no reactions to the challenge patches. The second subject had all negative scores except for a score of 2 (erythema and papules) at the 72-h reading after the second challenge. This reaction was considered to be irritant in nature, since no edema was observed. The body lotion was neither a strong irritant nor a strong contact sensitizer.[129]

TABLE 3. Dermal Irritation and Sensitization (Clinical).

Ingredient	*Method*	*No. of Subjects*	*Results*	*Reference*
Retinyl Palmitate—1% in a moisturizer	Modified Draize RIPT[a]	100	All negative scores in 99; erythema in one subject after 8th and 9th insults—no reaction at challenge; nonirritating and non-fatiguing, possibly a sensitizer	128
Retinyl Palmitate—0.1% in a body lotion	Shelanski-Jordan RIPT	210	Not a strong irritant or a strong sensitizer	129
Retinyl Palmitate—0.1% in a moisturizer	Modified Draize-Shelanski RIPT	189	Nonirritating and non-sensitizing	130
Retinyl Palmitate—0.1% in a moisturizer	Modified Draize-Shelanski RIPT	108	Nonirritating and non-sensitizing	131
Retinyl Palmitate—0.1% in a body lotion	21-day cumulative irritation test	12	Base score of 58 (max = 630); "probably mild" in normal use; slight potential for mild cumulative irritation under test conditions	132
Retinyl Palmitate—0.1% in a moisturizer	21-day cumulative irritation test with challenge	20	Base score of 0 (max = 630); essentially nonirritating; nonsensitizing	133

[a]RIPT, Repeated Insult Patch Test.

A moisturizer containing 0.1% Retinyl Palmitate was evaluated for irritation and sensitization using a modified Draize-Shelanski RIPT. Occlusive patches were applied to the upper back of each of 189 subjects for 48 h, and sites were graded upon removal (max = 4). Applications were made on alternate days for a total of 10 applications. After a nontreatment period of 10–14 days, similar challenge patches were applied for 48 h and were graded upon removal. All reactions to application and challenge patches were negative. The moisturizer did not demonstrate any potential as a primary irritant or an allergic sensitizer.[130]

Another moisturizer containing 0.1% Retinyl Palmitate was evaluated for irritation and sensitization using a modified Draize-Shelanski RIPT. Occlusive patches containing 0.1 g samples of the moisturizer were applied to the upper backs of 108 subjects. Patches remained in place for 24 h, and the reactions were graded at 48 h (max = 4). Patches were applied on Monday, Wednesday, and Friday for 3 weeks and on Monday of the fourth week for a total of 10 applications. After a 2-week nontreatment period, two challenge patches were applied to each subject: one on the original site and one on a previously unpatched site. These patches remained in place for 48 h, and the reactions were graded upon removal. All but 1 of the subjects had negative scores throughout the study. This 1 subject had all negative scores except for a score of 1 (erythema only) after the challenge patch on a new site. The moisturizer did not appear to be a primary irritant or an allergic contact sensitizer.[131]

A body lotion and a moisturizer, each containing 0.1% Retinyl Palmitate, were evaluated for cumulative irritation using panels of 12 and 20 subjects, respectively. In each test, occlusive patches containing a sample of either product were applied to the backs of the subjects for 23 h. Reactions were graded at 24 h. Patches were applied daily to the same site for 21 consecutive days. The 12 subjects receiving applications of the body lotion had a total score of 70 (max = 756) and a base ($\eta = 10$) score of 58 (max = 630). The investigators considered the body lotion to be "probably mild" in normal use, since there was evidence of a slight potential for very mild cumulative irritation under conditions of the test.[132] The panelists used for testing the moisturizer received an additional challenge patch 2 weeks after the 21st consecutive patch. These patches remained in place for 24 h, and the reactions were graded at 48 and 96 h. The subjects had a total score of 0 (max = 1260) and a base ($\eta = 10$) score of 0 (max = 630) over the 21-day test period; the moisturizer was considered essentially nonirritating. Several panelists reacted to the challenge patch of some samples (more than one product were being simultaneously tested); however, since specific results were not given, it is not known whether any panelist reacted to the challenge patch with the moisturizer containing 0.1% Retinyl Palmitate. Most of these reactions subsided by 96 h and appeared to be due to skin fatigue or primary irritation, rather than contact sensitization.[133]

One case report of contact allergy to Retinyl Palmitate has been reported. A 55-year-old woman had a melanoma on her back removed and replaced by a skin graft. She developed eczematous dermatitis after the use of several topical applications on the donor site. Patch tests with a modification of the European Standard series were positive to nickel only. The patient was already

aware that she reacted to jewelry. A pharmaceutical and cosmetical test series including 20 substances was negative. When tested with the medicaments she had used, a positive reaction was obtained only with a magistral preparation. The individual ingredients of this cream were then tested, and a positive reaction was obtained with vitamin A (Retinyl Palmitate) oily solution Merck 10^6 U/g. Further testing with pure Retinyl Palmitate 10^6 U/g without additives as well as butyl hydroxy anisole and butyl hydroxy toluene, used as antioxidants, was positive only to pure Retinyl Palmitate. The pure Retinyl Palmitate was negative in 20 other patients. The investigator noted that Retinyl Palmitate may contain traces of nickel.[134]

Jordan et al.[135] reported that two male prison volunteers who reacted positively to all-trans-retinoic acid were negative when patch tested with 0.1% Retinol or Retinyl Palmitate in petrolatum. A retest conducted 8 months after the initial testing produced the same results.

Vitamin A and Cancer

Epidemiological Studies

The majority of epidemiological studies indicate that an inverse relationship exists between cancer risk and vitamin A consumption. Most of these studies have related dietary intake of vitamin A (estimated by the frequency of ingestion of foods known to have a high content of β-carotene or preformed Retinol) to the incidence of cancer, whereas others compared serum concentration of Retinol to the incidence of cancer. Both prospective and retrospective studies have been conducted. The reader is referred to Moon and Itri,[114] Kummet et al.,[136] and Peto et al.[137] for comprehensive reviews on the epidemiology of vitamin A.

Kummet et al.[136] reviewed 30 epidemiology studies on vitamin A and cancer. These studies were all encompassing: dietary,[17] serological,[13] prospective,[5] retrospective,[25] worldwide, and for multiple tumor types. They found that only 1/13 serological studies and 2/17 dietary studies did not show an inverse relationship between serum Retinol concentrations and cancer incidence and vitamin A consumption and cancer incidence, respectively. Furthermore, it was noted that the 1 negative serological study did not use controls, and one of the two negative dietary studies reported an inverse association between the intake of carotene-containing vegetables and cancer (although no association was made between total vitamin A intake and cancer). A dose–response effect was detected in many of the studies reviewed. Interestingly, both the prospective and retrospective serological studies indicated that the lower the serum viatmin A concentration, the greater the cancer risk, even for concentration within the normal range. The relative risk of low vitamin A intake or low serum Retinol averaged approximately 2 in these studies.

However, the controversy over this issue continues. Several recent prospective studies have produced no correlation between low serum Retinol and increased incidence of lung[138] and breast cancer.[139] The latter study, however, did show that those women who developed breast cancer generally had low serum concentrations of β-carotene, although this was not statisti-

cally significant. Greenwald et al.[140] noted that the mixed evidence from studies of serum vitamin A is not surprising, since hemostatic control maintains serum Retinol within a narrow range and may not reflect what is actually in the tissues.

Munoz et al.[141] conducted a randomized double-blind intervention trial in the People's Republic of China to determine if a combined treatment with 15 mg Retinol (50,000 IU), 200 mg riboflavin, and 50 mg zinc could lower the prevalence of precancerous lesions of the esophagus. The 610 subjects were randomized to receive either the combined treatment or a placebo once a week for 13.5 months. The combined treatment did not have an effect on the prevalence of esophageal lesions, as the incidence in the treated group was 48.9% and that in the placebo group was 45.3%.

It has been difficult to discern whether the observed anticancer effects are due to Retinol, β-carotene, some other dietary constituent, or a combination of these factors.[114,136] Peto et al.,[137] in reviewing 5 prospective and 15 retrospective dietary studies, suggested that β-carotene was of primary importance. On the other hand, most of the serological studies and in vitro laboratory work suggest that Retinol and its derivatives are primary factors.[136] A number of clinical studies and trials with retinoids are in progress in the field of oncology, including a large prospective study in 20,000 United States physicians to test the hypothesis that supplementary β-carotene reduces the risk of cancer.[31,114]

Stitch et al.[142] used the frequency of micronuclei in cells scraped from inside the human cheek as a measure of chromosome breakage in earlier cell divisions and as an indicator of carcinogenic stimuli (increased chromosome breakage indicates the presence of carcinogenic stimuli). They supplemented the diet of 40 rural Filipino betel nut and tobacco chewers with a sealed capsule of Retinol (100,000 IU/week) and β-carotene (300,000 IU/week) for 3 months and found a threefold decrease in the mean proportion of cells with micronuclei in 37/40 subjects. No large increases were noted in any subject, and the mean proportion of micronuclei did not change in a control group of 11. These data suggest that an increase in the dietary intake of Retinol or carotene may reduce the incidence of oral cancer in this population.

The present recommendation of the National Academy of Sciences[143] regarding vitamin A intake for cancer prevention is as follows:

> The epidemiological evidence is sufficient to suggest that foods rich in carotenes or vitamin A are associated with reduced risk of cancer. The toxicity of vitamin A in doses exceeding those required for optimum nutrition, and the difficulty of epidemiological studies to distinguish the effects of carotenes from those of vitamin A, argue against increasing vitamin A intake by the use of supplements.

Clinical Cancer Studies

Retinol has been used as a treatment (both orally and topically) for malignant tumors since the 1930s. However, most of the early clinical trials are difficult to assess for efficacy because of small patient numbers, lack of control groups, variability in response definitions, and imprecise data reporting. An

evaluation is further complicated by the fact that most of the patients also received other therapy, including surgery, irradiation, or hormones. Even so, evidence of activity is apparent in several malignant conditions. Numerous clinical studies are ongoing in this area, although most are using analogs of vitamin A because of more favorable therapeutic indices.[114]

The reader is referred to Bollag[144] and Moon and Itri[114] for reviews of the early and current literature on clinical cancer studies, respectively.

Teratogenic and Embryotoxic Effects

Numerous incidences have been reported of fetal malformations after maternal ingestion of high concentrations of vitamin A during pregnancy. Multiple fetal malformations of the central nervous system occurred in the child of a woman who had been treated (orally) with 150,000 IU vitamin A during gestation days 19–40.[145] Urinary tract malformations were observed in infants born of two women; the first had ingested daily doses of 25,000 IU vitamin A for the first 3 months and 50,000 IU for the last 6 months of pregnancy, and the second ingested 40,000 IU daily from the 6th to the 10th week of pregnancy.[88,105] Multiple craniofacial malformations occurred in the infant of a woman who ingested a very high dose of vitamin A in the second month of gravidity.[105]

Serum samples drawn 1 week postpartum from women who delivered children with spina bifida showed significantly increased vitamin A concentrations. The livers of fetuses with nervous system malformations also had increased vitamin A concentrations. The significance of the first finding was considered unclear because of the natural variation in serum vitamin A concentrations and the lack of specific information on serum vitamin A concentration in pregnancy. However, based on these data, vitamin A was concluded to be potentially embryotoxic in humans.[145]

Geelen,[105] after comprehensively reviewing hypervitaminosis A-induced teratogenicity, concluded that there is no definite proof of the teratogenic effect of vitamin A in humans. However, the well-documented teratogenic effects of vitamin A in different species of animals and the scarce data in humans suggest that hypervitaminosis A during pregnancy may adversely affect the developing embryo and fetus. FASEB[3] has noted that the lowest estimated no-effect level in animals (2500 IU/kg in mice) is more than 25 times greater than the estimated adult human intake from food sources (approximately 100 IU vitamin A/kg/day).

SUMMARY

Retinol is the primary naturally occurring form of vitamin A. Retinyl Palmitate is the ester of Retinol and palmitic acid, also known as vitamin A palmitate. These compounds are soluble in most organic solvents and insoluble in water. Retinol and Retinyl Palmitate have ultraviolet absorption maxima in the range of 324–328 nm.

Retinol and Retinyl Palmitate are produced today largely by commercial methods in which retinyl acetate is the end product. Retinol is also still obtained by concentration from animal fats and fish liver oils.

Retinol oxidizes readily and is inactivated by ultraviolet light, giving off a characteristic green fluorescence. Retinol is relatively heat stable and is more stable in alkaline than acid solution. The acetic and palmitic esters of Retinol are commercially important because of their greater degree of stability when compared to the alcohol.

High-pressure liquid chromatography (HPLC) has become the preferred method of retinoid analysis due to the stability of retinoids on most HPLC columns, its high resolution and sensitivity, and the rapidity of most analyses.

Cosmetic uses of Retinol and Retinyl Palmitate are primarily in hair, facial makeup, and skin care preparations. Retinol and Retinyl Palmitate were used in 138 and 102 formulations, respectively, in 1981. Generally, these ingredients were used at concentrations $\leq$ 1%.

Retinol and Retinyl Palmitate are both affirmed as GRAS (generally recognized as safe) food ingredients; their functional use in foods is as nutrient and dietary supplements. They are also used for this purpose in the veterinary field. Retinol sees further use in various pharmaceutical products and in the treatment of dermatoses.

Retinol is essential for the growthy, health, and life of higher animals. It is required for vision, reproduction, and for the maintenance of differentiated epithelia and of mucous secretion. The molecular mechanisms for its biological effects are largely unknown, with the exception of its role in the visual process.

The primary natural sources of vitamin A in the diet are certain plant carotenoid pigments, particularly β-carotene, and the long-chain retinyl esters found in animal tissues. β-Carotene is converted (50% maximum) to Retinol primarily in the intestinal mucosa, although conversion also is known to occur in the liver and other tissues.

Dietary retinyl esters, Retinol, and provitamin A carotenoids are dispersed and emulsified in the stomach during the gastric phase of lipid digestion. The esters are hydrolyzed in the intestinal lumen, and the resulting Retinol, as well as that obtained from the diet, is absorbed into the mucosal cell. Here, it is reesterified with long-chain, mainly saturated fatty acids, incorporated into chylomicrons and transported via the lymph into the general circulation. The chylomicrons are metabolized in extrahepatic tissues and reduced to smaller, cholesterol-rich particles containing essentially all of the original retinyl esters. These chylomicron remnants are removed from the circulation almost entirely by the liver.

Upon uptake by the liver, the retinyl esters are hydrolyzed, reesterified, and stored in the liver, primarily as Retinyl Palmitate. Vitamin A is mobilized from these hepatic stores as Retinol bound to a specific plasma transport protein retinol-binding protein (RBP) in a highly regulated process. A specific intracellular binding protein for Retinol has also been identified and designated as cellular retinol-binding protein (CRBP).

In a number of studies, radioactive metabolites of Retinol were excreted via urinary and fecal or biliary routes. The amount excreted in the urine

depended on the position of the radioactive atom. The urinary metabolites have been only partially characterized; they are mainly water-soluble and contain no detectable free Retinol or retinyl esters. The biliary and fecal metabolites have also not been characterized, with the exception of Retinol, retinoic acid, and their conjugates. The amount of the administered dose recovered in the bile and feces varies depending on the position of the radioactivity, the mode of administration, and on the quantity administered. Some Retinol is metabolized through retinoic acid. Therefore, the well-characterized metabolites of retinoic acid would also be metabolites of Retinol.

Retinol has long been known to interact with other micronutrients, including vitamin E, ascorbic acid (vitamin C), iron, and zinc. Vitamin E is generally believed to have a nonspecific antioxidant role. It has been suggested that Retinol influences the hepatic synthesis of ascorbic acid, whereas the latter acts as an antioxidant for excess hepatic Retinol. Iron may facilitate oxidative destruction of vitamin A active compounds in the intestine, whereas the vitamin may facilitate the mobilization of stored iron and its incorporation into erythrocytes. The hepatic mobilization of Retinol may also be impaired by zinc deficiency.

In other interaction studies, gonadal steroid and adrenocortical hormones have increased the hepatic mobilization of Retinol, DDT, and other drugs as well as xenobiotics have reduced hepatic stores of Retinol. Acute and chronic ingestion of alcohol has resulted in reduced plasma concentrations and hepatic storage (chronic only) of Retinol.

The retinoids have modified the growth and differentiation of both neoplastic and nonneoplastic cells in culture. Retinoids have various effects on the activity and synthesis of cellular enzymes and effectors as well as profoundly influencing the biosynthesis of all types of glycoconjugates. Two dominant theories of retinoid mechanism exist: the proposed cofactor role of retinoids in glycosyl transfer and the proposed steroid model for retinoid control of gene expression.

In studies on physiological effects, vitamin A affected multiple parameters of thyroid function. There appears to be an inverse relationship between thyroxine and vitamin A concentrations in the plasma. Retinyl Palmitate has decreased glucose tolerance in man.

In acute oral studies, Retinol was slightly toxic to mice, whereas Retinyl Palmitate was practically nontoxic in mice and rats. Large single doses can be lethal. Retinol was considerably more toxic than Retinyl Palmitate when administered IP.

Two cosmetic products, each containing 0.1% Retinyl Palmitate, were evaluated for dermal irritation in rabbits: one was no more irritating than the control product, whereas the second was quite irritating to rabbit skin. These same two products were relatively nonirritating to rabbit eyes.

Multiple low doses (severalfold greater than required intake level) of viatmin A can be toxic to laboratory animals. Characteristic symptoms of hypervitaminosis A include weight loss, erythema, hair loss, internal hemorrhage, and fractures. Many of these effects are reversible upon cessation of administration. The time to first appearance of clinical signs depends on the route of administration, the species and age of the animal, the duration of

treatment, the sign in question, and the dose size. In a review of current literature, the lowest reported adverse effect concentration in experimental animals was in the range of 25,000 to 60,000 IU Retinol per kg per day for periods of 3–5 weeks. Water-miscible vitamin A was more toxic than oil-soluble vitamin A because it was more readily absorbed.

In specific dermal studies, topical application of Retinol to rats for periods of 10–60 days produced acanthosis and approximately doubled the thickness of the epidermis. Subcutaneous injection of Retinol for up to 60 days induced no significant effect on the epidermis of rats. Topical application of Retinol to the nipples of guinea pigs for 10 days produced an acanthotic response. A drop of bear liver oil applied to the skin of mice daily for 14 days produced signs characteristic of hypervitaminosis A. Two drops of an oily vitamin A solution applied on alternate days to the surface of guinea pig skin wounds promoted healing and produced signs of local hypervitaminosis A. A body lotion containing 0.1% Retinyl Palmitate produced a mild dermatitis in all rabbits after daily administration of 6 mg/cm^2 for 90 days. No systemic toxicity was observed.

Retinyl Palmitate did not produce any adverse effects during 10 months of oral administration to dogs and rats at doses of up to 25,000 and 50,000 IU/kg/day, 5 days per week, respectively.

Vitamin A toxicity occurs when, due to excessive intake of vitamin A, Retinol begins to circulate in the plasma in a form other than bound to RBP.

Although it is recognized that Retinol is essential for reproduction, high intake of Retinol has produced adverse effects on several reproductive functions. These include decreased sperm motility and sperm survival in rabbits as well as testicular changes and inhibition of cyclic ovulatory activity in rats.

Vitamin A has produced more than 70 types of malformations in rats, mice, hamsters, guinea pigs, rabbits, dogs, pigs, and monkeys. The type and incidence of malformations depended on the dose and stage of pregnancy and, to a lesser extent, on species and strain. Abnormalities of the face, ears, eyes, and nervous system were most commonly observed. Minor brain defects, growth disturbances, and behavioral abnormalities have also developed postnatally after in utero exposure to Retinol. Appreciable amounts of vitamin A are transferred to suckling offspring through the maternal milk.

Vitamin A was nonmutagenic in the Ames test both with and without metabolic activation. Retinol also did not increase the frequency of sister chromatid exchanges or cell cycle delay in Chinese hamster cells either with or without metabolic activation. Retinol and Retinyl Palmitate have modified the effects of established mutagens, having both an inhibitory and a stimulatory effect (at low doses only).

There is no evidence that vitamin A is carcinogenic. However, the vitamin has both enhanced and inhibited responses to viral or chemical carcinogens. Results of many studies conducted in search of therapeutic roles for retinoids have indicated that retinoids can suppress the process of carcinogenesis in laboratory animals in vivo and the development of malignant phenotypes in vitro.

Retinoids have inhibited or stimulated the immune system: High doses have effectively inhibited both humoral (antibody-mediated) and cell-medi-

ated immunity, whereas subtoxic doses have been stimulatory. Timing, dose, and mode of administration play a major role in determining the effects of the retinoids on the immune system.

The RDA of vitamin A for humans varies between scientific groups. The NRC recommends 5000 and 4000 IU daily for male and female adults, respectively, lesser amounts for infants and children, and an increased 5000 and 6000 IU daily for pregnant and lactating women, respectively. The FAO/WHO has recommended lower daily intakes for all groups: 2500 IU for adults (including pregnant women), lesser amounts for infants and children, and an increased 4000 IU daily for lactating women. The requirement for vitamin A appears to be proportional to body weight. This proportion is believed to decline rapidly following birth and increase only slightly during the adolescent growth phase. Surveys of representative samples of the United States population (1971–1974) indicated that the daily intake of vitamin A from all food sources was 4774 IU for the overall ages 1–74 years.

In a review of hypervitaminosis A in humans dating from 1850 to 1979, 579 cases were reported. These indicated a wide variability in individual tolerance to Retinol. Acute toxicity is frequently reported when single doses of 100,000 IU vitamin A or more are given to infants or 300,000 IU or more to young children. Multiple doses of 200,000 IU orally or 100,000 IU intramuscularly on sequential days also may produce toxicity. Symptoms usually occur within a few hours after consumption and are of a transient nature, causing no permanent or adverse effects. The primary effects are on the central nervous system, and the gastrointestinal tract is secondarily affected.

Chronic hypervitaminosis A generally occurs when high doses, usually greater than 25,000 IU daily, have been administered over long periods of time. The most prominent signs of chronic toxicity are cutaneous, followed by gastrointestinal and central nervous system effects. Most of these signs disappear when the administration of vitamin A is discontinued. However, growth retardation caused by premature epiphysical closure has been reported in children. The least adverse effect intake in humans appears to be from 700 to 3000 IU Retinol/kg/day for several months, with most estimates skewed toward the upper end of this range. Daily intakes at this level would probably be attained only through supplementation, since the mean daily intake from usual dietary sources are of the order of 80 IU/kg for adults and 300 IU/kg for infants.

In repeated insult patch tests, cosmetic products containing 0.1–1% Retinyl Palmitate were at most slightly irritating and nonsensitizing in a total of 607 subjects. Results of cumulative irritation tests of two products containing 0.1% Retinyl Palmitate indicated that these products are "probably mild" in normal use and essentially nonirritating and nonsensitizing, respectively. One case of contact allergy to Retinyl Palmitate has been recorded.

The majority of epidemiology studies, both prospective and retrospective, have related dietary intake of vitamin A as well as serum concentration of Retinol to the incidence of cancer. Controversy exists as to whether the observed anticancer effects are due to Retinol, β-carotene, some other dietary constituent, or to a combination of these factors. The current recommendation of the National Academy of Sciences states that the epidemiological

evidence suggests that foods rich in carotenes or vitamin A are associated with reduced risk of cancer. However, due to the toxicity of vitamin A in doses exceeding those required for optimum nutrition as well as the difficulty in distinguishing the effects of carotene from vitamin A, the NAS does not recommend increasing vitamin A intake by the use of supplements.

Retinol has been used as a treatment (both orally and topically) for malignant tumors since the 1930s. Numerous clinical studies are ongoing in this area, although most are using analogs of vitamin A because of more favorable therapeutic indices.

Several cases have been reported of fetal malformations after maternal ingestion of high doses of vitamin A during pregnancy. These included malformations of the central nervous system, urinary tract, and craniofacial area. Hypervitaminosis A during pregnancy may adversely affect the developing embryo and fetus.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the CIR Expert Panel concludes that Retinyl Palmitate and Retinol are safe as cosmetic ingredients in the present practices of use and concentration.

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